

**Microbial Perspectives  
of the Methane Cycle in Permafrost Ecosystems  
in the Eastern Siberian Arctic**

***– Implications for the Global Methane Budget –***

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## PREFACE

This thesis (Habilitation) highlights the findings of several years of research work in permafrost environments of the Siberian Arctic. The studies were carried out to gain insights into the carbon dynamics of tundra wetlands – mainly the methane fluxes – and the contribution of the microbial processes and communities to the terrestrial methane cycle. Furthermore, the response of microorganisms to the environmental conditions of permafrost was investigated. Field research and sampling were carried out during eight expeditions to the Lena Delta and Cape Mamontov Klyk at the Laptev Sea coast. Experiments and analytical work was mainly accomplished at the Alfred Wegener Institute for Polar and Marine Research (AWI), Research Unit Potsdam. The study was financed through the AWI Research Program MARCOPOLI, and by the Bundesministerium für Bildung und Forschung (BMBF) and by the Deutsche Forschungsgemeinschaft (DFG) through the following projects:

- BMBF project: Russian-German Cooperation: SYSTEM LAPTEV SEA 2000: *“Balance of Trace Gas Fluxes and Process Studies within the Methane Cycle in Permafrost Regions”* (03G0534) by E.-M. Pfeiffer
- BMBF project: Russian-German Cooperation: SYSTEM LAPTEV SEA 2000 – Synthesis: *“Terrestrial Dynamics in the Laptev Sea Region”* (03G0534) by H.-W. Hubberten
- BMBF project: Process Studies on Permafrost Dynamics in the Laptev Sea: *„Microbial Processes in Permafrost: Anaerobic Microbial Carbon Decomposition“* (03G0589) by E.-M. Pfeiffer with the assistance of E. Spiack, and D. Wagner
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The thesis is composed of three parts: In the first part (chapters 1 and 2) an *Introduction* of the research topic and an *Overview* of the different studies, their present status and my personal contribution to each publication is given.

The second part, which is organized in four thematic chapters (3-6) comprises fourteen research article relevant to the topic of the thesis. Eleven articles were published or are in press in peer-reviewed international journals, complemented by three submitted manuscripts. I am the first author of five papers. To the other articles

(two of them are two-authored papers), I have made essential contributions in the form of text passages, field work, data sets or interpretations. The references of the *Introduction* and *Synthesis* are summarized with those of the publications at the end of the thesis, in order to avoid statement of the same references several times.

The thesis is terminated with a third part (chapters 7 and 8), in which the most important findings and conclusions are summarised. In the *Synthesis* the results of the publications to the microbially driven carbon dynamics and the tolerance of the microorganisms to extreme environmental condition are combined with other studies from permafrost regions and discussed in the scope of its significance for the global methane budget. Finally, the *Conclusions* derived from the studies are presented.

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## Abstract

The Arctic plays a key role in Earth's climate system as global warming is predicted to be most pronounced at high latitudes and because one third of the global carbon pool is stored in ecosystems of the northern latitudes. In order to improve our understanding of the present and future carbon dynamics in climate sensitive permafrost ecosystems, the present study concentrates on investigations of microbial controls of methane fluxes, on the activity and structure of the involved microbial communities, and on their response to changing environmental conditions. For this purpose an integrated research strategy was applied, which connects trace gas flux measurements to soil ecological characterisation of permafrost habitats and molecular ecological analyses of microbial populations. Furthermore, methanogenic archaea isolated from Siberian permafrost have been used as potential keystone organisms for studying and assessing life under extreme living conditions.

From 1998 to 2005, eight expeditions to the Lena Delta were carried out. Field work and sampling of different permafrost soils and sediments were mainly accomplished on Samoylov Island, central Lena Delta. In particular, the objectives of the study were: (1) to measure and balance methane fluxes from tundra environments, (2) to determine the soil ecological properties, (3) to gain more insights into the control functions of microorganisms, (4) to improve the knowledge of the abundance and biodiversity of microbial communities, and (5) to determine tolerance limits of methanogens under extreme living conditions.

Long-term studies on methane fluxes were carried out since 1998. These studies revealed considerable seasonal and spatial variations of methane emissions for the different landscape units ranging from 0 to  $362 \text{ mg m}^{-2} \text{ d}^{-1}$ . For the overall balance of methane emissions from the entire delta, the first land cover classification based on Landsat images was performed and applied for an upscaling of the methane flux data sets. The regionally weighted mean daily methane emissions of the Lena Delta ( $10 \text{ mg m}^{-2} \text{ d}^{-1}$ ) are only one fifth of the values calculated for other Arctic tundra environments. The calculated annual methane emission of the Lena Delta amounts to about 0.03 Tg. The low methane emission rates obtained in this study are the result of the used remotely sensed high-resolution data basis, which provides a more realistic estimation of the real methane emissions on a regional scale. Soil temperature and near soil surface atmospheric turbulence were identified as the driving parameters of methane emissions. A flux model based on these variables explained variations of the methane budget corresponding to continuous processes of microbial methane production and oxidation, and gas diffusion through soil and plants reasonably well. The results show that the Lena Delta contributes significantly to the global methane balance because of its extensive wetland areas.

The microbiological investigations showed that permafrost soils are colonized by high numbers of microorganisms. The total biomass is comparable to temperate soil ecosystems. Activities of methanogens and methanotrophs differed significantly in their rates and distribution patterns along both the vertical profiles and the different investigated soils. The methane production rates varied between 0.3 and 38.9 nmol h<sup>-1</sup> g<sup>-1</sup>, while the methane oxidation ranged from 0.2 to 7.0 nmol h<sup>-1</sup> g<sup>-1</sup>. Phylogenetic analyses of methanogenic communities revealed a distinct diversity of methanogens affiliated to *Methanomicrobiaceae*, *Methanosarcinaceae* and *Methanosaetaceae*, which partly form four specific permafrost clusters.

The results demonstrate the close relationship between methane fluxes and the fundamental microbiological processes in permafrost soils. The microorganisms do not only survive in their extreme habitat but also can be metabolic active under *in situ* conditions. It was shown that a slight increase of the temperature can lead to a substantial increase in methanogenic activity within perennially frozen deposits. In case of degradation, this would lead to an extensive expansion of the methane deposits with their subsequent impacts on total methane budget.

Further studies on the stress response of methanogenic archaea, especially *Methanosarcina* SMA-21, isolated from Siberian permafrost, revealed an unexpected resistance of the microorganisms against unfavourable living conditions. A better adaptation to environmental stress was observed at 4 °C compared to 28 °C. For the first time it could be demonstrated that methanogenic archaea from terrestrial permafrost even survived simulated Martian conditions. The results show that permafrost methanogens are more resistant than methanogens from non-permafrost environments under Mars-like climate conditions. Microorganisms comparable to methanogens from terrestrial permafrost can be seen as one of the most likely candidates for life on Mars due to their physiological potential and metabolic specificity.

## Zusammenfassung

Die Arktis spielt eine Schlüsselrolle im Klimasystem unserer Erde aus zweierlei Gründen. Zum einen wird vorausgesagt, dass die globale Erwärmung in den hohen Breiten am ausgeprägtesten sein wird. Zum anderen ist ein Drittel des globalen Kohlenstoffs in Ökosystemen der nördlichen Breiten gespeichert. Um ein besseres Verständnis der gegenwärtigen und zukünftigen Entwicklung der Kohlenstoffdynamik in klimaempfindlichen Permafrostökosystemen zu erlangen, konzentriert sich die vorliegende Arbeit auf Untersuchungen zur Kontrolle der Methanflüsse durch Mikroorganismen, auf die Aktivität und Struktur der beteiligten Mikroorganismengemeinschaften und auf ihre Reaktion auf sich ändernde Umweltbedingungen. Zu diesem Zweck wurde eine integrierte Forschungsstrategie entwickelt, die Spurengasmessungen mit boden- und molekularökologischen Untersuchungen der Mikroorganismengemeinschaften verknüpft. Ferner sind methanogene Archaeen aus Permafrostböden isoliert worden, um sie als Modellorganismen für die Untersuchungen des mikrobiellen Lebens unter extremen Lebensbedingungen zu verwenden.

Von 1998 bis 2005 wurden acht Expeditionen in das sibirische Lenadelta durchgeführt. Die Feldarbeiten und die Beprobung unterschiedlicher Permafrostböden und -sedimente wurden hauptsächlich auf der Insel Samoylov im zentralen Lenadelta durchgeführt. Die Zielsetzungen der vorliegenden Untersuchung im Detail waren: (1) die Methanfreisetzung aus Tundren zu messen und zu bilanzieren; (2) die bodenökologischen Kenngrößen zu bestimmen; (3) detaillierte Einblicke in die Funktionen der am Methanumsatz beteiligten Mikroorganismen zu erlangen; (4) die Abundanz und Biodiversität der Mikroorganismengemeinschaften zu untersuchen und (5) Toleranzgrenzen methanogener Archaeen unter extremen Lebensbedingungen zu ermitteln.

Langzeitmessungen zu den Methanflüssen werden seit 1998 durchgeführt. Diese Untersuchungen zeigten beträchtliche saisonale und räumliche Schwankungen der Methanemissionen auf, die zwischen 0 und  $362 \text{ mg m}^{-2} \text{ d}^{-1}$  für die untersuchten Landschaftseinheiten schwankten. Für die Bilanzierung der Methanemissionen für das gesamte Delta wurde erstmals eine Klassifikation der unterschiedlichen Landschaftseinheiten anhand von Landsat-Aufnahmen durchgeführt und für eine Hochrechnung der Methandaten genutzt. Die Mittelwerte der regional gewichteten täglichen Methanemissionen des Lenadeltas ( $10 \text{ mg m}^{-2} \text{ d}^{-1}$ ) sind nur ein Fünftel so hoch wie die berechneten Werte für andere arktische Tundren. Die errechnete jährliche Methanemission des Lenadeltas beträgt demnach ungefähr 0,03 Tg. Die geringen Methanemissionsraten dieser Studie können durch den bisher noch nicht realisierten integrativen Ansatz, der Langzeitmessungen und Landschaftsklassifizierungen beinhaltet, erklärt werden. Bodentemperatur und oberflächennahe

atmosphärische Turbulenzen wurden als die antreibenden Größen der Methanfreisetzung identifiziert. Ein Modell, das auf diesen Variablen basiert, erklärt die Veränderungen der Methanflüsse gemäß der dynamischen mikrobiellen Prozesse und der Diffusion von Methan durch den Boden und die Pflanzen zutreffend. Die Ergebnisse zeigen, dass das Lenadelta erheblich zur globalen Methanemission aufgrund seiner weitreichenden Feuchtgebiete beiträgt.

Die mikrobiologischen Untersuchungen zeigten, dass Permafrostböden durch eine hohe Anzahl von Mikroorganismen besiedelt wird. Die Gesamtbiomasse ist dabei mit Bodenökosystemen gemäßigter Klimate vergleichbar. Die Stoffwechselaktivitäten von methanogenen Archaeen und methanotrophen Bakterien unterschieden sich erheblich in ihrer Rate und Verteilung im Tiefenprofil sowie zwischen den verschiedenen untersuchten Böden. Die Methanbildungsrate schwankte dabei zwischen 0,3 und 38,9 nmol h<sup>-1</sup> g<sup>-1</sup>, während die Methanoxidation eine Rate von 0,2 bis 7,0 nmol h<sup>-1</sup> g<sup>-1</sup> aufwies. Phylogenetische Analysen der methanogenen Mikroorganismengemeinschaften zeigten eine ausgeprägte Diversität der methanogenen Archaeen auf. Die Umweltsequenzen bildeten vier spezifische Permafrostcluster aus, die den Gruppen *Methanomicrobiaceae*, *Methanosarcinaceae* und *Methanosaetaceae* zugeordnet werden konnten.

Die Ergebnisse zeigen, dass die Methanfreisetzung durch die zugrunde liegenden mikrobiologischen Prozesse im Permafrostboden gesteuert wird. Die beteiligten Mikroorganismen überleben nicht nur in ihrem extremen Habitat, sondern zeigten auch Stoffwechselaktivität unter in-situ-Bedingungen. Ferner konnte gezeigt werden, dass eine geringfügige Zunahme der Temperatur zu einer erheblichen Zunahme der Methanbildungsaktivität in den ständig gefrorenen Permafrostablagerungen führen kann. Im Falle der Permafrostdegradation würde dieses zu einer gesteigerten Freisetzung von Methan führen mit bisher unbekannten Auswirkungen auf das Gesamtbudget der Methanfreistzung aus arktischen Gebieten.

Weitere Untersuchungen zur Stresstoleranz von methanogenen Archaeen – insbesondere des neuen Permafrostisolates *Methanosarcina SMA-21* - weisen eine unerwartete Widerstandsfähigkeit der Mikroorganismen gegenüber ungünstigen Lebensbedingungen auf. Eine bessere Anpassung an Umweltstress wurde bei 4°C im Vergleich zu 28°C beobachtet. Zum ersten Mal konnte gezeigt werden, dass methanogene Archaeen aus terrestrischem Permafrost unter simulierten Marsbedingungen unbeschadet überleben. Die Ergebnisse zeigen, dass methanogene Archaeen aus Permafrostböden resistenter gegenüber Umweltstress und Marsbedingungen sind als entsprechende Mikroorganismen aus Habitaten, die nicht durch Permafrost gekennzeichnet sind. Mikroorganismen, die den Archaeen aus terrestrischen Permafrosthabitaten ähneln, können als die wahrscheinlichsten Kandidaten für mögliches Leben auf dem Mars angesehen werden.

# 1 General Introduction

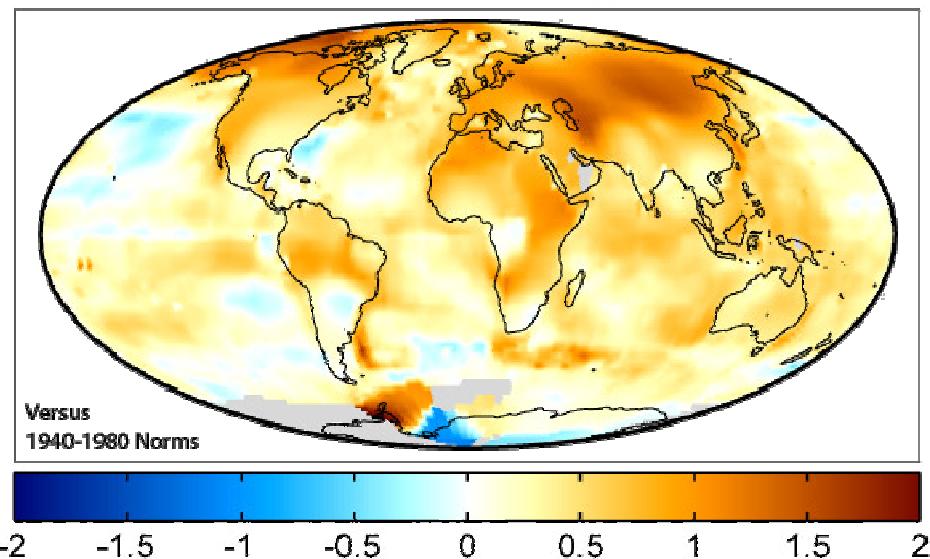
## 1.1 Significance of Permafrost Ecosystems for the Global Methane Budget

A better understanding of the terrestrial component of the global carbon cycle has become policy imperative, both nationally and worldwide. The Kyoto Protocol recognizes the role of terrestrial systems as carbon sinks and sources. Terrestrial and sub-marine permafrost is identified as one of the most vulnerable carbon pools in the Earth system (Osterkamp, 2001; Zimov et al., 2006). About one third of the global soil carbon is preserved in northern latitudes (Gorham, 1991), mainly in huge layers of frozen ground, which underlay around 24% of the exposed land area of the northern hemisphere (Zhang et al., 1999; Figure 1.1). This carbon reservoir plays a major role in the global carbon cycle, which is highlighted by currently observed climate changes in the Arctic (IPCC, 2001; Figure 1.2) and by climate models that predict significant changes in temperature and precipitation in the Northern Hemisphere (Kattenberg et al., 1996; Smith et al., 2002).



**Figure 1.1:** Permafrost distribution in the northern hemisphere (UNEP/GRID-Arendal and Landsat, 2000).

Global warming could result in a degradation of permafrost area up to 25% until 2100 (Anisimov *et al.* 1999). Thawing of permafrost could release large quantities of greenhouse gases into the atmosphere (Nelson 2003), thus further increasing global warming and transforming the Arctic tundra ecosystems from a carbon sink to a carbon source (Oechel *et al.* 1993). However, the processes of carbon release, their spatial distribution and their climate dependency are not yet adequately quantified and understood.



**Figure 1.2:** The figure shows the difference in surface temperatures between the periods 1995 through 2004 and “normal” temperatures at the same locations, defined to be the average over the interval 1940 to 1980 highlighting the immense warming of high north latitudes (modified after Robert A. Rohde, data source: Hansen *et al.*, 2001; Rayner, 2000; Reynolds *et al.*, 2002).

The world-wide wetland area has a size of about  $5.5 \times 10^6 \text{ km}^2$  (Aselman and Crutzen, 1989). About half of it is located in high-latitudes of the northern hemisphere ( $> 50^\circ\text{N}$ ). The atmospheric input of methane from tundra soils of this region has been estimated between 17 and 42 Tg CH<sub>4</sub> yr<sup>-1</sup> (Whalen and Reeburgh, 1992; Cao *et al.*, 1996; Joabsson and Christensen, 2001), corresponding to about 25 % of the methane emission from natural sources (Fung *et al.* 1991). Model calculations suggest that methane currently emitted from Arctic permafrost environments may enhance the greenhouse effect with a portion of approx. 20 % (Wuebbles and Hayhoe, 2002).

In the last decades, numerous studies on methane fluxes were focused on tundra environments in Northern America and Scandinavia (e.g. Svensson and Rosswall, 1984; Whalen and Reeburgh, 1988; Bartlett *et al.*, 1992; Liblik *et al.*, 1997; Reeburgh *et al.*, 1998; Christensen *et al.*, 2000). Since the political changes in the former Soviet Union in the early nineties, the large permafrost areas of Russia were integrated into

the circum-arctic flux studies (e.g. Christensen *et al.*, 1995; Samarkin *et al.* 1999; Panikov and Dedysh, 2000; Tsuyuzaki *et al.*, 2001). All these studies reveal temporal and spatial variability of methane fluxes, ranging between  $-1.9$  and  $360 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ . To understand these dramatic fluctuations, some studies focused on the environmental conditions and soil characteristics, comprising the water table position, soil moisture and temperature, type of substrate and vegetation as well as availability of organic carbon (e.g. Torn and Chapin, 1993; Vourlitis *et al.*, 1993; Bubier *et al.*, 1995; Oberbauer *et al.*, 1998; Joabsson *et al.*, 1999; Yavitt *et al.*, 2000). These factors influence the methane dynamics of tundra environments. Although 80 to 90% of total methane emissions originate from microbial activity (Ehhalt and Schmidt, 1978), only a few investigations dealt with methane production and methane oxidation caused by basically microbiological processes in the course of carbon dynamics (Slobodkin *et al.*, 1992; Vecherskaya *et al.*, 1993; Samarkin *et al.*, 1994; Schimel and Guldge, 1998; Segers, 1998; Frenzel and Karofeld, 2000; Wagner *et al.*, 2001).

Microbial methane production (methanogenesis) is one of the most prominent microbiological processes during the anaerobic decomposition of organic matter. Methanogenesis is solely driven by a small group of strictly anaerobic organisms called methanogenic archaea, which belong to the kingdom *Euryarchaeota* (Garcia *et al.*, 2000). They can be found either in temperate habitats like paddy fields (Grosskopf *et al.*, 1998a), lakes (Jurgens *et al.*, 2000; Keough *et al.*, 2003), freshwater sediments (Chan *et al.*, 2005) and the gastrointestinal tract of animals (Lin *et al.*, 1997), or in extreme habitats such as hydrothermal vents (Jeanthon *et al.*, 1999), hypersaline habitats (Mathrani & Boone, 1995) or permafrost soils and sediments (Kobabe *et al.*, 2004). In cold environments two main pathways of energy-metabolism dominate: (i) the reduction of  $\text{CO}_2$  to  $\text{CH}_4$  using  $\text{H}_2$  as a reductant and (ii) the fermentation of acetate to  $\text{CH}_4$  and  $\text{CO}_2$  (Conrad, 2005). However, only a few psychrophilic (cold-adapted) strains of methanogenic archaea have been described so far (Simankova *et al.*, 2003; Cavicchioli, 2006).

The biological oxidation of methane by methane oxidizing (methanotrophic) bacteria, which represent very specialized *Proteobacteria*, is the major sink for methane in terrestrial habitats. They are using methane as the sole carbon source, while energy is gained by the oxidation of  $\text{CH}_4$  to  $\text{CO}_2$ . Between 43 and 90% of the methane produced in the soil is oxidised before reaching the atmosphere (Frenzel *et al.*, 1990; Le Mer & Roger 2001, van Bodegom *et al.*, 2001). Methanotrophic bacteria are common in almost all environments, where they can survive under unfavourable living conditions by the formation of spores.

Since methanogenic archaea and methane oxidizing bacteria are sensitive to temperature variations, methane fluxes from tundra environments are expected to

increase with temperature rise (Hassol, 2004). This response would imply a positive feedback to climate change from permafrost methane sources.

Although the metabolism of both groups of microorganisms is well studied, little is known about their impact on carbon fluxes in Arctic environments, about the role of microbial diversity for the functioning and stability of the system and about the reaction of these microorganisms to changing environmental conditions. Hence, the knowledge on current trace gas fluxes, their control by the microbial communities, and their reaction to environmental change is crucial for understanding current carbon dynamics and the prediction of the future development of permafrost environments as a source of greenhouse gases.

## 1.2 Astrobiological Aspects of Permafrost Environments

Apart from the global relevance of permafrost as a large carbon reservoir, this extreme environment is also of particular interest in the scope of astrobiological research as an analogue for extraterrestrial permafrost habitats, which is a common phenomenon in our solar system.

Terrestrial permafrost is characterised by extreme environmental conditions, such as sub-zero temperatures, aridity, and long-lasting levels of back-ground radiation as a result of an accumulation over geological time scales. Despite these harsh conditions, terrestrial permafrost is colonized by high numbers of chemotrophic bacteria as well as microbes such as methanogenic archaea (Shi et al., 1997). Because of the specific adaptations of methanogens to conditions like on early Earth (e.g. no oxygen, no or less organic compounds) and their phylogenetic origin, they are considered as one of the most probable model organisms for life in extraterrestrial permafrost such as on Mars.

In our solar system Mars is considered as one of the most similar planets to Earth (Goldsmith and Owen, 1980), even if Mars is characterized by extreme coldness and dryness today. Various paleo-climate models of the early Mars showed that prior to 3.8 Ga, Mars was characterised by moderate temperatures, the presence of liquid water and an anoxic atmosphere comparable to those conditions on early Earth (Durham et al., 1989; McKay et al., 1992), where the evolution of microorganisms had already started (Schopf, 1993). Assuming that early life developed on Mars as well, Martian life must have adapted to drastically changing environmental conditions or became extinct, whereas living conditions remained suitable for life on Earth. The present Mars is characterized by extreme dryness, permafrost and a temperature regime which ranges between  $-123^{\circ}\text{C}$  and  $+20^{\circ}\text{C}$ . One possibility for survival of Martian microorganisms might be the presence of subsurface lithoautotrophic ecosystems like deep sediments. Comparable environments exist in polar regions on earth, described e.g. in Antarctic ice cores of several thousand meter depth (Abyzov

et al., 1998) and in Siberian permafrost cores of about 50 meter depth (Gilichinsky et al., 1993), where microorganisms exist independently of photosynthetic energy production. These microorganisms derive their energy by chemical reactions, oxidizing H<sub>2</sub>S, CH<sub>4</sub> or reduced nitrogen compounds. Compared to these ecosystems, an adaptation of life on Mars in combination with a retreat in niches under the surface does not seem any longer impossible.

One of the most important biophysical requirements for life is water. Actually, the mid- to low-latitudes of Martian surface at present may include up to 10 wt% of water (Feldman et al., 2002). Furthermore, the current mission of the European Space Agency (ESA) *Mars Express* has for the first time demonstrated the presence of methane in the Martian atmosphere (Formisano, 2004). On Earth methane is formed by methanogenic archaea from carbon dioxide and hydrogen, which are also compounds of the Martian atmosphere (Krasnopolsky and Feldman, 2001). Further data obtained by *Mars Express* showed that surface water or ice and methane gas are concentrated in the same regions of the Martian atmosphere (European Space Agency, 2004). This finding may have important implications for the possibility that microbial life, possibly similar to methanogenic archaea on Earth, could exist on Mars. To evaluate the hypothesis of present life on Mars, the tolerance of methanogenic archaea to extreme environmental conditions and their response to Martian thermo-physical conditions is under focus of astrobiological research.

### 1.3 Objectives and Research Strategy

The main objective of this thesis is to fill fundamental gaps in our knowledge on carbon dynamics in Arctic permafrost ecosystems and the involved microbial communities. In particular, the study focuses on the microbial controls of methane fluxes as well as on the activity and structure of the archaeal and methanotrophic communities and their response to changing environmental conditions. For this purpose an integrated research strategy was applied, which connects trace gas flux measurements to soil ecological characterisation of the different habitats and microbiological/molecular ecological analyses of microbial communities. Furthermore, methanogenic archaea isolated from Siberian permafrost have been used as potential keystone organisms for studying life under Martian permafrost conditions.

To achieve the objectives, the following topics have been identified and specific approaches have been chosen for detailed analyses:

1. **Balance of methane emissions:** The quantification of the methane fluxes from tundra environments and their seasonal variability provides the basis for any balance of this important greenhouse gas. To study methane fluxes on the ecosystem scale and to identify the main biological and physical parameters which control trace gas fluxes from permafrost environments, high-resolution

eddy covariance measurements were established on Samoylov Island (chapter 3.1). Closed chambers were used to study the importance of the vegetation for the transport of methane from the soils to the atmosphere (chapter 3.2). The results of the trace gas flux studies have been used in combination with a land cover classification to estimate the methane emission for the entire Lena Delta (chapter 3.3). In order to be able to study the effect of climatic change on carbon dynamics, long-term studies on methane fluxes have been carried out on Samoylov Island since 1998. The evaluations of these data are still under progress.

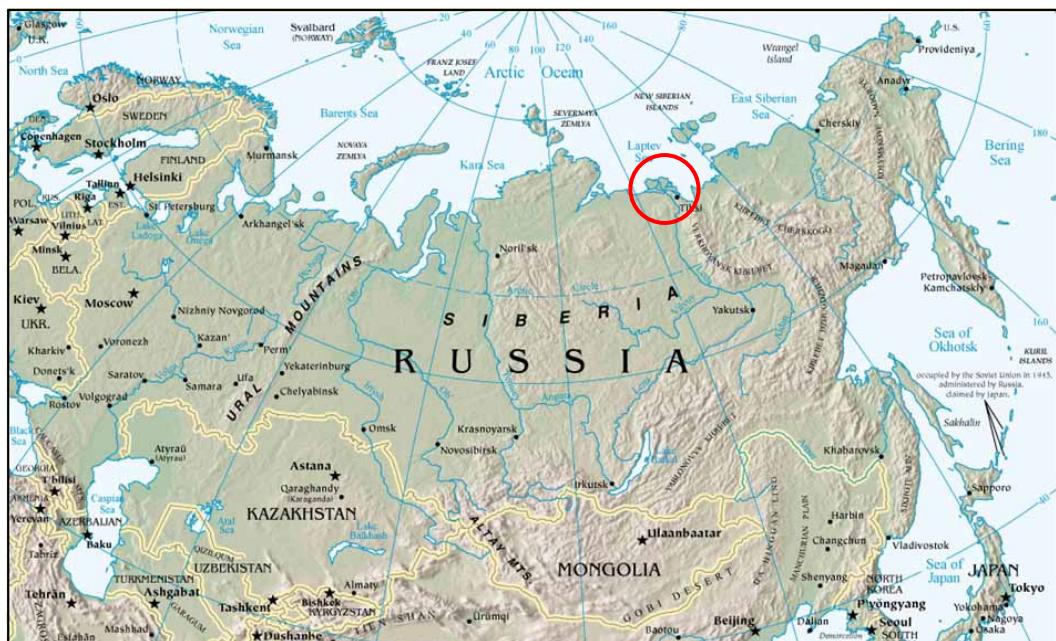
2. **Microbial processes of the methane cycle:** For the understanding of the annual and seasonal variability of the methane fluxes, the habitat properties and the microbial processes were characterized for different soil profiles at representative localities. As one of the main controlling factors for the microbial methane production and oxidation the redox potential was determined and redox sensitive elements (Mn, Fe, P) were analysed along a transect from the elevated rim to the depressed center of the polygon (chapter 4.1). The activity of the involved microorganisms were analysed under *in situ* conditions at the same sites (chapter 4.2). Special emphasis was placed on the abundance and potential activity of methanotrophs as the only sink for methane in permafrost ecosystems (chapter 4.3). For the prediction of the future contribution of permafrost environments to the global methane budget, the activity and biomass in perennially frozen ground was studied (chapter 4.4). On basis of the obtained data, a conceptional model was developed which shows the proportion of the different processes responsible for the methane fluxes in the active layer and its changes during the season (chapter 7.3).
3. **Microbial community structure:** Carbon mineralization in tundra environments is carried out exclusively by highly specialized microorganisms such as methanogenic archaea and methane oxidizing bacteria. To estimate the impact of global warming on these microorganisms and for the prediction of their reaction under changing environmental conditions, a detailed knowledge of the structure and composition of the microbial communities is needed. The population of active microorganisms was characterized by fluorescence *in situ* hybridisation (FISH) and by phospholipid (PLFA, PLEL) profiling for the main study site (chapters 5.1 and 5.2). On basis of these data the composition of the methanogenic community was studied by 16S rRNA gene fingerprints in three different permafrost soils (chapter 5.3)
4. **Microbial life under extreme conditions:** Permafrost in polar regions is characterized by extreme environmental condition. In order to preserve their viability the microorganisms had to develop strategies to resist salt stress,

physical damage by ice crystals and long-lasting background radiation. From the astrobiological point of view, terrestrial permafrost is considered to be a model for Martian permafrost and methanogenic archaea are suitable model organisms for studying life under Martian conditions (chapter 6.1). For the investigation of the methane formation within the active layer, which is characterized by large gradients in temperature and geochemistry during the annual freezing thawing cycles, a permafrost microcosmos was developed (chapter 6.2). Methanogenic archaea isolated from Siberian permafrost were used to study their tolerance limits under unfavourable living conditions (chapter 6.3) and their survival under simulated Martian conditions (chapter 6.4). The results showed that methanogenic archaea from permafrost can be seen as one of the most likely candidates for life on Mars (chapter 7.4).

## 1.4 Study Area

Samoylov Island (Siberia), the main study site of this thesis, is located within the Lena Delta, which represents the largest delta of the circum-arctic land masses. The Lena Delta is located at the Laptev Sea coast between the Taimyr Peninsula and the New Siberian Islands (Figure 1.3).

The delta occupies an area of about 29,000 km<sup>2</sup> (Schneider, 2005) and is characterized by a network of smaller and larger rivers and channels as well as more than 1500 islands. The delta can be divided into three geomorphologically different terraces (Are and Reimnitz, 2000; Schwamborn et al., 2002): (i) the oldest terrace was formed in the middle to late Pleistocene and is fragmentarily exposed (30–55 m a.s.l.) in the southern part of the delta. The terrace consists of ice-complexes containing fine-grained silty sediments with a high content of segregated ice. The ice-complex moreover includes enormous layers of organic-rich material and less decomposed peaty material, (ii) Arga Island, the western part of the delta (20–30 m a.s.l.) is characterized by coarse-grained sandy sediments and an abundant of deep lakes, which were formed in the late Pleistocene to late Holocene, (iii) the eastern terrace formed since the early Holocene is the currently still active part of the Lena Delta. This terrace is covered by several modern flood plains (1–12 m a.s.l.). The landscape of the delta is characterized by the patterned ground of ice-wedge polygons in different development stages (Müller, 1997). The entire delta is situated in the zone of continuous permafrost with a thickness of about 500–600 m (Zhang et al., 1999).

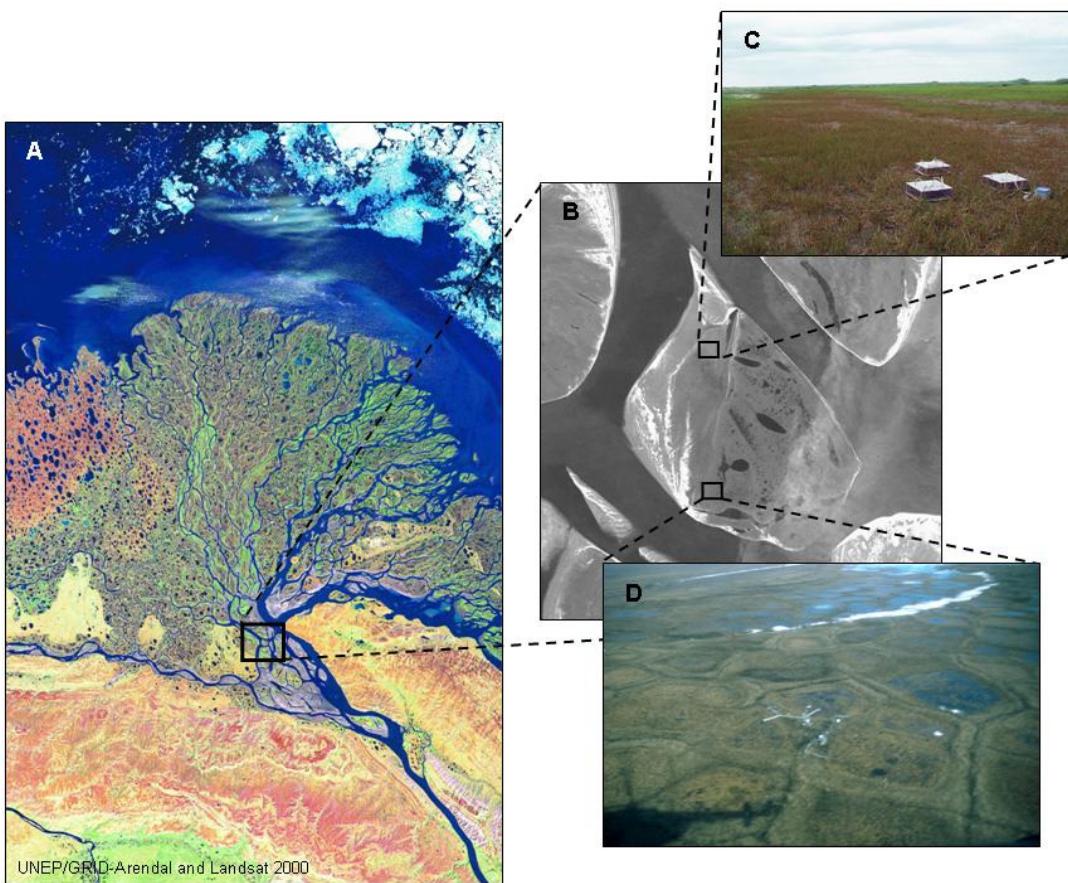


**Figure 1.3:** Map showing the Lena Delta (red cycle) located at the Laptev Sea coast between the Taymyr Peninsula and the New Siberian Islands (modified after Indo-European Documentation Center, The University of Texas at Austin).

Samoylov is a representative island in the active and youngest part of the Lena Delta and covers an area of about 5 km<sup>2</sup> (Figure 1.4 A and 1.4 B). The western coast of the island is characterized by modern accumulation processes (fluvial and aeolian sedimentation). Three flood plains can be distinguished (e.g. Figure 1.4 C), which differ in their flooding frequency and vegetation coverage. The texture of accumulated sediments is dominated by the sand fraction (fine to medium).

The soil of the flood plain site in the north of the island is classified as *Typic Aquorthel*, according to the US Soil Taxonomy (Soil Survey Staff, 2003). The periodically flooding (once a year) of the site causes a water level near the soil surface. Therefore, the soil is anoxic with the exception of the upper soil horizon (Ah). The vegetation is dominated by *Arctophila fulva*. This plant is characterized by a large aerenchyma, which is responsible for the oxygen transport into the rhizosphere.

In contrast to the floodplain site, the eastern coast of Samoylov is dominated by erosion processes, which form an abrasion coast. This part is composed of middle to early Holocene deposits, which cover about 70% of the total area of the island. Most studies on trace gas fluxes (CH<sub>4</sub> and CO<sub>2</sub>) and microbial processes and community structures were carried out within this terrace, which is dominated by active ice wedges with low-centered polygons (Figure 1.4 D). The topography is determined by this patterned ground and shows a distinct micro-relief of polygon rims and polygon centers (Figure 1.5).

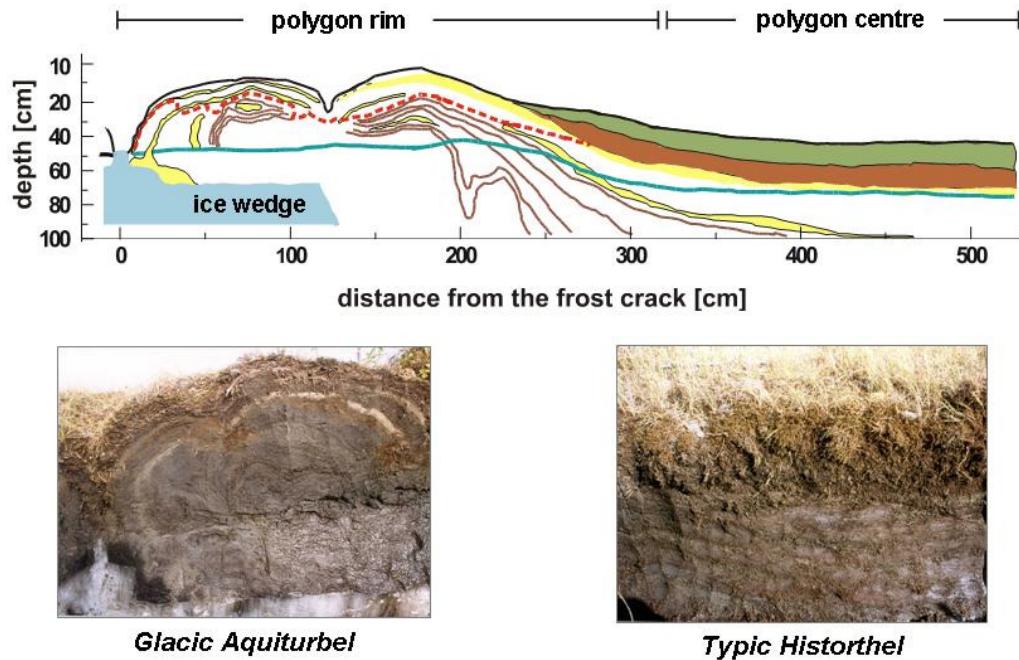


**Figure 1.4:** The Lena Delta (A) with the location of the investigation area on Samoylov Island (B; 72°22'N / 126°29'E) and a view from the helicopter on the long-term study sites (C, D).

Soil and vegetation characteristics show great variation over small distances owing to the geomorphological situation of the polygonal tundra (Fiedler et al., 2004; Kutzbach et al., 2004). The soils of the this site are characterized by very homogeneously spread soil units: the polygon rims are dominated by *Glacic Aquiturbels*, whereas the prevalent soil type of the polygon depressions are *Typic Historthels*. The peaty soils of the polygon depression are characterized by a water level near the soil surface and the predominantly anaerobic accumulation of organic matter. The drier soils of the polygon rim show a distinctly deeper water level, lower accumulation of organic matter and pronounced cryoturbation properties. The vegetation of the polygon rim is dominated by the dwarf shrub *Dryas punctata* and the mosses *Hylocomium splendens* and *Timmia austriaca*, whereas the polygon depression is dominated by hydrophytes like various *Carex* species and different moss species (e.g. *Limprichtia revolvens*, *Meesia longiseta*). In August, the thaw depth of the soils amounted to 30 and 45 cm in the rim and in the depression, respectively.

The dry continental Arctic climate of the southern Lena Delta with the Island Samoylov is characterized by a low mean annual air temperature (-14.8 °C) and a summer precipitation of about 200 mm (HMCR, 2004). The winter season lasts nine

months, from the end of September to the end of May ( $T_{\text{avg}} = -30 \text{ }^{\circ}\text{C}$ ,  $T_{\text{min}} = -48 \text{ }^{\circ}\text{C}$ ) with insufficient light (polar night) and heavy snowstorms ( $140 \text{ km h}^{-1}$ , Wein 1999). The summer period of almost 12 weeks is characterized by warm temperatures ( $T_{\text{avg}} = 7 \text{ }^{\circ}\text{C}$ ,  $T_{\text{max}} = 18 \text{ }^{\circ}\text{C}$ ) and by permanent light (polar day).



**Figure 1.5:** Cross-section of a typical low-center ice-wedge polygon on Samoylov Island. The photos show the typical soils for the polygon rim and center, respectively, on the main study site (photos L. Kutzbach, AWI).

## 1.5 Expeditions

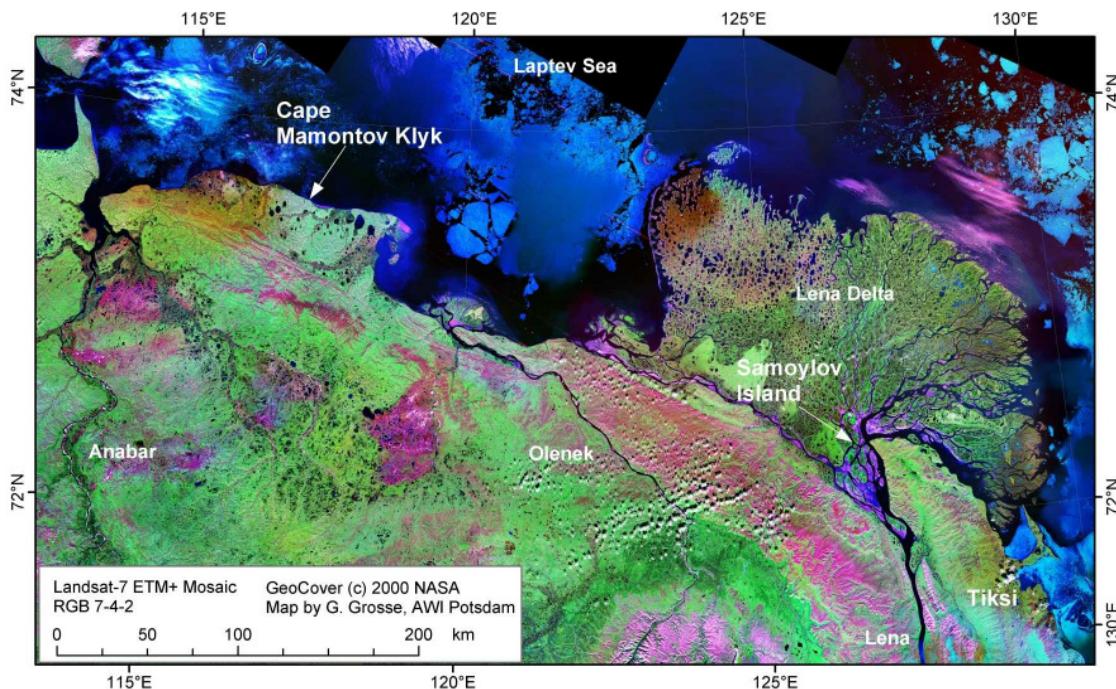
From 1998 to 2005, eight expeditions to the Lena Delta and the Laptev Sea coast were carried out. Most of the investigations presented in this study were accomplished in the context of the expeditions to Samoylov Island, Lena Delta. Additional studies on methanogenesis were done in the framework of the expedition LENA-ANABAR 2003 on Cape Mamontov Klyk (Figure 1.6).

The accomplished work during the different expeditions focused on various aspects of the methane cycle in permafrost environments ranging from habitat characteristics to microbial diversity and function. In the following the main research programs of the individual expeditions are briefly presented:

- During the first expedition **LENA DELTA 1998** (Rachold and Grigoriev, 1999) the long-term study site for methane flux measurements was established on Samoylov Island. This site is located in an area of low-centered ice-wedge polygons, which are typical patterned ground for the Siberian Arctic. Each five

stainless steel frames were permanently installed as a base for the closed chamber flux measurements and a catwalk was built, in order to protect the study area against influences during the measurements (chapter 3).

- The main objectives of the expedition **LENA 1999** (Rachold and Grigoriev, 2000) were the analyses of methane emission from permafrost soils for the main vegetation period and the plant-mediated transport of methane to the atmosphere. These investigations are still on-going. Furthermore, the soils of the long-term study site were classified according to the US Soil Taxonomy (Soil Survey Staff, 1998) and samples for geochemical and physical characterization had been taken (chapters 3 and 4).



**Figure 1.6:** Geographical map of the Laptev Sea coast with the location of the study sites.

- In the course of the expeditions **LENA 2000** (Rachold and Grigoriev, 2001) and **LENA 2001** (Pfeiffer and Grigoriev, 2002), the microbial processes of methane production and oxidation were studied under field conditions within the active layer of the long-term study site. Moreover, during LENA 2001 expedition the first drilling campaign was accomplished with the main focus on Holocene permafrost deposits on Samoylov Island (chapters 4 and 5).
- A second drilling campaign focussing on Late Pleistocene permafrost deposits was carried out during the expedition **LENA 2002** (Grigoriev et al., 2003) on Kurungnakh Island (manuscript in preparation). Furthermore, samples for studying methane oxidation were taken on Samoylov Island (chapter 4).

- In the scope of the expedition **LENA-ANABAR 2003** (Schirrmeister et al., 2004) soil samples for studying the diversity of the methanogenic community were taken from Cape Mamontov Klyk and from Samoylov Island (chapter 5).
- The expedition **LENA 2004** (Wagner and Bolshiyanov, 2006) was the first campaign within the scope of the DFG project *Tolerance Limits of Methanogenic Life in Terrestrial Permafrost* (WA 1554/1-1) contributing to astrobiological research. Different stress experiments were carried out and samples for further studies on the response of methanogenic archaea under simulated Martian conditions had been taken (chapter 6).
- During the expedition **LENA 2005** (Schirrmeister and Wagner, 2007), field experiments were devoted to the activity, diversity and stability of the methane producing and oxidizing communities were accomplished and samples for further molecular ecological analyses of both groups of microorganisms had been taken (chapter 4).

## 2 Overview of the Publications

In the following chapters (3-6) recently published results to the microbial methane cycle in permafrost ecosystems in the Eastern Siberian Arctic are presented in form of altogether fourteen publications. The manuscripts were arranged into four thematic chapters as followed:

### Chapter 3: Methane Release from Siberian Tundra Environments

This chapter shows published results of the long-term analyses of methane emission from polygonal tundra and the contribution of the plant-mediated transport of methane from the soil to the atmosphere.

- 3.1 Wille C., Kutzbach L., Sachs T., **Wagner D.** and Pfeiffer E.-M. (2007) Methane emission from Siberian Arctic polygonal tundra: eddy covariance measurements and modeling. *Global Change Biology*, submitted.

*The paper was mainly written and compiled by Wille under the assistance of the coauthors. The concept of this study was developed by Kutzbach and Pfeiffer. Wagner coordinated the fieldwork during the expeditions LENA-ANABAR 2003 and LENA 2004 and contributed with valuable discussion about the effect of the microbial processes on the methane fluxes.*

- 3.2 Kutzbach L., **Wagner D.** and Pfeiffer E.-M. (2004) Effects of microrelief and vegetation on methane emission from wet polygonal tundra, Lena Delta, Northern Siberia. *Biogeochemistry* 69, 341-362.

*The paper was mainly written and compiled by Kutzbach under the assistance of the coauthors. The concept of this study was developed by Wagner (90%) and partially interpreted (20%). Pfeiffer contributed with valuable discussion to the interpretation of the results.*

- 3.3 Schneider J., Grosse G. and **Wagner D.** (2007) Land cover classification of tundra environments in the Arctic Lena Delta based on Landsat 7 ETM+ data and its application for upscaling of methane emissions. *Remote Sensing of Environment*, submitted.

*The paper was mainly written and compiled by Schneider in the scope of her diploma thesis under the assistance of the coauthors. The concept of this study*

*was developed by Wagner (80%) and partial interpreted (20%). Grosse assisted during the processing of the satellite images (40%) and contributed with valuable discussion to the interpretation of the classification.*

## Chapter 4: Permafrost Ecosystems and Their Microbial Processes

Publications in this chapter describe the microrelief and soils of low-center ice-wedge polygons, which are typical patterned ground in the Siberian Arctic. The different soils as the habitat for the microorganisms were geochemically and physically characterized. Furthermore, the microbial processes of methane production and oxidation were studied by activity measurements under *in situ* conditions and cell counting.

- 4.1 Fiedler S., **Wagner D.**, Kutzbach L. and Pfeiffer E.-M. (2004) Element redistribution along hydraulic and redox gradients of low-centered polygons, Lena Delta, Northern Siberia. *Soil Science Society of America Journal* 68, 1002-1011.

*The paper was mainly written by Fiedler under the assistance of Wagner (text 20%, illustrations 40%, interpretation 30%). Furthermore, Wagner carried out the redox measurements and the sampling during the expeditions LENA 1999 and 2000, and provided geochemical and physical analyses (60%). Kutzbach provided data on soil and vegetation characteristics. Pfeiffer was leader of the project.*

- 4.2 **Wagner D.**, Kobabe S., Pfeiffer E.-M. and Hubberten H.-W. (2003a) Microbial controls on methane fluxes from a polygonal tundra of the Lena Delta, Siberia. *Permafrost and Periglacial Processes* 14, 173-185.

*Wagner was responsible for the field laboratory and carried out most of the field work (80%). Furthermore, text and compilation of the data (100%), figures (100%) and interpretation (80%). Coauthors were partly involved in the field work and assisted in the interpretation of the data.*

- 4.3 Liebner S. and **Wagner D.** (2007) Abundance, Distribution and Potential Activity of Methane Oxidizing Bacteria in Permafrost Soils from the Lena Delta, Siberia. *Environmental Microbiology* 9, 107-117.

*The paper was mainly written and compiled by Liebner in the scope of her PhD project under the supervision of Wagner. Furthermore, Wagner contributed with*

*the description and sampling of the floodplain soil during the expedition LENA 2002, geochemical data (10%) and the interpretation of the data (30%).*

**Wagner D.**, Gattinger A., Embacher A., Pfeiffer E.-M., Schloter M. and Lipski A. (2007) Methanogenic activity and biomass in Holocene permafrost deposits of the Lena Delta, Siberian Arctic and its implication for the global methane budget. *Global Change Biology*, accepted <sup>#</sup>.

*Wagner was responsible for the sample preparation, geochemical, physical and microbial analyses as well as text (90%), illustration (100%) and interpretation (80%). Gattinger provided total lipid extractions and PLEL analyses, text (10%), interpretation (20%) and statistics (90%). Lipski carried out PLFA analyses. Embacher was responsible for organic matter analyses and Pfeiffer for the permafrost drilling during the expedition LENA 2001. Schloter contributed with valuable discussion to the interpretation of the results.*

## Chapter 5: Microbial Community Structure in Permafrost Ecosystems

Publications in this chapter report studies on the abundance and biodiversity of microbial communities involved in the anaerobic decomposition of organic carbon in different permafrost ecosystems. The microbial communities were phenotypical and genotypical characterized by culture-independent methods.

5.1 Kobabe S., **Wagner D.** and Pfeiffer E.-M. (2004) Characterization of microbial community composition of a Siberian tundra soil by fluorescence *in situ* hybridization. *FEMS Microbiology Ecology* 50, 13-23.

*The paper was mainly written and compiled by Kobabe in the scope of her PhD project under the supervision of Pfeiffer. Wagner initiated this study (100%), advised the field work during the expedition LENA 2001 (80%) and significantly contributed to the discussion of the results (40%).*

5.2 **Wagner D.**, Lipski A., Embacher A. and Gattinger A. (2005) Methane fluxes in permafrost habitats of the Lena Delta: effects of microbial community structure and organic matter quality. *Environmental Microbiology* 7, 1582-1592.

*Wagner was responsible for the field work, analyses of the microbial activity, text (80%), illustrations (75%), and interpretation (80%). Gattinger provided total lipid*

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<sup>#</sup> since the submission of the thesis the paper is published in *Global Change Biology* 13, 1089-1099 (2007)

*extractions and PLEL analyses, text (20%) and interpretation (20%). Lipski carried out PLFA analyses and illustration (25%). Embacher was responsible for organic matter analyses.*

- 5.3 Ganzert L., Jurgens G., Münster U. and **Wagner D.** (2006) Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. *FEMS Microbiology Ecology* #, doi: 10.1111/j.1574-6941.2006.00205.x 59

*The paper was written, compiled and illustrated by Ganzert (50%) and Wagner (50%). The concept of this study was developed by Wagner (70%). Activity measurements and DNA-based analyses were performed by Ganzert. Jurgens and Münster provided the phylogenetic trees.*

## **Chapter 6: Methanogenic Archaea as Model Organisms for Life in Extreme Habitats and Their Relevance for Astrobiological Research**

Publications in this chapter deal with methanogenic archaea from Siberian permafrost as model organisms for possible life in extraterrestrial permafrost like on Mars. The tolerance against different environmental stress (low temperature, high salinity, radiation, starvation, desiccation) was tested. Furthermore, the survival of methanogenic archaea from permafrost and non-permafrost habitats was analysed under simulated Martian thermo-physical conditions.

- 6.1 **Wagner D.**, Speck E., Bock E. and Pfeiffer E.-M. (2001) Microbial life in terrestrial permafrost: Methanogenesis and nitrification in gelisols as potentials for exobiological processes. In: Horneck, G. & Baumstark-Khan, C. (eds.): *Astrobiologie-the quest for the conditions of life*, Springer-Verlag Berlin, 143-159.

*The paper was mainly written and compiled (90%) as well as illustrated (60%) by Wagner. Results on methanogenesis were provided by Wagner, while results on nitrification were delivered by Speck. Bock and Pfeiffer contributed with valuable discussion.*

- 6.2 **Wagner D.**, Wille C., Kobabe S. and Pfeiffer E.-M. (2003b) Simulation of freezing thawing cycles in a permafrost microcosm for assessing microbial methane production under extreme conditions. *Permafrost and Periglacial Processes* 14, 367-374.

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# the paper is now published in *FEMS Microbiology Ecology* 59, 476-488 (2007)

*The paper was mainly written and compiled (90%) as well as illustrated (60%) by Wagner. The field measurements on methane emission as well as the methane production activity was analysed by Wagner. Wille was responsible for the technical realization of the permafrost microcosms, text (10%) and illustration (40%). Kobabe provided the preliminary test sequence with the microcosm and Pfeiffer contributed with valuable discussion.*

- 6.3 Morozova D. and **Wagner D.** (2007) Stress response of methanogenic Archaea from Siberian permafrost compared to methanogens from non-permafrost habitats. *FEMS Microbiology Ecology* <sup>#</sup>, awaiting acceptance.

*The paper was mainly written and compiled by Morozova in the scope of her PhD project under the supervision of Wagner. Wagner initiated this study (100%), was mainly involved in the enrichment and isolation of methanogenic archaea from Siberian permafrost soils (70%) and significantly contributed to the discussion of the results (30%).*

- 6.4 Morozova D., Möhlmann D. and **Wagner D.** (2006) Survival of methanogenic archaea from Siberian permafrost under simulated Martian thermal conditions. *Origin of Life and Evolution of Biospheres*\*, doi: 10.1007/s11084-006-9024-7.

*The paper was mainly written and compiled by Morozova in the scope of her PhD project under the supervision of Wagner. Wagner was mainly involved in the enrichment and isolation of methanogens from permafrost soils (70%) and significantly contributed to the discussion of the results (30%). Möhlmann made the Mars simulation facility available.*

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# since the submission of the thesis the paper is published in *FEMS Microbiology Ecology* 61, 16-25 (2007)

\* the paper is now published in *Origin of Life and Evolution of Biospheres* 37, 189-200 (2007)

### 3 Methane Release from Siberian Tundra Environments

Global Change Biology, submitted

#### 3.1

##### Methane emission from Siberian Arctic polygonal tundra: eddy covariance measurements and modeling

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##### Abstract

Eddy covariance measurements of methane flux were carried out in an arctic tundra landscape in the central Lena River Delta at 72 °N. The measurements covered the seasonal course of mid-summer to early winter in 2003 and early spring to mid-summer in 2004, including the periods of spring thaw and autumnal freeze back. The study site is characterized by very cold and deep permafrost and a continental climate with a mean annual air temperature of -14.7 °C. The surface is characterized by wet polygonal tundra, with a pronounced micro-relief consisting of depressed water-logged and raised moist to dry sites. We found relatively low fluxes of typically 30 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> during mid-summer and identified soil temperature and near-surface turbulence as the driving parameters of methane emission. A model based on these variables explained variations of methane flux corresponding to continuous processes of microbial methane generation and oxidation, and diffusion through soil and plants reasonably well. Transitory processes related to spring thaw and turbulence- and pressure-induced ebullition were estimated to contribute about 10 % to the measured flux. The relationship found between methane flux and soil temperature was extrapolated to estimate the methane emission during the winter. Based on this estimate, the annual methane flux was 3 g m<sup>-2</sup>. This is low compared to values reported for similar ecosystems. Reason for this were thought to be (a) the very low permafrost temperature in the study region, (b) the sandy soil texture and low bio-availability of nutrients in the soils, and (c) the high surface coverage of moist to dry micro-sites. The methane emission accounted for about 13 % of the annual ecosystem carbon balance. Considering the global warming potential of methane, the methane emission turned the tundra into an effective source of greenhouse gases.

*Keywords:* methane, eddy covariance, tundra, carbon balance

##### Introduction

Approximately 24 % of the Northern Hemisphere's exposed land area is underlain by permafrost (Zhang *et al.*, 1999). These permafrost affected landscapes store about one third of the global organic

carbon pool near the surface (Gorham, 1991). Because of the high sensitivity of high-latitude ecosystems to climate changes, as well as their large proportion of the earth surface, these landscapes are critically important for the Earth System, in particular for the global carbon cycle (Chapin *et al.*, 2000).

Arctic tundra environments account for 13-15 % of the global organic soil carbon pool (Post *et al.*, 1982) and are estimated to form the largest single source of methane, contributing about 20 % of the annual natural emissions (Fung *et al.*, 1991; Cao *et al.*, 1996; Christensen *et al.*, 1996). With the growing concern about climate change and the need to quantify emissions on a large scale, the greenhouse gas (GHG) budget of arctic wetlands have come into the focus of attention. Because methane has a 23-fold global warming potential compared to carbon dioxide (time horizon of 100 years; Houghton *et al.*, 2001), it has a strong influence on the GHG budgets of these landscapes (Friborg *et al.*, 2003; Corradi *et al.*, 2005). Furthermore, global climate models rely on predictions of future GHG concentrations, which require the ability to accurately model sinks and sources of methane as a powerful greenhouse gas.

However, there is still much uncertainty about the source strength and the driving forces of methane flux of tundra landscapes. Existing studies of high latitude methane fluxes were mostly based on the closed-chamber technique. Due to the high temporal and spatial variability of methane fluxes (Christensen *et al.*, 1995; Christensen *et al.*, 2000; Wagner *et al.*, 2003; Kutzbach *et al.*, 2004), this technique alone does not give reliable information on landscape scale fluxes. In addition, during chamber measurements the soil surface is isolated from the atmosphere so that the coupling of atmosphere and methane emission can not be studied. The eddy covariance technique provides non-intrusive spatially integrated flux data at the landscape scale. However, to our knowledge only three studies reported eddy covariance methane flux data from arctic tundra ecosystems, namely Fan *et al.* (1992) from Alaska, Friborg *et al.* (2000) from Greenland, and Hargreaves *et al.* (2001) from Finland.

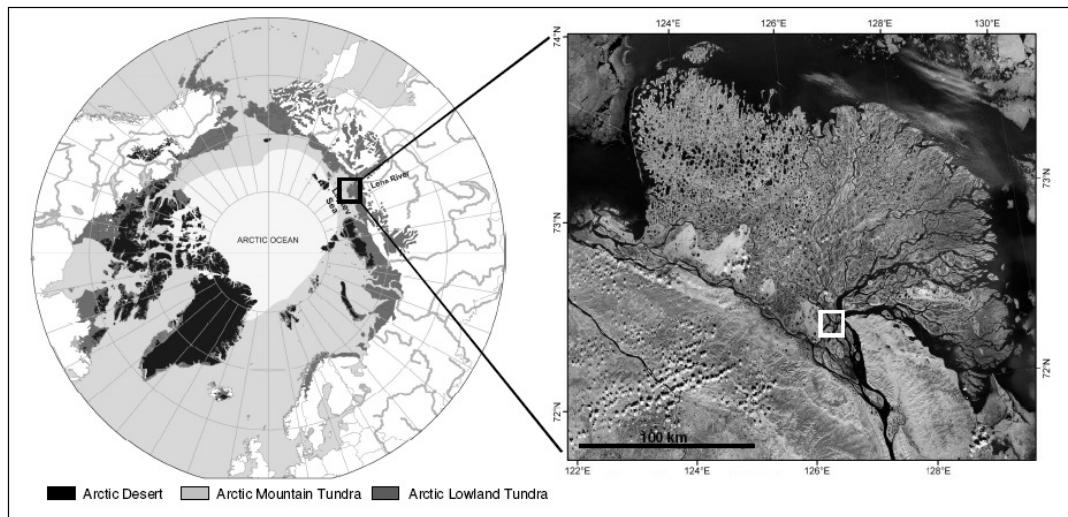
Here, we present the first eddy covariance methane flux data from a Siberian Arctic tundra landscape. The objective of this study was to quantify the methane emission over the full course of the “active” season from early spring to early winter, to analyze the contribution of different parts of the vegetation period, particularly spring thaw and soil re-freeze, to identify the biological and physical parameters which control the methane fluxes, and to estimate the annual methane emission. Together with the fluxes of carbon dioxide, which were measured concurrently and analyzed elsewhere (Kutzbach, 2006), a complete picture of the GHG budget of the tundra was gained.

## Material and methods

### Study site

The investigation site was located on Samoylov Island in the Lena River Delta at 72° 22'N, 126° 30'E (Fig. 1). The Lena River Delta is located in the zone of continuous permafrost with permafrost depths of 500 - 600 m (Zhang *et al.*, 1999; NSIDC, 2003) and permafrost temperatures between -11 and -13 °C (Kotlyakov and Khromova, 2002). Samoylov Island is situated in the southern central part of the river delta, approximately 120 km south of the Arctic Ocean. The central delta region has a dry continental arctic climate, which is characterized by very low temperatures and low precipitation. The 30-year (1961-1999) averages of annual air temperature and precipitation measured at the meteorological station in Tiksi about 110 km east of Samoylov Island are -13.6 °C and 319 mm, respectively (ROSHYDROMET, 2004). Data from the meteorological station on Samoylov Island from the period 1999-2005 showed a mean annual air temperature of -14.7 °C and a highly variable total summer precipitation (rain) between 72 and 208 mm (mean 137 mm; Boike and Wille, 2006). The polar night lasts from November 15 to January 28, and the polar day from May 7 to August 7. The snow melt typically starts at around the beginning of June and the growing season typically lasts from the middle of June to the middle of September. During spring, summer and autumn, the weather in the

central delta region is characterized by the rapid change between the advection of arctic cold and moist air masses from the north and continental warm and dry air masses from the south.

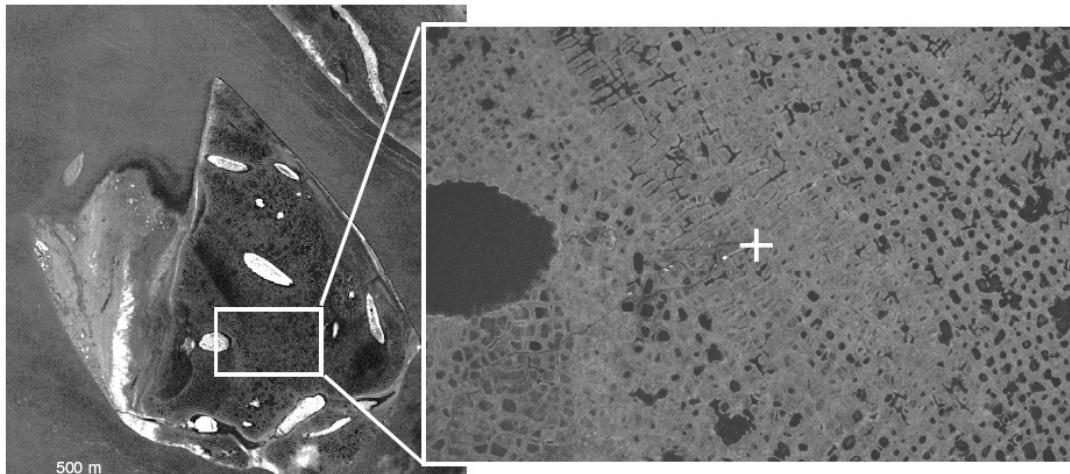


**Fig. 1** Overview map of the investigation area. Left: Vegetation zones in the Arctic (modified after UNEP/GRID-Arendal, CAFF 1996). Right: Satellite image of the Lena River Delta (Landsat 7 ETM+ GeoCover 2000, NASA); the location of the investigation area Samoylov Island is marked by a white square.

The flux measurements were carried out on the eastern part of Samoylov Island (Fig. 2), which is characterized by wet polygonal tundra. It represents the Late-Holocene river terrace which is one of the main geomorphological units in the Lena River Delta, occupying about 65 % of the total delta area (Grigoriev, 1993; Are and Reimnitz, 2000). The elevation of the eastern part of Samoylov Island ranges from 10 to 16 m a.s.l. During the annual spring flood only the low lying lakes in the south-eastern part of the island are flooded. The macro-relief of the island is level with slope gradients < 0.2 %. Larger elevation differences up to 2.5 m occur only along the shorelines of the large lakes. However, the surface of the terrace is structured by a regular micro-relief with elevation differences of up to 0.5 m within a few meters distance, which is caused by the genesis of low-centered ice wedge polygons (Washburn, 1979; French, 1996; Meyer, 2003). In the depressed polygon centers, drainage is impeded by the underlying permafrost, hence the soils are water-saturated and small ponds frequently occur. In contrast, the elevated polygon rims are characterized by a moderately moist water regime. The typical soil types are Typic Historthels in the polygon centers and Glacic or Typic Aquiturbels at the polygon rims (Soil Survey Staff, 1998). The vegetation in the polygon centers and at the edge of ponds is dominated by hydrophytic sedges (*Carex aquatilis*, *Carex chordorrhiza*, *Carex rariflora*) and mosses (e.g. *Limprichtia revolvens*, *Meesia longisetosa*, *Aulacomnium turgidum*). The vegetation on polygon rims is dominated by mesophytic dwarf shrubs (e.g. *Dryas octopetala*, *Salix glauca*), forbs (e.g. *Astragalus frigidus*) and mosses (e.g. *Hylocomium splendens*, *Timmia austriaca*). For more information on soil types and vegetation of the polygonal tundra on Samoylov Island see Pfeiffer *et al.* (2002), Kutzbach *et al.* (2003, 2004), and Fiedler *et al.* (2004). Aerial photography in July 2003 and subsequent surface classification showed that the surface fraction taken by elevated dry sites (polygon rims) and depressed wet sites (polygon centers and troughs) in the area surrounding the flux tower was about 60 % and 40 %, respectively (Wille, 2004; Schneider *et al.*, 2006).

During the last years, Samoylov Island has been the focus of several studies in the field of microbiology, soil science, and surface-atmosphere fluxes of carbon, energy and water (Hubberten *et al.*, 2006). Since 1998, meteorology and soil data has been measured nearly continuously on a monitoring site on the southern part of the island and has been used in studies of the energy and water balance of the permafrost soils (Boike *et al.*, 2003; Boike and Wille, 2006). Since 1999, fluxes of methane have been measured on the same monitoring site using the closed-chamber technique and

investigated with respect to the driving soil and vegetation parameters (Wagner *et al.*, 2003; Kutzbach *et al.*, 2004). For the assessment of the microbial activity as the driver of methane fluxes, process studies of methane production and oxidation and molecular-biological studies of the microbial community structure were carried out (Kobabe *et al.*, 2004; Wagner *et al.*, 2005; Liebner and Wagner, 2006; Ganzert *et al.*, 2006). Finally, a high-resolution time series of the fluxes of energy, water, and carbon dioxide over a full growing season, measured concurrently with the here described methane fluxes, were presented by Kutzbach (2006).



**Fig. 2** Satellite and aerial images of the investigation site. Left: CORONA satellite image of Samoylov Island taken during the spring flood in June 1964. Right: Aerial image of the central part of Samoylov Island, taken in July 2003. The position of the micrometeorological tower is marked by (+).

#### *Experimental set-up*

Eddy covariance measurements of methane flux were carried out in the periods 19 July -22 October 2003 (96 days), and 31 May - 21 July 2004 (52 days). The eddy covariance system was set up at a central position of the eastern part of Samoylov Island (Fig. 2). Wet polygonal tundra of the river terrace extended for 600 m around the tower, with several large lakes protruding into the periphery of the otherwise relatively homogeneous fetch area. The wind vector and sonic temperature were measured with a three-dimensional sonic anemometer (Solent R3, Gill Instruments Ltd., UK) which was mounted on top of a 3 m aluminum tower. The effective measurement height was 3.65 m. From a sample intake 15 cm below the anemometer measurement point, the sample air was pulled at a rate of 20 L min<sup>-1</sup> through a CO<sub>2</sub>/H<sub>2</sub>O gas analyzer (LI-7000, LI-COR Inc., USA), a membrane gas dryer (PD-200T-48SS, Perma Pure Inc., USA), and the methane gas analyzer, all of which were housed in a temperature regulated case at the foot of the tower. The methane gas analyzer was a tunable diode laser spectrometer (TGA100, Campbell Scientific Inc., USA). The concentrations of methane, carbon dioxide, and water vapor were output as an analog signal and digitized by the anemometer at a rate of 20 Hz. Data was logged using a laptop PC running the software EdiSol (J. Massheder, Univ. of Edinburgh, UK).

The tower was equipped with additional instruments for the measurement of air temperature and relative humidity (MP103A, ROTRONIC AG, Switzerland), incoming and outgoing solar and infrared radiation (CNR1, Kipp and Zonen B.V., The Netherlands), and barometric pressure (RPT410, Druck Messtechnik GmbH, Germany). Measurements of the water level were carried out at 3 points in the vicinity of the flux tower at intervals of 1 - 3 days. Precipitation, snow height, and soil temperature data was taken from the long-term monitoring station, which is situated about 700 m south-west of the flux tower (Boike and Wille, 2006). The thaw depth was measured by probing the soil with a steel rod

at 150 regularly spaced grid points near the long-term monitoring station at intervals of 3 to 7 days (Kutzbach *et al.*, 2004a).

#### *Data processing*

Analysis of raw data and calculation of turbulent fluxes were done using the software EdiRe (R. Clement, University of Edinburgh, UK). The averaging interval for the calculation of fluxes was set to 60 minutes. This is twice the value commonly used in flux studies. However, choosing such a long averaging interval was necessary to increase the signal-to noise ratio of the correlation calculation, which was frequently low due to the relatively low methane emission and high wind velocities. Two coordinate rotations were performed on the wind components measured by the sonic anemometer, so that the mean transverse and vertical wind components were reduced to zero for each averaging period (McMillen, 1988). The mean absolute value of the angle of the second rotation was  $1.0 \pm 0.9$  degrees; hence the error introduced to turbulent fluxes by the rotation should be well below 10 % for most measurements (Foken & Wichura, 1996). For each averaging period, the time lag between wind and methane concentration measurements was determined and removed from the concentration time series. Before the calculation of fluxes, a recursive high pass filter with a filter constant of 10 s (cut-off wavelength 63 s) was applied to the methane concentration time series. This effectively removed strong signal intensities at wavelengths  $> 50$  s in the spectra of the methane concentration signal which were attributed to the effects of instrument drift and instationary conditions. A correction was applied to the calculated methane flux to account for the mismatch of the frequency spectrum of the turbulent flux and the spectral response of the measurement system. In detail, the correction compensated for the effects of the spectral response of the gas analyzer, the separation of the anemometer and gas analyzer sampling points, the gas sampling through the tube, and the detrending filter (Moore *et al.*, 1986; Moncrieff *et al.*, 1997). On average, 41 % were added to the calculated flux, of which typically about 23 % and 13 % were related to the effect of the spectral response of the gas analyzer and the strong high pass filtering of the methane concentration data, respectively.

The calculated flux data was screened thoroughly. The cross correlation function of vertical wind and methane concentration was used to determine if the measurement was disturbed by excessive noise. Data points were discarded if any peaks in the cross correlation function greater than the flux peak occurred. The standard deviation of the cross correlation function at time shifts between 100 and 200 seconds was used to estimate the measurement error. This method accounts for the gaussian error of the individual measurements as well as the uncertainty in the stationarity during the averaging period (Kormann *et al.*, 2001). On average, the error calculated using this method was  $1.3 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ . Finally, the data was screened using an integral turbulence characteristics test (Foken & Wichura, 1996). In 2003, altogether 34 % of the measurements were rejected using the criteria named above. This value is similar to data coverage rates of studies of ecosystem fluxes of carbon dioxide, for instance in the EUROFLUX network (Falge *et al.*, 2001). In 2004, due to technical problems during the first half of the measurement campaign the rejection rate was about 72 %.

A footprint analysis following Schuepp *et al.* (1990) was carried out for the assessment of the fetch area size of the flux measurements. The 80 % cumulative footprint, i.e. the upwind distance from which 80 % of the observed methane flux originated, was on average 508 m during the combined measurement periods. The point of origin of the maximum contribution to the measured flux was on average 113 m.

#### *Flux modeling*

In order to identify the factors which control the methane flux, the relationship between the flux and environmental variables was studied. Because the time scale of interest was a full year, daily averages of all data were used rather than the measured hourly data. As a first step, the Pearson coefficient of correlation between methane flux and environmental variables was calculated. Variables which showed a strong correlation or had been identified by previous studies as important drivers of methane flux were studied more closely and included in a modeling approach similar to the one conducted by

Friborg *et al.* (2000). The underlying idea of this approach is the existence of an ecosystem reference flux which is associated with a set of average environmental variables. The actual flux is then a product of the reference flux and a regulation factor specific for each environmental variable. Hence, the general form of the equation used for fitting measured data to environmental variables was

$$FCH_4 = FCH_{4,ref} b^{(T-T_{ref}/10)} f_1(X_1) f_2(X_2) \dots \quad (1)$$

In equation (1)  $FCH_4$  is the time series of methane flux, and  $FCH_{4,ref}$  is the reference flux determined through the fit process. The first exponential term with  $b$  as fit parameter is the flux regulation factor for soil temperature  $T$  and is based on the well-described dependency of soil microbiological activity on temperature (e.g. Conrad, 1989). The terms  $f(X)$  describe the regulation of the flux by environmental variables  $X$ , where  $f$  can be a linear or exponential function term. For the fit process, a weighting factor of  $1/(\sigma_{FCH_4})^2$  was applied to the methane flux, where  $\sigma_{FCH_4}$  is the daily mean of the errors of the hourly flux data points.

## Results

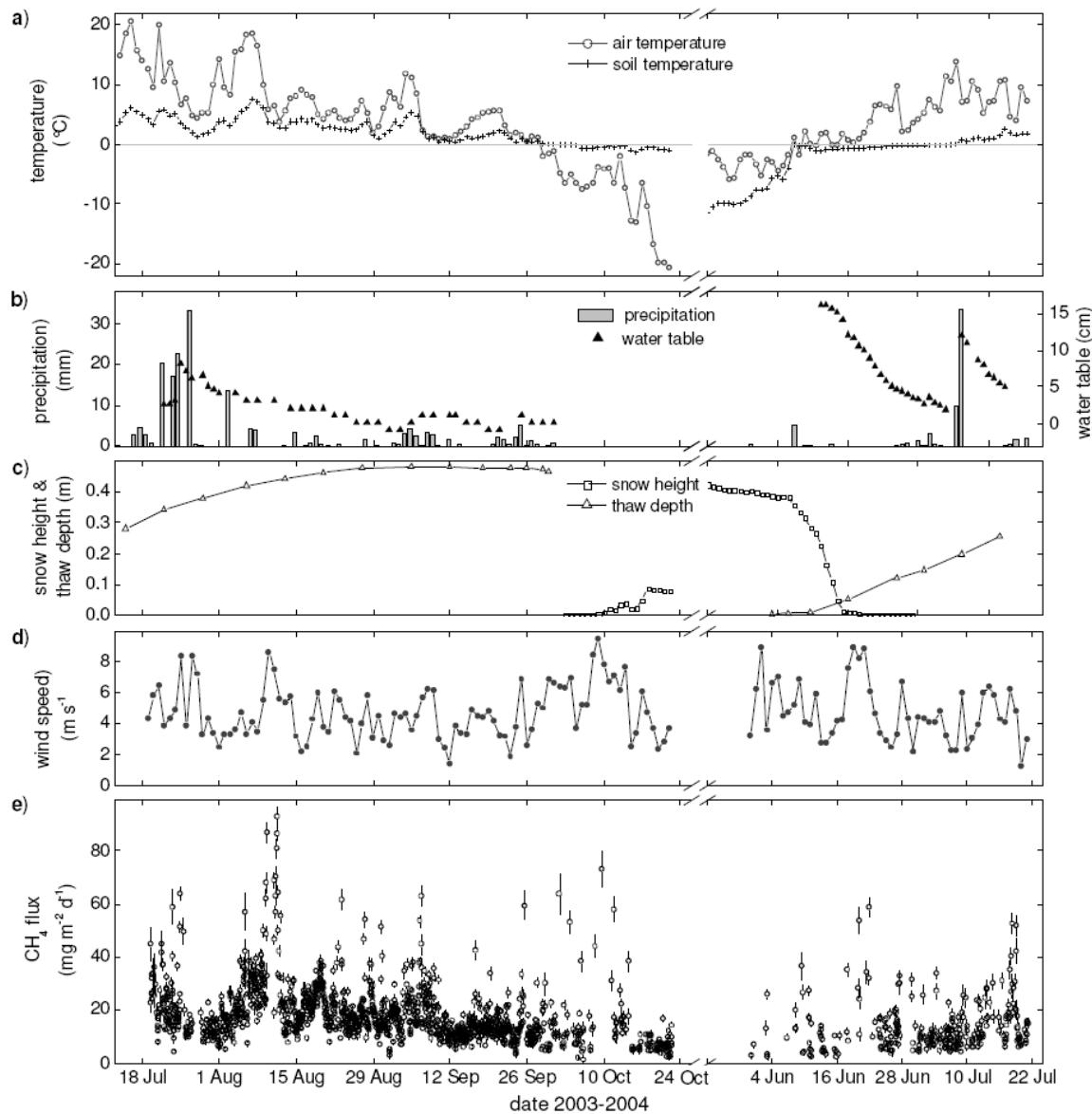
### *Meteorological conditions*

The summer and autumn of 2003 were characterized by above-average temperatures and precipitation (Fig. 3). Advection of warm continental air from the south leads to unusually high temperatures during the middle of July, at the beginning of August and during large parts of September. This leads to a considerable delay of the freeze-back of the soils. The daily average soil temperature measured at 0.15 m depth at a semi-moist micro-site reached a maximum of 7.6 °C on 7 August. From there it declined slowly until the isothermal state of the thawed layer was reached and re-freeze of the soil began on 30 September. At the end of the measurement campaign the soil temperature at 0.15 m depth was around -1 °C. Soil thaw depth was 0.3 m at the beginning of measurements in 2003 and reached a maximum of 0.48 m at the beginning of September. No measurements of thaw depth could be carried out after September 30 due to the freezing of the top soil layer. However, the temperature profile measurements showed that the soil was not completely frozen until after the middle of November. At 168 mm, the total amount of rainfall during the measurement period was exceptionally large. A great part of the rainfall occurred within one week at the end of July (94 mm), which caused the water table in the investigated polygons close to the eddy tower to rise well above the soil surface. Following a slow decrease, the water level stayed within ± 1 cm of the soil surface after the end of August. Snow started to accumulate at the beginning of October. By the end of the measurement campaign, the snow cover had reached a height of 0.15 - 0.25 m in the polygon centers and just a few centimeters on the polygon rims. The daily average wind speed over the measurement campaign 2003 was 4.7 m s⁻¹. There was no single predominant wind direction; however, wind directions east-north-east, south, and south-west occurred more frequently than other directions (data not shown).

When methane flux measurements started on 31 May 2004, the ground at the eddy tower site was completely covered with snow. The snow height had already started to decrease but was still 0.4 - 0.5 m in the polygon centers and about 0.1 m on the polygon rims. The daily average air temperature was in the range -5 to -2 °C, and the soil temperature in 15 cm depth was -10 °C. The snow thaw period started on 8 June with the occurrence of the first significant rainfall and the air and soil temperatures reaching 0 °C. The snow height decreased rapidly, and the polygon rims were largely free of snow after 2 days. Snow thaw in the polygon centers continued until 18 June. Towards the end of the snow thaw, the thaw of the soils and of polygon ponds and lakes started. Thawing of polygon ponds lasted until about 25 June, and the thaw depth of the soils increased by about 5 cm per week. At the end of the measurement campaign, daily average air and soil temperatures (15 cm depth) were around 8 °C and 2 °C respectively, and the soil thaw depth had reached about 30 cm (linear extrapolation from measurements). The water table in the polygon centers was generally higher than in 2003 and never

fell below the soil surface. The total rainfall up to 21 July was 60 mm. The average wind speed of the measurement period in 2004 was  $4.7 \text{ m s}^{-1}$ . Unlike 2003, there was a clear dominance of easterly winds, followed by winds from north-westerly directions (data not shown).

#### *Methane flux*



**Fig. 3** Data of measurement campaigns 19 July - 22 October 2003 and 31 May - 21 July 2004. (a) daily averages of air temperature at 2 m above ground and soil temperature at a semi-moist micro-site at 0.15 m depth, (b) daily sum of precipitation (rain only), and water table with respect to soil surface in a depressed polygon center, (c) daily average of snow height in a depressed polygon center, and soil thaw depth, (d) daily average of wind speed from sonic anemometer at 3.65 m above ground, (e) hourly methane flux as measured by eddy covariance. Air temperature data before 19 July 2003 and 31 May 2004 was taken from the long-term monitoring station.

The long-term variations of methane flux roughly followed the variations in soil temperature. However, there were great short-term variations in flux which partly correlated with wind speed. During days with strong wind, the methane flux could rise by a factor of 3 compared to fluxes measured during calm periods directly before and after, as for instance on 10 August 2003 (Fig. 3). In the first week of measurements in 2003, methane fluxes were on average  $23 \text{ mg m}^{-2} \text{ d}^{-1}$ . During the

following cold and rainy period, the fluxes dropped markedly but subsequent warming and further thawing of soils lead to the highest fluxes of on average  $30 \text{ mg m}^{-2} \text{ d}^{-1}$  being measured during the second week of August. From the middle of August until 10 September, the methane flux stayed at a high level of on average  $20 \text{ mg m}^{-2} \text{ d}^{-1}$ . Afterwards, methane fluxes started to decrease slowly. Also variations in flux decreased. No marked influence of the freezing of the top soil layer at the end of September on the methane flux was visible. Average fluxes measured during the first week of October and during the last week of measurements, when snow had accumulated on the ground, were  $13$  and  $7 \text{ mg m}^{-2} \text{ d}^{-1}$ , respectively.

**Table 1** Pearson coefficient for correlation between  $\text{CH}_4$  flux and environmental parameters during the combined measurement period 2003-2004

soil temperature depth (m)	r	friction velocity r	thaw depth r	water table r
0.15	0.52	0.67	0.22	-0.07

The average methane flux measured during the thaw period up to 18 June was  $10 \text{ mg m}^{-2} \text{ d}^{-1}$ . However, the variation in the flux data during this time was large. Low flux values of about  $4 \text{ mg m}^{-2} \text{ d}^{-1}$  occurred frequently throughout this period, but at the beginning of the snow melt methane fluxes of about  $30 \text{ mg m}^{-2} \text{ d}^{-1}$  were repeatedly measured. The high variability of the fluxes continued until about 28 June. After this date, the fluxes stabilized at a low level and increased slowly, following the increase of soil temperature. The average flux until the end of measurements on 21 July was  $14 \text{ mg m}^{-2} \text{ d}^{-1}$ .

**Table 2** Input and model parameters for the combined period 2003-2004 using equation 2

$T_{ref}$ (°C)	$u^*_{ref}$ ( $\text{m s}^{-1}$ )	a ( $\text{mg m}^{-2} \text{ d}^{-1}$ )	b	c	$R^2$
1.35	0.28	$14.44 \pm 0.11$	$3.14 \pm 0.10$	$11.88 \pm 1.03$	0.75

The analysis of methane flux data and environmental data showed a strong correlation between methane flux and friction velocity and soil temperature (Table 1). A good agreement ( $R^2 = 0.75$ ; cf. Table 2) with measured data of the combined period 2003-2004 was found when the daily mean methane flux was modeled using the equation

$$FCH_4 = a b^{((T-T_{ref})/10)} c^{(u^*-u^*_{ref})} \quad (2)$$

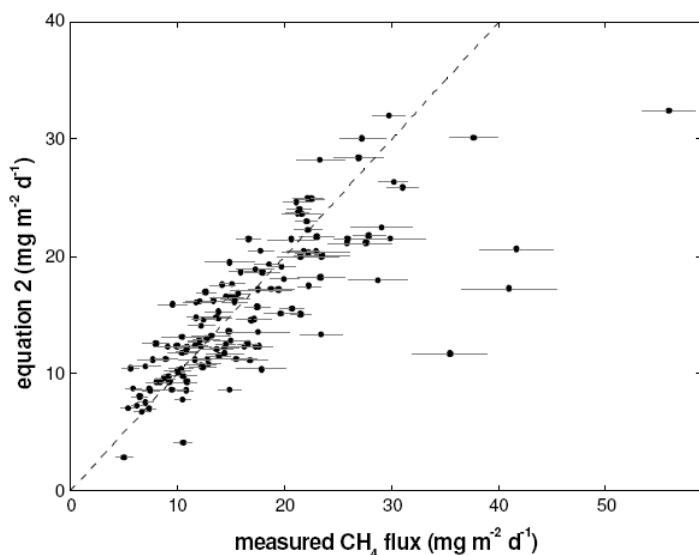
where  $T$  is the soil temperature at a semi-moist micro-site at  $0.15 \text{ m}$  depth,  $u^*$  is the friction velocity, and  $T_{ref}$  and  $u^*_{ref}$  are the mean values of the respective variables during the measurement period. There was only a very weak correlation between methane flux and thaw depth or water table position, and expanding the model to include these variables did not improve the fit. Measured fluxes and those modeled by equation (2) agree reasonably well over the whole range of measured flux values (Fig. 4), however, there is a relatively large number of residuals  $> 5 \text{ mg m}^{-2} \text{ d}^{-1}$ , i.e. where the model significantly underestimates the measured fluxes. This is better illustrated by Figure 5 which shows the time series of measured and modeled daily methane flux. Significant underestimation of the measured data by the model correlated with events of increased wind speed after calm periods (e.g. 9 and 19 August 2003, 25 September 2003) and with events of very low air pressure values near or below  $100 \text{ kPa}$  (e.g. 27 August, 6 September, and 13 October 2003, 27 June 2004, air pressure data not shown). The model also strongly underestimated methane fluxes on days with a mean wind speed of more than  $7 \text{ m s}^{-1}$ , and during the thaw period.

By gap-filling the measurements using equation 2, the cumulative methane emission over the combined measurement periods was calculated to be  $2.4 \text{ g m}^{-2}$ .

## Discussion

### Drivers of methane flux

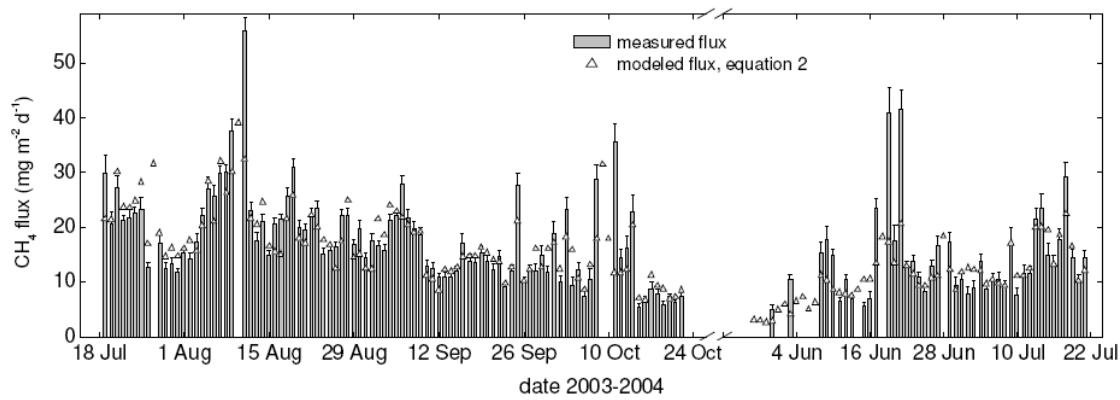
The combined measurement periods covered a whole vegetation period from spring thaw to refreeze of the soils and thus the most active period of methane emission. The wide range of environmental conditions covered allowed a detailed study of the driving forces of the methane flux. One of the two important parameters controlling methane emission was the soil temperature. The dependence of methane flux on soil temperature followed an exponential function. This reflects the fundamental dependence of (soil) microbiological activity on temperature and was confirmed by numerous studies of methane emission using closed chamber or eddy covariance techniques (e.g. Nakano *et al.*, 2000; Christensen *et al.*, 2001; Hargreaves *et al.*, 2001).



**Fig. 4** Modeled flux (equation 2) versus measured mean daily methane flux ( $N = 135$ ). The error bars are daily means of standard deviations of hourly flux data points.

The turbulence in the near-surface boundary layer was the second important driving factor of the methane flux in the polygonal tundra. Hargreaves *et al.* (2001) found a close relation between momentum flux and methane emission, but only for short periods up to one day. No other study was found to report a similar effect. There are two explanations for a dependence of methane flux on turbulence. One possible way of action of turbulence is to increase the fraction of methane which is bypassed around the oxidation process. The regulation of methane emissions by the balance of methane generation and oxidation in the soil was studied and described for wetland ecosystems by many authors (e.g. Panikov *et al.*, 2001; Wagner *et al.*, 2003). The bypass around the oxidation process is realized via plant-mediated transport of methane from deep soil layers directly to the atmosphere (e.g. Schütz *et al.*, 1991). Using the chamber technique, methane transport via *Carex aquatilis* was shown to account for between 27 and 66 % of overall methane emissions at the polygonal tundra on Samoylov Island (Kutzbach *et al.*, 2004). The same study suggested that diffusion across the dense root exodermes was the limiting factor of the gas transport. Increased turbulence could accelerate the gas exchange between the above-surface part of plants and the atmosphere, cause higher concentration gradients across the root exodermes and thus enhance diffusion across this interface. Increased turbulence could also lead to a better aeration of upper soil layers and dense moss layers. The result is a higher concentration gradient in the soil, leading to a higher diffusive flow of methane and hence a decrease of the fraction of methane oxidized by bacteria. These processes could be characterized as continuous, i.e. they should be consistent and proportional to the turbulence measured near the surface.

Another possible way of action of turbulence is the triggering of ebullition of small methane bubbles from water-logged vegetated areas or little ponds. Hargreaves *et al.* (2001) observed ebullition during periods of high wind speed following calm spells. Ebullition of small methane bubbles could also be caused by a sharp decline of air pressure. This mechanism has been suggested by other authors, e.g. Frolking and Crill (1994), and was observed during a detailed study of methane emission from lakes on Samoylov Island in 2002 (Spott, 2003). However, both effects are probably not continuous, because the ebullition of bubbles is thought to happen much faster than their generation by methanogenesis. Due to this transitory nature of ebullition, increased methane emission associated with ebullition events can not be expected to be adequately described by the model used in this study. This is supported by the underestimation of measured fluxes by the model observed on days of increased wind speed after calm periods and on occasions of very low air pressure. By integrating the large positive residuals of the model fit, the contribution of ebullition to methane emission during the combined measurement period is estimated to be 10 %.



**Fig. 5** Time series of measured and modeled daily averaged CH<sub>4</sub> fluxes during the periods 19 July - 22 October 2003 and 31 May - 21 July 2004. The error bars are daily means of standard deviations of hourly flux data points.

Many studies identified the thaw depth of soils as an important predictor of methane emission, spatially and temporally (e.g. Friberg *et al.*, 2000; Tsuyuzaki *et al.*, 2001; van Huissteden *et al.*, 2005). In our study, the thaw depth was only weakly correlated with methane flux and, when added as an additional variable, did not improve the performance of flux model based on soil temperature in 0.15 m depth and friction velocity. This indicates that during the warm period the majority of the methane emitted originated from the upper soil layers. Process studies showed that microbial methane production rates in soils of Samoylov Island at in-situ summer temperatures were between 5 and 20 times greater in the top of the active layer compared to the bottom (Wagner *et al.*, 2003). At the same time, it was shown that the microorganisms in deep soil layers are cold-adapted and have a high potential activity at temperatures close to 0 °C (Liebner and Wagner, 2006; Wagner *et al.*, 2006). Hence, the small contribution of deep soil layers to methane emissions can not be explained by the strong temperature gradient in the thawed soil, but is more likely caused by a substrate limitation of microbial activity. This is supported by Wagner *et al.* (2005) who reported a decreasing bioavailability of soil organic carbon with increasing depth in soils of Samoylov Island.

The water table position is another environmental variable which was identified by many studies as a main factor controlling methane emission (e.g. Friberg *et al.*, 2000; Suyker *et al.*, 1996). This was explained with the regulation of the methane production/oxidation balance through the ratio of the aerobic/anaerobic soil column depth. In the spatial domain, this regulation was also observed at our study site: Concurrent measurements of methane emissions by the closed-chamber technique showed that the methane fluxes from water logged polygon centers were larger by a factor of 8-10 compared to emissions from elevated, moderately moist polygon rims at any time during the measurement campaigns 2003 and 2004 (unpublished data). However, temporally, no significant influence of water

table on methane flux was detected, despite great variations of water table position during both years. This can be explained with respect to the two micro-sites prevalent in our study area: Firstly, in the polygon rims, the water table was always well below the soil surface, so the ratio of aerobic/anaerobic soil column was always high. Furthermore, process studies have shown that oxidation activity in these soils is greatest near the aerobic-anaerobic interface where the substrate provision is at its optimum (Liebner and Wagner, 2006). Hence, the methane flux from polygon rim areas did not respond strongly to variations of water table position, which is confirmed by the results of the closed-chamber measurements. Secondly, despite the variations in water table position, in most of the polygon centers the water table was distinctly above the soil surface during both measurement periods, so the change of water table position could not influence the methane production/oxidation balance significantly. However, extreme draught could lower the water level below the soil surface in many polygon centers and lead to increased oxidation and overall decreased methane flux. This “on-off switch” effect (Christensen *et al.*, 2001) was observed for single polygon center sites on Samoylov Island during the dry summer of 1999 (Wagner *et al.*, 2003; Kutzbach *et al.*, 2004).

#### *Seasonal dynamics of methane flux*

Despite the low data coverage during spring 2004, a description of the processes during the thaw period can be given with reasonable confidence. Large methane fluxes were measured on several occasions during the first days of snowmelt (8-13 June 2004), which indicate the release of methane from the snow cover during the metamorphosis and settling of snow which is associated with the initial stages of the thaw process (Boike *et al.*, 2003). Furthermore, during a period with strong wind directly after the snow thaw (18-21 June 2004), fluxes were observed which equalled those measured during the midsummer period of 2003. These large fluxes were very likely caused by the escape of methane trapped in ice covers of ponds and lakes which continued to thaw until at least 25 June 2004. Hargreaves *et al.* (2001) observed methane emissions in the range of maximum summer emissions during the thaw period in a Finnish mire which were associated with visible ebullition from thawing ice layers.

The average methane emission of the polygonal tundra on Samoylov Island during the “warm” months July, August, and September was 15.7, 22.3 and 15.2 mg m<sup>-2</sup> d<sup>-1</sup>, respectively. These values are lower compared to methane emissions reported by other eddy covariance flux studies from arctic wetlands. Friborg *et al.* (2000) reported methane fluxes of typically 50 mg m<sup>-2</sup> d<sup>-1</sup> during August from a north-east Greenland fen (74°N). Hargreaves *et al.* (2001) reported a mean methane emission of 38 mg m<sup>-2</sup> d<sup>-1</sup> during August from a Finnish mire (69°N). At these two sites, with 75 % and 70 % respectively, the surface area coverage of wet micro-sites was considerably larger compared to Samoylov Island. There exist numerous flux studies based on the closed-chamber method. However, when comparing results, the different scales covered by the measurements must be taken into consideration. Sites for chamber measurements are usually chosen to represent vegetation/soil-classes with contrasting properties so as to embrace the full range of emission values of the ecosystem. Without information on surface area coverage of the different classes, simple averages of flux values have to be used for comparison. The geographically closest study was conducted by Nakano *et al.* (2000) near Tiksi, about 120 km south-east of Samoylov Island, and reported a mean flux of 23 mg m<sup>-2</sup> d<sup>-1</sup> from a tundra site during July and August. This compares very well with the fluxes observed on Samoylov Island. The same study reported methane emissions of on average 140 mg m<sup>-2</sup> d<sup>-1</sup> from a floodplain of the Kolyma River (68.5°N) during July and August. Generally, flux studies from far north-east Siberia reported significantly higher methane emission. Tsuyuzaki *et al.* (2001) measured emissions of on average 105 mg m<sup>-2</sup> d<sup>-1</sup> in a north-east Siberian marshland near the Kolyma River (69°N) during July and August. Van Huissteden *et al.* (2005) studied emissions from a river terrace polygonal tundra of the Indigirka River floodplain (71°N), and reported an average flux of 103 mg m<sup>-2</sup> d<sup>-1</sup> in July. Corradi *et al.* (2005) measured the methane emission of tussock tundra in the floodplain of the Kolyma River (69°N) during July-September and reported a mean flux of 196 mg m<sup>-2</sup> d<sup>-1</sup>.

There are a number of reasons which could explain the differences in methane emission observed. Soil temperature regime, vegetation cover, hydrology and texture of soils, as well as bio-availability of nutrients play an important role in determining microbial activity in the soil and the gas exchange between soils and the atmosphere. The tundra soils at our study site are characterized by a sandy texture. Sand is known to be an unfavourable habitat for microbes (e.g. Wagner *et al.*, 1999). Furthermore, the availability of nutrients is limited because the organic matter in the soils is only weakly decomposed, and there is no input of organic carbon by recent flooding. These conditions appear to impede microbial methane production at our study site compared to other sites in north-east Siberia.

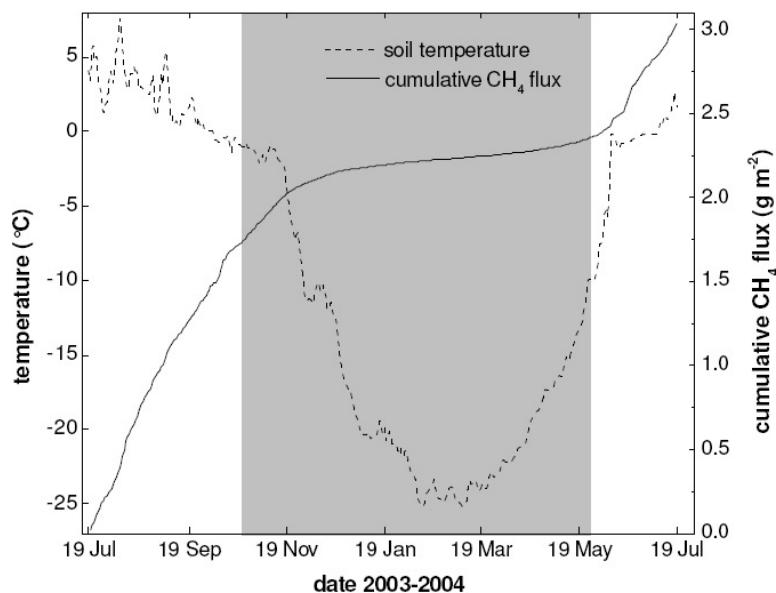
During autumn and early winter, the methane emission of the tundra on Samoylov Island decreased slowly, drastic changes in response to the refreeze of the top soil layer were not observed. Similar observations were made by Hargreaves *et al.* (2001), who linked the continuing emission of methane through the frozen top soil layer to vascular plants which, although senescent, kept providing a pathway for diffusion from deeper soil layers to the atmosphere. Kutzbach *et al.* (2004) showed that transport of methane through *Carex aquatilis* was driven by diffusion only, not by pressure-induced convection processes which would depend on the phenological status of the plant tissues. Moreover, also the accumulation of snow after the middle of October showed no marked influence on the methane flux. Both these findings are supported by the work of Corradi *et al.* (2005), who observed substantial emission of carbon dioxide from a north-east Siberian tundra during April, despite the completely frozen ground and a snow layer of about 0.6 m depth. Also, Panikov and Dedysh (2000) concluded from their study of cold season methane fluxes in a west Siberian peat bog that snow cover represented a passive layer only and gas flux into the atmosphere was controlled by production in the soil. However, the dependence of methane flux on turbulence observed during snow-free periods is expected to decrease with increasing snow cover, due to de-coupling of the turbulence from the soil surface and vegetation. Though, owing to the lack of data, this hypothesis could not be verified.

Similarly, due to a lack of data, methane emissions during winter, i.e. during the period of completely frozen soils and low sub-zero soil temperatures are uncertain. Many studies have stressed the importance of cold season fluxes in boreal wetlands. The contribution of cold season flux to the annual flux was reported to be 4-21 % at a Minnesota peatland at 47 °N (Dise, 1992), 3.5-11 % at a west Siberian peat bog at 57 °N (Panikov and Dedysh, 2000), 5-33 % at Finnish bogs and fens at 62-65 °N (Alm *et al.*, 1999), and 23 % at a Finnish mire at 69 °N (Hargreaves *et al.*, 2001). However, there exist few studies in permafrost regions which address the question of winter fluxes. Whalen and Reeburgh (1988) observed episodic methane emission from moss sites during winter which accounted for about 40 % of the annual flux, but which they attributed to physical processes during the soil freeze rather than microbial activity. Laboratory experiments have shown only recently that methanogenesis takes place in soils at sub-zero temperatures. Rivkina *et al.* (2004) reported methanogenesis in Siberian permafrost samples at temperatures down to -16.5 °C and concluded that the freezing of sediments is not a barrier to microbial activity. In a similar experiment, Wagner *et al.* (2006) detected methanogenesis in soil samples of Samoylov Island at a temperature of -6 °C. Hence, it is assumed that the relationship between soil temperature and methane emission based on the measurements in 2003-2004 can be extrapolated for the estimation of winter CH<sub>4</sub> emission. A similar approach was chosen by Corradi *et al.* (2005), who used a Lloyd-Taylor function (Lloyd and Taylor, 1994) based on data from the period July-October for the estimation of winter soil respiration. Using the soil temperature record from 0.15 m depth and equation 2, but omitting the *u*\*-term because of the expected de-coupling of methane flux and turbulence by the snow cover, the cumulative flux of the period 23 October 2003 - 30 May 2004 (221 days) was estimated to be 0.6 g m<sup>-2</sup> (Fig. 6). Based on this value, the integrated emission of the cold season October-May was 0.9 mg m<sup>-2</sup>, and its contribution to the annual emission was 30 %. This estimate is at the upper end of the range of results discussed above. The reason for the large contribution of the cold period at the Samoylov Site is the seasonal distribution of fluxes. During summer, methane emission from the tundra of Samoylov Island is generally low, while substantial emission continues well into early winter. Considering this

observation, the method of estimating cold season flux as the product of measured winter flux and number of days with sub-zero air temperature, as used e.g. by Panikov and Dedysh (2000), appears to be too simple and is likely to systematically underestimate the contribution of the cold season to the annual flux.

#### *Annual carbon fluxes and GHG budget*

Using the estimate of cold season flux, the annual methane flux of the tundra ecosystem during the period July 2003 – July 2004 was calculated to be  $3.0 \text{ g m}^{-2}$ . This value corresponds to 43-58 % of the annual emissions reported for ecosystems at similar latitudes. Hargreaves *et al.* (2001) gives an estimate of  $5.5 \text{ g m}^{-2}$  for a Finnish mire at  $69^\circ\text{N}$ , and Reeburgh *et al.* (1998) reported  $5.2 \text{ g m}^{-2}$  for wet tundra sites within the Alaskan Kuparuk River basin at  $69^\circ\text{N}$ . Friborg *et al.* (2000) reported a value of  $3.7 \text{ g m}^{-2}$  for a tundra site on Greenland ( $74^\circ\text{N}$ ) for the period June-August, compared to a value of  $1.6 \text{ g m}^{-2}$  for the same period at Samoylov Island. As discussed with respect to seasonal fluxes, there are many reasons which could explain the differences between annual fluxes observed. We hypothesize that the main reasons for the low annual flux observed are the low temperature and the low bio-availability of nutrients in the tundra soils of Samoylov Island.



**Fig. 6** Record of soil temperature at 0.15 m depth (dashed line) and cumulative methane flux (black line) for the period July 2003 - July 2004. The period with modeled data is shaded.

Measurement and modeling of the fluxes of carbon dioxide showed that the tundra was an annual sink of  $-72 \text{ g CO}_2 \text{ m}^{-2}$  during the period July 2003 - July 2004 (Kutzbach, 2006). Thus the overall carbon balance of the tundra was  $-17.4 \text{ g C m}^{-2}$ , and the methane emission accounted for about 13 % of the ecosystem carbon balance. A similar value of 19 % was given by Friborg *et al.* (2003) for a west Siberian peat bog, which had a carbon exchange about five times as high as the tundra on Samoylov Island. The high value of 25 % reported by Corradi *et al.* (2005) for a north-east Siberian tussock tundra was due to the high methane emission ( $10 \text{ g C m}^{-2}$  during 60 days in summer) compared to a moderate annual carbon uptake of  $-38 \text{ mg C m}^{-2}$ . Considering the global warming potential of methane compared to carbon dioxide (factor 23 per unit C mass for a time horizon of 100 years; Houghton, 2001), the greenhouse gas balance of the tundra in units of  $\text{CO}_2\text{-C}$  equivalents was  $+32 \text{ g C}_{\text{equiv}} \text{ m}^{-2}$ . Thus, although the methane emission had only a small influence on the tundra's capacity as a carbon sink, it turned the tundra into an effective source of greenhouse gases. This was also observed for other Siberian wetlands (Friborg *et al.*, 2003; Corradi *et al.*, 2005).

According to the ACIA (2004) climate model predictions, for the Lena River Delta an increase of annual air temperature of about 5 °C and an increase of summer precipitation of about 20 % is expected. A prediction of the response of the GHG source strength of the tundra to climate change is beyond the scope of this work. However, based on the climate model predictions and field observations the attention should be drawn to nonlinear effects in ecosystem response to climate change. During the summer of 2003, which was characterized by above average temperatures and precipitation, strong surface runoff and thermo-erosion were observed on Samoylov Island (Kutzbach, 2006; Boike and Wille, 2006). These processes lead to dry up of large areas which will certainly have a large influence on the GHG budget of the tundra. Hence, nonlinear processes must be taken into consideration in GHG budget models, especially in ice-rich permafrost ecosystems in north Siberia.

## Conclusions

- The study site in the Lena River Delta is one of the most northern sites where methane emissions on a landscape scale have been investigated using the eddy covariance technique, following Greenland (Friborg *et al.*, 2000). Furthermore, the study delivered the longest high-resolution time series of methane emission from a tundra ecosystem, covering one active season including spring thaw and autumn freeze back during two consecutive years.
- The methane emission at the wet polygonal tundra studied was low regarding daily summer fluxes (typically  $30 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ ) as well as the annual flux ( $3 \text{ g CH}_4 \text{ m}^{-2}$ ). Reason for this were thought to be (a) the very low permafrost temperature in the study region, (b) the sandy soil texture and low bio-availability of nutrients in the soils, and (c) the genesis of ice wedge polygons which lead to a strong spatial heterogeneity in soil and vegetation properties and to a high surface coverage of moist to dry micro-sites (> 50 %).
- The soil temperature and near-surface atmospheric turbulence were identified to be the main environmental variables controlling methane emission. A model based on these variables explained the variations of methane flux corresponding to continuous processes of microbial methane generation and oxidation, and diffusion through soil and plants reasonably well. Transitory processes related to spring thaw and turbulence- and pressure induced ebullition could not be modeled but were estimated to contribute about 10 % to the measured flux.
- The relationship between methane flux and soil temperature found during the period spring - early winter was extrapolated to estimate the methane emission during the winter when no measurements were performed. This approach has been used for the modeling of soil respiration  $\text{CO}_2$  fluxes of tundra in north-east Siberia (Corradi *et al.*, 2005). Recent findings on methanogenesis in permafrost soils at low sub-zero temperatures suggest that this approach should also be applicable to the modeling of winter methane fluxes. At 30 %, the modeled contribution of the winter period to the annual flux is very large. This is explained by the long cold period (October-May), in combination with generally moderate summer fluxes and continuing strong emission during the slow re-freeze of water-saturated tundra soils in early winter.
- By using the eddy covariance technique and measuring over a full active flux period, a more complete picture of the environmental variables controlling methane emissions in polygonal tundra was gained. Most importantly, with the identification of the near surface turbulence as a main flux driver, the close coupling of the soil and atmosphere systems became evident. The variables soil thaw depth and water table position, which were often identified as (spatial) flux predictors by short term flux studies using the closed-chamber technique were found to have only small effect in the temporal domain. These facts highlight the need for season-long measurement campaigns for a sound evaluation of the driving parameters of methane fluxes.
- During the period July 2003 - July 2004, the overall carbon balance of the tundra was  $-17.4 \text{ g C m}^{-2}$ , and the methane emission accounted for about 13 % of the ecosystem carbon balance. Considering the global warming potential of methane compared to carbon dioxide, the greenhouse gas balance of

the tundra in units of CO<sub>2</sub>-C equivalents was +32 g C<sub>equiv</sub> m<sup>-2</sup>. Thus, although the methane emission had only a small influence on the tundra's capacity as a carbon sink, it turned the tundra into an effective source of greenhouse gases.

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### 3.2 Effect of microrelief and vegetation on methane emission from wet polygonal tundra, Lena Delta, Northern Siberia

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**Key words:** Aerenchyma, *Carex aquatilis*, Methane emission, Microrelief, Plant-mediated gas transport, Polygonal tundra

**Abstract.** The effect of microrelief and vegetation on methane ( $\text{CH}_4$ ) emission was investigated in a wet polygonal tundra of the Lena Delta, Northern Siberia (72.37N, 126.47E). Total and plant-mediated  $\text{CH}_4$  fluxes were measured by closed-chamber techniques at two typical sites within a low-centred polygon. During the study period, total  $\text{CH}_4$  flux averaged  $28.0 \pm 5.4 \text{ mg m}^{-2} \text{ d}^{-1}$  in the depressed polygon centre and only  $4.3 \pm 0.8 \text{ mg m}^{-2} \text{ d}^{-1}$  at the elevated polygon rim. This substantial small-scale spatial variability of  $\text{CH}_4$  emission was caused by strong differences of hydrologic conditions within the microrelief of the polygon, which affected aeration status and organic matter content of the soils as well as the vegetation cover. Beside water table position, the vegetation cover was a major factor controlling  $\text{CH}_4$  emission from polygonal tundra. It was shown that the dominant vascular plant of the study area, *Carex aquatilis*, possesses large aerenchyma, which serve as pathways for substantial plant-mediated  $\text{CH}_4$  transport. The importance of plant-mediated  $\text{CH}_4$  flux was strongly influenced by the position of the water table relative to the main root horizon. Plant-mediated  $\text{CH}_4$  transport accounted for about two-thirds of the total flux in the wet polygon centre and for less than one-third of the total flux at the moist polygon rim. A clipping experiment and microscopic-anatomical studies suggested that plant-mediated  $\text{CH}_4$  transport via *C. aquatilis* plants is driven only by diffusion and is limited by the high diffusion resistance of the dense root exodermes.

#### Introduction

Northern wetlands play an important role within the global methane ( $\text{CH}_4$ ) cycle. Recent estimates of the  $\text{CH}_4$  source strength of northern wetlands, including tundra, range between 17 and 42 Tg  $\text{CH}_4 \text{ yr}^{-1}$  or from 3.5 to 8.5% of the total atmospheric budget (Whalen and Reeburgh 1992; Christensen 1993; Harriss et al. 1993, Roulet et al. 1994; Cao et al. 1996).

Anticipating global warming by an enhanced greenhouse effect, high-latitude ecosystems are expected to warm more rapidly and to a greater extent than the rest of the biosphere (Maxwell 1997; Intergovernmental Panel on Climate Change 2001). To assess the effects of climatic change on the sensitive arctic ecosystems

with regard to CH<sub>4</sub> emission and possible feedbacks to the atmospheric system, it is important to improve the understanding of how the CH<sub>4</sub> emission is controlled by the involved environmental variables, soil processes, and microbial communities. CH<sub>4</sub> emission from arctic wetlands results from the complex interaction between production, consumption, and transport of CH<sub>4</sub>. These processes are governed by a set of interrelated environmental factors, including microbial community structure, climatic conditions, soil properties, and vegetation characteristics.

The vegetation occupies a central position in this complex system. Plants can have both enhancing and attenuating effects on CH<sub>4</sub> emission. Through the aerenchyma of vascular plants, oxygen is transported from the atmosphere to the rhizosphere, thus stimulating CH<sub>4</sub> oxidation in otherwise anoxic soil horizons (Van der Nat and Middelburg 1998; Popp et al. 2000). In opposite direction, the aerenchyma are a major pathway for CH<sub>4</sub> transport from the anoxic horizons to the atmosphere, bypassing the oxic/anoxic interface in the soil, where CH<sub>4</sub> oxidation is prominent (Sebacher et al. 1985; Holzapfel-Pschorn et al. 1986; Schütz et al. 1991). Furthermore, the vegetation provides the substrates for methanogenesis as decaying plant material and fresh root exudates (Whiting and Chanton 1992; Joabsson et al. 1999). Most studies demonstrated that the enhancing effects of vegetation on CH<sub>4</sub> emission exceed the attenuating effects (e.g., Torn and Chapin 1993; Sorrell and Boon 1994; Thomas et al. 1996; King et al. 1998), but several other studies reported converse results (Grünfeld and Brix 1999; Roura-Carol and Freeman 1999).

Most studies on the effect of the vegetation on CH<sub>4</sub> emission from boreal and arctic ecosystems have been conducted in North America (e.g. Whiting and Chanton 1992; Morrissey et al. 1993; Torn and Chapin 1993; Schimel 1995; Waddington et al. 1996; Kelker and Chanton 1997; King et al. 1998). Despite an increasing number of studies on CH<sub>4</sub> fluxes from the vast wetlands of Siberia published in the last decade (Panikov et al. 1993; Christensen et al. 1995; Samarkin et al. 1999; Nakano et al. 2000; Wagner et al. 2003), only one investigation on the effect of vegetation on CH<sub>4</sub> emission from Siberian tundras has been reported so far (Tsuyuzaki et al. 2001).

This paper examines the effect of microrelief and vegetation on the small-scale variability of CH<sub>4</sub> emission from arctic wet polygonal tundra of the Lena Delta, Northern Siberia. Research focused on the plant-mediated CH<sub>4</sub> emission via individual culms of *Carex aquatilis* Wahlenb., the dominant vascular plant species of the examined tundra landscape. In detail, the purposes of this study were:

- to investigate the effects of microrelief and soil characteristics on the total (soil + plant-mediated) CH<sub>4</sub> flux from wet polygonal tundra;
- to assess the amount of plant-mediated CH<sub>4</sub> flux via the aerenchyma of *C. aquatilis* and its contribution to the total CH<sub>4</sub> flux;
- to examine the response of plant-mediated CH<sub>4</sub> flux to differing microtopography and hydrologic conditions;
- to get further information about the mechanism of gas transport through *C. aquatilis*.

### Study site

Within the scope of the joint Russian–German project ‘System Laptev Sea 2000’, an expedition was undertaken in August 1999 to the Lena Delta, Northern Siberia (Rachold and Grigoriev 2000). Field work was conducted on Samoylov, a typical island of the central part of the delta (72.37N, 126.47E). The Lena Delta with an area of 28,000 km<sup>2</sup> is one of the largest deltas in the world. It is located in the zone of continuous permafrost. The climate is true-arctic, continental, and characterised by very low temperatures and low precipitation. The mean annual temperature is –10.2°C, and the mean annual precipitation amounts to 140 mm (Müller 1997). The topography of the Lena Delta is flat, but well-structured by a prominent microrelief caused by the development of low-centred ice-wedge polygons. The depressed centres of these polygons are surrounded by elevated rims, which are situated above the ice-wedges. The polygon centres contribute about 45% and the rims about 55% to the total area of the polygonal tundra in the study area.

The investigation sites of this study were located within a typical low-centred polygon with a diameter of about 20 m. One investigation site was established in the polygon centre and the other at the polygon rim. Distance between these two sites was 10 m. The soil surface at the polygon rim was about 0.5 m higher than in the polygon centre. Wooden boardwalks were set up to minimise disturbance of the soils during investigations.

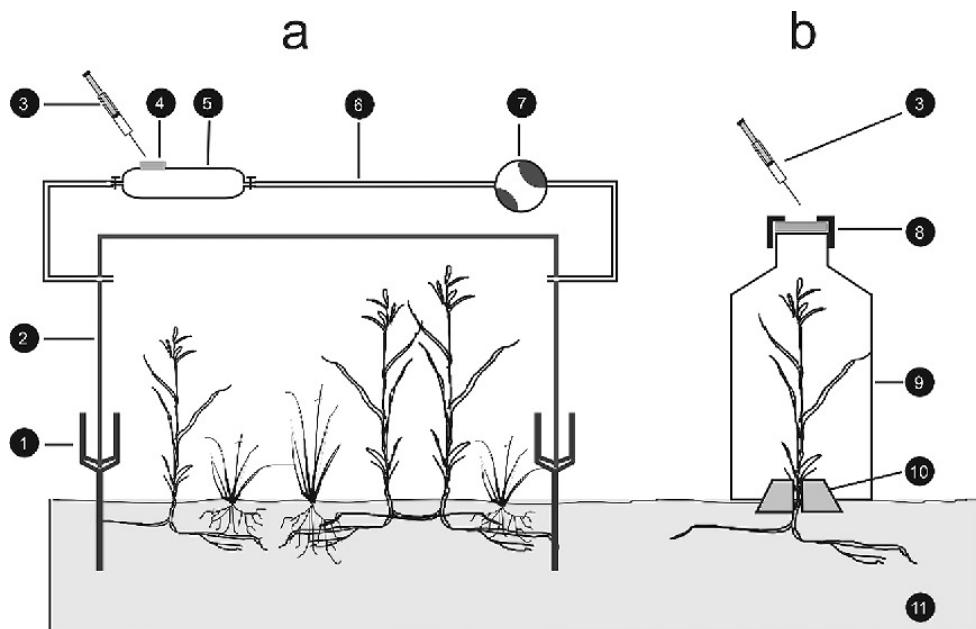
### Methods

#### *Characterisation of vegetation and soils*

The vegetation of the polygonal tundra was investigated according to the phytosociological approach of Braun-Blanquet (1964). The plant communities of the elevated rim and the depressed centre of the polygon were described at plots of 2 m<sup>2</sup>. Vascular plants were identified using Polunin (1959), mosses and lichens were identified by means of a reference herbarium provided by M.P. Zhurbenko and I.V. Czernyadeva (Komarov Botanical Institute, St. Petersburg). Species dominance was estimated as the percentage of basal area that was covered by the species.

The soils of the two study sites were described and sampled in small pits. Texture, colour, and quantity of roots in individual soil horizons were surveyed according to Schoeneberger et al. (1998). Redox status was characterised by means of  $\alpha\text{-}\alpha'$ -dipyridyl solution (Soil Survey Staff 1998), which was sprayed on freshly broken surfaces of field-wet soils. The reagent complexes ferrous iron to a complex which has a distinctive red colour. By proving the presence of soluble reduced iron ions, a positive  $\alpha\text{-}\alpha'$ -dipyridyl test indicates watersaturated and anoxic soil conditions. Bulk density, content of organic carbon and total nitrogen of soil samples were determined in the laboratory according to Schlichting et al. (1995). Soils were classified according to US Soil Taxonomy (Soil Survey Staff 1998) and the Russian system of Elovskaya (1987).

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**Figure 1.** Design of different  $\text{CH}_4$  flux chambers. (a) Total flux chamber, (b) plant-mediated transport chamber. Legend: 1, steel base with water-filled channel (permanently installed); 2, PVC top (removable); 3, syringe; 4, septum; 5, glass vessel; 6, tubing; 7, membrane pump; 8, cap with septum; 9, glass bottle; 10, rubber stopper with central channel; 11, soil.

Simultaneously to the  $\text{CH}_4$  flux measurements described below, soil conditions were recorded at each site as follows: depth of permafrost table was measured by driving a steel rod into the unfrozen soil until the hard frozen sediments were encountered. Water table was measured in perforated plastic pipes, which were installed in the soil active layer. Soil temperatures were recorded automatically at soil depths of 15 and 30 cm by thermistor probes (Campbell Scientific, Type 107) and a datalogger (Campbell Scientific Inc., CR10X).

#### $\text{CH}_4$ flux measurements

Three  $\text{CH}_4$  flux experiments were conducted in August 1999:

- (1) To provide a comparison between total (soil + plant-mediated)  $\text{CH}_4$  flux and plant-mediated  $\text{CH}_4$  flux, these fluxes were simultaneously determined by two different types of closed-chamber techniques. Measurements were conducted daily at midday during the period 9 August – 1 September (21 measurement days). At each site, three total-flux chambers (Figure 1(a)) and nine plant flux chambers (Figure 1(b)) were installed closely to each other within an area of  $2 \text{ m} \times 1 \text{ m}$ . Since *C. aquatilis* was the only aerenchymatous plant with a relevant dominance at the study site, examination of plant-mediated  $\text{CH}_4$  transport was restricted to *C. aquatilis*.

- (2) In the period 7–10 August, seven additional total-flux chambers were used at the investigated polygon (overall eight chambers in the centre and five at the rim). Obtained total CH<sub>4</sub> fluxes were related to areal densities of *Carex* culms, which were determined by counting the culms on the area that was covered by the chambers.
- (3) To get further information about the mechanism of plant-mediated CH<sub>4</sub> transport by *C. aquatilis*, a clipping experiment was conducted: at midday of 26 August, plant-mediated CH<sub>4</sub> fluxes through eight plants in the polygon centre were measured before and after clipping plants at the base of the culms 5 cm above the soil surface.

The total-flux chambers consisted of permanently installed bases of stainless steel and removable tops made of 6 mm thick, transparent PVC plates (Figure 1(a)). The chamber bases had the dimensions 0.5 m × 0.5 m × 0.15 m and isolated 0.25 m<sup>2</sup> of soil surface. The walls of the bases were inserted carefully in pre-cut grooves in the soil to a depth of 0.15 m at well-drained sites and 0.05 m at waterlogged sites, respectively. The chamber tops had the dimensions 0.5 m × 0.5 m × 0.05 m. For sample drawing, the chamber tops were sealed to the bases by a water-filled channel running around the top of each base. Air volume inside the chambers ranged from 12.5 l to 37.5 l depending on water table position. Flow-through circulation was provided by a small membrane pump connected with the chambers by Tygon® tubing. After 30 min deployment, gas samples were taken by means of glass vessels, which were integrated in the gas circulation system and could be sealed by taps.

For determination of plant-mediated CH<sub>4</sub> flux, special closed chambers were used, in which single culms of *C. aquatilis* could be enclosed (Figure 1(b)). The chambers were 0.5-l and 1.0-l glass bottles with a rubber septum put in the cap. In the bottom of the bottle was a hole, in which a rubber stopper could be fitted. For sample drawing, a rubber stopper with a hole drilled out and slit down the side was wrapped around the base of an individual plant culm. The chamber was then placed over the plant and onto the rubber stopper, sealing the system. After 30–60 min deployment 5-ml samples of the headspace gas were withdrawn through the septum with a gas-tight syringe and transferred into 10-ml glass tubes filled with saturated sodium chloride solution and sealed with rubber stoppers and twisted caps. The saturated sodium chloride solution prevented microbial activity and minimised solution processes of gases (Heyer and Suckow 1985).

In parallel to the deployment of chambers, ambient air was sampled directly above the soil surface. The CH<sub>4</sub> concentration of ambient air was used as an estimate for initial CH<sub>4</sub> concentrations in the chambers. To prove the reasonability of this estimation and the linearity of CH<sub>4</sub> accumulation with time, a series of test experiments ( $n = 6$  for each chamber type) were performed. Samples were drawn from the chambers at four points in time after sealing, at  $t_1 = 10$  min,  $t_2 = 20$  min,  $t_3 = 30$  min, and  $t_4 = 60$  min. The accumulation of CH<sub>4</sub> with time was analysed by least-square linear regression. During all tests, CH<sub>4</sub> accumulation was profoundly linear ( $r > 0.998$ ). Comparing the slopes of the linear regression lines with the

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two-point lines between ambient air and final chamber concentration, revealed an error of  $\pm 5\%$  introduced by applying the simplified two-point method.

Irregular release of CH<sub>4</sub> by bubbling was considered to be unlikely at the investigated tundra site because the water table was below the soil surface and the soil is densely vegetated. Under these conditions, the main gas transport process is diffusion, either through the soil pore system or via the aerenchyma of the wetland plants (Holzapfel-Pschorn et al. 1986; Chanton and Dacey 1991; Schütz et al. 1991).

### *Gas chromatography*

CH<sub>4</sub> concentrations in samples were analysed within 12 h of collection with a gas chromatograph (Chrompack, GC 9003) in the field laboratory. CH<sub>4</sub> was separated on a PoraPLOT Q capillary column (100/120 mesh, 20 m, Chrompack) operating at 80 °C with helium as carrier gas and was detected by a flame ionisation detector. The gas chromatograph was calibrated with standard gases. Measurement accuracy was 2% for CH<sub>4</sub> concentrations of 10 ppm. Sample concentrations ranged between 1.7 and 28 ppm.

### *Calculations*

CH<sub>4</sub> concentration measurements obtained from samples which were stored in glass tubes filled with NaCl solution were corrected for the systematic underestimation of CH<sub>4</sub> concentrations introduced by the partition of CH<sub>4</sub> between the aqueous and the gaseous phase as follows:

$$C_{\text{corr}} = C_{\text{orig}} * \frac{C_{\text{orig}} * V_H + C_{\text{orig}} * \beta * V_{\text{NaCl}}}{C_{\text{orig}} * V_H}, \quad (1)$$

where  $C_{\text{corr}}$  is the corrected CH<sub>4</sub> concentration value, and  $C_{\text{orig}}$  is the CH<sub>4</sub> concentration measured originally in the headspace above the NaCl solution in the tube.  $\beta$  is the solubility of CH<sub>4</sub> in saturated sodium chloride solution (0.00867 ml ml<sup>-1</sup> at 20 °C; Yamamoto et al. 1976; Seibt et al. 2000).  $V_H$  is the volume of the headspace, and  $V_{\text{NaCl}}$  is the volume of the NaCl solution. Since  $V_H$  and  $V_{\text{NaCl}}$  were equal (5 ml) in our experimental setup, Equation (1) can be rewritten as:

$$C_{\text{corr}} = C_{\text{orig}} * (1 + \beta) = C_{\text{orig}} * 1.00867. \quad (2)$$

CH<sub>4</sub> fluxes were calculated from the increase of CH<sub>4</sub> concentration in the enclosures using the following equation:

$$F = \frac{C_t - C_a}{t} * \frac{p * V * M}{R * T} \quad (3)$$

where  $F$  is the mass flux of CH<sub>4</sub>,  $C_t$  is the measured volume/volume CH<sub>4</sub> concentration in the chamber after the respective deployment time  $t$ , and  $C_a$  is the CH<sub>4</sub>

*Table 1.* Vegetation composition in the depressed centre of a low-centred polygon, Samoylov, Lena Delta.

Vascular plants stratum (height 30 cm)		Moss- and lichen stratum (height 5 cm)	
Species	Dominance <sup>1</sup>	Species	Dominance <sup>1</sup>
Total	30%	Total	95%
<i>Carex aquatilis</i>	25%	<i>Limprichtia revolvens</i>	25%
<i>C. rariflora</i>	3%	<i>Meesia longisetaria</i>	20%
<i>Arctagrostis latifolia</i>	++	<i>Calliergon megalophyllum</i>	20%
<i>Caltha palustris</i>	+	<i>Drepanocladus exannulatus</i>	15%
<i>Cardaminopsis tenuifolia</i>	+	<i>Calliergon giganteum</i>	5%
<i>Saxifraga cernua</i>	+	<i>Meesia triquetra</i>	5%
<i>Luzula confusa</i>	r	<i>Abietinella abietina</i>	++
<i>Equisetum variegatum</i>	r	<i>Aulacomnium palustre</i>	++
<i>Pedicularis sudetica</i>	r	<i>A. turgidum</i>	++
<i>Polygonum viviparum</i>	r	<i>Campylium stellatum</i>	++
<i>Salix glauca</i>	r	<i>Cinclidium latifolium</i>	++
<i>S. reptans</i>	r	<i>Cirriphyllum cirrosum</i>	++
		<i>Tomentypnum nitens</i>	+

<sup>1</sup>++: dominance 0.5–1.0%; +: dominance <0.5%; r: sporadic.

concentration of ambient air, which served as an estimate of initial CH<sub>4</sub> concentration in the chambers. *M* is the molecular weight of CH<sub>4</sub>, *p* is the barometric pressure, and *V* is the volume of the chamber. *T* is the air temperature (K), and *R* is the universal gas constant. Total CH<sub>4</sub> fluxes were referred to the area of soil surface from which the gas is emitted into the total-flux chamber. Estimated plant-mediated CH<sub>4</sub> flux on an areal basis was calculated by multiplying the average flux from *Carex* culms (*n* = 9 at each site) by the average areal density of *Carex* culms in the total-flux chambers (*n* = 3 at each site).

#### *Anatomical studies of C. aquatilis*

For anatomical characterisation of the aerenchyma of *C. aquatilis*, cross-sections of rhizomes, roots, and shoots of *C. aquatilis* were dissected with a razor blade. The sample pieces were dehumidified in an ascending ethanol sequence and then dried in a critical point dryer (BAL-TEC, CPD 030). They were coated with an approximately 50 nm thick gold film (BAL-TEC, SCD 050) and examined with a scanning electron microscope (Phillips, XL-20) at an accelerating voltage of 15 keV.

## Results

#### *Vegetation composition of the low-centred polygon*

The vegetation at the study sites, in the polygon centre (Table 1) as well as at the polygon rim (Table 2), was composed of two strata: a moss/lichen layer of about

**Table 2.** Vegetation composition at the summit of the elevated rim of a low-centred polygon, Samoylov, Lena Delta.

Vascular plants stratum (height: 20 cm)		Moss- and lichen stratum (height: 5 cm)	
Species	Dominance <sup>1</sup>	Species	Dominance <sup>1</sup>
Total	30%	Total	95%
<i>Carex aquatilis</i>	8%	<i>Hylocomium splendens</i>	70%
<i>Dryas octopetala</i>	6%	<i>Timmia austriaca</i>	7%
<i>Astragalus frigidus</i>	3%	<i>Climacium dendroides</i>	2%
<i>Salix glauca</i>	3%	<i>Distichium capillaceum</i>	2%
<i>S. reptans</i>	1%	<i>Tomentypnum nitens</i>	2%
<i>Lagotis glauca</i>	++	<i>Sanionia uncinata</i>	1%
<i>Luzula confusa</i>	++		
<i>L. nivalis</i>	++	<i>Peltigera aphtosa</i>	5%
<i>Poa arctica</i>	++	<i>Stereocaulon alpinum</i>	2%
<i>Pyrola rotundifolia</i>	++	<i>Cetraria laevigata</i>	1%
<i>Trisetum sibiricum</i>	++	<i>Dactylina arctica</i>	1%
<i>Polygonum viviparum</i>	+	<i>Flavocetraria cucullata</i>	1%
<i>Saxifraga hirculus</i>	+	<i>Peltigera sp.</i>	1%
<i>Koeleria asiatica</i>	r	<i>Cladonia pyxidata</i>	++
<i>Papaver radicatum</i>	r		
<i>Saxifraga cernua</i>	r		
<i>Stellaria sp.</i>	r		

<sup>1</sup>++: dominance 0.5–1.0%; +: dominance <0.5%; r: sporadic.

5 cm height and a vascular plant layer of 20–30 cm height. Whereas the total coverage of the moss/lichen layer was high with 95%, the total coverage of vascular plants was rather small with maximal 30%. The dominating vascular plant species at both sites was the sedge *C. aquatilis*. Its dominance was 25% in the polygon centre and 8% at the polygon rim. The density of *C. aquatilis* averaged 240 culms m<sup>-2</sup> in the centre and 72 culms m<sup>-2</sup> at the rim. The vegetation in the polygon centre (Table 1) could be assigned to the phytosociological association *Meesio triquetris–Caricetum stantis* (Matveyeva 1994). The vegetation at the polygon rim (Table 2) was considered to be a transient type between the associations *M. triquetris–C. stantis* and *Carici arctisibiricae–Hylocomietum alaskinii*. The latter was described as the typical ‘zonal’ association for Northern Siberia by Matveyeva (1994). Despite the over-all dominance of *C. aquatilis*, a pronounced vegetation zonation along the microtopographical gradient could be observed regarding species composition and dominance ratios. Most of the species that grew on the rim were not found in the polygon centre and vice versa. While the vegetation of the polygon centre was composed exclusively of hydrophytes like *C. aquatilis* and the mosses *Limprechtia revolvens* and *Meesia longiseta*, at the polygon rim mesophytes like the dwarf shrub *Dryas octopetala* and the mosses *Hylocomium splendens* and *Timmia austriaca* had high dominances.

### *Soil conditions*

Soil conditions varied greatly between plots at the elevated polygon rim and the depressed polygon centre (Table 3). The soils in the polygon centre were characterised by permafrost-induced waterlogging, predominantly reducing conditions in the pedon, anaerobic accumulation of organic matter, a sandy texture of the mineral soil, and the absence of cryoturbation. They were classified as *Typic Historthels* according to US Soil Taxonomy and as *Permafrost Peat-Gleys* according to the Russian system. By contrast, the soils of the polygon rim were characterised by a distinctly deeper water table, oxic conditions in the top soil, lower content of organic matter, a loamy soil texture, and pronounced cryoturbation properties. These soils were classified as *Typic Aquiturbels* (US Soil Taxonomy) and *Permafrost Turf Gleys* (Russian system), respectively. The main root horizon of the *Typic Historthels* of the polygon centre was situated in the water saturated soil zone with reducing conditions (OeBg, 11–26 cm, Table 3(a)). In the OeBg horizon, *C. aquatilis* had produced a dense mat of thick rhizomes, coarse perennial roots and a mass of fine branching roots. In the *Typic Aquiturbels* of the polygon rim, the main root horizon was situated in the oxic top soil (Ajj, 0–15 cm) while rooting density in the deeper horizons with reducing conditions was low (Table 3(b)).

During August 1999, permafrost table and water table did not show high temporal variation. In the *Typic Historthel* of the polygon centre, the permafrost table dropped gradually from 33.5 to 37.5 cm below soil surface, and the water table ranged between 0 and 4.5 cm below soil surface (Figure 2(b)). In the *Typic Aquiturbel* of the polygon rim, the permafrost table dropped from 36 to 40 cm below soil surface, and the water table was always situated directly (about 1 cm) above the permafrost table (Figure 3(b)). The soil temperature varied substantially during the study period. In the polygon centre, soil temperature 15 cm below soil surface ranged between 1.6 and 6.7 °C and averaged  $3.6 \pm 1.3$  °C; temperature at 30 cm depth averaged  $2.0 \pm 0.7$  °C (Figure 2(c)). At the polygon rim, soil temperature at 15 cm depth ranged between 2.6 °C and 7.6 °C and averaged  $4.7 \pm 1.4$  °C; temperature at 30 cm depth averaged  $3.4 \pm 1.0$  °C (Figure 3(c)).

### *CH<sub>4</sub> fluxes*

The strongly differing soil conditions at the centre and the rim of the polygon were reflected by the total (soil + plant-mediated) CH<sub>4</sub> fluxes. During the study period, total CH<sub>4</sub> flux averaged  $28.0 \pm 5.4$  mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> in the polygon centre and only  $4.3 \pm 0.8$  mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> at the polygon rim (Table 4). Within each site, temporal variability of total CH<sub>4</sub> flux was relatively low with only a few outliers (Figures 2(a) and Figure 3(a)).

Plant-mediated CH<sub>4</sub> flux via culms of *C. aquatilis* was of higher importance in the polygon centre than at the polygon rim. At the polygon centre, the estimated proportion of plant-mediated CH<sub>4</sub> flux ranged between 37 and 102% and averaged  $66 \pm 20\%$  of the total emission (Figure 2(a), Table 4). At the polygon rim it ranged

*Table 3.* Selected properties of the soils at the investigation sites. (a) *Typic Historthel* in the depressed polygon centre, (b) *Typic Aquiturbel* at the elevated rim of the low-centred polygon.

Soil type <sup>a</sup> (US and Russian Tax.)	Horizon <sup>b</sup>	Depth (cm)	Texture <sup>b</sup>	Munsell colour <sup>c</sup>	Reduc. conditions <sup>d</sup>	Roots <sup>e</sup>	Bulk Density <sup>f</sup> (g cm <sup>-3</sup> )	Org. C <sup>f</sup> (%)	C/N <sup>f</sup>
(a) <i>Typic Historthel</i> (Permafrost Peat Gley)	Oi	0–11	(Peat)	n.d.	No	1vf,f	0.4	22.1	43
	OeBg	11–26	(Peat)+ sand	10YR2/2	Yes	3vf,f,m	0.6	12.6	35
	Bg	26–31	Sand	2,5Y4/4	Yes	2vf,f,m	0.82	2.1	>100
	Bf	31–64	Sandy loam	10YR3/2	Yes	0	n.d.	4.2	30
(b) <i>Typic Aquiturbel</i> (Permafrost Turf Gley)	Ajj	0–15	Loamy sand	2,5Y3/2	No	3vf,2f,m	1.06	1.8	21
	Bjjg1	15–18	Sandy loam	2,5YR3/2	No	2vf,f,1m	1.21	2.2	21
	Bjjg2	18–32	Loam	10YR3/1	Yes	2vf,f,1m	1.23	3.4	25
	Bjjg3	32–46	Loam	10YR3/1	Yes	1vf,f	1.35	2.3	22
	Bjjf	46–90	Loam	10YR3/1	Yes	0	n.d.	3.0	20

<sup>a</sup>Classification according to Soil Survey Staff (1998) and – in parentheses – Elovskaya (1987).

<sup>b</sup>Soil horizon and texture designations according to Soil Survey Staff (1998).

<sup>c</sup>Soil colours were determined using the *Munsell® Soil Color Chart*.

<sup>d</sup>Reducing soil conditions were detected by the  $\alpha$ - $\alpha'$ -dipyridyl test (Soil Survey Staff 1998). A positive test indicates reducing and anoxic soil conditions by proving the presence of soluble ferrous iron.

<sup>e</sup>Root quantification codes according to Schoeneberger et al. (1998): 1, few; 2, common; 3, many; vf, very fine; f, fine; m, medium.

<sup>f</sup>Laboratory analyses were conducted according to Schlichting et al. (1995).

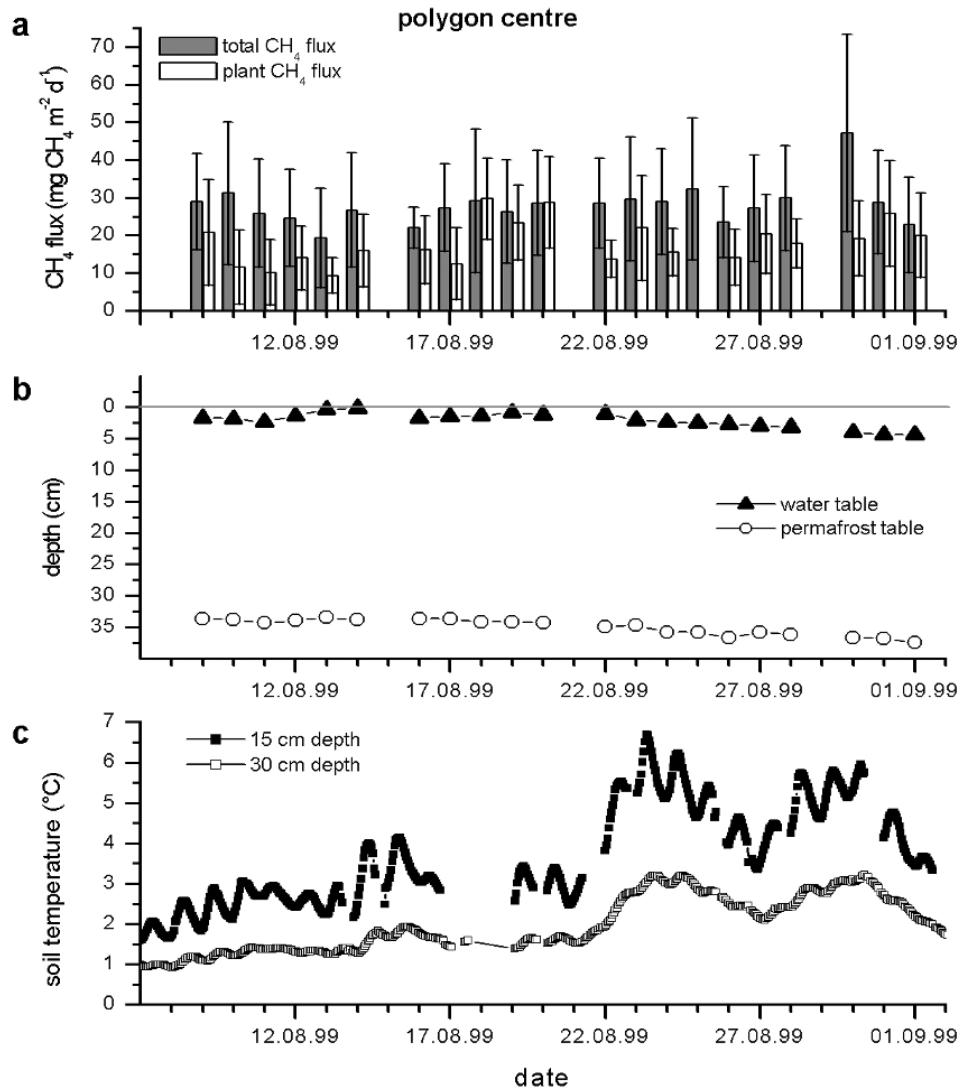


Figure 2.  $\text{CH}_4$  fluxes and soil conditions in the *centre of a low-centred polygon*, Lena Delta, Northern Siberia, August 1999. (a) Total  $\text{CH}_4$  flux (grey columns) and estimated plant-mediated  $\text{CH}_4$  flux (white columns). Each total flux column represents the average of fluxes from three  $0.25\text{-m}^2$ -plots. Estimated plant-mediated  $\text{CH}_4$  flux on an areal basis was calculated by multiplying the average flux from individual *C. aquatilis* culms ( $n=9$ ) by the average areal density of *C. aquatilis* culms in the total-flux chambers ( $n=3$ ). (b) Depth of water table (triangles) and permafrost table (circles) measured from soil surface. (c) Soil temperature at depths of 15 cm (filled squares) and 30 cm (open squares).

between 12 and 39% and averaged only  $27 \pm 9\%$  of the total emission (Figure 3(a), Table 4).

Total  $\text{CH}_4$  fluxes were strongly dependent on areal density of *C. aquatilis* culms. A positive correlation between culm density and total  $\text{CH}_4$  flux was found for the plots in the polygon centre. The respective least-square regression line was described by the linear function:  $\text{CH}_4$  emission =  $0.23 \text{ mg CH}_4 \text{ d}^{-1} \text{ culm}^{-1}$

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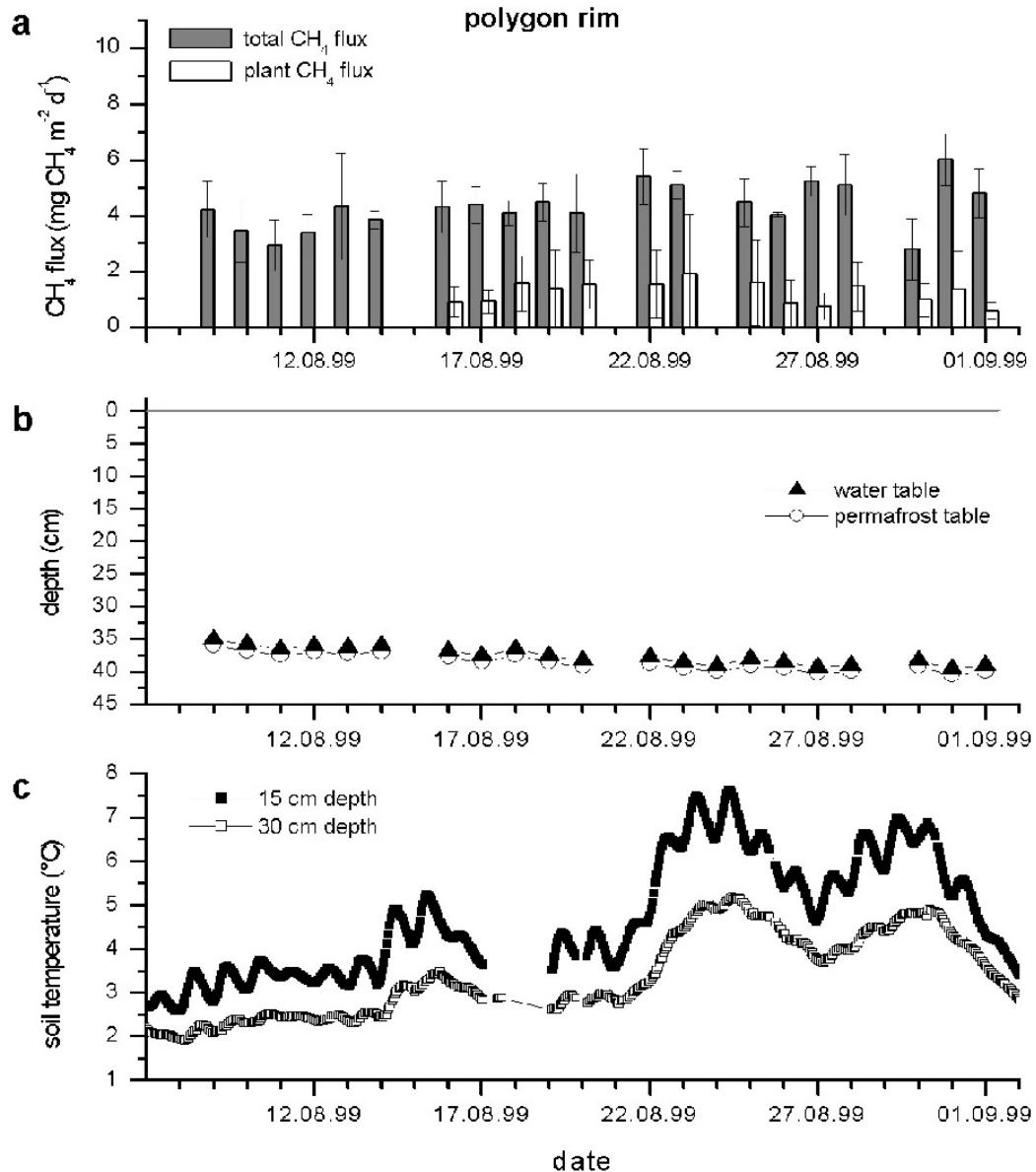


Figure 3.  $\text{CH}_4$  fluxes and soil conditions at the rim of a low-centred polygon, Lena Delta, Northern Siberia, August 1999. (a) Total  $\text{CH}_4$  flux (grey columns) and estimated plant-mediated  $\text{CH}_4$  flux (white columns). Each total flux column represents the average of fluxes from three  $0.25\text{-m}^2$ -plots. Estimated plant-mediated  $\text{CH}_4$  flux on an areal basis was calculated by multiplying the average flux from individual *C. aquatilis* culms ( $n=9$ ) by the average areal density of *C. aquatilis* culms in the total-flux chambers ( $n=3$ ). (b) Depth of water table (triangles) and permafrost table (circles) measured from soil surface. (c) Soil temperature at depths of 15 cm (filled squares) and 30 cm (open squares).

$-30.39 \text{ mg CH}_4 \text{ d}^{-1} \text{ m}^{-2}$  ( $r=0.88$ ,  $p=0.004$ ,  $n=8$ ). By contrast, a negative correlation between these variables was observed for the plots at the polygon rim. The respective regression line was described by the linear function:  $\text{CH}_4$  emission =  $-0.04 \text{ mg CH}_4 \text{ d}^{-1} \text{ culm density} + 6.92 \text{ mg CH}_4 \text{ d}^{-1} \text{ m}^{-2}$  ( $r=-0.97$ ,  $p=0.006$ ,  $n=5$ ).

**Table 4.** Total CH<sub>4</sub> flux and portion of plant-mediated CH<sub>4</sub> transport in wet polygonal tundra. Measurements were conducted in August 1999 on Samoylov Island (72.23N, 126.29E), Lena Delta, Northern Siberia.

Site	Total flux (mg m <sup>-2</sup> d <sup>-1</sup> ) <sup>a</sup>				Estimated portion of plant transport (%) <sup>b</sup>			
	Min	Max	Mean	n	Min	Max	Mean	n
Centre	19.2	47.2	28.0 ± 5.4	21	37	102	66 ± 20	20
Rim	2.8	6.0	4.3 ± 0.8	20	12	38	27 ± 9	14

<sup>a</sup>Total (soil + plant-mediated) CH<sub>4</sub> flux was measured by three closed chambers at each site with a footprint of 0.25 m<sup>2</sup>. The given values are minimums, maximums, means and standard deviations of daily means. n is number of measurement days.

<sup>b</sup>Plant-mediated CH<sub>4</sub> transport was measured by means of nine glass vessels at each site in which single *Carex* culms could be enclosed. The portion of plant-mediated CH<sub>4</sub> flux on an areal basis was estimated by multiplying the average flux through individual *Carex* culms by the average density of *Carex* culms in the total-flux chambers and setting the resulting value in relation to the total flux. The given values are minimums, maximums, means and standard of daily means. n is number of measurement days.

**Table 5.** Effect of clipping culms on plant-mediated CH<sub>4</sub> transport via *C. aquatilis*. CH<sub>4</sub> fluxes through eight individual culms were measured before and after clipping 5 cm above the soil surface. The experiment was conducted at midday of 26 August.

No.	CH <sub>4</sub> flux before clipping (mg d <sup>-1</sup> )	CH <sub>4</sub> flux after clipping (mg d <sup>-1</sup> )	Quotient after/ before clipping
1	0.051	0.041	0.80
2	0.073	0.067	0.91
3	0.027	0.031	1.14
4	0.076	0.076	1.00
5	0.051	0.044	0.87
6	0.069	0.061	0.89
7	0.037	0.042	1.13
8	0.057	0.061	1.08
Mean			0.98 ± 0.13

Clipping the culms 5 cm above the soil surface did not alter the amount of plant-mediated CH<sub>4</sub> flux by *C. aquatilis* significantly (Table 5).

#### *Aerenchyma in vegetative organs of C. aquatilis*

Extensive air spaces or lacunae (=aerenchyma) were observed in all vegetative organs of *C. aquatilis* by scanning electron microscopy (Figure 4). In particular, the extent of the lacunae in the fine roots of *C. aquatilis* was remarkable (Figure 4(a)): With the exception of a few regular arranged radial cell lines and the radial cell walls, the complete root cortex parenchyma was disintegrated and transformed into a large aerenchyma. The large aerenchyma was separated from the rhizosphere by a

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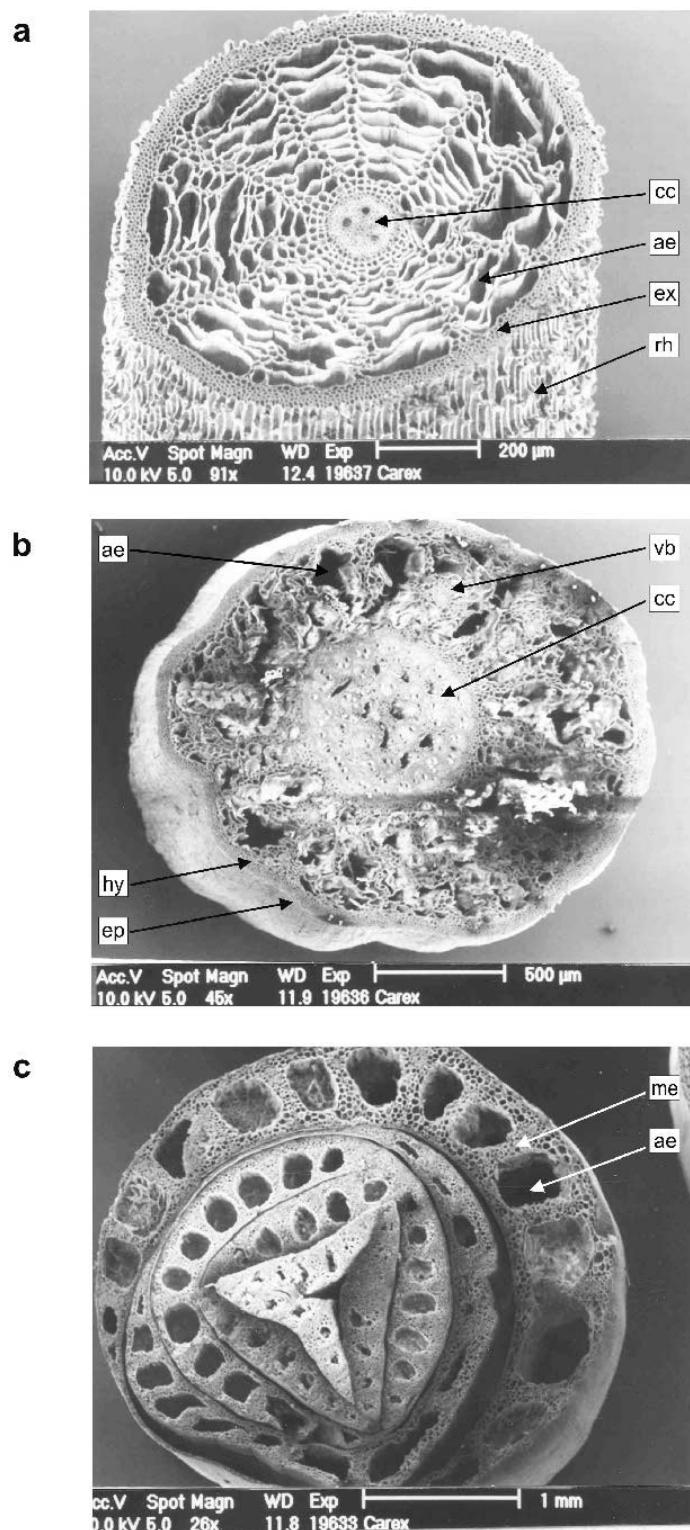


Figure 4. Transverse sections of vegetative organs of *C. aquatilis* Wahlenb. observed by scanning electron microscopy. (a) fine root, (b) rhizome, (c) culm (built up of nested leave sheaths). Legend: ae, aerenchyma; cc, central cylinder; ep, epidermis; ex, exodermis; hy, hypodermis; me, mesenchyme; rh, rhizodermis; vb, vascular bundle.

dense exodermis, which was built up of compactly packed hexagonal cells with thickened cell walls. In the rhizomes, lacunae were observed in the inner cortex (Figure 4(b)). They were less regular arranged and not as extensive as in the roots. In the leaves sheaths that build up the culms, large lacunae were observed that were embedded in the parenchyma and were arranged very regularly between the vascular bundles (Figure 4(c)).

## Discussion

### *Influence of microrelief and soil conditions on CH<sub>4</sub> fluxes*

Many tundra ecosystems are characterised by a complicated horizontal structure (Chernov and Matveyeva 1997). Cryogenic processes in permafrost soils lead to the formation of patterned ground with often a pronounced microrelief (French 1996). In the typical polygonal tundra of the central Lena Delta, the microrelief elements of the low-centred polygons and the respective soil and vegetation types are repeated in regular cyclic intervals of 10–30 m. Thus, soil conditions, vegetation characteristics, and consequently CH<sub>4</sub> fluxes in polygonal tundra are highly variable on the small scale (decimetres to metres) but rather homogenous on the large scale of (10<sup>2</sup> to 10<sup>4</sup> metres). In order to quantify CH<sub>4</sub> emission from tundra ecosystems on the regional scale, it is necessary to characterise the small-scale variability of CH<sub>4</sub> emission. During our study, CH<sub>4</sub> emission was 6–7 times greater in the depressed polygon centre than emission at the elevated polygon rim. In the *Typic Historthel* of the polygon centre, a high water table, anoxic conditions in most of the pedon, and high organic matter contents in the anoxic horizons stimulated CH<sub>4</sub> production. CH<sub>4</sub> emission was much lower at the polygon rim since in the *Typic Aquiturbel* of the polygon rim the water table was lower, organic matter contents in the anoxic horizons were less, and oxic horizons were more extensive (Table 3). Our results show the importance of the microrelief and the variability of hydrologic conditions as key control factors on CH<sub>4</sub> emissions from tundra soils in agreement with other CH<sub>4</sub> flux studies (e.g., Svensson and Rosswall 1984; Morrissey and Livingston 1992; Moore and Roulet 1993; Waddington et al. 1996; Grünfeld and Brix 1999). The water table position determines the relative extent of oxic and anoxic horizons within soils and consequently the ratio between CH<sub>4</sub> production and CH<sub>4</sub> oxidation, the fundamental microbial processes of the CH<sub>4</sub> cycle. Beside this direct effect, the water table position influences CH<sub>4</sub> fluxes indirectly by affecting soil genesis and vegetation composition, which are important additional control factors on CH<sub>4</sub> fluxes.

The CH<sub>4</sub> fluxes observed in our study were of the same order of magnitude, albeit slightly lower, as CH<sub>4</sub> fluxes reported by other investigators from polygonal tundra in Alaska (Morrissey and Livingston 1992; Christensen 1993) and Northern Siberia (Christensen et al. 1995; Samarkin et al. 1999). Wagner et al. (2003) measured total CH<sub>4</sub> fluxes during the complete summer season 1999 at the same low-centred polygon that we chose for our investigations. They observed much higher and more variable CH<sub>4</sub> emissions in the polygon centre during July ( $93 \pm 22 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ ),

when the water table was distinctly above the soil surface, than in August ( $25 \pm 7 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ ), when the water table was located a few centimetres below the soil surface. This decrease of  $\text{CH}_4$  emission was associated by an increase of  $\text{CH}_4$  oxidation activity in the soils. The effect of the water table position on  $\text{CH}_4$  emissions from wetlands can be compared with an on-off switch (Christensen et al. 2001). When the water table falls below the soil surface, microbial  $\text{CH}_4$  oxidation is drastically increased and  $\text{CH}_4$  emission is reduced. A narrow oxic soil zone can have a high capacity for  $\text{CH}_4$  oxidation (Whalen et al. 1996).

During our study, temporal variability of  $\text{CH}_4$  emission was low because the main control factors, water table position and thaw depth, were almost constant during the study period. Temporal fluctuations of soil temperature (Figures 2(c) and 3(c)) appeared to have only a minor effect on  $\text{CH}_4$  emission. This is in accordance with studies of Svensson and Rosswall (1984), Christensen (1993), and Nykänen et al. (1998). These authors found that  $\text{CH}_4$  emission correlates with soil temperature only at inundated sites, where the water table is distinctly above the soil surface. If the water table is positioned below the soil surface and  $\text{CH}_4$  oxidation gains importance, soil temperature is not expected to show a direct effect on  $\text{CH}_4$  emission.  $\text{CH}_4$  production has a stronger temperature response with reported  $Q_{10}$  values of 2.7–20.5 by comparison with  $\text{CH}_4$  oxidation with  $Q_{10}$  values of only 1.2–2.1 (e.g., Svensson and Rosswall 1984; King and Adamsen 1992; Dunfield et al. 1993; Moosavi and Crill 1998), but the upper soil horizons, where  $\text{CH}_4$  oxidation occurs, are more exposed to temperature changes than the subsurface horizons of methanogenesis. Thus, it can be assumed that the effect of soil temperature fluctuations on microbial  $\text{CH}_4$  production is compensated by the temperature effect on  $\text{CH}_4$  oxidation (Christensen 1993; Whalen et al. 1996).

#### *Effects of vegetation on $\text{CH}_4$ fluxes*

Numerous studies demonstrated the importance of the vegetation as a major control factor on  $\text{CH}_4$  emissions from wetlands (e.g., Schütz et al. 1991; Whiting and Chanton 1992; Grünfeld and Brix 1999; Joabsson et al. 1999; Roura-Carol and Freeman 1999). Tsuyuzaki et al. (2001) found that  $\text{CH}_4$  emission from grassy marshlands near the Kolyma River in the tundra/taiga transition zone of North-east Siberia was strongly dependent on the vegetation type. At our study site, the strongly differing hydrological conditions within the microrelief of the polygonal tundra caused substantial differences in vegetation cover between the rim and the centre of the polygon. These vegetation differences had a direct effect on the small-scale variability of  $\text{CH}_4$  emission. Sites in the polygon centre with high densities of *C. aquatilis* culms emitted distinctly more  $\text{CH}_4$  than sites at the polygon rim with low densities of *C. aquatilis*. The enhancing effect of *C. aquatilis* on  $\text{CH}_4$  emission is due to the capability of its aer-enchyma to serve as conduits for plant-mediated  $\text{CH}_4$  transport. Plant-mediated  $\text{CH}_4$  transport accounted for about two-thirds of the total flux in the polygon centre and for less than one-third of the total at the polygon rim. It is assumed that *C. aquatilis* plants can have an additional positive effect on  $\text{CH}_4$  emission by providing fresh substrates

for the methanogenesis as described for several vascular plants by other investigators (Schütz et al. 1991; Whiting and Chanton 1992; Joabsson et al. 1999).

The influence of *C. aquatilis* on CH<sub>4</sub> fluxes has to be valued separately for the different microrelief elements of the low-centred polygon. Total CH<sub>4</sub> flux (soil + plant flux) was positively correlated to areal density of *C. aquatilis* culms at sites in the polygon centre, while no such relationship could be found at the sites at the polygon rim. At the polygon rim, the presence of *C. aquatilis* culms appeared to have even a small attenuating effect on the total CH<sub>4</sub> flux. Previous studies showed that the influence of vascular plants can differ substantially between different sites. Some authors found significant positive correlations between CH<sub>4</sub> emission and plant biomass (Morrissey and Livingston 1992; Whiting and Chanton 1992) or culm density (Christensen 1993; Schimel 1995). At other sites, a negative effect of vascular plants on CH<sub>4</sub> emission was confirmed (Grünfeld and Brix 1999; Roura-Carol and Freeman 1999). At these sites and likewise at our study site at the polygon rim, the stimulation of rhizospheric CH<sub>4</sub> oxidation by vascular plants appeared to have a greater effect on CH<sub>4</sub> emission than the enhancing effects, that is, plant-mediated CH<sub>4</sub> transport and the supply of substrates for methanogenesis.

The plant-mediated proportion of the total CH<sub>4</sub> emission from the polygon centre (about 2/3) lay in the same range as results obtained by Schimel (1995) for arctic wet meadow tundra in Alaska, which was characterised by a water table below the soil surface. In inundated tundra wetlands of Alaska, the proportion of plant-mediated CH<sub>4</sub> transport was reported to be considerably higher with 90–98% of the total flux (Morrissey and Livingston 1992; Whiting and Chanton 1992; Torn and Chapin 1993). Our results and the comparison with other wetland studies suggest that the effect of plant-mediated CH<sub>4</sub> flux is substantially affected by water table position. The effect of plant-mediated CH<sub>4</sub> transport is greatest with a high water table and the bulk of roots growing in anoxic soil horizons (Waddington et al. 1996). The lower the water table, the less roots grow within the anoxic CH<sub>4</sub>-enriched soil horizons and can serve as conduits for CH<sub>4</sub> transport to the atmosphere. The better the pore system of a soil is aerated, the bigger is the portion of CH<sub>4</sub> that diffuses via the soil pores to the atmosphere. It has to be considered, that diffusion velocity of CH<sub>4</sub> is 10<sup>4</sup> times higher in air than in water (Schachtschabel et al. 1998). The dense root exodermes of *C. aquatilis* have an even higher diffusion resistance than water (Končalová 1990). Thus, a substantial CH<sub>4</sub> diffusion from the pore waters into the root aerenchyma through the exodermes only happens when a high CH<sub>4</sub> concentration gradient between pore waters and root aerenchyma is present and the diffusion via the soil pore system is hampered by water saturation.

#### *Mechanism of gas transport via C. aquatilis*

The microscopic-anatomical studies showed that large aerenchyma are present in the vegetative organs of *C. aquatilis* that can act as pathways of facilitated diffusion for CH<sub>4</sub> produced in anoxic soil horizons. Many monocotyledonous wetland plants develop aerenchyma as adaptation to soil waterlogging. The internal air spaces

provide a conduit for oxygen from the atmosphere to the roots and for CH<sub>4</sub> in the opposite direction. Particularly, the CH<sub>4</sub> transport processes in large emergent and floating wetland plants like *Phragmites australis*, *Typha latifolia*, or *Nuphar lutea* are thoroughly investigated (e.g., Große et al. 1991; Tornbjerg et al. 1994; Armstrong et al. 1996). In these plants, gas transport by pressure-induced convection could be verified. The exact mechanism of plant-mediated CH<sub>4</sub> flux through smaller monocotyledonous plants as sedges and grasses is more uncertain, although it was addressed by several studies in the last decade (Morrissey et al. 1993; Schimel 1995; Kelker and Chanton 1997; King et al. 1998).

The results of our clipping experiment suggest that plant-mediated CH<sub>4</sub> transport via *C. aquatilis* plants is driven only by facilitated diffusion. A pressure-induced active transport should have broken down after clipping. Furthermore, the experiment indicated that diffusion was not limited by the diffusion resistance of the above-ground portion of *Carex* plants but rather by a high diffusion resistance at the transition between the rhizosphere and the root aerenchyma. This conclusion was backed by the microscopic-anatomical studies of *Carex* roots: dense exodermes, that were built up of compactly packed hexagonal cells with thick cell walls, separated the root aerenchyma from the rhizosphere. Such exodermes act as effective diffusion barriers and reduce oxygen loss into the rhizosphere as well as CH<sub>4</sub> infiltration into the root aerenchyma (Končalová 1990; Schütz et al. 1991). In contrast to our results, Morrissey et al. (1993) and Schimel (1995) observed that the CH<sub>4</sub> release from arctic *Carex*-dominated wetlands was limited by the above-ground portion of the plant, that is, by stomatal control. On the other hand, Kelker and Chanton (1997) observed no clear and enduring increase of CH<sub>4</sub> flux after clipping sedges in a boreal fen and concluded that plant-mediated gas transport through *Carex* plants had to be regulated below ground, as is common among most other plant species (Armstrong 1979; Chanton and Dacey 1991). The different results between studies can be due to differences in factors other than leaf or root resistance, such as plant phenology, CH<sub>4</sub> concentration in pore waters of the respective soil, microclimate, or root uptake controlled by soil temperature (Morrissey et al. 1993). These factors should be considered in a more detailed continuative study to improve the understanding of plant-mediated CH<sub>4</sub> transport by *C. aquatilis* in tundra ecosystems.

### Perspectives

A multi-year study on the seasonal and interannual variability of CH<sub>4</sub> emission is in progress at the described polygonal tundra. Longer time series are needed to accurately quantify the impacts of environmental controls on CH<sub>4</sub> emission, as water table, soil temperature, thaw depth, or plant phenology. Beside the moist and wet soils, which were investigated in the presented study, polygonal ponds are an important landscape feature of polygonal tundra. The CH<sub>4</sub> dynamics of these ponds shall be studied in detail in the future. The relative areal extent of the ponds and the different soils shall be evaluated by the analysis of high-resolution remote sensing data to allow a reasonable regional quantification of CH<sub>4</sub> emission by upscaling.

## Conclusions

The presented study points out the high small-scale spatial variability of CH<sub>4</sub> fluxes in the polygonal tundra of the Lena Delta, Northern Siberia. The pronounced microrelief of polygonal tundra induces strongly differing hydrologic conditions within short distances that affect aeration status and organic matter content of soils as well as the vegetation cover. CH<sub>4</sub> emission is controlled by all these interdependent factors in a complex way. Beside water table position, the vegetation cover is of great importance in controlling CH<sub>4</sub> emission from polygonal tundra. It was shown that the dominant vascular plant of the study area, *C. aquatilis*, possesses large aerenchyma, which serve as pathways for substantial plant-mediated CH<sub>4</sub> transport. The importance of plant-mediated CH<sub>4</sub> flux is strongly influenced by the position of the water table relative to the main root horizon. Plant-mediated CH<sub>4</sub> transport accounted to about two-thirds of the total flux in the polygon centre and to less than one-third of the total flux at the polygon rim. The effect of plant-mediated CH<sub>4</sub> transport is greatest with a high water table and the bulk of roots growing in anoxic soil horizons. A clipping experiment and microscopic-anatomical studies suggested that plant-mediated CH<sub>4</sub> transport via *C. aquatilis* plants is driven only by diffusion and is limited by the high diffusion resistance of the dense root exodermes.

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### 3.3

## Land cover classification of tundra environments in the Arctic Lena Delta based on Landsat 7 ETM+ data and its application for upscaling of methane emissions

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### Abstract

The Lena River Delta, situated in Northern Siberia, is the largest arctic delta. Since natural deltas are characterised by complex geomorphological patterns and ecosystems, high-resolution information on the distribution and extend of the delta environments is necessary for an accurate spatial quantification of biogeochemical processes as the emission of greenhouse gases from tundra soils. In this study, the first land cover classification for the entire Lena Delta based on Landsat 7 ETM+ images was conducted and used for the quantification of methane emissions from the delta ecosystems on the regional scale. Nine land cover classes of aquatic and terrestrial ecosystems in the clearly wetland dominated Lena Delta could be defined by this classification approach. The use of the high-resolution land cover maps for regional upscaling of methane emissions reduces the error potential of the upscalings.

**Keywords:** Land cover classification; Methane emission; Upscaling; Tundra environments; Lena River Delta

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### 1. Introduction

Beside carbon dioxide and water vapour, the atmospheric trace gas methane is one of the most important greenhouse gases. Methane is chemically very reactive and more efficient in absorbing infrared radiation than carbon dioxide. Its contribution to the radiative forcing from pre-industrial to present time is estimated with about 20 % of all greenhouse gases (IPCC, 2001; Le Mer & Roger, 2001).

Methane has a wide variety of natural and anthropogenic sources (Wuebbles & Hayhoe, 2002). Although the major sources of atmospheric methane are known, the quantification of the methane emissions from these sources is difficult due to high spatial and temporal variability (IPCC, 2001). The most important natural sources are wetlands (Bartlett & Harriss, 1993; Wuebbles & Hayhoe, 2002). They cover about 4-6 % of the Earth's surface (Mitsch et al., 1994). 28 % of these wetlands are located in the high latitudes north of 60°N in the Arctic and Subarctic climate zone (Matthews & Fung, 1987). Wetlands emit about 100 Tg methane annually, or about 20 % of overall global emissions of 450-550 Tg a<sup>-1</sup> (Matthews, 2000). Estimates of the methane emissions of the arctic and subarctic wetlands range between 10 and 39 Tg a<sup>-1</sup>, or between 2.2 and 8.6 % of the overall global methane emissions (Bartlett & Harriss, 1993; Bartlett et al., 1992).

Biogene methane emissions from wetlands are determined by two different microbial processes: methane production and methane oxidation (Cao et al., 1998; Wagner et al., 2003). The major controlling factors of both processes are the availability of oxygen, temperature, amount and quality of

organic matter, vegetation, and pH (Bartlett et al., 1992; Morrissey & Livingston, 1992; Christensen et al., 1995; Whalen et al., 1996; MacDonald et al., 1998; Wagner et al., 2005). These factors are of high temporal and spatial variability within the active layer of permafrost soils and thus also are the CH<sub>4</sub> emissions.

For the calculation of the global methane emission often a simple multiplication of mean emission rates from small individual study sites by the area of the ecosystem (Panikov et al., 1993; Harriss et al., 1993), or by the global wetland area is conducted (Matthews & Fung, 1987; Bartlett & Harriss, 1993). These calculations do not consider small-scale spatial variations like complex vegetation patterns or variations in soil moisture.

Optical remote sensing is a direct method for the observation of Earth's surface. The multispectral data of the Landsat-7 ETM+ sensor have proven potential for classification of vegetation, geological structures, and soils. These are also major factors determining the methane emission from the arctic tundra (Bartlett et al., 1992; Gross et al., 1990; Morrissey & Livingston, 1992; Christensen et al., 2000). The strong correlation between methane emissions and the prevailing vegetation cover and soil moisture is vital for the land cover classification focusing on the quantification of methane emission from tundra wetlands. Extensive field knowledge of the individual land cover classes in the investigation area allows the upscaling of the methane emission rates from individual study sites to the entire study regions considering the influencing factors in this biogeochemical process.

Although land cover classifications are a standard application of remote sensing data, recent and high-resolution land cover maps are still lacking for large Arctic regions. Within the last 15 years, some studies on land cover classifications utilizing remote sensing data with different thematic focus have been conducted in Alaska and Canada (Ferguson, 1991; Gross et al., 1990; Joria & Jorgenson, 1996; Muller et al., 1999; Stow et al., 1998; Brook & Kenkel, 2002). Land cover classifications of Arctic investigation areas in Russia are rare and most of them have been done in the last years (Rees et al., 2003; Takeuchi et al., 2003; Virtanen et al., 2004; Grosse et al., 2006).

Our study applying remote sensing techniques for the quantification of methane emission focuses on the tundra region of the Lena Delta in Arctic Siberia. The purposes of this study were: (i) to classify the land cover of the Lena Delta with regard to its methane emission rates based on the Landsat-7 ETM+ satellite data; (ii) to determine the spatial distribution and coverage of the various land cover classes; (iii) to balance the methane emissions of the individual land cover classes and of the Lena Delta in total. The methane flux data were inferred from long-term closed chamber measurements.

## 2. Study area

The study area is the Lena Delta, located in Northern Siberia at the Laptev Sea coast between the Taimyr Peninsula and the New Siberian Islands. Occupying an area of about 29.000 km<sup>2</sup>, it is the largest delta in the Arctic and one of the largest in the world. The delta is characterised by a network of small and large rivers and channels, and more than 1000 islands. The Lena Delta can be divided into three geomorphologically different terraces and active floodplain levels (Are & Reimnitz, 2000; Schwamborn et al., 2002; Fig. 1). The active floodplain and the first terrace (1-12 m a.s.l.) are the youngest parts of the Lena Delta. The first terrace was formed during the middle Holocene and mainly occupies the eastern part of the Lena Delta. It is characterised by the patterned ground of ice-wedge polygons. The second terrace (11-30 m a.s.l.), formed between the late Pleistocene and early Holocene age, occupies about 23 % of the delta and differs from other terrace levels in sedimentological composition, geomorphological habit, and the character of the vegetation cover. The second terrace is characterised by sandy sediments with low ice content. The polygonal microrelief is less expressed; thermokarst-lake assemblages are typical. The third terrace (30-60 m a.s.l.) is the oldest terrace in the Lena Delta. It is not a fluvial-deltaic deposition but an erosional remnant of a Late Pleistocene accumulation plain of fine-grained, organic- and ice-rich sediments in front of the Chekanovsky and Kharaulakh mountain ridges in the southern zone of the study area (Schirrmeyer et al., 2003). The surface of the third terrace is characterised by polygonal ground and thermokarst processes.

The Lena Delta is covered by tundra vegetation of various types. Major components are grasses, sedges, mosses, lichens, and dwarf shrubs.

The region is characterised by an Arctic continental climate with low mean annual air temperatures of  $-13^{\circ}\text{C}$ , a mean temperature in January of  $-32^{\circ}\text{C}$ , and a mean temperature in July of  $6.5^{\circ}\text{C}$ . The mean annual precipitation is low and amounts to about 190 mm (WWIS, 2004). The Lena Delta is located in the zone of continuous permafrost with a thickness of about 500-600 m (Romanovskii & Hubberten, 2001). The thickness of active layer is usually in the range of 30-50 cm during summer.

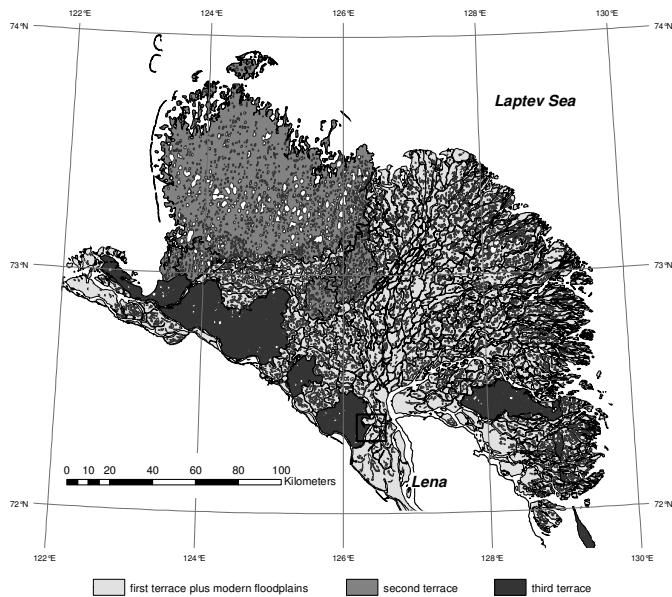


Fig. 1. Map of the geomorphological units of the Lena Delta according to Schwamborn (2002).

The island Samoylov, situated in the central delta ( $72^{\circ}22' \text{ N}$ ,  $126^{\circ}29' \text{ E}$ ), is the main study site for the methane emission measurements in the Lena Delta since 1998. Samoylov covers an area of about  $5 \text{ km}^2$  and is representative for the first terrace and the floodplains. The western part of Samoylov is formed by recent fluvial and aeolian processes. Three floodplain levels can be distinguished by inundation frequency and vegetation cover. The sediments are characterised by fine to coarse sands. The middle Holocene deposits of the first terrace cover about  $3 \text{ km}^2$  in the eastern part of Samoylov. This area is dominated by active ice-wedge formation, low-center polygons and small thermokarst ponds. The vegetation and soil patterns are complex due to high lateral variability of the polygonal microrelief with polygon rims and polygon depressions.

### 3. Materials and methods

#### 3.1. Image data and processing

The study was based on the land cover classification of three Landsat-7 ETM+ satellite images. The acquisition dates are 27 July 2000 (path 131, row 8 and 9) and 26 July 2001 (path 135, row 8), both dates which are within the main vegetation period. ERDAS Imagine software was used to carry out all image processing tasks. In addition to the ETM+ satellite imagery, we acquired and utilized numerous other ancillary data for determination of typical land cover classes and field training sites: vegetation field data, soil information, field and aerial photography.

The three Landsat-7 images were rectified using ground control points from three already orthorectified Landsat-7 ETM+ images (August 2000, path 130, row 9; July 2001, path 133, row 8 and 9) and by applying a first-order polynomial transformation. The scenes were resampled to  $30 \text{ m} \times 30 \text{ m}$  pixels using the nearest neighbour approach. The RMS error was less than 1 pixel, while the base imagery has a horizontal accuracy of approximately 50 m. To minimize the effects of the

atmosphere, sun illumination geometry, and instrument calibration on the image data, a radiometric and image-based atmospheric correction was applied (Chavez, 1996). As a result the image digital numbers (DN) were converted to reflectance values and the radiometric differences between the three scenes due to atmospheric conditions were lowered. The reflectance images are more appropriate for land cover analysis than DN images (Huang et al., 1998). Finally, the three scenes were projected to UTM Zone 52 with the geodetic datum WGS 1984 and a mosaic of the Lena Delta was composed.

Image classifications were conducted using both unsupervised and supervised techniques. Cloud cover was identified by an unsupervised classification and masked out from the image mosaic. The unsupervised classification was also used to identify spectrally similar areas and possible training sites for the supervised classification. Nevertheless, most of the spectral classes determined in the unsupervised classification did not represent homogeneous land cover classes. Therefore, a supervised classification was carried out using the spectral bands 1-5 and 7 (VIS, NIR, SWIR). For the supervised classification, the *minimum distance algorithm* was used, because it can be more effective than the often used *maximum likelihood algorithm* when the number of training sites per class is limited (Richards & Jia, 1999). Ancillary data, like local thematic maps, field knowledge, and aerial and field photography, were used to select the training areas for each class. This process resulted in 34 training areas for 10 land cover classes. A detailed accuracy assessment (Story & Congalton, 1986; Congalton, 1991) was not obtained for the land cover classification of the Lena Delta area, as necessary area-wide independent data is not available for this large region. Nevertheless, a first assessment with field photographs and new field knowledge from a Lena Delta expedition in summer 2005 indicates a good accuracy of the classification result. A quantitative accuracy assessment is planned using hyperspectral Chris-Proba data and field spectrometry conducted during this and subsequent expeditions.

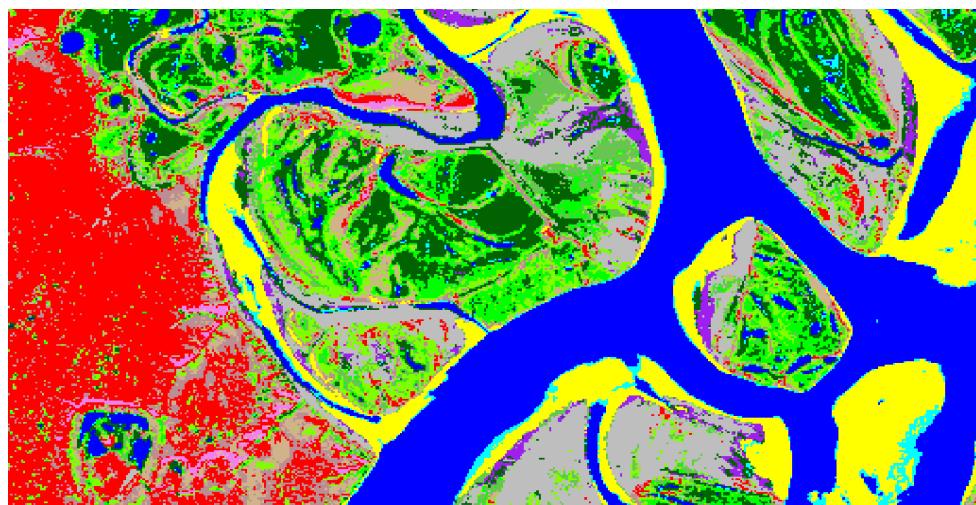


Fig. 2. Supervised classification of the Lena delta (detailed section with Samoylov Island in the right center). Colour code: dark blue = water; light blue = shallow water; dark green = wet sedges and mosses dominated tundra; light green = moist sedges and mosses dominated tundra; yellow = mainly non-vegetated areas; gray = dry to moist dwarf shrubs dominated tundra; red = dry grasses and dwarf shrubs dominated tundra; light brown = dry sandy mosses, sedges and dwarf shrubs dominated tundra; violet = dry grasses dominated tundra; pink = dry tussock tundra.

### 3.2. Methane emission measurements and upscaling

The methane emission rates of individual study sites were determined by closed chamber measurements. The measurements were carried out within the scope of long-term investigations of the trace gas emissions in the Lena Delta during the years 1999 to 2006. Methane concentrations were determined with a gas chromatograph in the field laboratory (Wagner et al., 2003).

Currently, long-term measurements (more than 1 month) of methane emissions in the Lena Delta have been conducted on polygonal tundra sites and sites located on the lower floodplain vegetated by dwarf willows or cotton grass. The results for all other classes depend either on calculations based on the methane emission rates which have been measured in a shorter period, or on estimations derived from the literature. For moist and dry habitats the measurements continued less than 1 month. For the dry habitats we used methane emission rates measured by Kutzbach & Kurchatova (2002) in the Lena Delta. We also have assumed that emission rates of all dry classes are equal because all of these classes have the dry substrate in common. The characteristics of the non-vegetated areas point to very low methane emission rates, so we assume that it is nearly zero. This assumption is confirmed by a measurement of Kutzbach & Kurchatova (2002) at a sandy deflation cliff in the delta. The *shallow water* study sites appear either as a vegetated site with high emission rates (e.g. vegetated lake margins) or as a non-vegetated site with very low methane emission rates. It was not possible to separate these two habitat types properly with the available methods. To avoid miscalculations, we do not include the methane emissions of this land cover class into the balance of the methane emissions of the Lena Delta area.

The different habitat types of the class *water bodies* (e.g. rivers, lakes, and coastal waters) result in variations of the methane emission potential within the class. The area covered by the land cover class *water bodies* is nearly 8900 km<sup>2</sup>. The lakes > 1 ha occupy around 2800 km<sup>2</sup> of the Lena Delta (Morgenstern, 2005), or about 31.5 % of the open water area. We used only the methane emission rates of the lakes to determine the balance of the methane emission of the Lena Delta. Due to lacking measurements of the methane emission rates of thermokarst lakes in the Lena Delta, we assumed that the rates are as high as those of the lakes in Alaska north of 68°N measured by Morrissey and Livingston (1992).

Table 4 shows the methane emission rates we used for the calculation of the methane emissions of the Lena Delta. These are the mean values measured in July; only the rates for the land cover class *moist to dry dwarf shrub-dominated tundra* have been measured in June.

We used equation (1) for the calculation of the daily methane emissions of the Lena Delta. There, **n** is the number of land cover classes, **A<sub>i</sub>** is the area of the individual classes and **E<sub>di</sub>** is the daily methane emission rate for each class.

$$Ed = \sum_{i=1}^n A_i E_{di} \quad (1)$$

We used equation (2) for the calculation of the annual methane emissions of the Lena Delta area, where **n** is the number of land cover classes, **A<sub>i</sub>** is area of the individual classes and **E<sub>ai</sub>** is the methane emission rate of each class within the period from June to October.

$$Ea = \sum_{i=1}^n A_i E_{ai} \quad (2)$$

## 4. Results

### 4.1. Supervised classification

Nine land cover classes characterised by their vegetation, surface moisture, and topography plus one cloud mask class could be defined for the Lena Delta area:

Water bodies (WB): water bodies include the open water of lakes, rivers, streams and coastal waters.

Shallow water (SW): this class consists of recurrent or steadily shallow inundated areas: a) shallow coastal waters, shallow waters of riverbanks, and mainly barren sand bars, or b) shallow parts of lakes and rivers with typical vegetation of sedges and hydrophilic grasses.

Mainly non-vegetated areas (NV): barren or partially vegetated areas on active river bars, along the coast line, or deflation cliffs. These sites are mostly sandy and vary in soil moisture.

Wet sedge- and moss-dominated tundra (WT): sites with water-saturated substrate and a nearly continuous cover of sedges, especially *Carex aquatilis*, and other hydrophilic graminoids growing in shallow water (e.g. *Eriophorum scheuchzeri*) or mosses.

Moist grass- and moss-dominated tundra (MT): areas are characterised by moist tundra on poorly drained soils and a continuous vegetation cover of grasses, mosses and dwarf shrubs (*Betula nana*, *Salix spp.*).

Dry moss-, sedge- and dwarf shrub-dominated tundra (DMSD): well drained sites with sand as predominant substrate, found often close to cliffs. The vegetation cover can vary: there are sites dominated by sedges, and cotton grass and mosses as dominant vegetation with isolated occurring lichens and dwarf shrubs, other sites are dominated by dwarf shrubs and lichens.

Moist to dry dwarf shrub-dominated tundra (DMD): this class is dominated by dwarf shrubs and is found on moist to dry sites. It occupies large areas of the lower floodplain and is dominated by dwarf willows; on moist sites cotton grass occurs. Seasonal inundations of these areas result in a high content of nutrients in the soils and a dense vegetation cover.

Dry grass-dominated tundra (DG): this cover type occurs predominantly on the lower floodplain, the substrates are mostly dry and temporary moist after the inundation. The areas are characterised by grasses (e.g. *Deschampsia brevifolia*), some sites are only sparsely vegetated.

Dry tussock tundra (DT): this land cover class is characteristic for dry, very well-drained sites of upper slopes and pingos. The vegetation cover consists of *Eriophorum vaginatum* tussocks.

Cloud mask: clouds and cloud shadows.

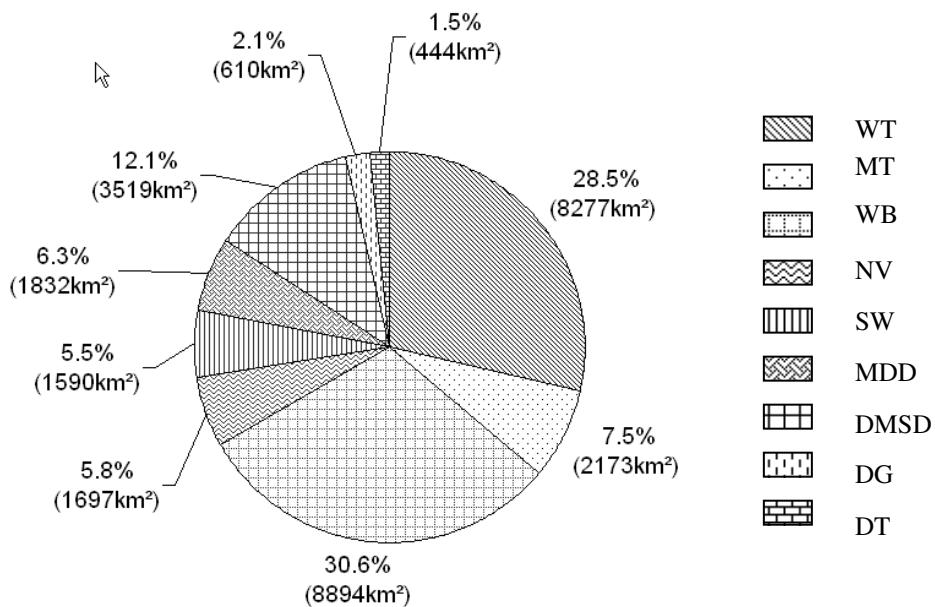


Fig. 3. Percentage of surface coverage and area estimates of the land cover classes in the Lena delta, based on the original resolution (30m\*30m) of the LANDSAT-7 ETM+ images.

The land cover classification reflects the different river terraces and floodplains of the Lena Delta. A detailed image of the classification of a part of the Lena Delta is shown in Figure 2. Nearly 1/3 of the total area of the Lena Delta is occupied by water bodies (WB, 30.6 %). Together with the land cover classes SW (5.5 %), WT (28.5 %) and MT (7.5 %) this amount to 72.1 % of the Lena Delta area, indicating the dominance and importance of wetland areas for the delta ecosystem. The composition and distribution of land cover classes varies for the three main river terraces. In contrast to the first and the third terrace, the second terrace is dominated by classes indicating mainly dry conditions. Area estimates for each land cover class are given in Figure 3.

#### 4.2. Upscaling of methane emissions: case study for the land cover class wet sedge- and moss-dominated tundra

Within the scope of this study the most detailed measurements were done for the land cover class *wet sedge- and moss-dominated tundra* (WT). There are several reasons for concentrating on detailed and systematic investigation of methane emissions from this class. First, from our previous measurement campaigns we know that this extent land cover class is the most important source of methane in the Lena Delta. Second, extensive ancillary data about the soil composition, soil moisture, soil physics, vegetation, microrelief, and microbiology are available for the systematic investigation of the determining factors for methane emission. This allows the investigation of small-scale heterogeneities of these parameters and their influence on the methane emission from this individual land cover class. The land cover class *wet sedge-and moss-dominated tundra* (WT) consists of polygonal microrelief and lakes of different sizes with high emission rates in wet polygon centers, and lower emission rates from drier polygon rims, open water ponds, and the vegetated lake margins. For this class we used methane emission rates measured at habitat-scale (Table 1). The weighted calculation shows that methane emission rates of this class range from 10.8 to 23.2 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup>, with a mean at 16.8 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup>

Table 1

Habitat types of the land cover class *wet sedge- and moss-dominated tundra* and the belonging daily methane emission rates in the months June to October. The percentage of habitat type cover was determined by aerial image analysis of key sites on the first delta terrace.

Habitat type	Cover %	Methane emission (mg CH <sub>4</sub> m <sup>-2</sup> d <sup>-1</sup> )				
		June	July	August	September	October
Very wet sites	7.8	Mean	54.1	93.7	44	17.9
		Min	13.7	60.3	32.9	7
		Max	89.4	119.6	72.6	25.8
Dry sites	62.2	Mean	2.5	4.7	6.1	2.1
		Min	0.7	3.3	3.1	0.6
		Max	4.6	6.2	11.4	4
Water	15.2	Mean	4.1	4.1	7.9	4.1
		Min	2.0	2.0	3.3	2.0
		Max	7.9	7.9	15.7	7.9
Overgrown water	14.8	Mean	40.3	40.3	48.1	40.3
		Min	25.6	25.6	31.9	25.6
		Max	59.9	59.9	67.1	59.9

Table 2

Methane emissions of the land cover class *wet sedge- and moss-dominated tundra* in the months June to October (weighted calculation).

Wet sedge- and moss-dominated tundra	Methane emission (mg CH <sub>4</sub> m <sup>-2</sup> d <sup>-1</sup> )				
	June	July	August	September	October
Mean	12.32	16.75	15.4	9.28	8.46
Minimum	5.63	10.79	9.6	4.97	4.7
Maximum	19.79	26.16	24.83	14.51	14.43

Temporal variations during the vegetation period are another central objective of long-term investigation of methane emissions in the Lena Delta. While methane emission rates for the polygon rims and centers have been determined in the field in the period from June to October, the rates for the open water and vegetated rims of lakes have been determined only in July and August. For the estimation of methane emission for the land cover class *wet sedge- and moss-dominated tundra* we

used the July methane emission rates for missing periods. The maximum, mean and minimum values of the methane emissions from each of the habitats are summarised in Table 1. Based on these assumptions, we calculated the methane emission rates for the different months for this land cover class; they are listed in Table 2. The fast increase of emissions in June, the maximum in July, and the following slow decrease of methane emission rates are obvious.

#### 4.3 Upscaling of methane emissions in the Lena Delta

The daily methane emission rates vary strongly among the individual land cover classes. While the highest amounts are emitted by the class *moist to dry dwarf shrub-dominated tundra*, followed by the *moist grass- and moss-dominated tundra*, the lowest rates are emitted by the “dry” classes. The land cover classes *wet sedge- and moss-dominated tundra* and *moist grass- and moss-dominated tundra* represent the wetlands of the Lena Delta. The methane emission rate of the wetlands is  $16.8 \text{ mg CH}_4 \text{ m}^{-2}\text{d}^{-1}$  (weighted calculation).

Table 3 shows the results of the calculation of the daily methane emissions in the Lena Delta (after equation 1). Upscaling is based on the original resolution (30m x 30m) of the land cover classification.

Table 3

Methane emission rates of individual land cover classes used for the calculation of the methane emission of the Lena delta and the daily methane emission of each class in July

Land cover class	Area (km <sup>2</sup> )	Methane emission	
		(mg m <sup>-2</sup> d <sup>-1</sup> )	(10 <sup>6</sup> g d <sup>-1</sup> )
Wet sedge- and moss-dominated tundra	8277	16.8 <sup>1</sup>	139.1
Moist grass- and moss-dominated tundra	2173	17.2 <sup>1</sup>	37.4
Open water	8894		
- lakes (> 1ha)	2805	3.1 <sup>2</sup>	8.7
- rivers, coastal waters and lakes < 1ha	6089	0	0
Mainly non-vegetated areas	1697	0	0
Shallow water	1590	0	0
Moist to dry dwarf shrubs-dominated tundra	1832	58.4 <sup>1</sup>	107
Dry moss-, sedge- and dwarf shrub-dominated tundra & Dry grass-dominated tundra & Dry tussock tundra	4573	0.4 <sup>3</sup>	1.8
Total	29036	10.1	294

<sup>1</sup> Mean values measured in the Lena Delta, <sup>2</sup> Assumption after Morrissey & Livingston (1992), <sup>3</sup> Assumption after Kutzbach & Kurchatova (2002)

In comparison to the daily methane emission rates, the annual emission rates are of higher importance for the calculation of the methane emissions of the Lena Delta area. Due to our measurements in early winter (Oct. 2003), we assume that the methane emission in winter is about zero. Within the scope of our study, our calculations could only determine temporal trends of methane emissions for the class *wet sedge- and moss-dominated tundra* (Table 2). The methane emissions of the class *wet sedge- and moss-dominated tundra* for the period from June-October amounts to  $1907 \text{ mg CH}_4 \text{ m}^{-2}$ . Based on this calculation, we tested different assumptions regarding the length of the vegetation period. Assuming that the length of the vegetation period is 120 days, the methane

emission of the land cover class *wet sedge- and moss-dominated tundra* is  $2010 \text{ mg CH}_4 \text{ m}^{-2}$ , compared to  $1675 \text{ mg CH}_4 \text{ m}^{-2}$  for a vegetation period of 100 days. Therefore, in the first case the calculated methane emission is 5.4 % higher and in the second case 12.2 % lower than  $1907 \text{ mg CH}_4 \text{ m}^{-2}$ . This calculation confirmed our decision to use the calculated trend and not the assumptions of the length of the vegetation period for further investigations.

The calculation of the methane emissions from all other land cover classes during the vegetation period is based on the ratio of the methane emissions of each individual land cover class and the methane emission of the class *wet sedge- and moss-dominated tundra* (equation 2).

Table 4 shows the results of the upscaling for the individual land cover classes and for the Lena Delta. The upscaling for the Lena Delta is a weighted calculation using the methane emission rates of the individual classes. The highest amounts of methane are emitted by the classes *wet sedge- and moss-dominated tundra* ( $15.8 \times 10^9 \text{ g CH}_4$  per year) and *moist to dry dwarf shrub-dominated tundra* ( $12.1 \times 10^9 \text{ g CH}_4$  per year), followed by *moist grass- and moss-dominated tundra* ( $4.2 \times 10^9 \text{ g CH}_4$ ). Although the share of the land cover classes *dry tundra* with different vegetation cover in the total area of the Lena Delta is relatively large, the contribution of these classes to the total methane emission of the delta is low ( $0.2 \times 10^9 \text{ g CH}_4$  per year).

Table 4

Annual methane emissions of the individual land cover classes and their total methane emission in the Lena delta

Land cover class	Methane emission ( $\text{mg m}^{-2}\text{a}^{-1}$ )	Area ( $\text{km}^2$ )	Methane emission ( $10^6 \text{ g}$ )
Wet sedge- and moss-dominated tundra	1906.9	8277	15783.4
Moist grass- and moss-dominated tundra	1952.3	2173	4242.3
Mainly non-vegetated areas	0	1697	0
Shallow waters	0	1590	0
Moist to dry dwarf shrub-dominated tundra	6628.7	1832	12143.8
Dry moss-, sedge- and dwarf shrub-dominated tundra &	44.1	4573	201.7
Dry grass-dominated tundra & Dry tussock tundra			
Open water (only lakes > 1ha)	352.7	2805	989.3
Other (lakes < 1ha, rivers, streams, coastal waters)	n.a.	n.a.	n.a.
Total	1149	29036	33360.5

n.a. = not analysed

## 5. Discussion

### 5.1. Land cover classification

Whalen & Reeburgh (1990), Bartlett et al. (1992) and Christensen et al. (2000) have used land cover classifications for the upscaling of methane emissions within their studies. Heikkinen et al. (2004) did an upscaling of methane and carbon dioxide emissions for the catchment of the Lek Vorkuta River, East European Russia. All these balances of methane and carbon dioxide are based on land cover classifications with only five to six land cover classes.

A common method for a general land cover classification of large heterogeneous datasets is the automatic unsupervised classification based on a chain algorithm and the subsequent labelling of land

cover classes with real land cover features (Joria & Jorgenson, 1996; Stow et al., 1998; Cihlar, 2000). Such unsupervised classifications with a very large number of classes proved unsuitable for the land cover classification of wetlands with a focus on methane balancing, as usually only a limited number of measurement sites are available. Furthermore, the classes obtained with such an approach are ecologically very heterogeneous and thus unsuitable for site-upscaling. Thus, we used a supervised classification approach based on a relatively small number of classes for the classification of the Lena Delta. We obtained the best results with the supervised *minimum distance algorithm* using nine classes. The Landsat 7 derived classes reflect especially the local soil moisture and vegetation conditions, both are important parameters for the methane emissions of a site (MacDonald et al., 1998; Wagner et al., 2003; Kutzbach et al., 2004). Therefore, the land cover classes could be related to locally measured methane emissions.

Within this study we used class area calculations based on the land cover classification of a Landsat 7 image mosaic from July 2000 and 2001. This mosaic provides a snapshot of the mid-summer situation in the highly dynamic environment of the Lena Delta. Seasonal variations, e.g. like changes in vegetation cover density, soil moisture, and the annual inundation of the floodplain levels during the spring flood, are not considered.

Commonly, three types of potential errors for a land cover classification are distinguished (Felix & Binney, 1989). The first possible error is misclassification that describes the discrepancy between a classification-derived and the field-observed land cover. The second is the cutpoint error, where the field-observed land cover is a transition zone between two land cover classes with similar characteristics. The third possible error is the wrong or incomplete description of the land cover classes. To a certain amount, all these types of errors occur in our classification. Cutpoint errors occur mostly among the spectrally similar classes *wet sedge- and moss-dominated tundra* and *moist grass- and moss-dominated tundra*, which predominantly differ in their soil moisture. On the other hand, there is almost no misclassification between the class open water and all other classes. Considering the moderate quality of ancillary data for some areas of the large Lena Delta, we assume that there are misclassification errors between some of the vegetation classes. Some of the class descriptions will need modification. These errors are currently not quantifiable unless further ground truth is done in the Lena Delta.

Nevertheless, we were able to classify the land cover of the Lena Delta regarding the balance of the methane emission despite the described problems. This land cover classification is the first encompassing the entire Lena Delta at high-resolution. The total number of the classes of our land cover classification is nine.

Based on some general Russian thematic maps, fieldwork experience, and general knowledge of the landscape structure and field photographs we assume that the classification of the Lena Delta has a good accuracy.

## 5.2. Case study for the land cover class wet sedge- and moss-dominated tundra

The Lena Delta is characterised by small-scaled heterogeneity of vegetation cover, soils and water balance. These factors have a direct effect on the methanogenesis and on the amount of the emitted methane. The most detailed balance of methane emissions could be realised for the land cover class *wet sedge- and moss-dominated tundra*. These sites mostly appear as polygonal tundra and cover large areas of the Lena Delta. In this study, we demonstrated that the drier habitats of polygon rims dominate the polygonal tundra, covering nearly 62 % of these sites. These results are confirmed by the study of Kutzbach et al. (2004). More than 50 % of the area of their study site was covered by polygon rims.

In the following, we compare the emission rates of the different habitats (polygon rims, polygon centers, lakes and vegetated lake margins) of the land cover class *wet, sedge- and moss-dominated tundra* with the results of methane measurements of other studies. Bartlett et al. (1992) and Christensen et al. (1995) measured less methane emission for dry tundra sites of polygonal tundra than in this study. Heikkinen et al. (2004) reported nearly the 8-fold amount of methane emission of a thermokarst lake in northeast Russia comparing to the amounts measured in the Lena delta. Further investigations on methane emissions from lakes have been done by Kling et al. (1992) in Alaska and by Zimov et al. (1997) in Siberia. The mean daily methane emission rates of the polygonal lakes of the

Lena Delta are noticeably lower than the methane emissions of the polygonal lakes of other study sites. The importance of lakes for the methane emissions from tundra and the global methane emissions is not yet sufficiently clarified. According to Semiletov et al. (1996), the limnic ecosystems of the tundra are one of the most important recent methane sources to the atmosphere. Morrissey & Livingston (1992) determined that lakes have a considerably lower methane emission potential than other habitats in the tundra. Bartlett et al. (1992) suggest a close connection between methane emissions and the size of the lakes. Small lakes (less than 10 km<sup>2</sup>) emit significantly more methane than the large lakes (more than 10 km<sup>2</sup>). In contrary, Kling et al. (1992) reported in their study that there is no correlation between the methane emission of a lake and its size, depth, or its latitudinal location. However, the importance of the vegetated lake margins for the methane balance of the tundra is well known. The mean daily methane emissions estimated by Bartlett et al. (1992) and Morrissey & Livingston (1992) amount double the methane emissions of vegetated lake margins in our study. Differences in methane emissions between these studies appear largely due to differences in the location of the study sites. The study site of Morrissey & Livingston (1992) was located south of 71°N (the most southern sampling area at 68°35'N), while the site of Bartlett et al. King et al. (1998) determined a correlation between temperature and plant mediated transport of methane in the vegetated lake margins. The results of King et al. (1998) probably explain why the methane emissions of vegetated lake margins found by Bartlett et al. (1992) and Morrissey and Livingston (1992) were higher than the results of this study. The mean daily methane emission rate of wet polygon centers in the Lena Delta is 93.7 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup>. About one third more was measured by Bartlett et al. (1992) at wet meadow sites, and nearly half of this amount by Christensen et al. (1995) at wet tundra sites. Both sites are comparable to the sites in the Lena Delta because these sites are also characterised by water saturated soils and vegetation of *Carex spp.* and *Eriophorum spp.* The measurements in the Lena Delta and those of Whalen & Reeburgh (1990) at wet tundra sites are highly consistent. The methane emissions reported by Nakano et al. (2000) for water saturated sites in the tundra are much higher than the methane emissions in our study. Differences between the measurements appear also due to the different length of the investigation period. If the study period is short, the methane emissions strongly reflect the weather conditions (Christensen, 1993). The methane emission rates in this study have been measured during field trips of several weeks since 1998. Summarizing, the measured methane emissions from the various habitats of the land cover class *moist grass- and moss-dominated tundra* in the Lena Delta are similar to those reported from analogous areas in the high latitudes.

The mean daily methane emission of the class *wet sedge- and moss-dominated tundra* amounts to 16.8 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup>. The high emission rates of the wet polygon centers are not reflected in the emission of this class due to the low percentage (7.8 %) of the area of this class. In contrast, the low methane emissions of the drier habitats and lakes have a strong influence on the methane emission of this class.

Generally, the length of the measurement period is an important factor for the quality and the applicability of measured methane emission rates for temporal and spatial upscaling. Most of the previous studies were conducted during July-August only (Whalen & Reeburgh, 1990; Bartlett et al., 1992; Martens et al., 1992; Christensen et al., 1995). Some provide only imprecise information about the investigation period (Morrissey & Livingston, 1992; Nakano et al., 2000; Takeuchi et al., 2003). This also results in difficulties when comparing these methane emission rates to our long-term multi-annual measurements of the class *wet sedge- and moss-dominated tundra*. A study by Christensen et al. (2000) covering an investigation period from the middle of June to the end of August is an exception. Their study covers the high-Arctic Zackenberg Valley in Greenland, which strongly differs in climatic and substrate conditions to the sites in the Lena Delta. In Zackenberg Valley, the fast increase of the emissions at the beginning of the vegetation period is missing and the methane emission is nearly zero mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup> in June. Methane emission rates throughout the year are considerably lower than in the Lena Delta.

### 5.3. Upscaling of methane emissions in the Lena Delta

The small-scaled heterogeneity in vegetation and soil moisture and, thus, in methane emission could be analysed only within the land cover class *wet sedge- and moss-dominated tundra*. The coverage of this class in the Lena Delta is 28.5 % and the percentage of methane emission is 47.3 %

(Fig. 4). The small-scaled mosaic of the class *moist grass- and moss-dominated tundra* could not be investigated with the same level of detail. The coverage of this class in the Lena Delta is 7.5 % and the percentage of methane emission is about 12.7 % (Fig. 4). Further investigations of small-scaled and seasonal variability of methane emissions within the class *moist to dry dwarf shrub-dominated tundra* are necessary. In this study, this land cover class emits 36.4 % of the methane in the Lena Delta but covers only 6.3 % of the delta (Fig. 4). Because the class *shallow water* could not be separated into its different habitat types, it was not included in the methane balance of the Lena Delta. Its influence on the overall methane emission of the delta seems to be negligible because large parts of the class consist of low-emission sites (non-vegetated shallow water).

Furthermore, the methane emission rates of large lakes and rivers in the Lena Delta are estimated to be low. The results of the studies by Heikkinen et al. (2004) and Whalen & Reeburg (1990) show, that the arctic rivers and thermokarst lakes are important methane sources. In comparison, the study of CH<sub>4</sub> in the surface arctic waters of the Lena Delta by Semiletov et al. (1996) shows that the rivers and coastal waters are not the significant factors in the present methane budget of the Lena Delta area. The mean daily methane emissions of lakes in Alaska (Whalen & Reeburgh, 1990) and in European Russia (Heikkinen et al., 2004) were at least 7-fold of the methane emission reported in our study. Kling et al. (1992) estimated an average methane emission of 5.76 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup> for Kuparuk River in Alaska and Heikkinen et al. (2004) 9.9 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup> for Lek Vorkuta River, which is higher than the methane emissions in the Lena Delta. At these study sites, the methane emissions of lakes and rivers play an important role in the balance of methane emissions. Although the open water habitats cover 30 % of the Lena Delta, their total share on the methane emission is only 3 % (Fig. 4).

The mean daily methane emissions (0.4 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup>) of dry sites in the Lena Delta are low as well. According to Heikkinen et al. (2004), the methane emission rates of the dry sites are in general very low or negative. In the Lek Vorkuta catchment they estimated methane emissions between -8.1 and 10.5 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup> and an average emission around zero. The dry sites cover nearly a quarter of the delta area, and the methane emission amounts to only 0.6 % of the total Lena Delta emission (Fig. 4).

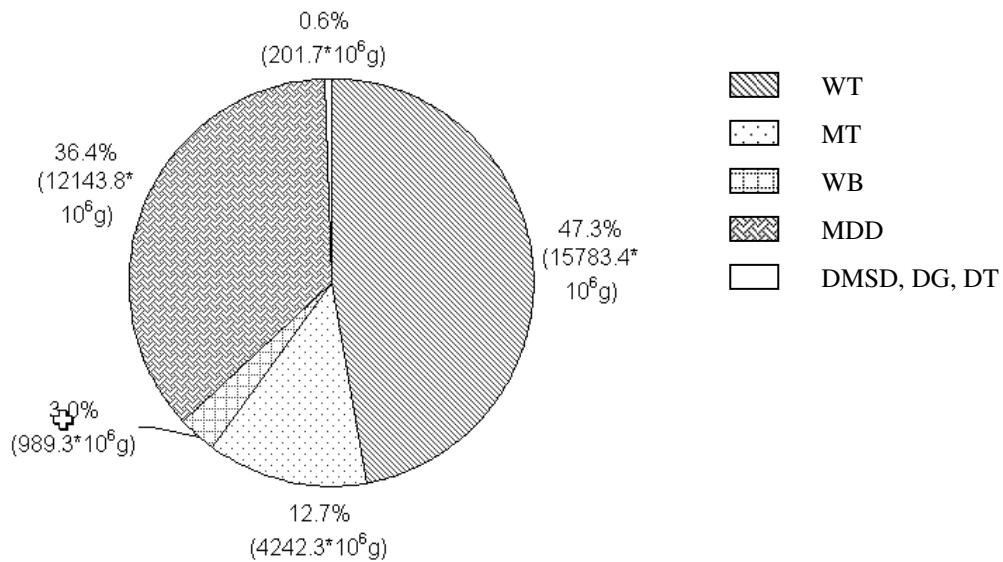


Fig. 4. Percentage of methane emissions of individual land cover classes based on the total methane emission of the Lena Delta

The mean daily methane emission of the Lena Delta is 10.1 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup>. This value is about 20 % of the value for the arctic tundra calculated by Whalen & Reeburgh (1990) (52 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup>). The mean daily methane emissions of the wetlands in the Lena Delta amount to 16.8 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup>. That is below the range of 40 to 50 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup> estimated by Christensen et al. (1995) for northern

wetlands. Earlier estimations have been much higher, for example the estimation by Matthews & Fung (1987) of about 200 mg CH<sub>4</sub> m<sup>-2</sup>d<sup>-1</sup>.

The methane emissions presented here are based on measurements in the period from June to October. We did not measure during the whole winter time. We assume that the methane emission during the cold season is nearly zero due to the low temperatures. This assumption is based on measurements in October, which show the methane emission rates decreasing to zero (Ganzert et al. 2004). The discussion about the amount of methane emitted in winter is still ongoing. Winter methane fluxes have been estimated only in North America and West Siberia (Whalen & Reeburgh, 1988; Dise, 1992; Melloh & Crill, 1996; Panikov & Dedysh, 2000). The reported winter emission rates amounted from about 4 to 41 % of the annual methane fluxes. Zimov et al. (1997) demonstrated that methane is produced in arctic lakes under ice during winter. The gas is largely released to the atmosphere from holes in the ice during winter or during water column circulation after the spring ice melt. According to Zimov et al. (1997), the north Siberian lakes could release about 75 % of their annual methane emission during winter. Christensen et al. (1995) underline, that the methane emissions in winter are not well investigated and may contribute significantly to the total emission of permafrost environments.

The results of a study by Worthy et al. (2000) in the Hudson Bay Lowland correspond to our assumptions. The largest emissions occur in the months July and August. The emissions drop off in September and become very weak in October. The emissions become observable again in June and are around zero or negative in the winter period.

Although the conditions of the study site of Reeburgh et al. (1998) are comparable to our site, the results of the methane emission measurements are incomparable. Their study site is the catchment of the Kuparuk River in North Alaska. It has a size of 26000 km<sup>2</sup> and is situated north of 68°N. They estimated that the class wet tundra of the Kuparuk catchment emitted 5171 mg CH<sub>4</sub> m<sup>-2</sup>a<sup>-1</sup>. This is nearly threefold of the methane emissions of the class *wet sedge- and moss-dominated tundra* in the Lena Delta. The methane emissions of the lakes and dry sites in the Lena Delta are significantly lower than those of the Kuparuk catchment and this reflects in the mean annual methane emissions, the emissions of the Lena Delta (1149 mg CH<sub>4</sub> m<sup>-2</sup>a<sup>-1</sup>) are half of the emissions of the Kuparuk River catchment (2390 mg CH<sub>4</sub> m<sup>-2</sup>a<sup>-1</sup>).

The annual methane emission of the Lena Delta amounts to about 0.03 Tg. The emissions presented here most probably underestimate the annual gas release since we did not include emissions from lakes < 1 ha and rivers due to lacking base data, nor possible emissions during the winter. Further investigations of the small-scaled heterogeneity of the vegetation cover and soil moisture have to be done. A comparison of the annual methane emission of the Lena Delta with those of other study sites is difficult due to lacking upscaling efforts of the methane emissions from measurement sites to larger study areas (Whalen & Reeburgh, 1988; Morrissey & Livingston, 1992; Christensen, 1993; MacDonald et al., 1998; Christensen et al., 2000).

## 6. Conclusions

The results of this study show that remote sensing and supervised image classification are powerful tools for the upscaling of local methane emission measurements in high-latitude landscapes. The supervised classification of the Landsat 7 ETM+ images is particularly suitable for detection of ecosystems in the Lena Delta. The methane emission of tundra environments is influenced by numerous factors, e.g. microrelief, soil moisture, temperature, amount and quality of organic matter, thickness of active layer, availability of oxygen and nutrients, and vegetation. Tundra land cover type is directly or indirectly influenced by these parameters, enabling the correlation of local methane measurements with land cover classes and the upscaling of emission rates to the entire Lena Delta. The applied supervised *minimum distance* classification was very effective with the few ancillary data that were available for training site selection. The three main river terraces of the Lena Delta were found to have different associations of land cover classes. The first terrace is characterised by wet sites and lakes, the second appear to be drier and differ also in vegetation and the third terrace is characterised by moist sites. Accordingly, the first terrace has the highest methane emission potential. There is a strong variation in between the individual land cover classes regarding the methane

emissions. The methane emissions of the classes in the Lena Delta are within the currently known natural range of emissions from tundra habitats. Taking our multi-scale approach into account, the methane source strength of certain tundra wetland types is expected to be lower than calculations based on coarser scales. This study is the first attempt to assess the methane emission of the Lena Delta based on satellite data and field measurements. Despite the uncertainties, the results suggest that the Lena Delta contributes significantly to the global methane emission because of its extensive wetland areas.

The approach we used for the balance of methane emission can contribute to the improvement of the recent global balance of methane emissions and there is still large potential for intensifying research in terms of validation of methane measurements for different land cover types and varying habitats in them.

### Acknowledgments

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## 4 Permafrost Ecosystems and their Microbial Processes

### 4.1 Element Redistribution along Hydraulic and Redox Gradients of Low-Centered Polygons, Lena Delta, Northern Siberia

S. Fiedler,\* D. Wagner, L. Kutzbach, and E.-M. Pfeiffer

#### ABSTRACT

**Wetland soils affected by permafrost are extensive in subarctic and arctic tundra.** However, this fact does not imply these soils have been sufficiently investigated. In particular, studies of element translocation processes are scarce. This study was conducted (i) to determine the relationship between water and redox regimes in wetland soils in the Siberian tundra, and (ii) to investigate their influence on the distribution of redox sensitive and associate elements (Mn, Fe, P). Major geomorphic units were chosen (microhigh, polygon rim and slope; microlow, polygon center) from two low-centered polygons in the Lena Delta. Within polygons, redox potential, permafrost, and water level were measured during summer in 1999 and 2000 and (related) compared with element distribution. Manganese, Fe, and P accumulations were preferentially observed in aerobic microhighs. Anaerobic conditions in the microlows lead to a mobilization of Mn, Fe, and P. The elements migrate via water and are immobilized at the microhigh, which acts as an oxidative barrier. The element pattern, indicating an upward flux via water along redox gradients, is explained by higher evapotranspiration from soils and vegetation of the microhighs (*Typic Aquiturbel*) compared with soils and vegetation of the microlows (*Typic Historthel*). However, in further research this upward transport should be validated using labeled elements.

**S**OILS IN SUBARCTIC and arctic tundra affected by permafrost occupy a total area of  $1.5 \times 10^9 \text{ km}^2$  (Harris et al., 1993). Permafrost soils represent the largest group of natural wetlands, which are an important source of the greenhouse gases methane and carbon dioxide (Aselmann and Crutzen, 1989; Christensen et al., 1995). They are C sinks (Bliss, 1997) as well, storing about 30% of the global soil organic C (SOC) (Michaelson et al., 1996). With respect to the predicted global temperature increase, it is assumed these soils can switch from sinks to sources of C (Grulke et al., 1990; Oechel et al., 1993). Therefore, recent studies primarily deal with C budgets, especially with the microbial processes of methane production and methane oxidation (Wagner et al., 2001a, 2003). In addition, permafrost soils are of special interest for current astrobiological research. Permafrost areas and the Mars surface have shown similar morphological structures, which suggest their development is based on comparable processes (Wagner et al., 2001b). The typical patterned ground in permafrost regions is composed of recurrent symmetrically formed ice wedge polygons that have emerged from annual freeze-thaw

processes (Brown, 1967; Tedrow, 1977; Tarnocai and Zoltai, 1978; Washburn, 1979). During winter, ice wedges crack, releasing contraction tension. During spring, melt water seeps into these cracks, eventually freezing and continuing the process. Expansion of the surface layer is caused by increasing surface temperatures during summer, which leads to a typical microrelief. Some parts are elevated (polygon rim and slope = microhigh), whereas others are depressed (polygon center = microlow). Upturning of permafrost strata by plastic deformation leads to transport of the solid soil phase. This process is reflected by twisted and mixed soil horizons as well as buried organic matter (Bockheim and Tarnocai, 1998) (Fig. 1). It is generally agreed cryoturbation is the dominant pedogenetic process of the polar regions (Van Vliet-Lanoë, 1991; Bockheim et al., 1999, 2003). However, in addition to twisted layers, continuous bands of Fe can also be found. These features follow the buckling surface regularly and indicate element transport via liquid phase is an important pedogenetic process within the polygons (Fig. 1).

Although arctic studies have added to an understanding of arctic soils (Feustel et al., 1939; Tedrow et al., 1958; Gersper et al., 1980; Rieger, 1983; Bliss, 1997; Korotaev, 1986), investigations in element translocation processes are still scarce. Results are predominantly of descriptive nature lacking pedogenetic information. To provide a better understanding of element translocation processes as a key to arctic soil genesis, a typical catena of polygonic soils along the island of Samoylov in the Lena Delta (Siberia) was investigated. The objective of this investigation was to identify and expound on the principal of element redistribution processes. It was hypothesized that within polygons (i) recent element redistribution via liquid phase caused by mobilization, transport, and immobilization processes was taking place and that (ii) these processes led to depletion and accumulation zones in the solid phase of polygonic soils. The controlling factors of these processes (iii) are hydraulic and redox gradients between microhighs and microlows.

#### MATERIALS AND METHODS

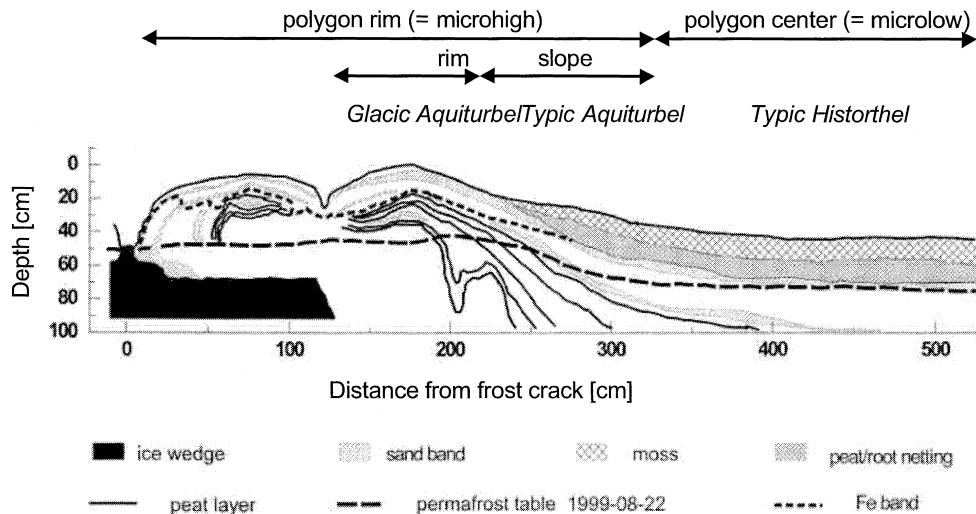
##### Environmental Setting of the Study Sites

Samoylov island ( $72^\circ 22' \text{ N}$  Latitude,  $126^\circ 28' \text{ E}$  Long.) in the Lena River Delta (approximately  $32000 \text{ km}^2$ , Are and

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**Abbreviations:** subscript [d], dithionite citrate bicarbonate extractable;  $D_b$ , bulk density; Dpd,  $\alpha$ - $\alpha$ -dipyridil;  $E_{\text{H}}$ , oxidation-reduction (redox) potential;  $Fe_t$ , total iron;  $K_{\text{cal}}$ , calcium lactate acetate extractable K; MC, matrix color;  $Mn_t$ , total manganese;  $N_t$ , total nitrogen; subscript [o], ammonium oxalate extractable; subscript [p], Na-pyro-phosphate extractable;  $P_{\text{cal}}$ , calcium lactate acetate extractable phosphorus (=plant available); PG1, Polygon 1; PG2, Polygon 2;  $P_i$ , phosphorus; SOC, soil organic carbon.

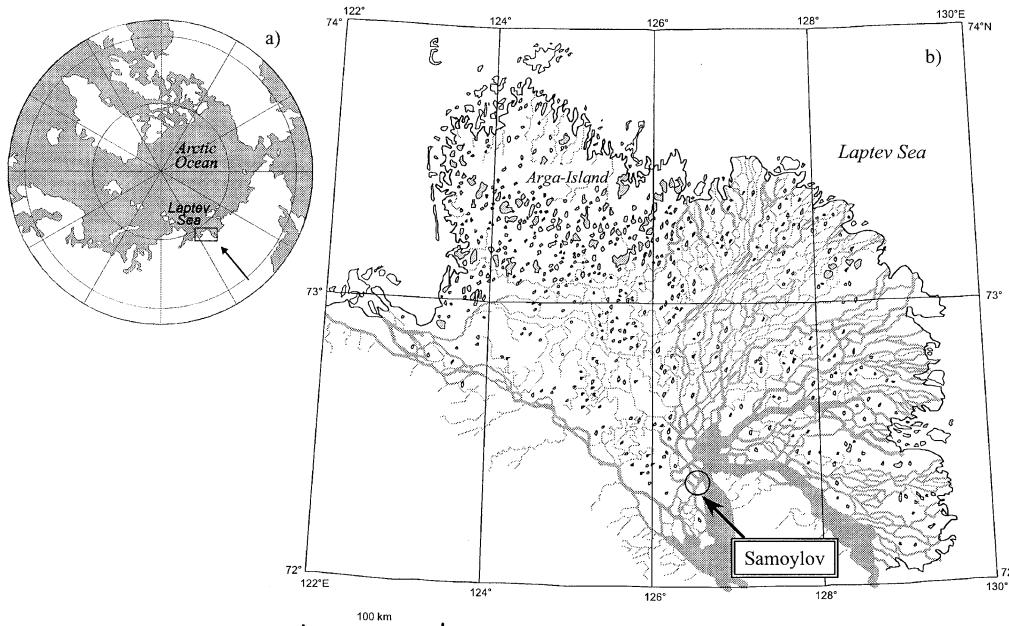


**Fig. 1.** Cross-section of a typical low-center polygon (Lena Delta, Northern Siberia).

Reimnitz, 2000) covers an area of approximately 1200 ha (Fig. 2) and portrays the active and youngest (approximately 8000–9000 yr) part of the delta (Schwamborn et al., 2002). Maximum altitude above mean sea level is 12 m, representing the oldest part of the island. Shore sites with elevations of about 4 m above mean sea level are the lowest areas. Geomorphology of the island can be structured as follows (Akhmadeeva et al., 1999): the western part is characterized by recent depositional processes (sandy fluvial and aeolian sediments) and the eastern part is dominated by erosion processes that have formed an abrasion coast. Due to changes in river levels, four terraces were formed. Investigations were performed on the Middle-Holocene terrace, which was dominated by active ice wedge growth with low and high centered polygons and thermokarst lakes.

Two major topographic units (microhigh = rim, microlow = center) of two low-centered polygons (Polygon 1 = PG1, Polygon 2 = PG2) were selected. In addition, the middle transect position polygon slope (= microhigh) of one of the selected polygons was analyzed (PG1, Fig. 1). Depending on its position, a variety of wet arctic tundra vegetation grows on Samoylov from mossy tundra to wet fen and flooded sedges in the center of the polygons (Kutzbach, 2000).

Well-defined climatic distinctions between seasons are characteristic for the Lena Delta, which belongs to the continental area of the Arctic. Winter lasts 9 mo (end of September–end of May,  $T_{\min} = -30^{\circ}\text{C}$  January), is characterized by insufficient light (polar night) and severe snowstorms ( $140 \text{ km h}^{-1}$ , Wein, 1999). During the arctic summer of almost 12 wk, temperatures are above freezing point ( $T_{\max} = 7^{\circ}\text{C}$  July). Mean annual air



**Fig. 2.** The investigation site on the island Samoylov. (a) Map showing the location of the Lena Delta, and (b) location of the study area on the island Samoylov.

temperature is  $-12^{\circ}\text{C}$ , and mean annual precipitation amounts to 190 mm. Approximately 25% of the annual precipitation is snow (<10 mm precipitation per month). Relative humidity is usually high (approximately 90%), and the annual evapotranspiration averages at 100 mm.

The investigations were performed during the summer of 1999 and 2000 within the framework of the joint cooperative Russian-German research project "Laptev Sea System 2000" (Pfeiffer et al., 2000; Wagner et al., 2001a). More details of the study sites have been described by Rachold and Grigoriev (1999, 2000, 2001).

### Field Methods

Every second day, thaw depth of the soil (active layer) and water levels were determined. Thaw depth was measured by pushing a steel rod into the soil until permafrost was encountered. Water table depths were measured in a series of slotted wells (polyvinylchloride, 6-cm i.d.). Redox potential ( $E_{\text{h}}$ ) was measured using Pt electrodes (90% Pt, 10% Ir, diameter of wires = 0.5 mm, wire length = 10 mm) as described by Fiedler (1997). Transects (from rim to center of polygon) along the two investigated polygons were equipped with a set of 30 Pt electrodes installed at a depth of 5 cm and directly above the permafrost. An Ag/AgCl electrode was used as a reference cell (Farrell et al., 1991). A data-logger (Delta-T-Devices LTD, Burwell, Cambridge, UK) was used for automatic readings at hourly intervals. Measurements were performed during summer (Period 1 = 29 June–2 Sept. 1999, Period 2 = 1 Aug.–21 Aug. 2000), the time with the highest biological activity. Redox data were corrected for the potential of the standard hydrogen electrode by adding 215 mV ( $10^{\circ}\text{C}$ ) to experimental readings. In addition, anaerobic conditions (presence of  $\text{Fe}^{2+}$ ) were verified in field using the  $\alpha$ - $\alpha$ -dipyridyl (Dpd) test (Bartlett and James, 1995) during the soil description. Soil solution at different depths (polygon rim 14, 27, 39 cm; polygon center 9, 15 cm below surface) was collected three times during each measurement period by suction lysimeters. Manganese and Fe concentrations were determined by atomic absorption spectroscopy (AAS, Varian SpectraAA-200, Mulgrave Victoria, Australia).

### Soil Characterization

Soil classification was determined according to U.S. Soil Taxonomy (Soil Survey Staff, 1999) and the World Reference Base of Soil Resources (ISSS-ISRIC-FAO, 1998). Microhighs were dominated by Typic Aquiturbels (Gleyi-Turbic Cryosols), whereas the prevalent soil type of the microlows was Typic Historthel (Gleyi-Histic Cryosol). Bulk density was measured using undisturbed soil cores ( $100 \text{ cm}^3$ , Schlichting et al., 1995, Method 5.3.1.1). Standard soil analyses were performed on the fine-earth fraction (<2 mm): particle-size distribution (Schlichting et al., 1995, Method 5.4.1.3), pH (CaCl<sub>2</sub>) (Schlichting et al., 1995, Method 5.4.5.1.2), and total carbon (C<sub>t</sub>, dry combustion, Leco CN-2000, Leco Instruments GmbH, Krefeld, Germany). In all soils investigated, SOC was equivalent to C<sub>t</sub>. Plant available potassium (K<sub>cal</sub>), and phosphorus (P<sub>cal</sub>) were determined by extraction with 0.1 M calcium lactate plus 0.1 M calcium acetate plus 0.3 M acetic acid (CAL, Schüller, 1969). Potassium in the extract was analyzed using flame emission spectroscopy (Elex 6361, Eppendorf, Hamburg, Germany). Determination of P was done by the molybdenum blue method of John (1970). Phosphorus was measured as molybdate-phosphate complex at  $\epsilon = 880 \text{ nm}$  using a spectrophotometer (Zeiss PL2DL, Germany). Pedogenetic oxides were extracted by dithionite citrate bicarbonate (Mn<sub>d</sub>, Fe<sub>d</sub>,

Schlichting et al., 1995, Method 5.5.5.3) (Mehra and Jackson, 1960) and ammonium oxalate (Mn<sub>o</sub>, Fe<sub>o</sub>, Schlichting et al., 1995, Method 5.5.5.2) (Schwertmann, 1964), and analyzed by atomic absorption spectrometry. The portion of organically bound Mn<sub>p</sub> and Fe<sub>p</sub> was extracted by Na-phyrophosphate at pH 10 (von Zezschwitz et al., 1973). Total element analysis (Mn, Fe<sub>b</sub>, P<sub>b</sub>) was performed with X-ray fluorescence (Siemens SRS-200, Bruker AXS, Karlsruhe, Germany).

### Calculation

Element masses per total soil volume of the active layer were calculated as follows (Sommer et al., 1997):

$$M_x = \sum_{i=1}^n \left( x_i D_b y_i \frac{100 - c f_i}{10000} \right)$$

$M_x$  is the mass of P, Fe, or Mn in the pedon fine-earth ( $\text{kg m}^{-2}$  profile depth $^{-1}$ );  $X_i$  is P, Fe, or Mn content (<2 mm) in horizon  $i$  ( $\text{g kg}^{-1}$  fine-earth);  $N$  is the number of horizons to profile depth;  $D_b$  is bulk density ( $\text{g m}^{-3}$ );  $Y_i$  is the thickness of the horizon  $i$  (cm);  $C f_i$  is the coarse fraction (>2 mm) of the horizon  $i$  (vol.%).

## RESULTS AND DISCUSSION

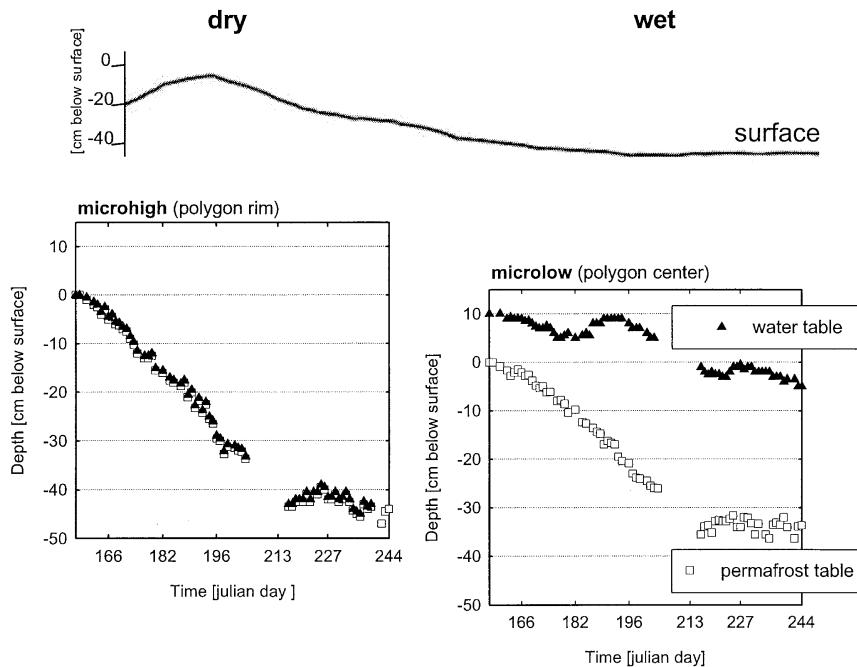
### Water Level and Permafrost Table

The two investigated polygons were characterized by very flat relief ( $\Delta$ height, PG1 = 37, PG2 = 22 cm over a distance of approximately 600 cm) (Fig. 4). According to Boike (1997) and Boike et al. (1998), seasonal hydrology can be described as follows: at the beginning of snowmelt, water flows laterally from microhighs to microlows. This leads to a supersaturation zone in the polygon center (water table ranged from +10 to  $-5 \text{ cm}$  below surface, Fig. 3), which lasts until freezing in autumn. On the contrary, the water table of microhighs rapidly drops to the permafrost table from spring to summer (0 to  $-40 \text{ cm}$  below surface).

The ground thaws rapidly until mid-June (after 18 June, Julian day 169) after which thaw rates are somewhat slower (Fig. 3). Thickness of the active layer varies from year to year in response to climatic conditions. Within the polygons, permafrost is deepest under the rim at the end of measurement periods (August 1999 vs. 2000, 44 vs. 38 cm) and most shallow in the polygon center (35 vs. 28 cm below surface). Maximum thaw depth was reached at the beginning of September. Summer in 1999 was warm and dry (August, average air temperature =  $7.8^{\circ}\text{C}$ ,  $T_{\min} = 0.6^{\circ}\text{C}$ ,  $T_{\max} = 23^{\circ}\text{C}$ , precipitation = 11.1 mm), while the same period in 2000 was characterized by lower temperature (average =  $5.5^{\circ}\text{C}$ ,  $T_{\min} = 2.5^{\circ}\text{C}$ ,  $T_{\max} = 10.5^{\circ}\text{C}$ ) and higher precipitation (20 mm). For both measurement periods, high evapotranspiration favored by continuous and strong winds was presumed.

### Redox Regime

Pronounced differences in soil moisture characteristics depending on topography were coupled with regular patterns of redox conditions (Fig. 4). Permanently submerged microlows showed strongly reducing conditions without an intrapedon redox gradient (topsoil approxi-

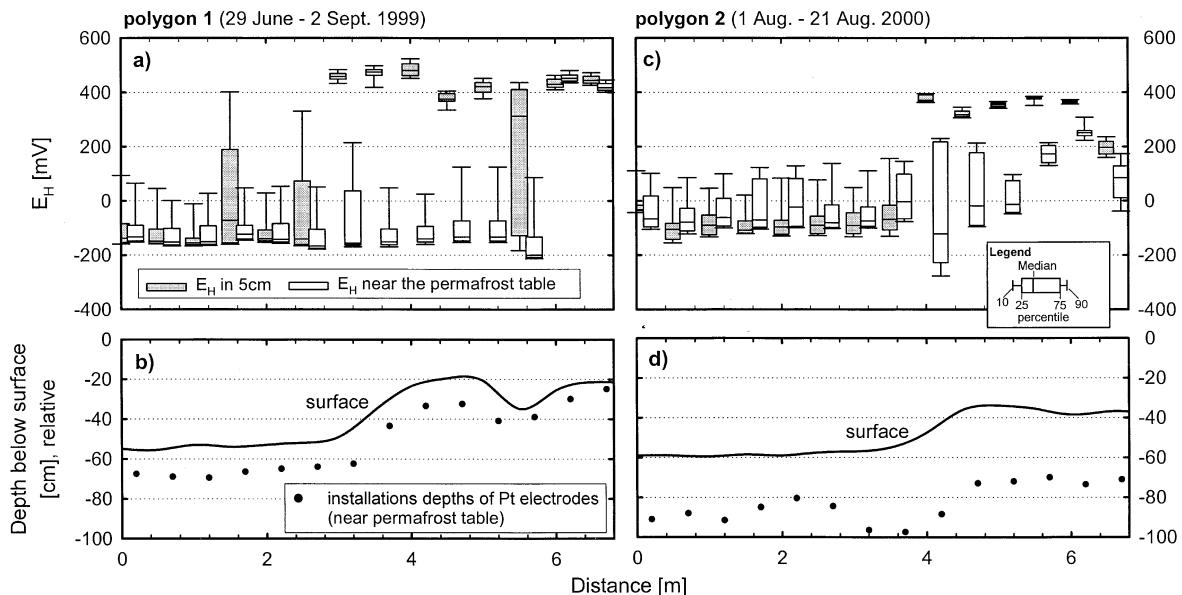


**Fig. 3.** Permafrost and water table of the low-center Polygon 1 (measurement period = 6 June to 1 Sept. 1999).

mately  $-50$  mV, just above the permafrost table approximately  $-90$  mV). Microhighs were distinguished into an oxidative layer at a depth of  $5$  cm below surface (approximately  $300$ – $400$  mV) and a reductive layer near the permafrost (approximately  $180$ – $0$  mV) (= intrapedon upward gradient of  $E_H$ ). Abrupt  $E_H$  changes could be observed from microlows to microhighs (= interpedon

gradient of  $E_H$ ). These changes were only registered in the upper soil zone ( $5$  cm). Measurements near the permafrost table indicated uniform reducing conditions in all microtopographical units. The slope had a transient position and was dominated by higher  $E_H$  fluctuations than other positions (Fig. 4c).

The redox conditions must be regarded as a result



**Fig. 4.** Relationship between topography and redox regime. The oxidation-reduction values distribution in form of box plots (see legend) (a) Polygon 1 and (c) Polygon 2. Cross section through studied low-center polygons with morphological units and installation depths (points filled in black) of the lower electrodes (directly above the permafrost table), (b) Polygon 1 (day of installation 28 June 1999), (d) Polygon 2 (day of installation 30 July 2000).

of microbial processes, which can be demonstrated by methane dynamics. Under anoxic conditions, as in the polygon center and permafrost boundary of the polygon rim, C decomposition in the course of microbial methane formation by strictly anaerobic organisms (methanogenic archaea) dominates. Within overlying oxic horizons so-called methane-oxidizing bacteria use the energy of transforming CH<sub>4</sub> to CO<sub>2</sub>. The relationship between redox potential and involved microbial processes gives reason for methane fluxes in the Lena Delta being largely dependent on topographic position (Kutzbach 2000; Wagner et al., 2003). The latter authors noticed higher methane emission from the polygon center (25–75 mg CH<sub>4</sub> d<sup>-1</sup> m<sup>-2</sup>) than from the rim (up to 6 mg CH<sub>4</sub> d<sup>-1</sup> m<sup>-2</sup>). While descriptive reports of E<sub>H</sub> from permafrost soil studies are numerous (Bleich and Stahr, 1978), E<sub>H</sub> measurements within cryogenic soils are rare. Limited information is available on E<sub>H</sub> measurements over a longer period than a couple of hours (Clark and Ping, 1997). However, single measurements of other studies have shown similar trends. Beyer et al. (1995) registered E<sub>H</sub> values (after 12 h of installation) between 600 mV (topsoil) and approximately 200 mV (18 cm below surface) in two Histosols within Antarctica. Mueller (1997) indicated that, at the slope of a comparably wet polygon in the Lena Delta, E<sub>H</sub> values were approximately 130 mV in the zone near the permafrost table (immediately after installation). The oxidation-reduction potential data presented here tended to be lower than those in both of the latter studies, which can easily be explained by the different measurement durations. It is well known that single E<sub>H</sub> measurements can lead to false conclusions (Böttcher and Strelbel, 1988). In this study, high daily fluctuations ( $\Delta E_H = 200$  mV) as well as the synchronous decrease of E<sub>H</sub> (polygon center near the permafrost table 200 → -100 mV) were observed in the first 5 d after installation followed by relatively constant values.

### Soil Properties and Element Distribution

All soils exhibited typical properties of alluvial soils (in this case, fluvial >> aeolian sedimentation): (i) high amounts of sand and silt, (ii) noticeable changes in texture and (iii) irregular C contents from one horizon to another (Table 1). The two polygons showed a high spatial variability of C content, which has been documented by numerous studies in polar regions (Brown, 1967; Beyer et al., 2000; Bockheim et al., 2003). Carbon/N ratios of rim horizons ranged from 14 to 16 (PG2) and from 21 to 24 (PG1). Values of center horizons ranged from 24 to 25 (PG2) and from 35 to 42 (PG1). Higher ratios of the anoxic center profiles coincided with a higher C accumulation (PG1 = 17 kg SOC m<sup>-2</sup>) compared with oxic rim profiles (11 kg SOC m<sup>-2</sup>).

Investigated polygons were nutrient-limited (input << biorecycling) and adhere to findings of Alexander and Schell (1973) and Haag (1974) in Alaska and Canada. The contents of plant available P only amounted to half of the content in soils investigated by Mueller (1997) in the same area. The storage of P<sub>cal</sub> ranged between 5 g m<sup>-2</sup>

(microhighs) and 2.4 g m<sup>-2</sup> (microlows) in this study. Potassium contents were comparable in both studies. In general, K stock was higher on microhighs (22 g m<sup>-2</sup>) compared with the microlows (15 g m<sup>-2</sup>). Low temperature and insufficiency of N (N<sub>t</sub> ranged from 0.2 to 6 g kg<sup>-1</sup>, Table 1) and P (P ranged from 2 to 30 mg kg<sup>-1</sup>) led to restricted production of phytomass. According to Kutzbach (2000), phytomass amounted to 1100 ± 40 g m<sup>-2</sup> (dry mass) in the center and 420 ± 50 g m<sup>-2</sup> on the polygon rim. Development of dense root systems is characteristic for tundra vegetation and indicates adaptation to a restricted nutrient supply and saturated/reducing conditions (McCown, 1978).

Total Mn and Fe within the active layer ranged from 208 to 920 mg Mn kg<sup>-1</sup> and 17 to 30 g Fe kg<sup>-1</sup> soil in the rim of PG1 vs. 320 to 430 mg Mn kg<sup>-1</sup> and 17 to 27 g Fe kg<sup>-1</sup> of PG2; values in the center of PG1 ranged from 225 to 400 mg Mn kg<sup>-1</sup> and 14 to 19 g Fe kg<sup>-1</sup> compared with 215 to 321 mg Mn kg<sup>-1</sup> and 16 to 18 g Fe kg<sup>-1</sup> in PG2 (Table 1). A parallel trend of Mn<sub>t</sub> as well as Fe<sub>t</sub> and dithionite-extractable Mn and Fe (Mn<sub>d</sub>, Fe<sub>d</sub>) was recognized. Manganese and Fe accumulated above the water table, in unsaturated well-drained horizons, which can be inferred by higher values of Fe<sub>d</sub> and Mn<sub>d</sub> (10.7 g Fe kg<sup>-1</sup>, 443 mg Mn kg<sup>-1</sup> in Bjg1, PG1) (Table 1). In contrast, Mn<sub>t</sub>, Fe<sub>t</sub>, and Mn<sub>d</sub>, Fe<sub>d</sub> was lowest in the very poorly drained active layer at the polygon center (3.7 g Fe<sub>d</sub> kg<sup>-1</sup>, <50 mg Mn<sub>d</sub> kg<sup>-1</sup> in Bg, PG1). Along the microrelief, a stepwise decline of the portion of organic-bound Fe and Mn within A and O horizons from the center (PG1, Fe<sub>p</sub>/Mn<sub>p</sub> ~ 0.4 g kg<sup>-1</sup>/63 mg kg<sup>-1</sup>) to the slope (Fe<sub>p</sub>/Mn<sub>p</sub> ~ 0.7 g kg<sup>-1</sup>/150 mg kg<sup>-1</sup>) to the rim (Fe<sub>p</sub>/Mn<sub>p</sub> ~ 2 g kg<sup>-1</sup>/200 mg kg<sup>-1</sup>) was observed. Additionally, with exception of the Typic Aquiturbel at the rim of PG2, a weak element enrichment in the permafrost fringe occurred (PG1, polygon center, Bg = 15.2 vs. Bf = 19.7 g Fe<sub>t</sub> kg<sup>-1</sup>).

All soils contained redoximorphic features corresponding to element distribution reflected by distinct redox states. Soils on the rim were hydromorphic with brownish, Mn- and Fe-enriched horizons above blue-greyish Fe-impoverished subsoil horizons close to the permafrost table. The Dpd test indicated oxic conditions over great depths (PG1/PG2, 32/33 cm below surface). In polygon center soils, the typical blue-greyish color (2.5Y 4/4) indicated reducing conditions just below the surface (PG1/PG2, 26/22 cm below surface) (Table 1).

### Mechanisms of Element (Matter) Redistribution Downward-Translocation

Element redistribution via solid soil phase was observed only within microhighs. These geomorphic units were characterized by twisted and mixed soil horizons (Turbel, Fig. 1). Cryoturbation plays an important role in mixing surface organic matter into the subsoil (Bockheim and Tarnocai, 1998; Bockheim et al., 1999, 2003). Based on radiocarbon dating (Mueller, 1997), the maximum age of buried organic matter at a 1-m depth was dated between 1530 and 1570 yr. It can be assumed that

**Table 1.** Selected soil properties of the study sites.<sup>†</sup>

Horizon <sup>‡</sup>	Depth	MC	Dpd	D <sub>b</sub> cm	Texture			pH	SOC	N <sub>t</sub>	P <sub>t</sub>	Fe <sub>t</sub>	Fe <sub>d</sub>	Fe <sub>o</sub>	Fe <sub>p</sub>	Mn <sub>t</sub>	Mn <sub>d</sub>	Mn <sub>p</sub>											
					Sand g cm <sup>-3</sup>	Silt g kg <sup>-1</sup>	Clay CaCl <sub>2</sub>																						
					Polygon 1 (location: 72°22.2'N, 126°28.5'E)																								
<b>Polygon rim Gleyi-Turbic Cryosol\$/Typic Aquiturbel   (altitude = 12.7 m above mean sea level)</b>																													
Ajj1	0–8	2.5YR 3/2		0.91	770	164	66	5.6	19	0.9	0.53	17.5	4.2	1.3	0.5	326	122	69											
Ajj2	–15	2.5YR 3/2		1.20	705	234	61	6.3	16	0.7	0.52	16.8	4.4	1.2	0.3	324	152	56											
Bjjg1	–18	2.5YR 3/2		1.21	596	302	102	6.2	22	1.0	0.63	30.0	10.7	5.6	1.0	920	443	75											
Bjjg2	–24	10YR 3/1		1.22	457	412	132	5.4	34	1.4	0.62	23.4	6.0	3.4	1.4	295	67	26											
Bjjg3	–28	10YR 3/1		1.24	527	362	110	5.4	20	0.8	0.56	21.9	5.3	2.9	1.1	306	67	46											
Bjjg4	–32	10YR 3/1	§+	1.3	784	143	73	5.3	4	0.2	0.47	22.5	10.2	1.3	0.2	208	<50	15											
Bjjg5	–46	10YR 3/1	#+	1.35	436	428	136	5.5	23	1.0	0.62	21.1	3.2	1.5	0.9	252	<50	16											
Bjjf	–90	10YR 3/1				6.0	30	1.5	0.60	22.5	3.7	2.0	1.0	265	<50	41													
<b>Polygon slope Gleyi-Turbic Cryosol\$/Typic Aquiturbel   (altitude = 12.6 m above mean sea level)</b>																													
Oi	0–10			0.7	n.d.	n.d.	n.d.	n.d.	236	4.2	0.66	15.6	1.1	2.2	0.9	338	170	180											
A	–21	10YR 2/2		0.93	n.d.	n.d.	n.d.	5.3	12	2.9	0.51	15.8	4.2	1.4	0.6	326	110	115											
B(jj)g1	–27	2.5Y 4/4		1.14	871	88	410	6.2	12	0.6	0.46	15.3	3.4	1.0	0.3	310	118	66											
B(jj)g2	–38	10YR 3/2	#+	1.00	673	255	72	4.9	25	0.8	0.54	19.2	12.4	1.6	1.2	305	150	76											
B(jj)g3	–44	2.5Y 4/4	#+	1.3	946	330	21	5.6	4	0.2	0.42	14.2	3.2	1.1	0.2	216	<50	<15											
B(jj)f	–80	10YR 3/2			705	228	67	4.6	23	0.6	0.51	18.3	5.3	2.0	2.1	237	<50	38											
<b>Polygon center Gleyi-Histic Cryosol\$/Typic Historthel   (altitude = 12.3 m above mean sea level)</b>																													
Oi	0–11			0.4	n.d.	n.d.	n.d.	5.0	221	5.1	0.65	14.2	1.8	2.7	1.6	400	280	272											
A	–26	10YR 2/2	#+	0.6	n.d.	n.d.	n.d.	4.8	126	3.6	0.60	19.8	5.0	1.9	2.7	325	133	126											
Bg	–31	2.5Y 4/4	#+	0.82	859	100	41	4.8	21	0.2	0.45	15.2	3.7	1.4	1.0	225	<50	<15											
Bf	–64	10YR 3/2			635	297	68	5.1	42	1.4	0.54	19.7	6.0	3.4	1.6	317	119	75											
<b>Polygon 2 (72°22.2'N, 126°28.5'E)</b>																													
<b>Polygon rim Gleyi-Turbic Cryosol\$/Typic Aquiturbel   (altitude = 12.7 m above mean sea level)</b>																													
Ajj	0–8	10YR 3/1		1.20	833	129	38	6.0	25	1.5	0.55	17.4	4.6	1.2	0.3	329	114	50.2											
Oe1	–11	10YR 3/1		1.2	777	176	47	5.7	20	1.3	0.56	18.9	4.2	1.8	0.5	354	139	50.6											
Oe2	–19	10YR 2/2		1.34	772	202	27	4.4	14	0.9	0.50	18.2	4.8	2.9	1.2	319	87	72.6											
Bf1	–22	5Y 3/1		1.3	742	199	59	4.3	19	1.3	0.55	18.9	5.2	2.8	1.3	379	129	128											
Bf2	–33	5Y 4/1	††+	1.26	664	255	81	4.9	19	1.3	0.59	23.2	5.8	4.6	1.4	428	155	107											
Bf3	–49	5Y 4/1	††+	1.3	310	589	101	5.3	22	1.5	0.63	26.9	8.6	5.6	1.7	383	126	105											
Bf4	–66	5Y 4/1	††+		458	407	135	6.2	32	2.0	0.58	21.2	2.7	1.7	0.7	623	290	323											
<b>Polygon center Gleyi-Histic Cryosol\$/Typic Historthel   (altitude = 12.5 m above mean sea level)</b>																													
Oi	0–14			0.3	n.d.	n.d.	n.d.	4.7	216	6.4	0.67	20.3	8.1	5.7	2.1	919	603	187											
Ajj	–22	7.5YR 2/0	††+	0.9	793	166	41	4.5	45	1.8	0.50	17.7	4.8	2.6	1.5	321	97	118											
Oi	–27	10YR 2/1	††+	1.1	858	96	46	4.5	19	0.8	0.40	13.4	3.1	1.4	0.7	215	37	41.1											
Bg	–31	7.5YR 3/0	††+	0.80	310	589	101	4.5	30	1.2	0.47	15.9	3.3	1.9	0.8	234	19	32.3											
Oef	–57	10YR 3/1	††+	0.96	691	241	68	4.4	30	1.3	0.55	18.6	4.6	3.0	1.2	230	18	20											

† MC = matrix color; Dpd = α- $\alpha$ -Dipyridil-test (+) = positive, day of description; D<sub>b</sub> = bulk density; Fe<sub>o</sub> = ammonium oxalate extractable iron; Fe<sub>p</sub>, Mn<sub>p</sub> = pyrophosphate extractable iron and manganese; n.d. = not determined; italic letters = horizons in the range of permafrost.

‡ Horizon nomenclature according to Soil Survey Staff (1999).

§ Soil classification according to ISSA-ISRIC-FAO (1998).

|| Soil classification according to Soil Survey Staff (1999).

# 22 Aug. 1999.

†† 19 Aug. 2000.

buried and twisted organic layers (Fig. 1) which now are parts of the perennial frozen layer were once close to the surface. Cryoturbation of organic and mineral material into the subsoils results in a relative enrichment of elements and organic matter (Fig. 5a). The sedimentation layers within the polygon center were not deformed, which was indicated by their horizontal orientation (Orthel).

A weak element enrichment at the permafrost fringe was recorded in all geomorphic units. This suggested a downward migration of ions (solutional phase) via gravitation to the contact with the frozen ground (Fig. 5b). Lundin and Johnsson (1994) also found a similar movement of mass flow by thermal gradients during the snowmelt. Boike (1997) and Boike et al. (1998) found up to 9% of liquid water in permafrost (–12°C) on the Taymyr Peninsula (Siberia). Overduin and Young (1997) explained the element enrichment by cumulative effect of solute exclusion over a repeated freeze-thaw cycles, and to the accumulation of solutes through convective transport of soil water to the freezing front due to ice lens formation.

## Upward-Translocation

The lateral upward-translocation of water-soluble elements in continental climates was described by Arndt and Richardson (1989), and was generalized by Sommer and Schlichting (1997). This translocation process affecting the entire catena was restricted to a very flat relief. The driving factor was the upward gradient of the water potential between microlows and microhighs. This gradient occurred during summer and was principally caused by higher evapotranspiration from soils of the microhighs (higher surface/volume ratio) compared with soils of the microlows. In addition, vegetation on microhighs acts as a large water pump resulting in a lowering of the water table (Richardson et al., 2001). Furthermore, it can be assumed the gradient was additionally supported by higher proportions of fine macro- and mesopores at the rim (PG1, in a depth of 28 cm = 37%) compared with the polygon center (in a depth of 31 cm = 20%) (Kutzbach, 2000). When soils are dry the matrix potential increases and the soil water moves via capillary transporting solutes (Richardson et al., 2001). The latter

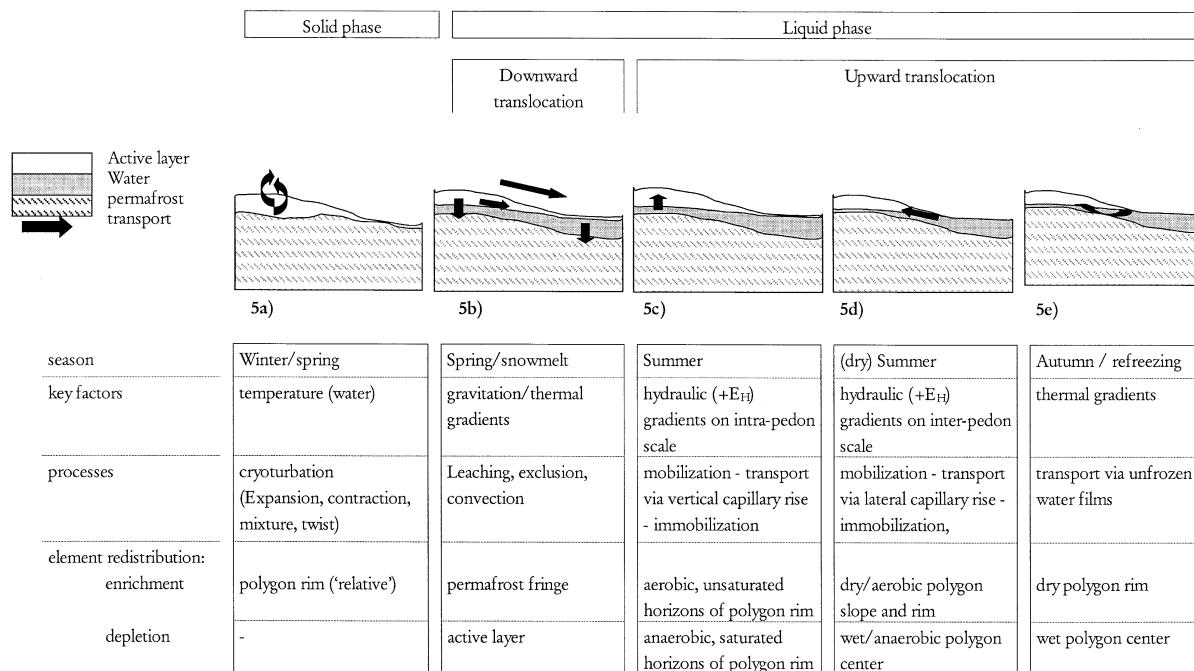


Fig. 5. Model of different kinds of element redistribution within low-center polygons.

process can be reconstructed using the contents of K. Potassium can easily be leached in soils with poor clay contents, because its bondage to organic matter is negligible (Schachtschabel et al., 1998). Consequently, water soluble K can migrate along water potentials resulting in a higher K storage in microhighs (PG1, polygon rim,  $22 \text{ g m}^{-2}$ ) than in the microlows ( $15 \text{ g m}^{-2}$ ). The results are supported by many other studies, which focused on the upward-translocation of salts (Kellogg, 1934; Douglas and Tedrow, 1960; Whittig and Janitzky, 1963; Wiegand et al., 1966; MacLean and Pawluck, 1975; Hallsworth et al., 1982) and gypsum (Steinwand and Richardson, 1989) within very flat geomorphic units.

Parallel to K, an upward-translocation of redox-sensitive elements (Mn, Fe) was also recorded. Migration of these elements is coupled to changes in valence caused by electron transfer. Thus, redox (and hydraulic) gradi-

ents control the element redistribution within low-centered polygons. Continuous redox measurements demonstrated strong redox gradients, which lead to an upward element transport along these gradients. After element mobilization in the reducing center and, subsequent, upward transport along  $E_H$  gradients via capillary rise, immobilization processes occurred in the oxic microhighs. The latter can be explained by an abrupt change in the redox environment (Fig. 4). Consequently, in both polygons, a higher absolute element mass was calculated in the well-drained microhighs (PG1 rim/slope;  $\text{Fe}_t = 11.7/7 \text{ kg m}^{-2}$ ,  $\text{Fe}_d = 2.9/2.3 \text{ kg m}^{-2}$ ,  $\text{Fe}_o = 1.1/0.6 \text{ kg m}^{-2}$ ) compared with the microlows ( $\text{Fe}_t = 3 \text{ kg m}^{-2}$ ,  $\text{Fe}_d = 0.7 \text{ kg m}^{-2}$ ,  $\text{Fe}_o = 0.3 \text{ kg m}^{-2}$ , Table 2). On the contrary, the liquid phase of the center profiles was characterized by higher Mn and Fe concentrations than that of the rim profiles (approximately  $4 \text{ mg Mn L}^{-1}$  soil solution, approximately  $2.9 \text{ mg Fe L}^{-1}$ , vs. approximately  $1.2 \text{ mg Mn L}^{-1}$ , and  $0.2 \text{ mg Fe L}^{-1}$ , Table 3). The Fe accumulation at the polygon rim was visible in as continuous Fe-band, which regularly followed the buckling surface (Fig. 1). This element accumulation due to upward migration from bottom of the drainage way to a slightly higher elevation along the adjacent slope was referred to in the literature as edge effect (Steinwand and Richardson, 1989; Sommer and Schlichting, 1997). Similar research (Everett and Parkinson, 1977; Everett and Brown, 1982) described an upward Fe translocation along a microcatena (Cryaquept-Cryofibrist) of Alaskan tundra soils. Researchers observed an Fe accumulation solely in microhighs and explained this phenomenon by Fe flux via groundwater combined with a redox

Table 2. Absolute mass based on active layer ( $\text{g m}^{-2}$ ) of soils studied.<sup>†</sup>

$P_t$	$\text{Fe}_t$	$\text{Fe}_d$	$\text{Fe}_o$	$\text{Fe}_p$	$Mn_t$	$Mn_d$	$Mn_p$
<b>PG1, polygon rim Gleyi-Turbic Cryosol<sup>‡</sup>/Typic Aquiturbel<sup>§</sup></b>							
320	11 730	2 896	1 146	435	179	46	20
<b>PG1, polygon slope Gleyi-Turbic Cryosol<sup>‡</sup>/Typic Aquiturbel<sup>§</sup></b>							
212	6 967	2 355	630	296	128	47	37
<b>PG1, polygon center Gleyi-Histic Cryosol<sup>‡</sup>/Typic Historthel<sup>§</sup></b>							
101	3 032	682	341	355	56	24	23
<b>PG2, polygon rim Gleyi-Turbic Cryosol<sup>‡</sup>/Typic Aquiturbel<sup>§</sup></b>							
360	13 850	3 905	2 408	774	232	78	56
<b>PG2, polygon center Gleyi-Histic Cryosol<sup>‡</sup>/Typic Historthel<sup>§</sup></b>							
101	3 373	960	564	258	81	35	20

<sup>†</sup>  $P_t$ ,  $\text{Fe}_t$ ,  $Mn_t$  = total phosphorus, iron, manganese;  $\text{Fe}_d$ ,  $Mn_d$  = dithionite citrate bicarbonate extractable iron and manganese,  $\text{Fe}_o$  = ammonium oxalate extractable iron;  $\text{Fe}_p$ ,  $Mn_p$  = pyrophosphate extractable iron and manganese.

<sup>‡</sup> Soil classification according to ISSA-ISRIC-FAO (1998).

<sup>§</sup> Soil classification according to Soil Survey Staff (1999).

**Table 3.** Characterization of soil solution (Polygon 1[PG1]).

Position	Depth	Sampling date												
		7 July 1999		14 July 1999		21 July 1999		7 Aug. 2000		14 Aug. 2000		21 Aug. 2000		
cm	Fe	Mn	Fe	Mn	Fe	Mn	Fe	Mn	Fe	Mn	Fe	Mn	Fe	Mn
Rim	14	n.d. <sup>†</sup>	n.d.	0.12	0.20	n.d.	n.d.	0.08	0.06	0.07	0.05	0.08	0.07	
Rim	27	0.09	0.13	0.10	0.67	0.14	0.79	0.09	1.98	0.11	1.61	0.19	1.66	
Rim	36	0.20	0.63	0.27	1.51	0.38	1.92	0.42	2.74	0.41	3.07	0.54	2.81	
Center	9	n.d.	n.d.	0.33	4.28	n.d.	n.d.	1.71	1.35	10.43	8.24	0.36	9.74	
Center	15	4.27	4.89	0.41	2.24	0.71	2.16	0.57	2.65	0.49	2.59	0.43	2.65	

<sup>†</sup> not determined.

gradient (oxic conditions in microhighs, anoxic conditions in microlows).

Soil P exists in forms of organic P, the fixed mineral P, and orthophosphate (ortho-P). Fixed mineral form of ortho-P is bound to Al and Fe oxides/hydroxides (Gisbury et al., 1965). The transformation of fixed mineral P into soluble ortho-P is controlled by redox processes (Vepraskas and Faulkner, 2001). As a consequence of reducing conditions in the polygon center, P bound to Fe was mobilized, transported, and immobilized in microhighs. Thus, Fe as well as P storage was higher in microhighs (PG1, 11.7 kg Fe<sub>t</sub> m<sup>-2</sup>, 320 mg P<sub>t</sub> m<sup>-2</sup>) than in microlows (PG1, 3 kg Fe<sub>t</sub> m<sup>-2</sup>, 101 mg P<sub>t</sub> m<sup>-2</sup>) (Table 2).

In addition to the interpedon (center to rim, Fig. 5d), an intrahorizon/pedon upward-translocation (Fig. 5c) was assumed. Distribution of pedogenetic oxides at the polygon rims are typical for gleyzation processes (Schlichting, 1973) and are in agreement with observed E<sub>H</sub> gradients. The transmission zone of an oxic/anoxic environment, verified by the use of Dpd, was characterized by enrichment of Mn and Fe (Table 1).

More frequent than upward transport of the liquid phase, the ionic migration during refreezing of the active layer in autumn (Fig. 5e) was described in literature (Chuvilin et al., 1998a, 1998b). Controlling factors of this mechanism are temperature gradients (Hinkel and Outcalt, 1994) that promote upward moisture and ionic transfers. As a result of decreasing air temperature in autumn, the heat flux is directly outward, cooling the active layer from above. A downward-moving freezing front evolves, which is delayed by increases in soil water contents or by water transport to the freezing front (Boike, 1997). Consequently, the polygon rim with lower water content and latent heat initially freezes somewhat faster than the water saturated polygon center. Thus, an upward water and ionic transport from the saturated center to the frozen polygon rim via unfrozen water films is enforced.

## CONCLUSIONS

Low-centered polygons are one kind of a typical form of pattern ground in the arctic tundra, which can be divided topographically into microhighs (rims and slopes of the polygon) and microlows (polygon center). The controlling factors of their formation are cryogenetic processes reflected by twisted and mixed soil horizons within elevated parts of the relief. This solid soil phase transloca-

tion as well as liquid phase translocation, accompanied by element redistribution, is a result of the complex interplay of thermal, hydraulic, and redox gradients. The strongly varying soil-climatic conditions within the microrelief of polygons determine the direction of gradient (downward vs. upward). Importance of the different transport processes could not be differentiated.

Element redistribution along redox gradients, a phenomenon of which scarce information is provided in literature, was examined. Topography was closely linked to hydrology and the oxidation-reduction environment. Within the microrelief, each unit corresponded via solution fluxes with other units. This linkage was a fundamental part of the catena concept, based on the principles of mobilization, transport and immobilization (Sommer and Schlichting, 1997). Based on saturated/reducing conditions, soils in depressions were deemed as mobilization areas. Element transport was due to (hydro)geochemical gradients. Immobilization of elements was caused by drastic changes in environmental conditions (anaerobic to aerobic milieu). For future research, this upward transport should be validated using radioactive labeled elements. Although the presented study adds to the understanding of element redistribution processes in arctic soils, it does not imply these soils have been sufficiently investigated. All relevant factors of redistribution should be correlated, depending on seasonal and annual range of the permafrost table. This presupposes a long-term investigation to monitor climate, water balance, redox conditions and element migration.

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## 4.3

# Abundance, distribution and potential activity of methane oxidizing bacteria in permafrost soils from the Lena Delta, Siberia

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## Summary

The methane oxidation potential of active layer profiles of permafrost soils from the Lena Delta, Siberia, was studied with regard to its respond to temperature, and abundance and distribution of type I and type II methanotrophs. Our results indicate vertical shifts within the optimal methane oxidation temperature and within the distribution of type I and type II methanotrophs. In the upper active layer, maximum methane oxidation potentials were detected at 21°C. Deep active layer zones that are constantly exposed to temperatures below 2°C showed a maximum potential to oxidize methane at 4°C. Our results indicate a dominance of psychrophilic methanotrophs close to the permafrost table. Type I methanotrophs dominated throughout the active layer profiles but their number strongly fluctuated with depth. In contrast, type II methanotrophs were constantly abundant through the whole active layer and displaced type I methanotrophs close to the permafrost table. No correlation between *in situ* temperatures and the distribution of type I and type II methanotrophs was found. However, the distribution of type I and type II methanotrophs correlated significantly with *in situ* methane concentrations. Beside vertical fluctuations, the abundance of methane oxidizers also fluctuated according to different geomorphic units. Similar methanotroph cell counts were detected in samples of a flood plain and a polygon rim, whereas cell counts in samples of a polygon centre were up to 100 times lower.

## Introduction

The Arctic is of major interest in the context of global climatic change for two reasons. First, one-third of the global carbon pool is stored in northern latitudes (Post *et al.*, 1982), mainly in huge layers of frozen ground, termed permafrost, which cover around 24% of the exposed land area of the Northern Hemisphere (Zhang *et al.*, 1999). Second, the Arctic is observed to warm more rapidly and to a greater extent than the rest of the earth surface (IPCC, 2001). Serreze and colleagues (2000) refer to evidence of increased plant growth and northward migration of the tree line and conclude that permafrost has warmed in Alaska and Russia.

Northern wetlands such as the Lena Delta in north-east Siberia are significant natural sources of methane (Friborg *et al.*, 2003; Smith *et al.*, 2004; Corradi *et al.*, 2005). As a consequence of the harsh winter climate, decomposition processes in northern wetlands are inhibited leading to an accumulation of organic matter. The organic matter is partly decomposed under water-saturated, anaerobic conditions during the short summer period. The terminal step in the anaerobic decomposition of organic matter is the microbial formation of methane (methanogenesis). Several studies estimated the methane source strength of northern wetlands, including tundra, to range from 17 to 42 Tg CH<sub>4</sub> year<sup>-1</sup> (Whalen and Reeburgh, 1992; Cao *et al.*, 1996; Joabsson and Christensen, 2001; Wagner *et al.*, 2003). This corresponds to about 25% of the methane release from natural sources (Fung *et al.*, 1991).

Global warming could thaw 25% of the permafrost area by 2100 (Anisimov *et al.*, 1999), exposing huge amounts of currently fixed organic carbon to aerobic as well as anaerobic decomposition processes. Also, higher temperatures are likely to reinforce methanogenesis and therefore increase the methane source strength of Arctic wetlands (Wuebbles and Hayhoe, 2002). Additional methane would have a positive feedback on the atmospheric warming process because methane is both on a mass and a molecule level 23 times more effective as a greenhouse gas than CO<sub>2</sub> (IPCC, 2001).

The biological oxidation of methane by methane oxidizing (methanotrophic) bacteria, which belong to the

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$\alpha$ - (type II methanotrophs) and  $\gamma$ - (type I methanotrophs) *Proteobacteria*, is the major sink for methane in terrestrial habitats. Between 43% and 90% of the methane produced in the soil is oxidized before reaching the atmosphere (Roslev and King, 1996; Le Mer and Roger, 2001). Hence, it is crucial to investigate methanotrophic communities and their response to global change in particular in climatic sensitive regions like the Lena Delta.

Our study determines abundance and distribution of methanotrophic bacteria within morphologically characteristic sites on Samoylov Island. Samoylov Island is located in the central part of the Lena Delta and is representative for the polygonal tundra, which is typical for the patterned ground of permafrost. We will also give insights into how the extreme environmental conditions of Siberian permafrost influence potential methane oxidation rates. Particularly, the temperature response of potential methane oxidation rates in soils from the Lena Delta was investigated as temperature is the most extreme parameter in permafrost soils and it is known that low temperatures induce processes of microbial adaptation and specialization (Georlette *et al.*, 2004).

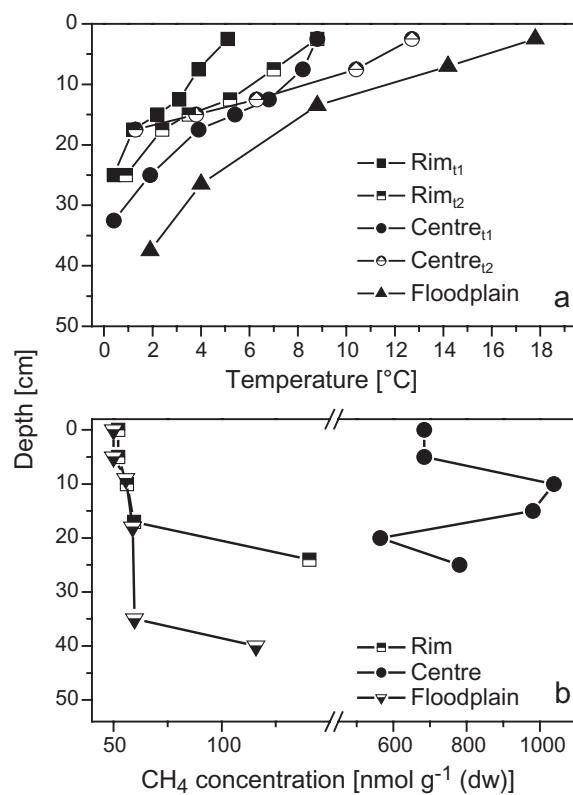
## Results

### Soil characteristics

The microrelief of the polygonal tundra, which results from annual freezing and thawing processes, determines steep environmental gradients in particular within the active layer (seasonally thawed layer) of permafrost. Three sites were investigated in this study: a polygon rim, a polygon centre and a flood plain soil. Temperature and methane gradients through the active layer profiles of the three sampling sites were determined during the sampling periods and are shown in Fig. 1. In the uppermost 5 cm mean temperature values reached up to 5–12°C in the polygon rim and centre, and 18°C in the flood plain. In all profiles, temperatures decreased rapidly to almost 0°C in 25–40 cm depth close to the permafrost table. Temperatures in the uppermost soil layers fluctuated at greater amplitude than in layers close to the permafrost table, where they remained constantly around 0°C.

The methane concentration profiles of the polygon rim and the flood plain showed a steep gradient between the upper and the deeper active layer. Within both profiles, methane concentrations increased rapidly from around 50 nmol g<sup>-1</sup> (dw) in the uppermost 18 cm to 140–180 nmol g<sup>-1</sup> (dw) close to the permafrost table. Compared with the flood plain and the polygon rim, the methane concentrations in the polygon centre were up to 10 times higher and did not show a vertical gradient.

Additional soil properties of the three sites are summarized in Table 1. The organic carbon content did not



**Fig. 1.** Vertical profiles of (a) *in situ* temperatures and (b) *in situ* methane concentrations in the active layer of a polygon rim, a polygon centre and a flood plain soil on Samoylov Island, Lena Delta. Temperatures represent mean values ( $n = 3$ ) measured around noon (11 AM to 1 PM) on 21st July (t1) and 2nd August 2005 (t2) (polygon rim and polygon centre) and on 22nd July 2002 (flood plain). Methane concentrations represent mean values ( $n = 3$ ) of active layer cores sampled around noon on 22nd July 2005 (polygon rim and polygon centre) and on 22nd July 2002 (flood plain).

exceed 3.0% and 3.1%, respectively, in the polygon rim and the flood plain but reached up to 16.1% in the polygon centre. In contrast to the polygon rim, which was dominated by sandy material, the flood plain mainly consisted of silty material. The grain size fraction of the polygon centre could not be determined due to its high content of organic soil matter.

### Cell numbers

Total and methanotroph cell counts were determined for all sites. Additionally, cell counts of *Bacteria* were determined for the polygon rim and the polygon centre. All cell numbers are shown in Fig. 2. Cell counts of *Bacteria* and methanotrophs relative to total cell counts (TCC) are summarized in Table 2.

Within the upper active layer profiles (0–10 cm), TCC were highest in the polygon rim [ $20.1 \times 10^8$  cells g<sup>-1</sup> (dw)].

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**Table 1.** Selected soil properties of a polygon rim, a polygon centre and a flood plain soil on Samoylov Island, Lena Delta.

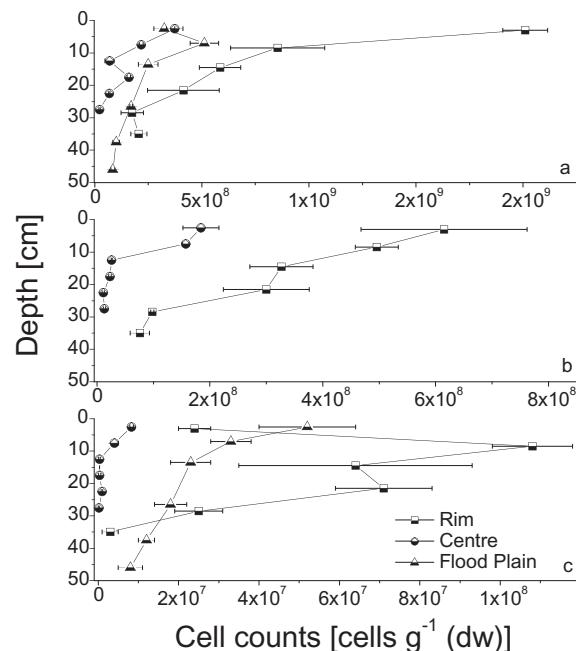
Depth (cm)	H <sub>2</sub> O content (%)	C <sub>org</sub> (%)	N (%)	Grain size fraction (%)		
				Clay	Silt	Sand
<b>Rim</b>						
0–6	26.2	3.0	0.2	2.4	10.6	87.0
6–11	15.7	2.1	0.1	2.3	9.1	88.5
11–18	24.1	2.3	0.1	1.7	17.5	80.7
18–25	24.8	2.0	0.1	10.0	45.7	44.3
25–32	25.2	1.2	0.0	3.0	11.1	85.9
32–38	16.6	2.8	0.1	0.5	21.5	78.1
<b>Centre</b>						
0–5	85.7	15.5	0.7	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>
5–10	77.3	15.1	0.4	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>
10–15	80.6	16.1	0.4	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>
15–20	73.4	7.3	0.2	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>
20–25	58.9	2.2	0.2	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>
25–30	68.5	4.7	0.2	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>
<b>Flood plain</b>						
0–5	30.1	3.1	0.4	11.1	64.8	24.2
5–9	31.9	1.1	0.2	20.2	61.4	18.4
9–18	28.3	2.2	0.3	18.3	63.5	18.2
18–35	35.4	2.8	0.4	20.2	62.7	17.1
35–40	32.4	2.4	0.3	20.4	55.6	24.0
40–52	31.8	1.7	0.2	17.6	67.7	14.7

a. Was not determined due to the high content of organic soil matter.

Total cell counts were in the same range in the polygon centre and in the flood plain [ $3.7 \times 10^8$ , respectively,  $5.1 \times 10^8$  cells g<sup>-1</sup> (dw)]. Close to the permafrost table, TCC were similar in all sites and ranged between

$1.7 \times 10^8$  cells g<sup>-1</sup> (dw) in the polygon rim and  $0.2 \times 10^8$  cells g<sup>-1</sup> (dw) in the polygon centre.

Cell numbers detected with probe EUB338, which identified members of the domain *Bacteria*, were 5–10 times



**Fig. 2.** (a) Total, (b) *Bacteria* and (c) methanotroph cell counts of a polygon rim, a polygon centre and a flood plain soil on Samoylov Island, Lena Delta.

**Table 2.** Ratio of type I to type II methanotrophs and cell counts of *Bacteria* and methanotrophs relative to TCC of a polygon rim, a polygon centre and a flood plain soil on Samoylov Island, Lena Delta.

Depth (cm)	Ratio Type I/Type II MOB <sup>a</sup> (%)	Relative to TCC (%) (mean ± SD)	
		<i>Bacteria</i>	MOB <sup>a</sup>
<b>Rim</b>			
0–6	56.9/43.1	30.5 ± 7.3	1.2 ± 0.2
6–11	88.5/11.5	58.1 ± 4.5	12.7 ± 1.2
11–18	60.3/39.7	55.9 ± 9.6	11.0 ± 5.0
18–25	95.6/4.4	72.4 ± 18.5	17.3 ± 2.9
25–32	50.0/50.0	56.2 ± 2.1	14.6 ± 3.5
32–38	< d.l./100.0	37.2 ± 8.5	1.7 ± 1.1
<b>Centre</b>			
0–5	n.d.	49.3 ± 8.6	0.2 ± 0.1
5–10	n.d.	72.3 ± 2.9	1.9 ± 0.8
10–15	n.d.	38.5 ± 1.4	0.5 ± 0.3
15–20	n.d.	14.9 ± 1.6	0.2 ± 0.1
20–25	n.d.	16.2 ± 3.7	1.4 ± 0.3
25–30	n.d.	57.3 ± 10.7	0.7 ± 0.5
<b>Flood plain</b>			
0–5	93.7/6.3	n.d.	16.1 ± 3.8
5–9	69.5/30.5	n.d.	6.4 ± 1.1
9–18	64.5/35.5	n.d.	9.3 ± 2.3
18–35	84.9/15.1	n.d.	10.8 ± 2.6
35–40	38.3/61.7	n.d.	12.1 ± 2.7
40–52	25.8/74.2	n.d.	9.5 ± 4.7

a. Methane oxidizing bacteria.  
n.d., not determined.

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higher in the polygon rim than in the polygon centre. They varied between  $6.1 \times 10^8$  (0 and 6 cm) and  $0.7 \times 10^8$  cells g<sup>-1</sup> (dw) (32–38 cm) in the polygon rim and between  $1.8 \times 10^8$  (0 and 5 cm) and  $0.1 \times 10^8$  cells g<sup>-1</sup> (dw) (25–30 cm) in the polygon centre. Hence, their contribution to TCC was 30.5–72.4% at the polygon rim and 14.9–72.3% at the polygon centre.

Methanotroph cell counts were highest in the polygon rim where they ranged between  $1.0 \times 10^8$  (6 and 11 cm) and  $3.0 \times 10^6$  cells g<sup>-1</sup> (dw) (32–38 cm). Methanotroph cell counts in the polygon rim accounted for 1.7–17.3% to the TCC. Methanotroph cell counts of the polygon centre were two orders of magnitude lower than in the polygon rim and in the flood plain and accounted for only 0.2% to at most 1.9% to TCC. In the flood plain, cell counts of methanotrophs varied between  $5.0 \times 10^7$  (0 and 5 cm) and  $8.0 \times 10^6$  cells g<sup>-1</sup> (dw) (40–52 cm) and accounted for 6.4–16.1% to TCC.

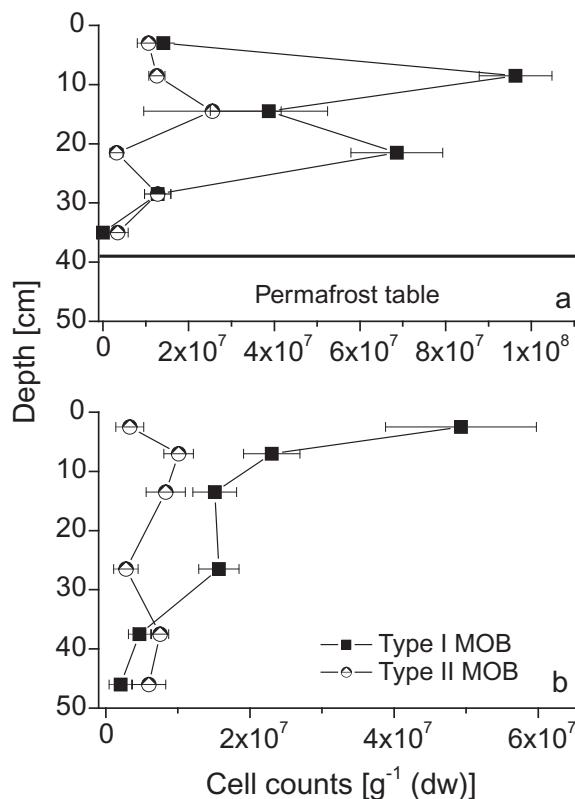
#### Distribution of type I and type II methanotrophs

The vertical distribution of type I and type II methanotrophs was determined for the polygon rim and the flood plain soil (Fig. 3). Within both profiles, type I methanotrophs dominated through the active layer but their abundance strongly fluctuated with depth. Type II methanotrophs were less abundant than type I methanotrophs and their cell numbers fluctuated less with depth. Type II methanotrophs displaced type I methanotrophs close to the permafrost table. The relative abundance of type I and type II to total methanotroph cells (Table 2) resulted in a significant sigmoidal correlation (Boltzmann model) with the methane concentrations *in situ* for both profiles (rim:  $r^2 = 0.993$ ,  $\chi^2 = 28.99$ ,  $n = 6$ ; flood plain:  $r^2 = 0.819$ ,  $\chi^2 = 26.98$ ,  $n = 6$ ). A correlation between distribution of type I and type II methanotrophs and *in situ* temperatures could not be detected.

#### Potential methane oxidation rates

Incubation experiments (based on  $^{14}\text{CH}_4$ ) were carried out at 0, 4, 12, 21, 28 and 38°C with soil slurries of the polygon rim and the polygon centre. Another incubation experiment (based on the linear regression of  $\text{CH}_4$  in the headspace determined by gas chromatography) was carried out with soil slurries of the flood plain at 0, 4, 12 and 21°C.

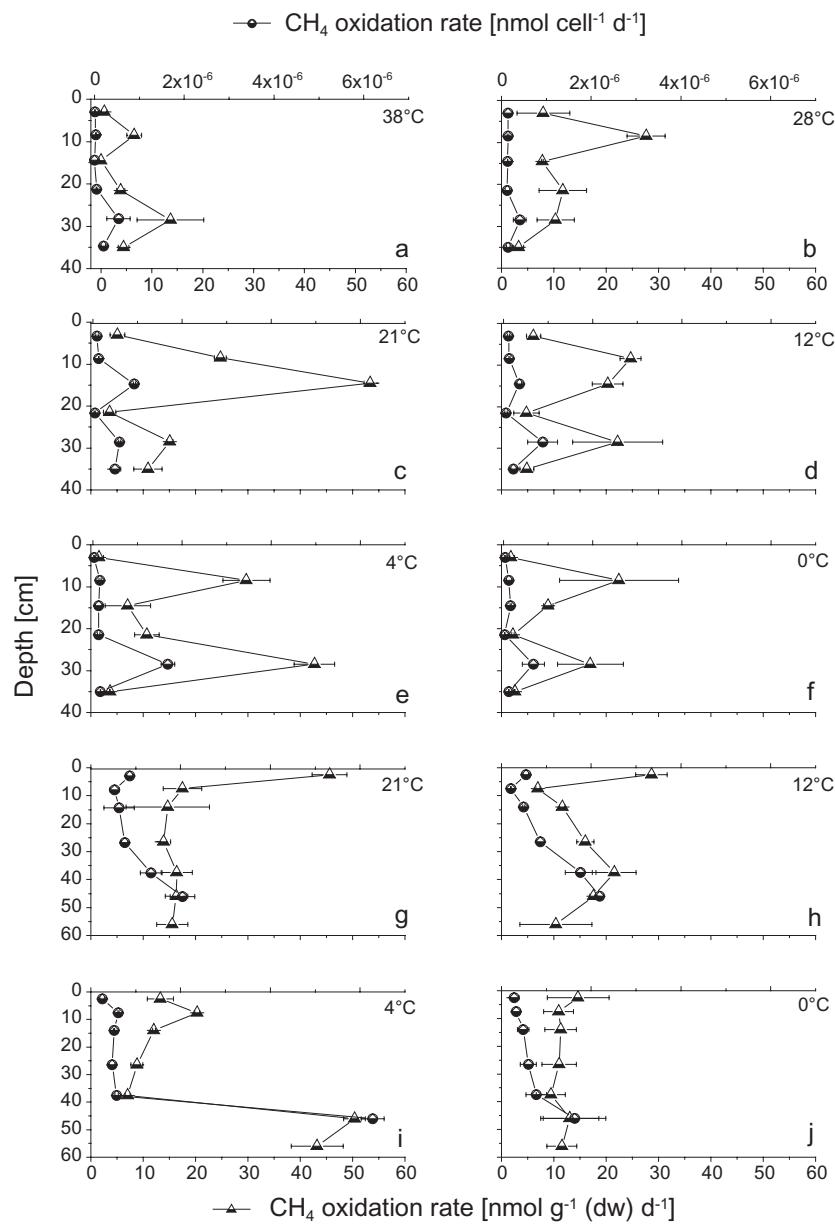
The potential to oxidize methane at different incubation temperatures was similar in samples of the polygon rim and the flood plain soil. Maximum rates of around 50 nmol g<sup>-1</sup> (dw) day<sup>-1</sup> were detected in samples of both sites. There was a clear shift of the temperature optimum from 21°C in upper active layer zones to 4°C in deeper active layer zones in both profiles (Fig. 4). In samples of



**Fig. 3.** Vertical distribution of type I and type II methanotrophic bacteria through the active layer of (a) a polygon rim and (b) a flood plain soil on Samoylov Island, Lena Delta.

the polygon rim and the flood plain, the potential methane oxidation rates per gram dry weight of deep soil layers at 4°C were similar to those of upper layers at 21°C. Based on the cell counts determined by fluorescence *in situ* hybridization (FISH) and on the potential oxidation rates measured at various incubation temperatures, the potential methane oxidation rates per methanotroph cell and day were calculated. At 4°C the potential methane oxidation rates per methanotroph cell detected near the permafrost table exceeded cell activities in the other horizons by one order of magnitude (Fig. 4). Independently of the temperature, cell activities increased by 50–150% compared with upper soil layers at 25 cm in the polygon rim and at 40 cm in the flood plain. Lowest rates [ $< 23 \text{ nmol g}^{-1} (\text{dw}) \text{ day}^{-1}$ ] through the active layer profiles were detected at 0°C at both sites and at 38°C in samples of the polygon rim (Fig. 4). Soil horizons with the highest abundance of methanotrophs did not show any temperature response (polygon rim: 6–11 cm and 18–25 cm; flood plain: 5–40 cm). In these horizons the methane oxidation potential did not change significantly at the different incubation temperatures. The methane oxidizing potential through the entire active layer of the polygon centre was

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**Fig. 4.** Potential methane oxidation rates at different incubation temperatures of soil slurries of (a–f) a polygon rim (0–38°C) and (g–j) a flood plain soil (0–21°C) on Samoylov Island, Lena Delta.

about two orders of magnitude lower than in samples of the rim and the flood plain (data not shown).

### Discussion

Soil ecosystems of the Siberian Arctic are characterized by small-scale variations both within the microrelief of the polygonal tundra and within vertical profiles of the active layer. Within the active layer, temperature is the most

extreme environmental factor with a distinct gradient from the surface to the permafrost table.

At different sites examined in this study, the potential to oxidize methane of soil horizons close to the permafrost table was greatest at 4°C. The methane oxidation potentials per methanotroph cell near the permafrost table was significantly higher compared with both the cell activities near the surface at the same temperature (4°C) but also compared with cell activities in the same depth at different

a more efficient carbon assimilation of type I methanotrophs (Hanson and Hanson, 1996). Several studies investigated differences in substrate affinities of type I and type II methanotrophs but so far the results do not consistently show one or the other group to clearly prefer either high- or low-substrate concentrations. According to studies on rice field soils, type I methanotrophs seem to out-compete type II at very low *in situ* methane concentrations (Henckel *et al.*, 2000) and type II methanotrophs are strongly related to soil porewater methane concentrations (Macalady *et al.*, 2002), which indicates a higher substrate affinity of type I compared with type II methanotrophs. Other studies (Horz *et al.*, 2002; Knie and Dunfield, 2005; Knie *et al.*, 2006) show members of the type II group as the most oligotrophic methanotrophs. They suggest that type II methanotrophs might be responsible for atmospheric methane consumption. According to our results based on methane concentrations higher than atmospheric but lower than in high-affinity environments, type I methanotrophs dominate in particular in the upper active layer. A dominance of type I methanotrophs in active layers of Siberian permafrost soils from the Lena Delta was already suggested by Wagner and colleagues (2005) who used marker fatty acid analysis to distinguish between type I and type II methanotrophs.

Differences between the methanotrophic communities could not only be shown with respect to active layer depth but also with respect to different geomorphic units. Polygon rim and flood plain seem to provide favourable conditions for methane oxidizing bacteria. Cell counts between  $10^7$  and  $10^8$  per gram dry soil even exceed cell counts of methanotrophs in temperate soils located in Europe by at least one order of magnitude (Horz *et al.*, 2002; Eller *et al.*, 2004). We have to consider, though, that cell counts in these studies were obtained by the most probable number and not by direct cell counting. The highest activity of methanotrophs in the flood plain soil compared with the other two sites studied could result from its high proportion of silt and clay material. The surface area and the amount of negative charges determine the sorptive activity for microorganisms and nutrients (Stotzky, 1966; Heijnen *et al.*, 1992). Hence, clay and silt support availability and uptake of substrates.

Significant cell numbers of methanotrophs were detected in deep soil layers that are, according to Fiedler and colleagues (2004), exposed to reduced *in situ* conditions. This is consistent with methane oxidation potentials observed under *in situ* conditions near the permanently frozen ground of a polygon rim also located on Samoylov Island (Wagner *et al.*, 2005). Methane oxidation can occur under microaerophilic (Bodegom *et al.*, 2001) and oxygen-limiting conditions (Roslev and King, 1996). Besides, root exodermis can provide oxygen in deep active layer zones and can therefore prevent methano-

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trophs from oxygen deprivation. Hence, methanotrophs in deep and reduced soil layers should be equally accounted for in models on methane fluxes.

In contrast to polygon rim and flood plain soil, potential oxidation rates and cell counts indicate unfavourable conditions for the methanotrophic community within the polygon centre despite significantly higher methane concentrations. A hampered process of methane oxidation in the polygon centres is in accordance with significantly higher methane emission rates from the centre of ice-wedge polygons compared with the rim (Wagner *et al.*, 2003; Kutzbach *et al.*, 2004). The unfavourable conditions for methanotrophs in the polygon centre may result from constant water saturation supplemented by a lack of oxygen input.

#### Conclusions and prospects

We could show that abundance, distribution and eco-physiology of methane oxidizing bacteria in permafrost affected soils from the Lena Delta are determined by microrelief as well as environmental gradients within the active layer. Because the microbial methane oxidation is an essential part of models on methane emissions from wetlands (Walter and Heimann, 2000), these models should consider small-scale variations within the methanotrophic community as observed in our study. However, until now, methane oxidation rates in these models are based on general parameters like Michaelis-Menten kinetics ( $K_m$ ) and  $Q_{10}$ -values but differences in substrate affinities and enzyme kinetics of methanotrophs as well as spatial fluctuations of their cell numbers are not considered. Although our study gives a first insight into the importance of these small-scale variations within the active layer, further studies are needed to supply reliable input data for modelling of methane fluxes.

In addition to abundance and distribution, changes within the methanotrophic community composition need to be studied. Cavigelli and Robertson (2001) suggested influences of the change of the microbial community composition on the function of a terrestrial ecosystem in the context of denitrification. It is likely that shifts within the methane oxidizing community composition will affect its function as a sink for methane as the group of methane oxidizers forms the physiologically 'narrowest' group of all trace gas processors. This allows for a clear demonstration of ecosystem-level influences (Schimel and Guldledge, 1998).

Finally, we should aim at understanding the stability of the methanotrophic community in soils from the Lena Delta in the context of global change. For this purpose it is necessary to extend the usage of molecular tools and to combine our data with an analysis of the diversity of the seasonally active methanotrophic 'keyplayers'.

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## Experimental procedure

### Study site and soil properties

With an area of about 32 000 km<sup>2</sup> the Lena Delta is the second largest delta in the world (Are and Reimnitz, 2000). It is located in the zone of continuous permafrost and characterized by arctic continental climate with a mean annual air temperature of -11.9°C over the 2001–2003 period and a mean precipitation during the same period of about 233 mm (measured by the Russian weather station Stolb Island). Our study site is located in the youngest and presently most active part of the delta on Samoylov Island (N 72°22', E 126°28'). Detailed descriptions of the geomorphology of Samoylov Island and the whole delta were given previously by Schwamborn and colleagues (2002). Samoylov Island covers an area of only 1200 ha with the highest elevation at 12 m above sea level. The island is dominated by the typical permafrost pattern of low-centred ice-wedge polygons covering at least 70% of the island area. The soils in the Lena Delta are entirely frozen for at least 8 months every year leaving only a shallow active layer of about 20–50 cm unfrozen during the summer months.

Expeditions to Samoylov Island were carried out in summers 2002 and 2005 in the frame of the Russian-German cooperation 'System Laptev Sea 2000'. Samoylov was defined with respect to different characteristic geomorphic units. Exemplarily, a polygon rim, a polygon centre and a flood plain soil were chosen for sampling. We defined our sampling sites according to soil horizons following Schöneberger and colleagues (2002). These soil horizons are characterized according to soil genesis, physical and chemical parameters. Given that bacteria are associated with mineral and organic soil particles (Christensen *et al.*, 1999), it is reasonable to assign microbial communities to soil horizons. It is noteworthy that cryoturbation, a common phenomenon in permafrost affected soils hampering a static view on active layer profiles, is negligible through all our studied profiles.

The two profiles at the rim and at the centre of a low-centred polygon were located in the eastern part of the island. The distance between these two profiles was approximately 7 m and the difference in elevation between the rim and the depressed centre was approximately 0.4 m. At the time of sampling (July 2005) the standing water level was in a depth of approximately 38 cm at the rim and at approximately 10 cm above the surface of the polygon centre. The permafrost table was in a depth of 38 cm at the polygon rim and in a depth of 30 cm at the polygon centre. The third profile was located on a flood plain in the northern part of the island. At this location, annual flooding leads to a continuing accumulation of fluvial sediments. At the time of sampling (July 2002) the permafrost table was in a depth of 54 cm. Soil samples were taken horizontally stepwise, stored in Nalgene boxes, frozen immediately after sampling and transported to Germany for further processing. *In situ* methane concentrations and temperatures were determined in the field according to Wagner and colleagues (2005). Additional soil characteristics (grain size fraction, content of organic carbon, nitrogen and water) were analysed according to Schlichting and colleagues (1995).

### Fixation of cells for hybridization

Fresh soil samples of each horizon were fixed according to Pernthaler and colleagues (2001). Subsamples (0.5 ml) were fixed with 1.5 ml freshly prepared 4% paraformaldehyde/phosphate-buffered saline (PBS) solution (pH 7.2–7.4) for 4–5 h at 4°C. Fixed samples were diluted with 0.1% sodium pyrophosphate in distilled water to obtain 100–300 cells (total) per microscopic field of view (63 × 100 objective). The dilution was treated with mild sonication using an MS73 probe (Sonoplus HD70; Bandelin, Berlin, Germany) at a setting of 20 s to separate cells from soil particles. As a result of the comparatively much higher background fluorescence of soil particles observed after hybridization on membrane filters (own observations) the dispersed soil samples were spotted on gelatine-coated Teflon-laminated slides (Zarda *et al.*, 1997) with 10 wells. Replicates of 10 µl of fixed and dispersed soil sample and 2 µl of 0.2% sodium dodecyl sulfate (SDS) were dropped onto each well resulting in full coverage of the well. Slides were dried at 45°C for 15 min and dehydrated in 50%, 80% and 96% ethanol.

### Fluorescence in situ hybridization (FISH) and DAPI staining

The FISH method was used directly in soil samples because extraction of bacterial cells from soil is difficult to perform due to the exclusion of bacteria associated with soil particles (Christensen *et al.*, 1999).

All oligonucleotide probes used in this study were purchased from Interactiva (Ulm, Germany). They were all labelled with the cyanine dye Cy3. Probes for the domain *Bacteria* and the families *Methylococcaceae* (type I methanotrophs) and *Methylocystaceae* (type II methanotrophs) were used. Probe names, details and references are summarized in Table 3. For *in situ* hybridization, a 10 µl aliquot of hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl; pH 8.0, 0.02% SDS), formamide in concentrations according to Table 3, and 30 ng µl<sup>-1</sup> of probe were dropped onto each well. The slides were transferred to an equilibrated 50 ml polypropylene top tube and incubated at 46°C for 120 min. Slides were then washed at 48°C for 10 min in washing buffer (20 mM Tris-HCl; pH 8.0, 5 mM EDTA, 0.01% SDS w/v and 225 mM NaCl according to a formamide concentration of 20% in the hybridization buffer). Afterwards they were washed in ice-cold double distilled water for a few seconds and quickly dried in an air stream. Subsequently, 10 µl of 4'6-Diamidino-2-phenylindole (DAPI, 1 µg ml<sup>-1</sup> working solution) was dropped onto each well and incubated in the dark at room temperature for 10–15 min. Slides were then washed in ice-cold double-distilled water and allowed to air-dry. Finally, slides were embedded in Citifluor AF1 antifadent (Plano; Wetzlar, Germany) and covered with a coverslip.

### Determination of cell counts

Microscopy was carried out with a Zeiss Axioskop 2 equipped with filters 02 (DAPI), 10 (FLUOS, DTAf) and 20 (Cy3), a mercury-arc lamp and an AxioCam digital camera. The counting was done manually. For each hybridization approach and

**Table 3.** rRNA-targeted oligonucleotide probes used for FISH.

Probe	Target group	Target site <sup>a</sup>	FA <sup>b</sup> (%)	Reference
EUB338	Domain <i>Bacteria</i>	16S rRNA (338)	0–50	Amann and colleagues (1990)
EUB338 II	Domain <i>Bacteria</i>	16S rRNA (338)	0–50	Daims and colleagues (1999)
EUB338 III	Domain <i>Bacteria</i>	16S rRNA (338)	0–50	Daims and colleagues (1999)
NON338	Control probe complementary to EUB338	16S rRNA	n.d.	Wallner and colleagues (1993)
Ma450	Type II MOB <sup>c</sup>	16S rRNA (450)	20	Eller and colleagues (2001)
Mg705	Type I MOB <sup>c</sup>	16S rRNA (705)	20	Eller and colleagues (2001)
Mg84	Type I MOB <sup>c</sup>	16S rRNA (84)	20	Eller and colleagues (2001)

<sup>a</sup> *Escherichia coli* numbering.<sup>b</sup> Percentage (v/v) of formamide in the hybridization buffer.<sup>c</sup> Methane oxidizing bacteria.

n.d., not determined.

sample at least 800 DAPI stained cells were counted on 30 randomly chosen counting squares. Microscopy was carried out using  $63 \times 100$  magnification giving an area of  $3.9204 \times 10^{-2} \text{ mm}^2$  per counting square. Using FISH, only cells with a sufficient number of ribosomes are detected (Amann *et al.*, 1995). The number of these cells was calculated by counting probe-specific positive signals relatively to DAPI counts. Counting results were always corrected by subtracting signals obtained with the probe NON338. Unspecific cell counts were in the range of  $3.53 \times 10^5$ – $2.7 \times 10^6$  cells g<sup>-1</sup> (dw). For calculating the number of cells per cubic centimetre of slurry (bacterial counts per volume, BC<sub>v</sub>), the mean count of bacteria per counting area (B), the microscope factor (area of sample spot/area of counting field, M), the dilution factor (D) and the volume of the fixed sample used for hybridization (V) were determined and arranged in the equation:

$$\text{BC}_v = B M D V^{-1} \quad (1)$$

Finally, the bacterial counts per millilitre of slurry were converted into cells per gram of soil (dw) according to the equation:

$$\text{BC}_w = \text{BC}_v (1 + \text{WC}/100) D \quad (2)$$

where BC<sub>w</sub> are the cells per gram of soil (dw), WC is the water content of the slurries and D is the density of the dried soil.

#### Potential methane oxidation rates

The methane oxidation rates of the polygon rim and the polygon centre were determined in incubations without headspace via the conversion of  $^{14}\text{CH}_4$ – $^{14}\text{CO}_2$  modified according to Iversen and Blackburn (1981). Before the tracer experiment, thoroughly homogenized subsamples (160 g) of each soil horizon were mixed with autoclaved tap water at the ratio of 1:1 (w/v) and incubated in 1 l glass bottles at 4°C with 3% CH<sub>4</sub>. The slurries were shaken continuously at 120 rpm and CH<sub>4</sub> concentrations were determined daily using gas chromatography. Subsequent radiotracer analysis was compared according to incubations with and without headspace. Incubations without headspace: three replicates per slurry and temperature were distributed to 5 ml Hungate tubes and sealed with butyl-rubber stoppers and screw caps leaving no gas bubbles inside the tube. Anaerobically stored  $^{14}\text{CH}_4$  tracer (Fa. Amersham) was injected. Replicates were incubated at six different temperatures, namely 0, 4, 12, 21, 28 and 38°C,

for 13 h at methane concentrations between 50 and 1200 nmol g<sup>-1</sup> (dw) according to the methane concentrations determined *in situ*. Incubations with headspace: three replicates per slurry were distributed to 16 ml Hungate tubes leaving 3 ml of headspace and  $^{14}\text{CH}_4$  tracer was injected. Replicates were incubated for 72 h to allow sufficient tracer to dissolve into the sample. Near surface samples were incubated at 21°C and near permafrost samples were incubated at 4°C, because previous tests had shown that maximum activities were detected at these temperatures at the according depths. Methane oxidation rates (MOR) were calculated as nanomoles of CH<sub>4</sub> oxidized per gram dry weight (dw) and day according to the equation:

$$\text{MOR} = [\text{CH}_4] a/(A t) \quad (3)$$

where [CH<sub>4</sub>] is the sediment concentration of methane in nmol cm<sup>-3</sup> dry volume (dv), a are the counts recovered as  $^{14}\text{CO}_2$ , A are the counts recovered as (remaining)  $^{14}\text{CH}_4$  and t is the incubation time (days). Rates are based on three replicates and were corrected according to five blanks for each temperature running the same analysis. The potential methane oxidation rates in incubations without headspace were comparable to those in incubations with headspace (data not shown) so that we could exclude possible oxygen deficits limiting the process of methane oxidation in incubations without headspace.

The potential methane oxidation of the flood plain profile was determined by gas chromatography with the aid of difluoromethane (CH<sub>2</sub>F<sub>2</sub>) inhibiting the process of methane oxidation (Krueger *et al.*, 2002). Thoroughly homogenized subsamples (30 g per horizon) were divided into three replicates, filled into sterile serum bottles (120 ml), mixed with autoclaved tap water at the ratio of 1:1 (w/w) and vortexed for 20 s. The slurries were incubated over night at 0, 4, 12 and 21°C. The supernatant was decanted and the bottles were closed with a screw cap containing a septum. Subsequently, methane concentrations between 50 and 200 nmol g<sup>-1</sup> (dw) were adjusted according to the methane concentrations determined *in situ* and the samples were again incubated at the accordant temperature. The methane concentration in the headspace was determined twice per day for a period of 6 days. Afterwards, the bottles were evacuated and again incubated as described but additionally with CH<sub>2</sub>F<sub>2</sub> (8000 ppm). Gas analysis was carried out as described below. Potential methane oxidation rates were calculated from the linear regression of methane concentrations in the

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headspace taking into account methane production rates in samples incubated with CH<sub>2</sub>F<sub>2</sub>.

$$\text{MOR} = -\text{MOR}_{\text{with\_inhibitor}} + \text{MOR}_{\text{without\_inhibitor}} \quad (4).$$

**Gas analysis**

Gas analysis was carried out with a gas chromatograph (Agilent 6890, Fa. Agilent Technologies) equipped with a Carbonplot capillary column ( $\varnothing$  0.53 mm, 30 m length) and a flame ionization detector (FID). Oven as well as injector temperature was 45°C. The temperature of the detector was 250°C. Helium served as carrier gas.

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## 4.4

### Methanogenic activity and biomass in Holocene permafrost deposits of the Lena Delta, Siberian Arctic and its implication for the global methane budget

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#### Abstract

Permafrost environments within the Siberian Arctic are natural sources of the climate relevant trace gas methane. In order to improve our understanding of the present and future carbon dynamics in high latitudes we studied the methane concentration, the quantity and quality of organic matter, and the activity and biomass of the methanogenic community in permafrost deposits. For these investigations a permafrost core of Holocene age was drilled in the Lena Delta (72°22' N, 126°28' E). The organic carbon of the permafrost sediments varied between 0.6 and 4.9 % and was characterized by an increasing humification index with permafrost depth. A high CH<sub>4</sub> concentration was found in the upper 4 m of the deposits, which correlates well with the methanogenic activity and archaeal biomass (expressed as PLEL concentration). Even the incubation of core material at -3 °C and -6 °C with and without substrates showed a significant CH<sub>4</sub> production (range: 0.04 – 0.78 nmol CH<sub>4</sub> h<sup>-1</sup> g<sup>-1</sup>). The results indicated that the methane in Holocene permafrost deposits of the Lena Delta originated from modern methanogenesis by cold-adapted methanogenic archaea. Microbial generated methane in permafrost sediments is so far an underestimated factor for the future climate development.

**Keywords:** methane, methanogenesis, psychrophiles, phospholipid biomarker, methane release, permafrost deposits

#### Introduction

Northern wetlands play an important role within the global methane cycle. Methane is chemically very reactive and more efficient in absorbing infrared radiation than carbon dioxide. Estimates of the methane emissions of arctic and sub-arctic wetlands range between 10 and 39 Tg a<sup>-1</sup>, or between 2.2 and 8.6 % of the global methane emission (Bartlett & Harriss 1993; Cao *et al.* 1998). Methane as a powerful greenhouse gas contributes to about 20 % of the global warming (IPCC, 2001).

Permafrost, which occurs mainly in polar and sub-polar regions, occurs within about 25 % of the

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land surface (Zhang *et al.* 1999). It can be divided into three temperature depth zones which characterize typical living conditions: (i) the active layer with an extreme temperature regime from about +15°C to -35°C, (ii) the correlated upper permafrost sediments (0.5-20 m thickness) with smaller seasonal temperature variation of about 0 °C to -15 °C above the zero annual amplitude and (iii) the deeper permafrost sediments which are characterized by a stable temperature regime of about -5 °C to -10 °C (French 1996).

A considerable amount of organic carbon is stored in the upper permafrost layers, indicating that the extreme Arctic climate condition reduces the organic carbon decomposition rate more than the net primary production rate (Oechel *et al.* 1997). The positive temperature trend in the Russian Arctic favours warming and thawing of terrestrial permafrost (Richter-Menge 2006). The degradation of permafrost and the associated release of climate relevant trace gases, as a consequence of an intensified turnover of organic carbon and from ancient methane reservoirs, represent a potential risk with respect to future global warming. At this point, the functioning of microbial communities and their reaction on changing environmental conditions are not adequately understood, neither the potential methane releases from frozen sediments are adequately quantified.

In general, temperature is one of the most important variables regulating the activity of microorganisms. The potential of growth as well as the molecular, physiological and ecological aspects of microbial life at low temperatures have been investigated in many studies (e.g. Russel & Hamamoto 1998; Gounot 1999; Cavicchioli 2006). Certain key processes of the methane cycles are carried out exclusively by highly specialized microorganisms such as methanogenic archaea and methane oxidizing bacteria. The microbial methane production (methanogenesis) in the active layer of permafrost is the terminal step during the anaerobic decomposition of organic matter, while the methane oxidation is the primary sink for methane in Arctic wetlands (Wagner *et al.* 2005). With recent findings it becomes evident that methanogenic archaea and methane oxidizing bacteria also exist in permafrost soils, with numbers comparable to those in moderate soil environments (Kobabe *et al.* 2004; Liebner & Wagner 2006).

However, there are only few studies investigating the geochemistry and microbiology of permafrost deposits, mainly in Siberia and Canada. Direct bacterial counts in the order of  $10^7$  to  $10^8$  were reported for permafrost deposits from Northeast Siberia (Rivkina *et al.* 1998). Furthermore, Shi *et al.* (1996) found viable bacteria in permafrost sediments up to 3 million years in age in the Kolyma-Indigirka lowlands. Most of the isolated bacteria showed mesophilic growth characteristics. In contrast, the minimum temperature for growth of permafrost bacteria was recently calculated with -20 °C (Rivkina *et al.* 2000). Furthermore, molecular life markers and low numbers of methanogens were found in the Mallik gas hydrate production research well (Colwell *et al.* 2005; Mangelsdorf *et al.* 2005). However, methanogenic activity could not be detected in the permafrost sediments by using radiolabelled  $^{14}\text{C}$ -substrates.

So far there is no proof for recent methanogenic activities in permafrost deposits. The main objective of this study was to identify the vertical position and quantify the methanogenic activity along a Holocene permafrost core from the Siberian Lena Delta, and correlate this activity with biotic and abiotic factors. A polyphasic cultivation-independent approach was used based on geochemical and microbiological methods to identify the origin of permafrost borne methane. As direct cultivation of methanogenic archaea from cold environments is limited to only very few species, we used etherlipids, which are a unique component of the archaeal cell membrane, as biomarkers to quantify methanogens along the permafrost core.

## Material and methods

### Investigation area

Within the scope of long-term studies on carbon dynamics in the Siberian Arctic, the LENA 2001 expedition was carried out by the Alfred Wegener Institute for Polar and Marine Research. The Lena

Delta lies at the Laptev Sea coast between the Taimyr Peninsula and the New Siberian Islands. Continuous permafrost, which occurs throughout the investigation area extends to depths of about 100-300 m (Yershov 1998). It is characterised by an arctic continental climate with low mean annual air temperature of -14.7 °C ( $T_{\min} = -48$  °C,  $T_{\max} = 18$  °C) and low summer precipitation of < 198 mm. The study site, Samoylov Island (72°22' N, 126°28' E) with the Russian-German Research Station Samoylov, is located in the active part of the Lena Delta (Hubberten *et al.* 2006). This part of the delta was formed during the Holocene with an age of about 9,300 years BP. Further details of the study site were described previously by Wagner *et al.* (2003a).

#### *Permafrost drilling and preparation*

During summer 2001 a permafrost core of 850 cm length was drilled in the depression of a low-centered ice-wedge polygon on Samoylov Island. The drilling was carried out with a portable gasoline powered permafrost corer without using any drilling fluid to avoid microbiological contamination of the permafrost samples. A mixing of the permafrost sediments was not be observed due to the frozen state of the core material. The individual core segments, which were up to 50 cm in length, were placed immediately after removal from the corer into plastic bags and stored at about -8 °C in the permafrost cellar of the Research Station Samoylov. After drilling of the core the borehole temperature was monitored with a string of 9 thermistors. The cores were transported in frozen conditions in insulated containers with cool packs to Potsdam, Germany. During the transport the temperatures in the containers were monitored by micro data loggers. The storage temperature in the Potsdam laboratory was -22 °C.

Core segments were split along their axis into two halves under aseptic conditions with a diamond saw in an ice laboratory at -22 °C. Afterwards, one half of the core was cleaned with a sterile knife for lithological and geocryologically descriptions. Subsequently, one half was cut into segments of about 10-30 cm length according the lithology and the geocryology. Small pieces (approx. 10 g) of each sub-sample were taken for analysing the methane concentration in the frozen sediments. The remaining material of each sub-sample was thawed at 4 °C and homogenized under anoxic and sterile conditions for analysis of the sediment properties and the microbial activities and biomarkers. Sub-samples for the different analyses were filled into sterile plastic Nalgene boxes. Separated samples were used directly for the experiments (e.g. methane production, biomarker analysis) or were refrozen for later analyses at -22 °C. The second half of the core is kept as an archive in the ice core storage at the Alfred Wegener Institute.

#### *Sediment properties*

Grain-size distribution was analysed on bulk sediment samples for each segment, on average every 20 cm. The sediments were oxidised using a 5 % H<sub>2</sub>O<sub>2</sub> solution to remove organic matter from each sample. The gravel (> 2 mm) and sand (0.063–2.0 mm) fractions were determined by wet sieving. The remaining silt (0.002–0.063) and clay (< 0.002 mm) material were separated by sedimentation in ammoniac water (10 ml NH<sub>3</sub> in 100 l deionised water). From the dry weight of gravel, sand, silt and clay, the weight percentages of the bulk dry sediment were calculated for each fraction.

Radiocarbon dating was carried out for three selected samples (289, 557 and 843 cm depth) with the Accelerator Mass Spectrometer (AMS) facility at the Leibniz Laboratory for Radiometric Dating and Stable Isotope Research, University of Kiel, Germany. Standard calibration techniques (Stuiver *et al.* 1998) were used to express the sediment ages in calendar years before the present. A more detailed description of the equipment and method was given by Nadeau *et al.* (1997, 1998).

The total carbon (TC) and total nitrogen (TN) contents were determined with an automatic element analyser (Elementar VARIO EL III). The total organic carbon (TOC) content was measured on corresponding samples after HCl (10%) acid digestion to remove the carbonate on the same analyser (Elementar VARIO EL III). Based on TOC and TN values, the C/N ratio was calculated. Analytical precision is ± 5% for element analyses.

The humification index, a criterion for organic matter quality, was determined in the water extractable fraction of organic carbon as described in Wagner et al. (2005). In brief, the obtained aqueous extracts from frozen soil samples were subjected to optical measurements (UV absorption and fluorescence emission intensity). Dissolved organic matter differs in fluorescence behaviour in accordance to its molecular complexity. Humified carbon is characterized by highly substituted aromatic structures and condensed unsaturated systems. While their fluorescence emission lie in longer wavelengths, fresh and low humified organic matter fluoresce in the shorter wavelengths (Senesi et al. 1989). To quantify the humification of dissolved carbon we measured the fluorescence emission intensity in 1nm steps between 300-480nm with an excitation wavelength of 254nm (Cary Eclipse F-4500, Varian®). Summarized intensities between 435-480 nm (upper quartile of the whole spectrum recording emission of more humified carbon) were divided by summarized intensities between 300-345 nm (lower quartile of the whole spectrum recording emission of less humified carbon), resulting in an dimensionless humification index (Zsolnay 2003). The higher the humification index, the more the organic carbon in the samples is humified. All extracts were adjusted to pH 2 since pH influences the fluorescence of organic molecules in solution (Zsolnay et al. 1999).

Vertical profiles of sediment CH<sub>4</sub> concentrations were obtained from each segment by extracting CH<sub>4</sub> from sediment pore water by thaw small frozen core material (approx. 10 g) in saturated NaCl solution. The samples were then placed in glass jars and sealed gas tight with black rubber stoppers. The thawed samples were shaken and the CH<sub>4</sub> headspace concentration was analysed with gas chromatography.

#### *CH<sub>4</sub> production*

The CH<sub>4</sub> production of permafrost sediments were analysed for each segment without any additional methanogenic substrate, or with acetate, and hydrogen as an energy source. Fresh sediments (20 g) were weighed in 100-ml glass jars and closed with a screw cap containing a septum. The samples were evacuated and flushed with ultra pure N<sub>2</sub>. Afterwards the samples were supplemented with 6 ml sterile and anoxic tap water for analysing methane production without substrate addition. In the case of potential CH<sub>4</sub> production 6 ml of acetate solution (10 mM) or sterile and anoxic tap water in combination with H<sub>2</sub>/CO<sub>2</sub> (80:20 v/v, pressurized 150 kPa) were added as substrates. Three replicates were used for each segment. The incubation temperature was 5 °C. CH<sub>4</sub> production was measured daily over a period of one week by sampling the headspace using a Hamilton gastight syringe. CH<sub>4</sub> production rates were calculated from the linear increase in CH<sub>4</sub> concentration.

#### *Methane production at sub-zero temperature*

The CH<sub>4</sub> production at sub-zero temperatures was analysed in samples from the upper part of the permafrost core (45-63 cm depth). The homogenized material (10 g) was directly weighed in 25-ml glass jars and closed with a screw cap containing a septum. The further preparation of the samples followed the description to the potential methane production activity. The prepared soil samples were incubated at -3 °C and -6 °C for 14 and 21 days, respectively.

#### *Methane analysis*

Gas analysis were performed with an Agilent 6890 gas chromatograph equipped with a Carbonplot capillary column (Ø 0.52 mm, 30 m) and a flame ionization detector (FID). Helium was used as the carrier gas. The injector, oven and detector temperatures were set at 45 °C, 45 °C and 250 °C, respectively. All gas sample analyses in the various experiments were done after calibration of the gas chromatograph with standard gases. After calibration the analytical reproducibility is > 98.5%. Details of CH<sub>4</sub> analysis were described previously in Wagner et al. (2003b).

#### *Determination of phospholipids fatty acids (PLFA) and phospholipids etherlipids (PLEL)*

Lipids were extracted from the freshly homogenized material from selected segments using an equivalent of about 30 g of dry weight, according to the Bligh-Dyer method (Zelles & Bai 1993). The

resulting lipid material was fractionated into neutral lipids, glycolipids and phospholipids on a silica-bonded phase column (SPE-SI; Bond Elute, Analytical Chem International, CA, USA) by elution with chloroform, acetone and methanol, respectively. An aliquot of the phospholipid fraction equivalent to 10 g soil dry weight (dw) was taken for phospholipid fatty acid (PLFA) analysis. After mild alkaline hydrolysis, the lipid extract was separated into OH-substituted ester-linked PLFA, non-OH substituted ester-linked PLFA and non saponifiable lipids following procedures described in Zelles & Bai (1993). The fraction of unsubstituted ester-linked PLFA was reduced to dryness under nitrogen and dissolved in 100 µl hexane supplemented with nonadecanoic methyl ester as internal standard. The analyses of the fatty acid methyl ester (FAME) extracts were performed by GC-MS as described in Lipski & Altendorf (1997). The position of double bonds of monounsaturated fatty acids was determined by analysing the dimethyl disulfide (DMDS) adducts (Nichols *et al.* 1986). The fraction of non-saponifiable lipids was cleaved during acidic alkaline hydrolysis and the resulting non-ester-linked PLFA were separated into OH-substituted non-ester-linked PLFA (UNOH) and non-OH substituted non-ester-linked PLFA (UNSA). Separation of the non-ester-linked PLFA, derivatization and measurement were performed according to Gattinger *et al.* (2002). Another aliquot of the phospholipid fraction equivalent to 20.0 g soil dw was used for phospholipid etherlipid (PLEL) analysis according to the method described by Gattinger *et al.* (2003). After the formation of ether core lipids, ether-linked isoprenoids were released following cleavage of ether bonds with HI and reductive dehalogenation with Zn in glacial acetic acid. The resulting isoprenoid hydrocarbons were dissolved in 100 µl internal standard solution (nonadecanoic methyl ester) and subjected to GC/MS analysis at operating conditions described elsewhere (Gattinger *et. al.* 2003). PLFA/PLEL concentrations are expressed in nmol g<sup>-1</sup> dry weight (dw).

#### Statistical analysis

Statistical analyses such as descriptive statistics and analysis of variance (ANOVA) were performed with SPSS software package release 12.0 (SPSS Inc., Chicago, USA). The Kolmogorov-Smirnow test was used to assess distribution fitting. The definition of the four core units was based on the results of a Constrained Incremental Sum of Squares (CONISS) cluster analysis of the grain size data (using ZONE software version 1.2).

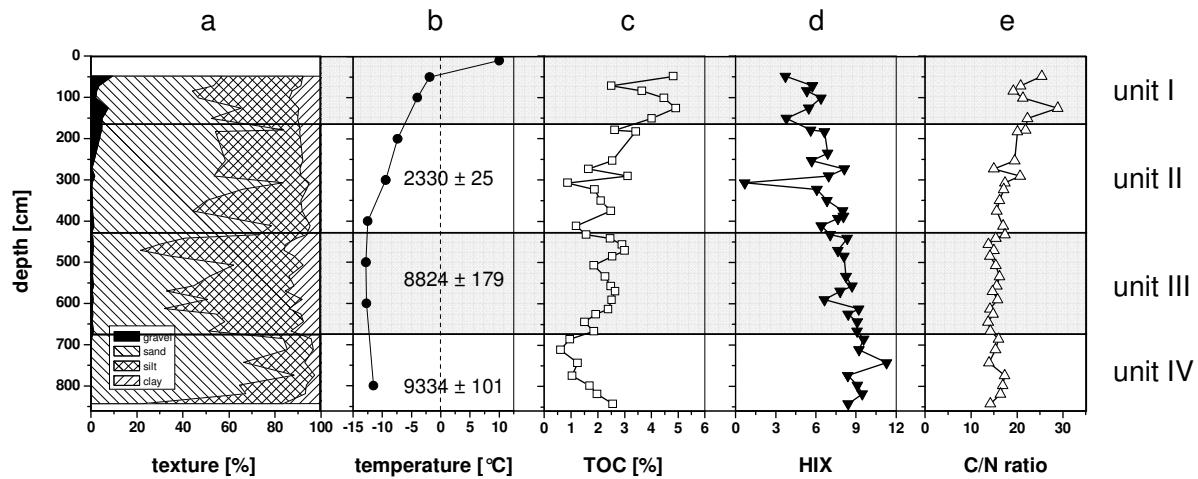
## Results

### Abiotic characteristics of the permafrost sediments

On basis of the grain size distribution and the subsequent CONISS analyses the permafrost core was separated into four units (I – IV). The further description of the results and the later discussion followed this classification.

The grain size analyses showed that gravel continuously decreased from the top to the bottom of the core (4.9 – 0.2 %). For the three other fractions (sand, silt and clay) a clear change between the different units was recognized. The average value of the sand fraction is highest in the Units II and IV (U-I = 48.2 %, U-II = 62.3 %, U-III = 42.6 %, U-IV = 68.2 %), while the highest values for silt (U-I = 36.4 %, U-II = 28.7 %, U-III = 44.7 %, U-IV = 24.9 %) and clay (U-I = 10.5 %, U-II = 7.6 %, U-III = 11.8 %, U-IV = 6.7 %) were determined in the Units I and III (Fig. 1a). A steep temperature gradient was observed in the permafrost core, which ranged between +10 °C (near surface) and -11.5 °C at 800 cm depth. The median values were -1.9, -9.4, -12.8 and -11.5 °C, for Units I to IV, respectively (Fig. 1b). The total organic carbon content (TOC) followed a depth gradient with median values between 4.2 and 1.3 % for Units I to IV, respectively, but with significant variations within the vertical profile (Fig. 1c). A depth-dependent relationship can also be seen from the humification index (HIX) and the C/N ratio. The HIX and C/N ratio are both descriptors for organic matter quality. The lowest HIX values were found in Unit I (on average 5.1) which corresponded with the highest C/N ratios (23.0).

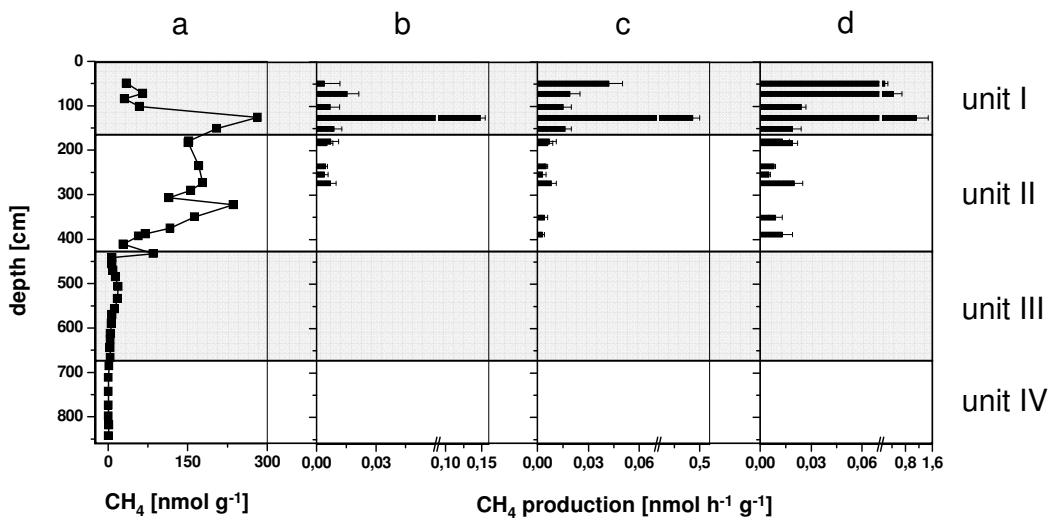
HIX was highest in unit IV (on average 9.4) in accordance to a relatively low C/N ratio of 15.7 (Fig. 1d, 1e).



**Fig. 1** Abiotic parameters of the Holocene permafrost deposits from Samoylov Island, Lena Delta (Siberian Arctic). (a) sediment texture, (b) bore hole temperature and calendar years of selected sediment layers, (c) total organic carbon content (TOC), (d) humification index (HIX, dimensionless) and (e) C/N ratio. Calendar years (BP) of selected sediment layers are denoted in graph (b). Unit I to IV based on CONISS (constrained incremental sum of squares) analysis of the different grain size fractions.

#### Methane concentrations and potential methanogenic activities

Methane was detected in all samples of the permafrost core. Highest concentrations were observed in Units I ( $281.5 \text{ nmol g}^{-1}$ ) and II ( $236.1 \text{ nmol g}^{-1}$ ). In Unit III, low values, ranging between  $3.5$  and  $19.1 \text{ nmol g}^{-1}$  were detected, while only traces in the bottom zone of the core were found ( $0.4 - 1.9 \text{ nmol g}^{-1}$ ; Fig. 2a).



**Fig. 2** Vertical profiles of methane concentration (a) and methane production rates determined at  $5^\circ\text{C}$  without any additional substrate (b) as well as with acetate (c) or hydrogen (d) as methanogenic substrates. Unit I to IV based on CONISS analysis of the different grain size fractions.

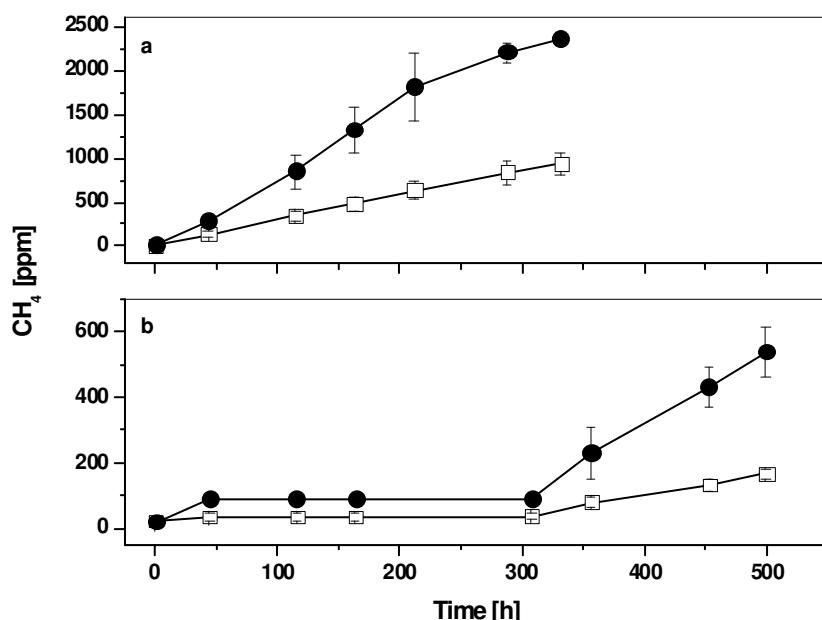
Methanogenic activity was determined at 5 °C with and without methanogenic substrates in selected sediment samples representative for the different core units. Methane production could be determined only in the two upper units (I, II), while in the lower two units (III, IV) no methane formation was detected before and after addition of methanogenic substrates (Fig. 2b-d). The methane production rates analysed in Unit I, were generally higher than in Unit II. In any case, the highest activity was observed in about 125 cm sediment depth, which corresponded on the highest concentration of methane (Fig. 2a-d).

**Table 1** AMS radiocarbon dating, calendar years and  $\delta^{13}\text{C}$  values of the organic carbon fraction from selected permafrost sediments of Samoylov Island

Reference number	Depth [cm]	Unit	$^{14}\text{C}$ age [years BP]	Calendar age [years BP]	$\delta^{13}\text{C}$ [%oo]
KIA20719	289	II	$2306 \pm 30$	$2330 \pm 25$	$-24.39 \pm 0.06$
KIA20720	557	III	$7970 \pm 56$	$8824 \pm 179$	$-24.63 \pm 0.04$
KIA20721	843	IV	$8295 \pm 50$	$9334 \pm 101$	$-23.06 \pm 0.03$

BP = years before the present

The incubation of permafrost samples from 45–63 cm depth at sub-zero temperatures with acetate and hydrogen as methanogenic substrates indicated a relatively high methane production rate under permafrost temperature conditions (Fig. 1b, 3). At a temperature of -3 °C a significant increase in methane production was found, which rose linearly to headspace concentrations of about 1000 ppm (with acetate) and 2500 ppm (with hydrogen) during 300 h after the initiation of the experiment. At a temperature of -6 °C methanogenesis was lower. However, after a lag phase of about 300 h a significant increase to 200 ppm (with acetate) and 500 ppm (with hydrogen) within 200 h was observed. The calculated activity of methanogenic archaea with hydrogen reached values of  $0.78 \pm 0.31 \text{ nmol CH}_4 \text{ h}^{-1} \text{ g}^{-1}$  and  $0.14 \text{ nmol CH}_4 \text{ h}^{-1} \text{ g}^{-1}$  at an incubation temperatures of -3 °C and -6 °C, respectively. This was 2.5 and 3.5 times higher compared to the activity with acetate ( $0.31 \pm 0.04 \text{ nmol CH}_4 \text{ h}^{-1} \text{ g}^{-1}$  and  $0.04 \pm 0.01 \text{ nmol CH}_4 \text{ h}^{-1} \text{ g}^{-1}$ ) at the corresponding temperatures.



**Fig 3** Methanogenesis in permafrost soils at sub-zero temperatures. Soil samples were incubated at -3 (a) and -6 °C (b) with hydrogen (circles) or acetate (squares) as a substrate (means  $\pm$  SE, n = 3).

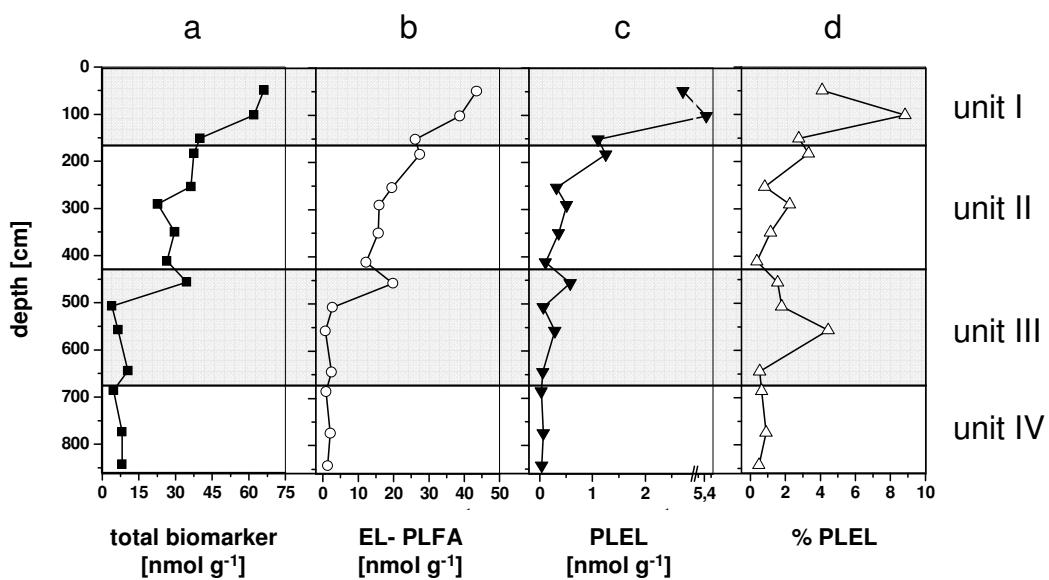
### Microbial biomarkers

Significant amounts of microbial biomarkers (bacterial and archaeal) were detected in sediment samples across all depth units (Fig. 4). Generally, microbial biomarkers were greatest in Unit I and lowest in Unit IV. Concentrations of total biomarker (PLFA, UNSFA, UNOH and PLEL) varied between 3.9 and 66.2 nmol g<sup>-1</sup>. Biomarker concentrations of ester-linked PLFA (EL-PLFA) ranged between 0.7 and 43.5 nmol g<sup>-1</sup> and for archaeal PLEL, between 0.03 and 5.4 nmol g<sup>-1</sup>. Archaeal PLEL accounted for 4.1 % of total biomarker in Unit I and for 0.7 % in Unit IV.

### Compilation of relevant data sets

The statistical analyses illustrate the relationships between organic matter quantity (TOC) and quality (HIX), methanogenic microorganisms (PLEL concentration) and activity, and the detected methane concentration in the permafrost sediments for the different core units (Fig. 5).

In Unit I five different PLEL derived isoprenoids were detected. In contrast, all other units contained only 1-3 different PLEL side chains. These chains were much lower concentrated (Fig. 5a). Furthermore, in the same unit the PLEL side chain i20:1, characteristic for *Methanosarcina* spp. (Gattinger *et. al.* 2002), accounted for 15.8 % of total PLEL. In the other core units this compound was only detectable in trace amounts. Unit I also showed the highest activity of methanogenic archaea (Fig. 5b and c). A positive correlation was found between the organic carbon content (TOC) and methane production activity with acetate ( $r = 0.541, P = 0.01$ ) and with hydrogen ( $r = 0.532, P = 0.01$ ), and with methane content ( $r = 0.434, P = 0.01$ ) in the sediment. The correlation between the amount of TOC and the humification index (HIX) was negative ( $r = -0.535, P = 0.01$ ).



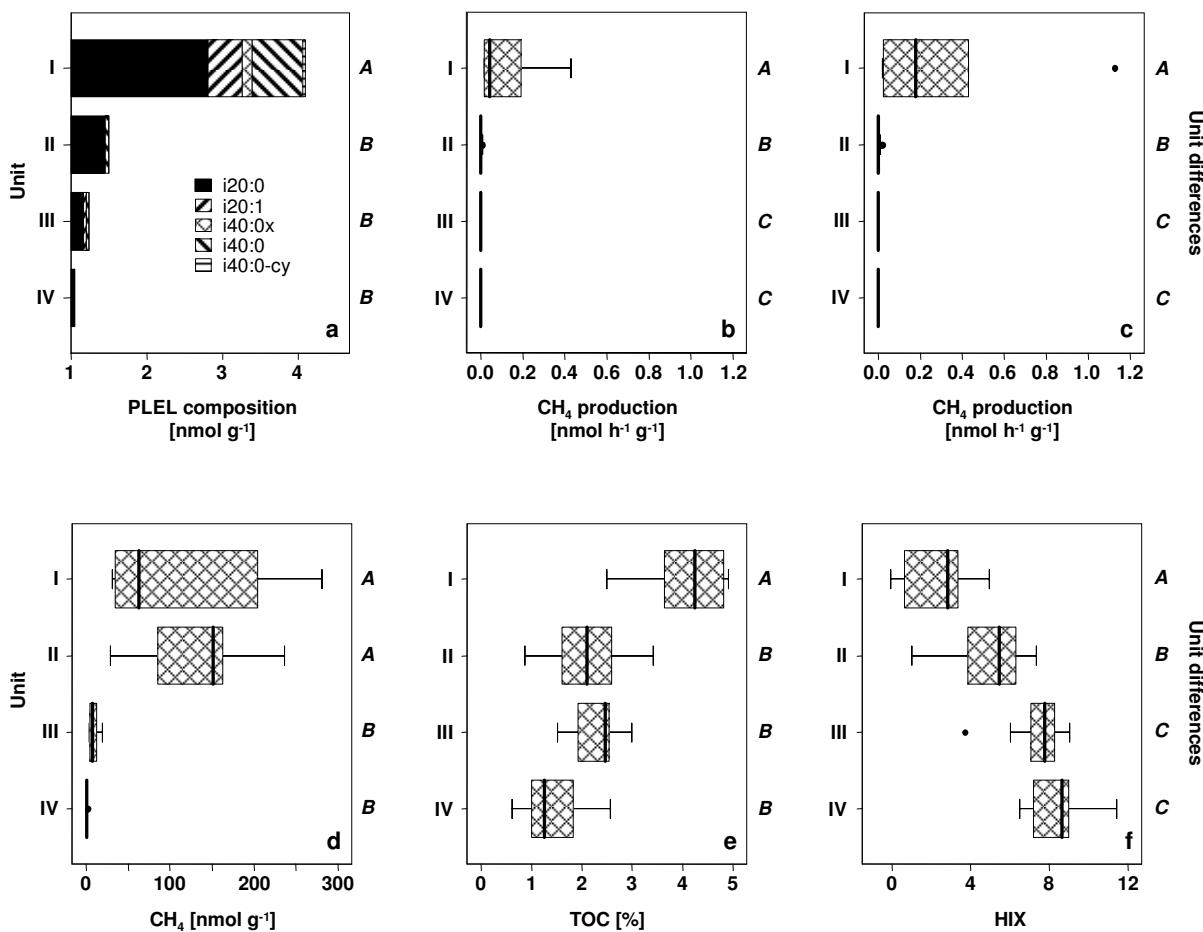
**Fig. 4** Lipid biomarker profiles within the Holocene permafrost deposits of Samoylov Island, Lena Delta (Siberian Arctic). (a) total biomarker, (b) ester-linked phospholipid fatty acids (EL-PLFA), (c) phospholipid ether lipids (PLEL) and (d) percentage of PLEL on total biomass. Unit I to IV based on CONISS analysis of the different grain size fractions.

### Discussion

Our results show significant amounts of methane in the first four meters of frozen sediments (Unit I and II, Late Holocene, 5000 yr BP until today) and only trace amounts of methane in the bottom section of the core (Unit III and IV; Middle Holocene, 9000 – 5000 yr BP; and Early Holocene, 11500 – 9000 yr BP). Different amounts of methane in different aged permafrost deposits from north-eastern Eurasia were also reported by Rivkina & Gilichinsky (1996). They detected methane in modern

(Holocene) and old permafrost deposits (Middle and Early Pleistocene, 1.8 – 0.78 mill. yr BP), but not in Late Pleistocene ice complexes (ice rich permafrost, 130000 – 11500 yr BP). They concluded from their findings that methane can not diffuse through permafrost sections. If methane is unable to diffuse through permafrost from deeper deposits, it must be either be entrapped during the deposition of the sediments or originate from recent methanogenesis in the frozen ground.

The investigation of phospholipids shows a vertical profile with the same trend as the methane concentration. Specifically, significant amounts of phospholipids were determined in the upper Late Holocene deposits (Unit I, II), which correlates ( $r = 0.632, P = 0.05$ ) with the highest amount of methane in the permafrost section. In contrast, the biomarker concentrations in the Middle and Early Holocene permafrost sediments (Unit III, IV) drastically decreased to values below 10 nmol g<sup>-1</sup> sediment, which corresponds with the detected traces of methane.



**Fig. 5** Relationship between archaeal biomarkers (a), methane production activities with acetate (b) and hydrogen (c), methane concentration (d), organic matter quantity (TOC) and quality (HIX) for the different core units of the Holocene permafrost deposits. Different capitals indicate differences at the significance level of  $P = 0.05$ .

Phospholipid fatty acids (PLFA) are molecular biomarkers for the domains of Bacteria and Eukarya. Phospholipid etherlipids (PLEL) are indicators for the domain Archaea. Phospholipids are compounds of the cell membranes that rapidly degraded after cell death (Harvey *et al.* 1986, White *et al.* 1979). They are regarded as appropriate biomarkers for viable microorganisms (e.g. Ringelberg *et al.* 1979; Zelles 1999). The detection of biomarkers for viable microorganisms does not necessarily indicate their activity status. Enclosed in deep permafrost deposits, they can represent completely or partially inactive or dormant microorganisms (Colwell *et al.* 2005). A further possibility is that the

detected PLFA and PLEL are well preserved remains of ancient microbes. However, the positive correlation of methane concentration with viable bacteria and archaea gives us the first strong evidence of recent methanogenesis under *in situ* conditions in permafrost deposits.

The analyses of methane production revealed activity only in permafrost layers with significant concentrations of both methane and microbial (particularly archaeal) biomarkers. Although the activity in Unit I was higher compared to Unit II (both Late Holocene), which was also still characterized by high concentrations of methane, an important finding from the activity analyses is that no methane production was detectable in the bottom part of the permafrost section (Unit III, IV) characterized only by traces of methane. This was also the case after addition of acetate or H<sub>2</sub>/CO<sub>2</sub> as energy and carbon source. This indicates the absence of methanogenesis does not depend on deficiency of methanogenic substrates in the Middle and Early Holocene deposits. Methane was only found in permafrost sediments with a considerable amount of viable microorganisms and verifiable methane production activity.

In Unit I (32-194 cm depth), archaeal biomass expressed as PLEL concentration, was highest and even exceeded values that were reported previously for the active layer at a related permafrost site (Wagner *et al.* 2005). In the present core, archaeal biomarkers accounted for 5.2 % of total phospholipids in the upper section and for 2.3 % of the entire profile. In addition, methanogenic PLEL chains (i20:1), indicative for *Methanosarcina* spp. (Gattinger *et al.* 2002), were detected in significant amounts only in Unit I. This finding was confirmed by isolates from the same study site identified as members of the genus *Methanosarcina*, which are characterized by extreme tolerance against various stress conditions (D. Morozova and D. Wagner, data under publication). This supports the hypothesis that *Methanosarcina*-like cells are better protected against damage caused by environmental stresses compared to other methanogens due to their typical formation of cell aggregates.

Although, only a few psychrophilic strains of methanogenic archaea have been isolated so far (Simankova *et al.* 2003; Cavicchioli 2006), there are some indications of methanogenic activity in cold permafrost environments (Kotsyurbenko *et al.* 1993; Wagner *et al.* 2003; Ganzert *et al.* 2006). However, this study actually revealed methane production under *in situ* permafrost temperature conditions of down to -6 °C. The methane production rate with acetate or hydrogen at sub-zero temperatures was only 10 times lower compared with the activity in the active layer of a permafrost soil from the same study site (Wagner *et al.* 2005). This indicates a tolerance of permafrost methanogens to their cold environment. This assumption is also supported by the finding of Ganzert *et al.* (2006) who reported increasing methane production activity close to the permafrost table at low *in situ* temperature conditions. The zone of high methane concentrations in the permafrost deposits was characterized by *in situ* temperatures between approx. -2 and -9 °C. This is the same temperature range used in the incubation experiments.

One prerequisite for any metabolic activity in frozen permafrost sediments is the availability of unfrozen water. The Late Holocene permafrost deposits at the study site were characterized by a sediment texture of loamy sand with a relatively high content of silt and clay. In permafrost soils with a prominent part of fine textured material, liquid water has been observed at temperatures down to -60 °C (Ananyan 1970). Biologically, the most important feature of unfrozen water in permafrost is the ability to transfer ions and nutrients (Ostroumov & Siegert 1996).

Additionally, the quality of organic carbon is a limiting factor in the microbial metabolism process. Our results reveal a high organic carbon content (on average 2.4 %) for the Holocene permafrost deposits. However, the quantity of organic matter in permafrost ecosystems provides no information on the quality, which determines the availability of organic compounds as energy and carbon sources for microorganisms (Hogg 1993; Bergmann *et al.* 2000). For this purpose qualitative parameters like the humification index (HIX) or the C/N ratio can give suitable information with regard to microbial metabolism. Wagner *et al.* (2005) demonstrated that the availability of organic carbon in permafrost soils decreased with increasing HIX. This is in agreement with the present study. It was shown for the permafrost sequence the HIX increased continuously with depth. At the same time the C/N ratio and the organic carbon content decreased with permafrost depth. In both cases, this

significantly correlates with the HIX. Consequently, at this point we can summarize that the zone with significant concentrations of methane and activity of methanogenic microorganisms is characterized by the highest concentration of high quality organic carbon.

In contrast to the results of the soil-ecological variables (methane production activity, PLEL biomarker concentration, TOC, HIX), we do not achieve any ideas for a possible entrapment process of methane during sedimentation from data of paleoclimate research (Andreev *et al.*, 2004; Andreev and Klimanov, 2005).

In the Early Holocene to approx. 8800 yr BP, the environmental conditions were relatively stable in comparison to the Late Holocene (5000 yr BP until today). This was shown by climate reconstruction based on pollen and chironomid records from the Lena Delta (Andreev *et al.*, 2004). They determined the Holocene climate optimum between 10300 to 9200 yr BP, which was characterized by warmer (up to 3°C) and wetter conditions than the present day. Between 9200 and 6000 yr BP, the climate was still relatively warm but more unstable concerning the temperature. From the climate reconstruction, it can be concluded that the environmental conditions during the Early Holocene until 8800 yr BP were favourable for methanogenic archaea and methanogenesis. The absence of methane in the permafrost sediments from this period indicates that the likely produced methane was emitted to the atmosphere before it could be entrapped by freezing of the sediments. It can be expected that under more unstable conditions methane production is low or no methanogenesis occurred. However, the highest methane concentrations were detected in sediments (< 8800 yr BP until present) deposited under such conditions, indicating an accumulation of the methane over longer periods by *in situ* activity. This gives us evidence for the prediction that the methane concentration profile rather depends on *in situ* activity of methanogenic archaea than on the inclusion of methane during sedimentation processes.

More than 20 percent of the terrestrial Arctic is characterized by ice rich permafrost (Zhang *et al.* 1999). Large areas, mainly dominated by continuous permafrost, exist in Siberia with thicknesses up to 900 m (Yershov 1998). The present study revealed considerable parts of these cold habitats as recent sites of methane production, probably catalyzed by specific cold-adapted methanogenic archaea. This increasing reservoir of climate relevant trace gases becomes of major importance against the background of global warming which could result from a thawing of permafrost area up to 25% until 2100 (Anisimov *et al.* 1999) and subsequent disposal of the methane reservoirs into the atmosphere. Today, more than  $75 \times 10^6 \text{ t a}^{-1}$  permafrost sediments are eroded at the Laptev Sea coast (Rachold *et al.* (2000), from which a theoretical methane release of about  $100 \text{ t CH}_4 \text{ a}^{-1}$  can be calculated. These quantities are not considered in regional and global greenhouse gas balances and modeling. This is of particular importance as our results reveal a substantial increase of the microbial methane production in the frozen ground, if the permafrost temperature arises from -6 °C to -3 °C.

## Conclusions

This work shows for the first time that microorganisms, particularly methanogenic archaea, do not only survive in permafrost habitats but also can be metabolic active under *in situ* conditions. Due to the sub-zero experiments and the *in situ* temperatures of permafrost sediments, we can conclude that the methanogenic community is dominated by psychrotolerant or even psychrophilic microorganisms. Despite this adaptation to cold environments, we show that a slight increase of the temperature can lead to a substantial increase of methanogenic activity. In case of degradation, this would lead to an extensive expansion of the methane deposits with their subsequent impacts on total methane emission. A future in-depth characterization of the metabolism of these cold-adapted methanogens will reveal biotic and abiotic factors which influence the methanogenic activity of these organisms.

The results further show that methane in permafrost, which originates from modern methanogenesis, represents contribution thus far not considered to the global methane budget. The methane is released to the atmosphere by permafrost degradation in form of thermokarst or coastal

erosion processes, which is an ongoing process in Arctic regions. Although the change of permafrost by global warming is examined in the framework of different international projects (e.g. ACD: Arctic Coastal Dynamics, CALM: Circumpolar Active Layer Monitoring), these investigations should be linked more strongly with microbiological process studies and biodiversity research. Thus a contribution could be made to understand the role of permafrost in the global system and possible feedbacks by material fluxes and greenhouse gas emissions.

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## 5 Microbial Community Structure in Permafrost Ecosystems



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### 5.1 Characterisation of microbial community composition of a Siberian tundra soil by fluorescence in situ hybridisation

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#### Abstract

The bacterial community composition of the active layer (0–45 cm) of a permafrost-affected tundra soil was analysed by fluorescence in situ hybridisation (FISH). Arctic tundra soils contain large amounts of organic carbon, accumulated in thick soil layers and are known as a major sink of atmospheric CO<sub>2</sub>. These soils are totally frozen throughout the year and only a thin active layer is unfrozen and shows biological activity during the short summer. To improve the understanding of how the carbon fluxes in the active layer are controlled, detailed analysis of composition, functionality and interaction of soil microorganisms was done. The FISH analyses of the active layer showed large variations in absolute cell numbers and in the composition of the active microbial community between the different horizons, which is caused by the different environmental conditions (e.g., soil temperature, amount of organic matter, aeration) in this vertically structured ecosystem. Universal protein stain 5-(4,6-dichlorotriazin-2-yl)aminofluorescein (DTAF) showed an exponential decrease of total cell counts from the top to the bottom of the active layer ( $2.3 \times 10^9$ – $1.2 \times 10^8$  cells per gram dry soil). Using FISH, up to 59% of the DTAF-detected cells could be detected in the surface horizon, and up to 84% of these FISH-detected cells could be affiliated to a known phylogenetic group. The amount of FISH-detectable cells decreased with increasing depth and so did the diversity of ascertained phylogenetic groups.

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**Keywords:** Fluorescence in situ hybridisation; Community composition; Active layer

#### 1. Introduction

Arctic tundra soils contain large amounts of organic carbon, accumulated in thick layers of soil organic matter [1,2] and are known as a major sink of atmospheric CO<sub>2</sub> during the Holocene [3,4]. The reason for the accumulation of organic material is a reduced microbial decomposition of organic matter due to the extreme climatic conditions. Generally, this decomposition is slow and incomplete with high moisture and low temperature [5]. Arctic tundra soils are totally frozen throughout the year and only a thin active layer is unfrozen and shows biological activity during the short summer. In this extreme environment microorganisms

have to be capable of withstanding low temperature and repeated freezing–thawing cycles. Due to the underlying ice shield large part of the soils is waterlogged and decomposition of organic matter takes place under anaerobic conditions. The final step in the process of anoxic decomposition of complex organic matter is methanogenesis. Therefore, tundra soils are one of the most important sources in the budget of atmospheric methane (CH<sub>4</sub>), which is the second significant greenhouse gas after carbon dioxide [6]. Tundra wetlands are estimated to emit between 20 and 40 Tg yr<sup>-1</sup> CH<sub>4</sub> [7]. It accounts for 20% of global methane emission [8].

The large carbon pool in the northern latitudes together with a predicted climate warming [9] leads to speculations about a possible feedback effect on global climate changes by increased decomposition of organic matter and possible increased methane emission [9,10].

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For a better prediction of how such changes in environmental conditions may affect the carbon pool, a detailed knowledge of composition, functionality and interaction of microorganisms involved in the carbon cycle is important.

In general, the number, diversity, and activity of soil organisms are influenced by soil organic matter properties (e.g., content, availability), soil texture, pH, moisture, temperature, aeration, and other factors [11]. Soil is a heterogeneous environment and soil aggregates, soil pores, and root environments (rhizosphere) provide numerous niches for different soil microbial communities. Because of the complex vertical structure of the soil and the physical and chemical differences between the horizons it is not possible to deduce a subsurface community structure from analysing surface soils. Deeper layers may contain microbial communities, which are specialised for their environment and differ from surface communities [12,13].

Cultivation-based methods usually fail to give a complete picture of the composition of complex communities [14–16]. Molecular, i.e., cultivation-independent techniques could give complementary insight into complex environments. The FISH method is based on the detection of rRNA. Because the rRNA content is associated with the metabolic state of the organism [17–19], FISH results are influenced by the activity of the cells [20–25]. Although FISH signals cannot directly be translated to cell activity status it is approved that there is a positive relationship between bacterial metabolic rates and the capacity to detect the active cells [26]. In ecological studies it could be an advantage to describe the composition of the more active, and hence ecologically relevant part of the community instead of the total existent population.

The aim of this study was to characterise the population of active microorganisms in the whole active layer of a permafrost-affected soil, by using FISH without previous cultivation.

## 2. Materials and methods

### 2.1. Study site and soil conditions

The investigation site is located on Samoylov (N 72°22', E 126°28'), a typical island of the central part of the Lena Delta, Siberia. Detailed description of the geomorphologic situation of the island and the whole delta was given previously [27–29]. With an area of about 32,000 km<sup>2</sup>, the Lena Delta is the second largest delta in the world. It is situated in the continuous permafrost zone. The mean annual air temperature in the period 2001–2003, measured by a Russian weather station on Stolb Island, which is approximately 8 km away from Samoylov Island, was –11.9 °C; mean annual

precipitation in the same period was about 233 mm [30]. The landscape of the delta is dominated by a microrelief of ice-wedge polygons, which develop due to annual freeze-thaw cycles. The soils are totally frozen for at least eight months every year and only a shallow active layer of about 20–50 cm is unfrozen during the summer months. Predominant landscape formations are low-centred polygons where the flat central parts are surrounded by raised rims. The investigated soils in the polygon centres are characterised by a water level near the soil surface, which, together with the cold climate conditions, leads to an accumulation of organic matter and formation of peat layers. The mainly anaerobic decomposition of soil organic matter generates high CH<sub>4</sub> production and emission rates from these sites. Mean flux rates of  $53.2 \pm 8.7 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$  were measured for the polygon centres for the period between the end of May and the beginning of September 1999 [27].

The soil in the centre of a typical polygon was described and sampled in August 2001. At this time the thaw depth of the active layer was up to 45 cm with the water table 18 cm below the surface. The vegetation in the polygon centres site was composed of a moss/lichen layer (total coverage 95%) and a vascular plant layer (total coverage 30%). The latter was dominated by the sedge, *Carex aquatilis* [31]. For soil description and sampling, a vertical soil profile was dug. Four different peaty, sandy loam textured soil horizons were distinguished. They were covered by a horizon consisting of weakly decomposed organic material. Subsamples for physio-chemical soil characteristics and biological analyses were taken in a horizontal way subsequently after the horizons were identified. Samples for biological analyses were placed into 250 ml Nalgene boxes, which were locked with a seal tape to prevent oxygen contamination of the samples. To ensure detailed investigation of the soil, horizons with a thickness of more than 10 cm were subdivided and subsamples were taken. Soil properties (Table 1, Fig. 1) were described according to Schoeneberger et al. [32] and laboratory analyses were done according to Schlichting et al. [33]. The soil was classified as Typic Historthel according to US Soil Taxonomy [34].

The active layer is subject to great temperature variations between the different horizons. Automatic soil temperature measurements were started in 1998 using Thermistor Soil Temperature Probes 107 (Campbell Scientific Ltd.). The temperature sensors were installed in different depths between soil surface and permafrost table [35]. The average, minimum and maximum temperatures in August for three years are given (Table 2). During summer the upper soil horizons had not only a higher average temperature, compared to the deeper horizons. They were also exposed to a strong diurnal fluctuation, decreasing with depth. The minimum winter

Table 1  
Selected soil physico-chemical characteristics of the investigated Typic Historthel<sup>a</sup>

Horizon <sup>b</sup>	Depth (cm)	Soil texture <sup>b</sup> (%)			Textural class <sup>b</sup>	Munsell color <sup>c</sup>	Roots <sup>b</sup>	Reducing soil conditions <sup>d</sup>
		Sand	Silt	Clay				
Oi	0–5				—	n.d.	Many	No
	5–10					n.d.	Many	No
	10–17					n.d.	Many	No
A	17–20	77	15	8	Sandy loam	10YR 2/1	Many	Yes
Bg 1	20–23	76	18	6	Sandy loam	2,5Y 4/4	Common	Yes
Bg 2	23–30	69	26	5	Sandy loam	10YR 3/2	Few	Yes
	30–35	68	27	5	Sandy loam	10YR 3/2	Few	Yes
Bg 3	35–40	65	29	6	Sandy loam	10YR 4/2	None	Yes
	40–45	60	33	7	Sandy loam	10YR 4/2	None	Yes

<sup>a</sup> Soil classification was done according to US Soil Taxonomy Soil Survey Staff [34].

<sup>b</sup> Classifications were done according to Schoeneberger et al. [32].

<sup>c</sup> Soil colour determination was done according to the Munsell soil colour charts [72].

<sup>d</sup> Reducing soil conditions were detected by using  $\alpha$ - $\alpha'$ -Dipyridil test. Soil Survey Staff [34].

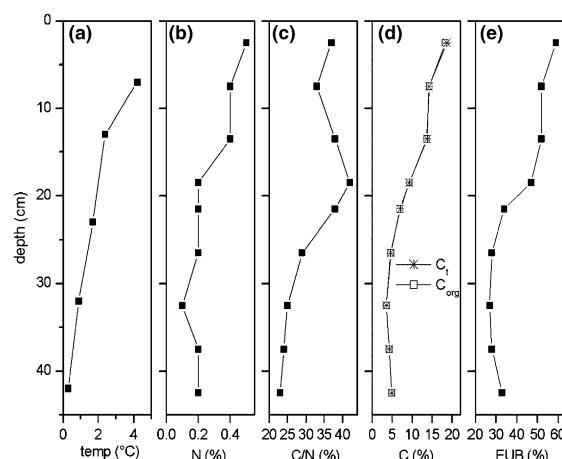


Fig. 1. Vertical profile of selected soil properties and EUBmix counts relative to total bacterial cell numbers. (a) Average soil temperature in August, (b) N content, (c) C/N ratio, (d) C content, and (e) fraction of TBC detected with probe EUBmix. C and N contents were measured using Vario EL III elemental analyzer (Elementar Analysensysteme, Germany).

temperature measured was  $-37^{\circ}\text{C}$  at a depth of 7 cm and increased up to  $-29^{\circ}\text{C}$  at a depth of 42 cm. The upper soil above 23 cm depth was frozen for 8 months,

while the deeper horizons were frozen for 9–10 months in the year.

## 2.2. Sample preparation for cell counts

Subsamples for cell counts by 5-(4,6-dichlorotriazin-2-yl)aminofluorescein (DTAF) staining and FISH were frozen immediately after sampling and were transported to Germany. After transportation they were thawed at  $3^{\circ}\text{C}$ . Immediately after thawing subsamples of 1 gram were either fixed directly in ethanol (96%) or in a freshly prepared, 4% paraformaldehyde/phosphate-buffered saline (PBS) solution (pH 7.2) for 4 h at  $5^{\circ}\text{C}$ . The paraformaldehyde-fixed samples were then washed with PBS and stored in ethanol–PBS (1:3) at  $-20^{\circ}\text{C}$ . Before application to slides, we diluted the samples with 0.1% sodium pyrophosphate in distilled water to obtain 100–200 cells per microscopic field of view. The dilution was dispersed by mild sonication with an MS73 probe (Sonopuls HD70; Bandelin, Berlin, Germany) at a setting of 20 for 30 s. Dispersed soil samples were spotted on gelatin-coated (0.1% gelatine, 0.01%  $\text{KCr}(\text{SO}_4)_2$ ) Teflon-laminated slides with ten wells. Twenty  $\mu\text{l}$  of fixed and dispersed soil sample were dropped onto each well, allowed to air dry, and dehydrated by serial immersion of the slides in 50%, 80%, and 96% ethanol. The FISH method was used directly in soil smears because

Table 2  
August temperatures in different soil depths of the investigated Typic Historthel

Depth (cm)	Temperature ( $^{\circ}\text{C}$ ) in August (avg. min./max.)			2001
	1998	1999	2001	
7	4.2	0.7/12.8	5.1	1.8/11.1
13	2.4	0.8/5.3	3.6	-0.6/8.6
23	1.7	0.0/3.2	—	—
32	0.9	0.4/1.7	1.9	-1.6/3.2
42	0.3	-0.6/0.6	0.9	0.4/4.1

—, No temperature was determined due to broken temperature sensor.

extraction of bacterial cells from soil is difficult to perform due to the exclusion of bacteria associated with soil particles [25].

### 2.3. Non-selective staining of all cells (DTAF staining)

Before the slides were prepared for FISH, they were stained with the universal protein stain DTAF. Freshly prepared stain solution consisted of 2 mg of DTAF dissolved in 10 ml of phosphate buffer (0.05M Na<sub>2</sub>HPO<sub>4</sub> with 0.85% NaCl, pH 9 [36]). The staining procedure was done as described by Bloem et al. [37]. A drop of stain solution was given on each well with dried soil film and incubated for 30 min at room temperature. After staining the slides were washed three times for 20 min each with phosphate buffer (pH 9). Finally they were passed through four water bathes each for a few seconds and air-dried.

### 2.4. Fluorescence in situ hybridisations and probe description

All oligonucleotide probes used in this study were purchased from Interactiva (Ulm, Germany). They were labelled with the cyanine dyes Cy3 or Cy5. Probes for the domains *Bacteria* and *Archaea* and specific probes for different phylogenetic groups of *Bacteria* were used. All probe sequences, formamide concentrations in hybridisation buffer, NaCl concentrations in washing buffer, and references are summarised in Table 3. For each hybridisation the bacterial probe EUbmix was used together with one specific probe marked with a

different dye. Therefore, the target cells of the group specific probes were marked with three different dyes.

In situ hybridisations with probes HGC69a and LGCmix were done on ethanol-fixed samples, while paraformaldehyde-fixed soil samples were used for probing gram-negative bacteria. Probe NON338 was used as a negative control for the ethanol-fixed as well as the paraformaldehyde-fixed samples. In situ hybridisations were performed similarly as described elsewhere [38,39]. A 10 µl aliquot of hybridisation buffer (0.9 M NaCl, 20 mM Tris-HCl; pH 8.0), 0.02% sodium dodecyl sulfate (SDS), formamide in concentrations as given in Table 3, and 30 ng/µl of probe was placed on each well. The slides were transferred to an equilibrated 50 ml polypropylene top tube [24] and incubated at 46 °C for 90 min. Slides were then washed at 48 °C for 10 min in washing buffer (20 mM Tris-HCl, pH 8.0), 5 mM EDTA, 0.01% SDS w/v, NaCl concentration as given in Table 3. Afterwards they were washed in ice-cold double-distilled water for a few seconds and quickly dried in an air stream. Finally slides were mounted in Citifluor AF1 antifadent (Plano; Wetzlar, Germany) and covered with a coverslip.

### 2.5. Microscopy and quantification

A Zeiss LSM 510 scanning confocal microscope equipped with an Ar ion laser (488 nm), and two HeNe lasers (543 and 633 nm) was used to record optical sections. Image handling was done with the LSM510 software (version 2.3). Small fluorescent pieces of the soil matrix hindered an automatic detection with our

Table 3  
rRNA-targeted oligonucleotide probes used for hybridisation

Probe	Target group	Sequence (5'-3') of probe	Target site <sup>a</sup>	FA <sup>b</sup> (%)	NaCl <sup>c</sup> (mM)	Reference
EUB338*	Domain <i>Bacteria</i>	GCTGCCTCCCGTAGGAGT	16S (338)	0-35	80-900	[73]
EUB338 II*	Domain <i>Bacteria</i>	GCAGGCCACCCGTAGGTGT	16S (338)	0-35	80-900	[74]
EUB338 III*	Domain <i>Bacteria</i>	GCTGCCACCCGTAGGTGT	16S (338)	0-35	80-900	[74]
NON338	Control probe complementary to EUB338	ACTCCTACGGGAGGCAGC	16S	0-35	80-900	[75]
ARC915	Domain <i>Archaea</i>	GTGCTCCCCGCCAATTCCCT	16S (915)	20	225	[39]
ALF968	α-subclass of <i>Proteobacteria</i>	GGTAAGGTTCTGCGCGTT	16S (968)	35	80	[76]
BET42a	β-subclass of <i>Proteobacteria</i>	GCCTTCCCACCTTCGTTT	23S (1027)	35	80	[77]
GAM42a	γ-subclass of <i>Proteobacteria</i>	GCCTTCCCACATCGTTT	23S (1027)	35	80	[77]
CF319A**	<i>Cytophaga-Flavobacterium</i> cluster of CFB-phylum	TGGTCCGTGTCTCAGTAC	16S (319)	35	80	[77]
CF319B**	Same as CF319A	TGGTCCGTATCTCAGTAC	16S (319)	35	80	[77]
HGC69a	Gram-positive bacteria with high GC content	TATAGTTACCACCGCCGT	23S (1901)	25	159	[78]
LGC354A***	Gram-positive bacteria with low GC content	TGGAAGATTCCCTACTGC	16S (354)	35	80	[79]
LGC354B***	Same as LGC354A	TGGAAGATTCCCTACTGC	16S (354)	35	80	[79]
LGC354C***	Same as LGC354A	TGGAAGATTCCCTACTGC	16S (354)	35	80	[79]

\*—\*\*, Probes marked with the same amount of \* are applied in equimolar mixtures.

<sup>a</sup> *Escherichia coli* numbering.

<sup>b</sup> Percentage (vol/vol) of formamide in the hybridisation buffer.

<sup>c</sup> Percentage (vol/vol) of NaCl in the washing buffer.

equipment, as was described, e.g., by Daims et al. [40], therefore the counting was done manually. For each hybridisation approach at least 1000 DTAF-stained cells were counted on at least 10 randomly chosen fields. For each horizon the rate of bacteria detectable by FISH (cells containing a sufficient number of ribosomes [16]) was calculated by counting EUBmix-positive signals relative to DTAF-counts on PFA-fixed samples. These calculations were corrected for samples with high proportions of gram-positive bacteria, according to Friedrich et al. [41]. This was done by adding the amount of bacteria detected by probes HGC69a and LGCMix in the ethanol-fixed samples to the EUBmix counts.

Group-specific cell counts were performed with DTAF-stained soil samples simultaneously hybridised with Cy5-labelled EUBmix and the respective group-specific Cy3-labelled probe. This procedure enabled a threefold staining of the target-cells. This was important because background signals of non-bacterial soil particles often hinder clearly convincing results in soil samples, when using a single fluorescent probe [42]. Quantification of specific cells was done relative to the number of EUBmix-hybridised cells. Counting results were always corrected by subtracting signals obtained with the probe NON338.

For calculating the number of cells per gram of soil (BC), the mean count of bacteria per counting area (B), the microscope factor (area of the sample spot/area of counting field), (M) the dilution factor (D) and the weight of fixed sample used for hybridisation (W) were determined and arranged in the equation BC = B × M × D × W<sup>-1</sup>.

### 3. Results and discussion

Total bacterial cell numbers (TBC) in the soil, determined by DTAF staining, ranged from 1.2 to

$23.0 \times 10^8$  cells g<sup>-1</sup>. These numbers were comparable to TBCs found in other arctic soils [43], and peat [44] and soils of other regions [45]. As shown in Table 4 and Fig. 1, TBC were highest ( $23.0 \times 10^8$  cells g<sup>-1</sup>) in the uppermost 5 cm layer of the soil and decreased with depth. In the deepest investigated horizon (40–45 cm)  $1.2 \times 10^8$  cells g<sup>-1</sup> were detected. It corresponds to 10% of the TBC detected in the surface horizon. TBC decreased exponentially with increasing depth ( $r^2 = 0.9711$ ), with the exception in the horizon between 17 and 20 cm soil depth, where cell counts in the same order as in the layer above were retrieved (Table 4). Total cell counts for the deepest sample of the active layer were in the same range as other studies found below the permafrost table in continuously frozen sediments in Russia using acridine orange [46], DTAF staining [47], or direct visual microscopic counting [48].

Not only the total cell numbers, but also the fraction of cells detectable with probe **EUBmix**, which identified members of the domain *Bacteria*, decreased by depth from 59% to around 30% (Table 4). This decrease in the fraction of detectable bacteria was due to a strong exponential decrease ( $r^2 = 0.963$ ) of absolute EUBmix counts from 13.6 to  $0.4 \times 10^8$  cells g<sup>-1</sup> (Fig. 2). There were only a few studies to compare our data. The soil samples in which Zarda et al. [49] and Christensen et al. [25] found 41% or only 5% of the TBC with probe EU338, were from the upper 10 cm of soils located in the temperate zone. A more comparable environment could be the Siberian peat samples investigated by Dedysh et al. [44], in which up to 65% of TBC were found with EU338. These results indicate that the extreme low temperatures in the investigated tundra soil did not lead to lower amounts of active bacteria than that from other soils. The high amounts of active bacteria found in our investigation of tundra soil as well as by Dedysh in the peat samples could be attributed to the very high content of organic matter, especially in the upper horizons. It is accepted and shown in other studies that

Table 4  
Total bacterial numbers in different horizons of the soil analysed after DTAF staining and relative percentage of hybridised cells with specific probes

Sample depth (cm)	Total cell counts (cells/g [ $10^8$ ]) (mean $\pm$ SD)	% of DTAF cells (mean $\pm$ SD) detected with specific probes <sup>a</sup>								% Affiliated Eubacteria <sup>b</sup>
		EUBmix	ALF968	BET42a	CF mix	GAM42a	HGC69a	LGCMix	ARC915	
0–5	$23.0 \pm 7.8$	$59 \pm 12$	$1.3 \pm 1.2$	$11.7 \pm 3.2$	$8.4 \pm 5.6$	$3.3 \pm 0.7$	$4.3 \pm 2.3$	$5.7 \pm 2.0$	$0.5 \pm 0.7$	34.7
5–10	$13.8 \pm 3.3$	$52 \pm 7$	$0.5 \pm 0.9$	$6.3 \pm 1.2$	$3.4 \pm 3.2$	$9.8 \pm 2.1$	$7.5 \pm 2.3$	$1.0 \pm 1.0$	$22.4 \pm 30.0$	29.0
10–17	$9.2 \pm 2.9$	$52 \pm 10$	$5.5 \pm 2.2$	$11.9 \pm 2.4$	$5.1 \pm 5.1$	$3.2 \pm 1.2$	$10.0 \pm 4.4$	$7.7 \pm 7.1$	$9.9 \pm 23.3$	44.0
17–20	$10.4 \pm 3.2$	$47 \pm 16$	$1.1 \pm 1.3$	$9.5 \pm 0.8$	$0.4 \pm 1.0$	$2.9 \pm 0.8$	$12.6 \pm 3.2$	$3.1 \pm 2.6$	$5.2 \pm 11.1$	31.9
20–23	$5.5 \pm 2.1$	$34 \pm 6$	$2.1 \pm 1.5$	$4.7 \pm 1.0$	$0.0 \pm 0.0$	$5.6 \pm 2.5$	$10.8 \pm 5.8$	$3.2 \pm 2.2$	$7.2 \pm 16.6$	28.5
23–30	$3.7 \pm 1.5$	$28 \pm 6$	$2.8 \pm 3.5$	$3.4 \pm 1.0$	$1.1 \pm 1.1$	$0.0 \pm 0.0$	$4.7 \pm 3.3$	$0.4 \pm 0.8$	$2.6 \pm 5.8$	12.2
30–35	$2.6 \pm 0.7$	$27 \pm 10$	$0.4 \pm 0.9$	$5.2 \pm 0.4$	$0.0 \pm 0.0$	$0.1 \pm 0.9$	$0.1 \pm 0.1$	$0.4 \pm 0.8$	$0.9 \pm 2.4$	17.2
35–40	$2.0 \pm 0.7$	$28 \pm 5$	$0.2 \pm 0.6$	$5.5 \pm 0.8$	$0.0 \pm 0.0$	$0.2 \pm 0.5$	$2.2 \pm 1.2$	$1.2 \pm 1.8$	$8.1 \pm 19.3$	15.2
40–45	$1.2 \pm 0.5$	$33 \pm 8$	$3.8 \pm 1.8$	$2.9 \pm 2.0$	$0.0 \pm 0.0$	$0.0 \pm 0.0$	$4.9 \pm 2.4$	$0.1 \pm 0.4$	$1.0 \pm 1.8$	12.2

<sup>a</sup> Percent detection compared to DTAF. Numbers have been corrected by subtracting NON338 counts. Probe names are abbreviations of those shown in Table 1.

<sup>b</sup> Results obtained from the addition of counts with all above named specific probes without probe ARC 915.

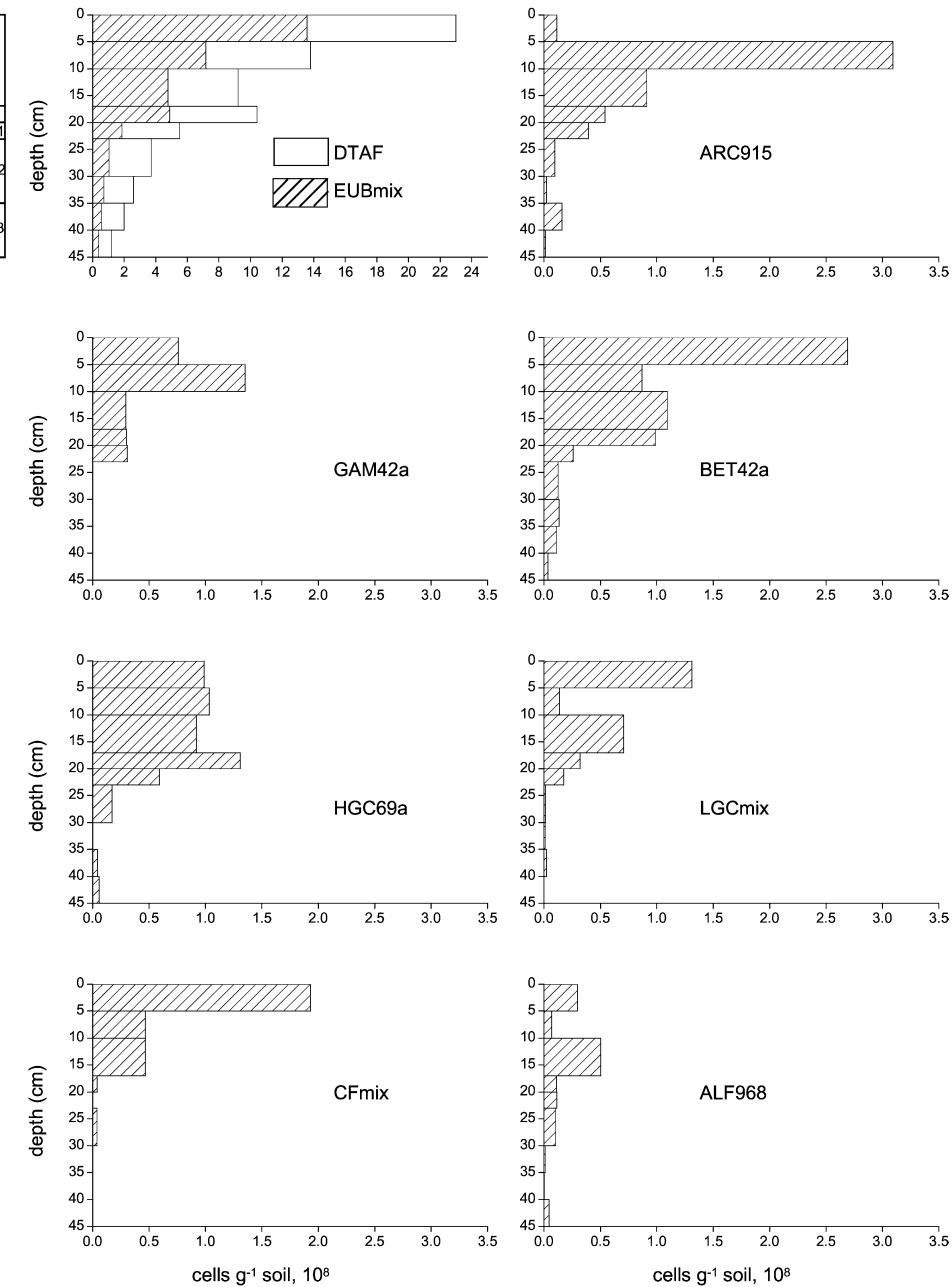


Fig. 2. Vertical profile of absolute numbers of bacteria detected with different probes in the investigated Typic Historthel. All data correspond to 1 g soil (dry weight). Probe names and target groups are explained in Table 3. Note the different x-axis range of the first diagram. Classification of the soil profile in different horizons is given on the left of the DTAF/EUBmix profile.

carbon availability is the most important limiting factor for microbial growth in soil [50–53]. Its possible influence on the activity of cells and hence on their ribosome content enhanced the detectability by FISH. Therefore the decreased EUB detection found in the deeper soil horizons could be attributed to the lower C content in

these horizons. The EUB detection rates throughout the soil profile showed a very strong correlation to  $C_{org}$  content ( $R^2 = 0.951, p = < 0.0001, N = 9$ ) (Fig. 1(d) and (e)). Considering the low temperatures, at which mesophilic organisms show a drastic decrease of metabolic activity, the EUBmix amounting of the TBC number in

the deeper horizons indicated the existence of psychrophilic or at least psychrotrophic organisms.

In contrast to the distribution observed for the domain *Bacteria*, none of the individual **major phylogenetic groups** showed a clear exponential decrease of cell numbers with depth. Notably, the highest abundance of the gram-positive *Bacteria* with a high G + C content, the  $\alpha$ -*Proteobacteria*, and the  $\gamma$ -*Proteobacteria* was found in horizons below the top 5 cm. However, all groups showed much higher abundances in the upper part of the profile and a significant reduction in cell numbers in depths below 23 cm. Apparent differences among the individual bacterial groups were the amount of cell numbers and their distribution in soil profile (Fig. 2). This can be explained by different habitat requirements for the bacterial groups as well as positive and negative influences among bacterial species.

For example, the *Cytophaga–Flavobacterium* cluster (CF group) of CFB phylum (*Cytophaga–Flavobacterium–Bacteroides* phylum) was found in the amount between  $2.0 \times 10^8$  and  $0.5 \times 10^8$  cells g $^{-1}$  in the upper 17 cm of soil. Most of the known members of this group are organotrophic, aerobic bacteria that are specialised in the degradation of complex macromolecules like proteins, pectin or cellulose [54,55]. The influence of water level and organic matter on the abundance of the *Cytophaga–Flavobacterium* group was obvious. In the aerobic upper 17 cm layer of the soil, the top 5 cm layer containing the highest amount of organic matter had the highest amount of cells of *Cytophaga–Flavobacterium* group whereas in the anaerobic zone, below the water table at 18 cm, nearly no cells were detected. This major difference in cell abundance between the aerobic and anaerobic horizons was not found for the other phylogenetic groups. For the high G + C group, cell counts were nearly equal (approximately  $1 \times 10^8$  cells g $^{-1}$ ) in the horizons between 0 and 20 cm whereas the gram-positive *Bacteria* with low G + C content showed a more varying distribution in the upper 20 cm layer with cell numbers between  $1.3$  and  $0.3 \times 10^8$  g $^{-1}$ .

The abundances of the phylogenetic groups as illustrated in Fig. 2 led to the different compositions of active *Bacteria* in the horizons as shown in Fig. 3. With our set of probes for major phyla within the domain *Bacteria* we could affiliate between 12.2% and 44.0% of the total DTAF counts (Table 4). In the upper 23 cm layer this ratio was between 28.5% and 44%, which means that at least in the upper horizons the majority (55–84%) of the FISH-detectable *Bacteria* could be affiliated to a known phylogenetic group. In the upper part of the soil, a more diverse composition of active *Bacteria* than in the lower horizons was found. Two dominant groups and at least two other groups with relevant cell numbers were found in each horizon. For example, in the upper 5 cm the  $\beta$ -*Proteobacteria* group was dominant (20% of all EUB-counts could be assigned to this phylogenetic group),

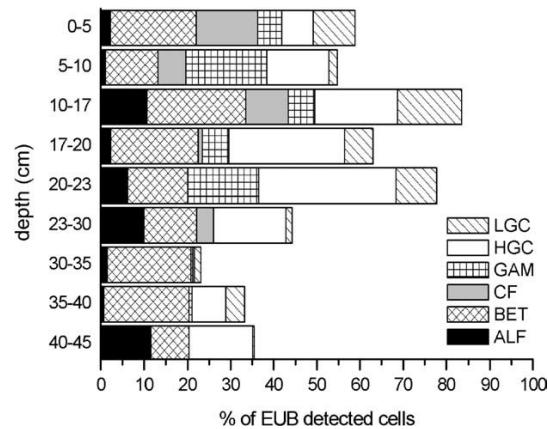


Fig. 3. Vertical profile of probe-specific counts relative to EUB-counts (percentages) in the Typic Historthel. Probe names are abbreviations of those shown in Table 3.

followed by the *Cytophaga–Flavobacterium* group (14%). Three other groups were found in ratios between 6% and 10%. The  $\beta$ -*Proteobacteria* and high G + C groups were the dominant throughout the soil profile. A direct comparison of the data with other studies was not possible due to the above-mentioned restriction of other FISH studies to the surface layer. Significant decrease in the diversity of the soil microbial communities with depth were also detected in other studies by using phospholipids fatty acid (PFLA) analysis or terminal restriction fragment length polymorphism (TRFLP) analysis [13,53,56]. The decrease of the amount of EUB-detected cells detectable by specific probes in depths below 23 cm suggested that a larger portion of species did not fit into the phylogenetic groups detected with our probes. However, the reason is most likely a lower activity of cells in deeper horizons and hence a lower rRNA content, which is not high enough for a detectable signal of the phylogenetic group probes. This assumption is supported by the observation that the signal intensity of the EUBmix probe decreased in deeper soil horizons.

The greater diversity and activity of cells in the upper horizons can be explained by higher temperatures and better substrate supply. In the upper layers the high root density may contribute, in addition to the high C content, to higher activity. While the upper 23 cm showed a high root density, only few roots were found between 23 and 35 cm, and none below 35 cm. The rhizosphere is known as a highly active soil compartment. Microbial activity and growth are stimulated through the release of compounds such as amino acids and sugars in plant root exudates [57]. Therefore microbial communities associated with the rhizosphere are often more active in relation to the microorganisms in bulk soil [58]. In addition to better substrate provision in the rhizosphere, another

effect, which could influence bacterial composition in the rhizosphere, is the transport of oxygen from the atmosphere to the roots through the aerenchyma. This transport is described for many wetland plants as adaptation to anaerobic soil conditions [59–61]. At the investigated soil site the large aerenchyma of *Carex aquatilis* serve as pathways for gas transport [31]. The presence of oxygen in the rhizosphere could enable the existence of aerobic species in an environment, which is anaerobic on the macroscopic scale. Altogether, the decrease of temperature, aeration, and substrate supply with depth leads to reduced activity of cells and eliminates those unable to withstand these harsher conditions.

Quantification of the domain **Archaea** was more difficult and showed higher standard deviations than that of the domain **Bacteria**. This was due to a strong formation of aggregates, as shown in Fig. 3. The basic requirement for the counting method is a homogeneous distribution of cells on each well. Cell counts are illustrated in Fig. 2, but one should keep in mind the statistical problems related with these data. Most *Archaea* were found in the Oi-horizon between 5 and 10 cm. The number of approximately  $3.0 \times 10^8$  cells g<sup>-1</sup> means that 22% of all DTAF cell counts could be assigned to the domain *Archaea* (Table 3). Also, the cell numbers from 10 to 23 cm were higher than in the upper 5 cm.

In the following, the cell counts retrieved by Arc915 were discussed as cell counts of methanogens. For justification, the subsamples of the upper 17 cm, where high amounts of cells were detected by probe Arc915, were additionally hybridised with probe MER 1, which is specific for 16SrRNA of methanogens [62]. Nearly the same number of cells hybridised with Arc915 as with MER1. Additionally, methane activity measurements (unpublished data) show a high methane production in the depth of 5–17 cm, where high numbers of Arc915 cells were found.

Soil depth, at which most methanogens were found, was a zone with changing oxygen conditions, which was above the water level during sampling time and not in the anaerobic zone of the profile. Oxygen content in this horizon is not optimal for methane production since methanogens are regarded as strictly anaerobic organisms [63] and can only survive in anaerobic microhabitats of this horizon. Other soil investigations also found methanogens in oxic soil horizons [64–66]. Wagner and Pfeiffer [64] demonstrated that aerobic and facultative anaerobic microflora in soil enable methane activity in the presence of oxygen in microscale anoxia. The reason why the methanogens seem to favour the upper horizons despite the suboptimal oxygen rates could be related to the better substrate provision in these horizons. The upper horizons of the *Typic Historthel* provide a high content of organic carbon and high cell numbers of Eubacteria, which convert high-weight macromolecules

to the substrates that can be used by the methanogens. Other studies also found a strong influence of the amount [67] and the quality [68] of fresh organic carbon in soil on the amount and activity of methanogens. Despite its high amount of organic matter, nearly no *Archaea* were found in the uppermost 5 cm. This upper layer is less compact and therefore better vented than the layers below and did not provide enough anaerobic microhabitats.

It should be noted that soils are spatiotemporal heterogeneous environments. Therefore the results of this study reflect the situation at the sampling point in the low-centred polygon at the time of sampling. In order to draw broader conclusions about the ecological situation in low-centred polygons further samplings at different times are necessary. Due to the time-consuming analysis only one profile was examined but attention was paid to that the characteristics of this polygon were typical for the surrounding landscape. This study can thus provide only an early insight into community structure in polygonal tundra soils.

#### 4. Conclusions

This study reveals the first analysis of the composition of an active community from tundra soil. By use of FISH we have shown that differences in soil parameters, like C content, water content, and root existence, caused differences in the composition of bacterial communities among the horizons of the *Typic Historthel*. For some phylogenetic groups such as the *Cytophaga–Flavobacterium* group, the most influential parameters were identified. Despite all differences in the requirements of the specific groups, which influence their abundances in soil, the total diversity and quantity of active cells was strongly related to the content of organic matter, an effect that was noted in soils of polygonal tundra also by other researchers [69,70]. Nevertheless, despite the harsh environmental conditions in the deeper horizons directly above the permafrost, there is evidence for high amount of cells ( $4 \times 10^7$  cells g<sup>-1</sup>) with at least minimal activity. Even in the deeper parts of the active layer at least a minimal turnover of organic matter, by probably psychrophilic organisms, can therefore be expected.

Using the described protocol for FISH, this technique was successfully used to describe the composition of active bacterial community of arctic soil. By applying three different dyes, every positive target cell was stained at least with two different dyes, which enabled a clear discrimination between bacterial cells and non-bacterial soil particles (Fig. 4). The relatively high standard deviation of cell counts obtained for some groups is attributed to the heterogeneity of soil. However, the deviations were in the same order as in other studies of sediment [71] and soil [49]. To better resolve the effect of

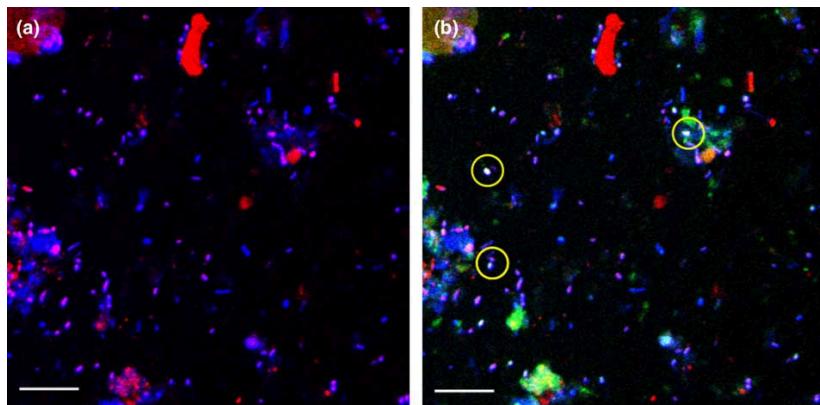


Fig. 4. Confocal laser scanning microscope (CLSM) images showing DTAF staining and in situ hybridisation of bacteria in a soil sample from the top 5 cm. Both pictures show identical microscopic fields, displayed in different artificial colours. (a) Hybridisation with probe EUBmix-Cy5 (red), additional all cells were stained with DTAF (blue). Due to double staining the target *Bacteria* cells appear purple. (b) Additional hybridisation with probe CFmix-Cy3 (green). Target *Cytophaga-Flavobacterium* cells appear yellow, due to threefold staining. Some of them were marked with yellow circles. The scale bars represent 10  $\mu$ m.

different community compositions on the carbon conversion, future investigations will combine community analyses with investigations of substrate conversion in different soil horizons.

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## 5.2

# Methane fluxes in permafrost habitats of the Lena Delta: effects of microbial community structure and organic matter quality

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## Summary

For the understanding and assessment of recent and future carbon dynamics of arctic permafrost soils the processes of CH<sub>4</sub> production and oxidation, the community structure and the quality of dissolved organic matter (DOM) were studied in two soils of a polygonal tundra. Activities of methanogens and methanotrophs differed significantly in their rates and distribution patterns among the two investigated profiles. Community structure analysis showed similarities between both soils for ester-linked phospholipid fatty acids (PLFAs) and differences in the fraction of unsaponifiable PLFAs and phospholipid ether lipids. Furthermore, a shift of the overall composition of the microbiota with depth at both sites was indicated by an increasing portion of iso- and anteiso-branched fatty acids related to the amount of straight-chain fatty acids. Although permafrost soils represent a large carbon pool, it was shown that the reduced quality of organic matter leads to a substrate limitation of the microbial metabolism. It can be concluded from our and previous findings first that microbial communities in the active layer of an Arctic polygon tundra are composed by members of all three domains of life, with a total biomass comparable to temperate soil ecosystems, and second that these microorganisms are well adapted to the extreme temperature gradient of their environment.

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## Introduction

More than 14% of the global terrestrial carbon is accumulated in soils and sediments of Arctic permafrost environments (Post *et al.*, 1982). Due to this carbon reservoir, tundra environments play a major role in the global carbon cycle, which is highlighted by current observed climate changes in the Arctic (IPCC, 2001) and by climate models that predict significant changes in temperature and precipitation in the Northern Hemisphere (Kattenberg *et al.*, 1996; Smith *et al.*, 2002). The atmospheric input of methane from tundra soils of high latitudes has been estimated between 17 and 42 Tg CH<sub>4</sub> year<sup>-1</sup> (Cao *et al.*, 1996; Christensen *et al.*, 1996), corresponding to about 25% of the methane release from natural sources (Fung *et al.*, 1991). Particularly, the degradation of permafrost and the associated release of climate relevant trace gases, like CH<sub>4</sub> and CO<sub>2</sub> from intensified microbial turnover of organic carbon, represent a potential environmental hazard.

Permafrost, which particularly occurs in the Northern Hemisphere, covers more than 25% of the Earth's land surface (Zhang *et al.*, 1999). These environments, which are under the influence of cryogenic processes, are characterized by patterned ground phenomena (Kessler and Werner, 2003). Low-centred ice-wedge polygons with a distinct microrelief (depressed centre, elevated rim) are one of the typical patterned grounds in tundra environments of northern Siberia. The microrelief affects the hydrological conditions as well as the organic matter contents and consequently the microbial processes.

The seasonal freezing and thawing leads to an extreme temperature regime in the upper active layer of permafrost. In spite of the extreme habitat conditions permafrost is colonized by high numbers of microorganisms including representatives of Archaea, Bacteria and Eukarya (Spirina and Fedorov-Davydov, 1998). In wet tundra soils methanogenesis is the terminal step during the anaerobic decomposition of organic matter, while the oxidation of methane by methanotrophic bacteria is the only sink for methane in these wetlands.

Generally, each habitat shows a characteristic composition of the microbial community, depending on the environmental conditions (Sundh *et al.*, 1997; Göttinger *et al.*, 2002a; Knief *et al.*, 2003). Only few studies deal with

ecosystem-scale differences in methanogenic and methanotrophic communities concerning trace gas dynamics (Dunfield *et al.*, 1993; Valentine *et al.*, 1993; Steudler *et al.*, 1996). Previous analysis of methane emission from polygonal tundra of the Lena Delta showed that the mean flux rate from the polygon depression was about 10 times higher compared with the CH<sub>4</sub> fluxes from the elevated polygon rim. These differences on the ecosystem level could be attributed to the different activity of the involved methanogenic and methanotrophic microflora as well as of the plant-mediated CH<sub>4</sub> transport (Wagner *et al.*, 2003a; Kutzbach *et al.*, 2004).

For the understanding and assessment of the recent and future carbon dynamic especially in sensitive high-latitude permafrost environments and the possible feedback to the atmospheric carbon budget the microbial processes have to be associated with the microbial community structure and functioning. The purpose of this study was to link methane production and oxidation activity with microbial community characteristics and the quality of dissolved organic matter in two different soils of a typical low-centred polygon. Special emphasis was given to the quantity and quality of water-extractable organic carbon (WEOC) and its function as a substrate for microorganisms. For community analysis of the polygon microbiota, we employed the polar lipid assay on samples from two soil profiles within the active layer of permafrost. The analyses included the determination of phospholipid fatty acids (PLFAs) and phospholipid ether lipids (PLELs) to enable detection of members of all three domains of the biosphere (Bacteria, Archaea and Eukarya).

## Results

### Soil characteristics

The microrelief formation of low-centred ice-wedge polygons leads to a small-scale variability in soil characteristics of the study site (Table 1).

The soils of the depressed polygon centre were dominated by *Typic Historthels*, whereas the prevalent soil type of the elevated polygon rim was classified as *Glacic Aquiturbel*. The thawing depth of both soils varied between 30 and 50 cm respectively. The peaty soil of the polygon centre was characterized by a water level near the soil surface and a soil texture of silty sand along with anaerobic accumulation of organic matter. Accordingly, large amounts of total organic carbon (TOC) and WEOC were determined, ranging between 36 and 183 mg g<sup>-1</sup> and between 337 and 2239 µg g<sup>-1</sup> dry weight (dw) respectively. The soils of the polygon rim were characterized by a soil texture of silty and loamy sand, pronounced cryoturbation properties, a distinctly lower water level causing oxic conditions in the top soil and a reduced organic matter accumulation. This is reflected by comparatively lower contents of TOC (21–33 mg g<sup>-1</sup>) and WEOC (238–309 µg g<sup>-1</sup>).

Analysis of the quality of WEOC revealed an increasing humification index (HIX) with increasing soil depth of the polygon centre. At the same time the bioavailable water-extractable organic carbon (BWEOC) content decreased (Fig. 1). Statistical analysis showed that both parameters were negatively correlated ( $r = -0.84$ ) at the significance level  $P < 0.01$ .

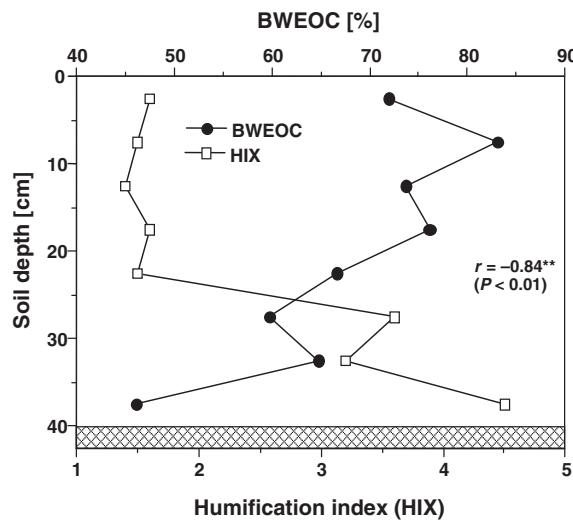
**Table 1.** Selected soil properties of the depressed polygonal centre and of the elevated polygonal rim.

Horizon	Depth (cm)	CH <sub>4</sub> concentration (µmol g <sup>-1</sup> )	T (°C)	H <sub>2</sub> O content (%)	pH	TOC (mg g <sup>-1</sup> )	N (%)	C/N	WEOC (µg g <sup>-1</sup> )	Sand (%)	Silt (%)	Clay (%)
<i>Polygon centre (Typic Historthel)</i>												
Oi1	0–5	0.15	7.5	72.2	n.d.	183	0.51	37.0	995	79.0	18.6	2.4
Oi2	5–10	13.19	5.8	67.4	7.9	138	0.43	33.1	2239	73.3	24.0	2.8
Aij1	10–15	24.37	4.0	60.7	7.4	137	0.36	38.3	663	78.8	18.6	2.6
Aij2	15–20	70.50	2.7	64.5	n.d.	93	0.23	41.5	349	76.6	15.4	7.9
Bg1	20–23	n.d.	1.2	60.3	n.d.	70	0.19	37.6	416	75.7	18.2	6.1
Bg2	23–30	163.24	0.4	55.0	n.d.	47	0.16	28.6	337	69.2	25.9	5.0
Bg2	30–35	328.87	n.d.	52.2	n.d.	36	0.15	24.6	440	67.7	27.0	5.3
Bg3	35–40	541.71	n.d.	52.6	n.d.	43	0.18	24.1	413	64.6	29.1	6.4
Bg3	40–45	n.d.	n.d.	47.9	n.d.	49	0.22	22.6	490	59.9	33.4	6.8
<i>Polygon rim (Glacic Aquiturbel)</i>												
Aij	0–5	0.40	6.4	30.1	n.d.	21	0.12	17.8	n.d.	85.7	10.4	3.9
Bijg1	5–12	0.29	5.0	27.5	n.d.	20	0.11	17.3	238	74.3	20.6	5.0
Bijg2	12–20	35.26	4.0	26.2	7.9	24	0.14	17.1	309	68.0	25.8	6.3
Bijg2	20–27	65.75	3.4	29.2	6.7	30	0.09	17.3	n.d.	63.7	30.3	6.0
Bijg2	27–35	153.51	2.4	25.8	6.8	24	0.07	16.5	294	56.5	34.5	9.1
Bijg3	35–42	224.71	1.7	26.1	n.d.	27	0.15	17.3	270	59.3	34.0	6.7
Bijg3	42–49	478.74	1.0	28.4	n.d.	33	0.18	18.1	n.d.	43.7	43.8	12.5

Horizon nomenclature and soil classification according to Soil Survey Staff (1998); T, *in situ* temperature; TOC, total organic carbon; WEOC, water-extractable organic carbon; n.d., not detected.

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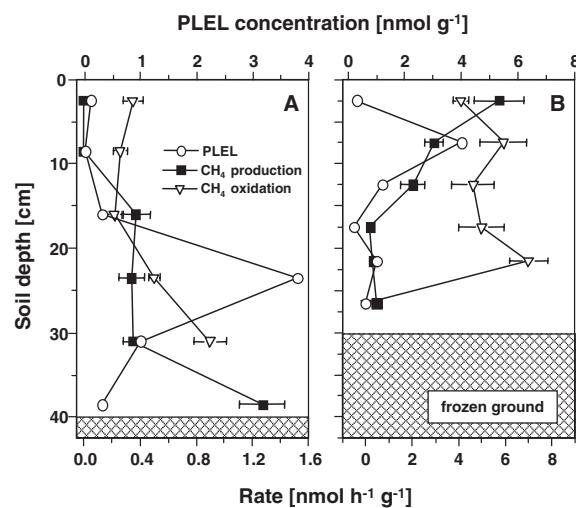


**Fig. 1.** Vertical profiles of bioavailable water-extractable organic carbon (BWEOC) and humification index (HIX, dimensionless) for the polygon centre. Bioavailable water-extractable organic carbon and HIX were negatively correlated at the significance level  $P < 0.01$ . Cross hatch indicates the frozen ground.

#### $\text{CH}_4$ production and oxidation

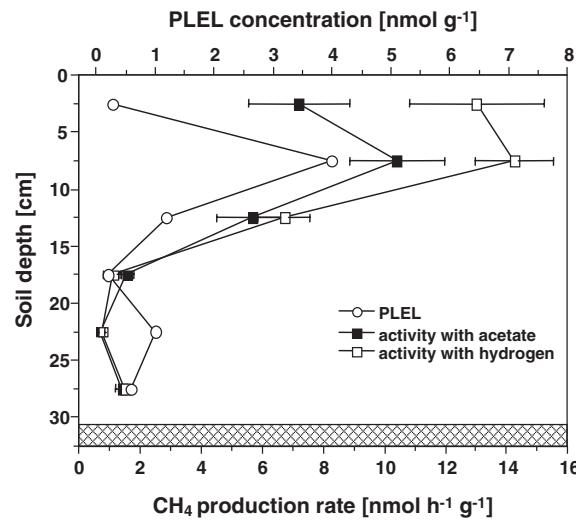
The microbial  $\text{CH}_4$  production and oxidation activity in the soil of the polygonal rim (*Glaci Aquiturbel*) was much lower and showed another distribution than those appearing in the soil of the polygon centre (*Typic Historthel*). No  $\text{CH}_4$  production was found in the upper soil layers (0–8 cm depth) of the elevated rim, which were dry and well aerated. The activity in the anoxic horizons (Bjjg) showed values from 0.3 to 1.3 nmol  $\text{CH}_4 \text{ h}^{-1} \text{ g}^{-1}$ . The highest  $\text{CH}_4$  production was detected at the boundary to the frozen ground at an *in situ* temperature of about 1°C (Fig. 2A, Table 1). The oxidation capacities in the same profile varied between 0.2 and 0.9 nmol  $\text{CH}_4 \text{ h}^{-1} \text{ g}^{-1}$ . The highest oxidation rates were observed in the soil layer between 23 and 31 cm depth, where significant  $\text{CH}_4$  production prevails. In contrast, the highest  $\text{CH}_4$  production in the polygon centre was found in the top layer (5.7 nmol  $\text{CH}_4 \text{ h}^{-1} \text{ g}^{-1}$ ), which decreased within the vertical profile and reached the lowest activity within the bottom zone with 0.2–0.3 nmol  $\text{CH}_4 \text{ h}^{-1} \text{ g}^{-1}$  (Fig. 2B). The  $\text{CH}_4$  oxidation capacity was determined for the whole profile, which varied between 4.1 and 7.0 nmol  $\text{CH}_4 \text{ h}^{-1} \text{ g}^{-1}$ , except for the boundary to the frozen ground, where no  $\text{CH}_4$  oxidation was detectable.

Methanogenic activity and concentration of archaeal PLELs followed the same trend in the polygon centre (Fig. 2B). However, no overall consistency between the  $\text{CH}_4$  production under *in situ* conditions and PLEL concentration was found in the polygon rim (Fig. 2A). A better correlation between methanogenic activity and archaeal



**Fig. 2.** Vertical profiles of  $\text{CH}_4$  production and oxidation under *in situ* conditions as well as phospholipid ether lipid (PLEL) concentrations for a low-centred ice-wedge polygon determined in July/August 2001. A. Polygon rim. B. Polygon centre.

PLELs could be obtained, when substrates like acetate or hydrogen were added to the soil samples, as shown for the polygon centre (Fig. 3). In general, the potential  $\text{CH}_4$  production rates were significantly higher compared with the activity under *in situ* conditions and reached values between 0.7 and 10.4 nmol  $\text{CH}_4 \text{ h}^{-1} \text{ g}^{-1}$  with acetate and 0.8–14.3  $\text{CH}_4 \text{ h}^{-1} \text{ g}^{-1}$  with hydrogen.



**Fig. 3.** Comparison of phospholipid ether lipid (PLEL) concentrations and potential  $\text{CH}_4$  production after addition of acetate (20 mM) and hydrogen (v:v; 80:20) as methanogenic substrates for the polygon centre. Cross hatch indicates the frozen ground.

Effects of microbial communities on  $\text{CH}_4$  fluxes in permafrost 1585*Phospholipid biomarker*

In the polygon centre concentration of total phospholipid biomarker (PLFA + PLEL) ranged between 15.2 and 851.6 nmol g<sup>-1</sup> dw (Table 2). The highest values were found in the depth between 5 and 10 cm, in contrast to the polygon rim, where highest concentration of total phospholipid biomarker was between 20 and 27 cm soil depths. In the polygon rim total phospholipid biomarker concentration varied between 20.5 and 105.5 nmol g<sup>-1</sup> dw.

For comparison of microbial community composition, PLFA and PLEL data were subjected to principal component analysis (PCA; Fig. 4). The profiles of ester-linked PLFAs, which are the dominating fraction of phospholipids, were similar for the centre and rim samples (Fig. 4A). In contrast, the fraction of the unsaponifiable PLFAs and the PLEL fraction showed different profiles for rim and centre samples (Fig. 4B). The most important phospholipid biomarkers responsible for this separation were the anteiso-branched unsaponifiable PLFAs (UNSFA-ant), the straight-chain unsaponifiable PLFAs (UNSFA-nor) and the  $\alpha$ -hydroxylated unsaponifiable PLFAs (UNOH- $\alpha$ ) (Table 2). While the UNSFA-nor and the UNOH- $\alpha$  fractions were more abundant in the centre profile, the UNSFA-ant fraction dominated the unsaponifiable PLFA fraction of the polygon rim.

The marker lipid for the type I methanotrophic family *Micrococcaceae*, *cis*-8-hexadecenoic acid (16:1 $\Delta$ cis8), was clearly detectable in both soils and showed its maximum concentration from 5 to 10 cm and from 20 to 27 cm depth in the polygon centre and rim respectively (Table 2). *Cis*-10-octadecenoic acid (18:1 $\Delta$ cis10), which is a marker lipid for the family *Methylcystaceae* (type II), was not detected in the polygon centre but at low concentrations in the upper two horizons (0–5 cm and 5–12 cm) of the polygon rim.

The fungal marker 18:2 $\Delta$ cis9,12 (Frostegard and Bååth, 1996) was detected in all investigated samples. The high-

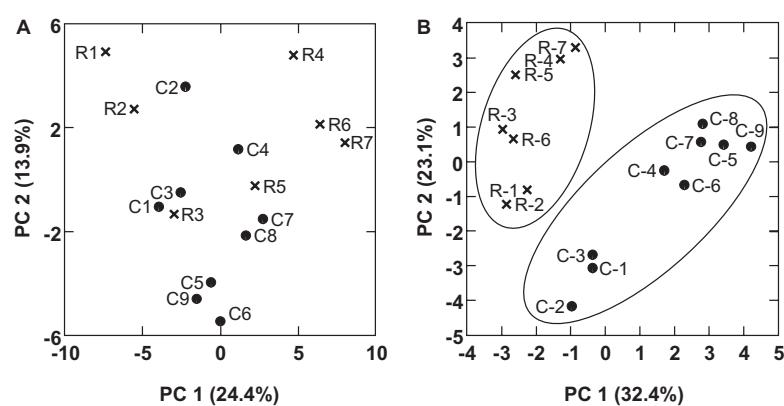
est percentages of this PLFA were detected at 10–15 cm soil depth for the polygon centre (zone of maximum plant root growth) with 10.4% and in the first 5 cm of the polygon rim with 9.2%.

A shift of the overall composition of the microbiota with depth at both sites was indicated by an increasing portion of iso- and anteiso-branched fatty acids related to the amount of straight-chain fatty acids. The ratio of 4.7 of straight-chain to iso- and anteiso-branched fatty acids at 0–5 cm depths for the centre sample and of 7.2 for the rim sample decreased to a ratio of 2.3 and 2.1, respectively, at the bottom of both soil profiles (Table 2).

Phospholipid ether lipid-derived isoprenoids (biomarker for archaea) were detected in all samples and were highest in the soil depths 5–10 cm (4.0 nmol g<sup>-1</sup>) for the polygon centre and 20–27 cm (3.8 nmol g<sup>-1</sup>) for the polygon rim (Table 2). Most of the samples contained only the two ubiquitous archaeal markers phytane and biphytane (i20:0 and i40:0). Only in the soil depth 5–10 cm from the polygon centre the side-chain i20:1 was found (data not shown), indicating the presence of acetoclastic methanogens (Gattinger *et al.*, 2002a). The PLEL biomarker in relation to the total phospholipid concentration increased in both vertical profiles with increasing soil depth and reached a maximum of 3.4% near the bottom layer of the centre and 3.6% in 20–27 cm soil depth of the rim (Table 2).

## Discussion

Permafrost, a common phenomenon in the Siberian Arctic, is controlled by climatic factors and characterized by extreme terrain conditions and landforms (Wagner *et al.*, 2001). The seasonal unfrozen part of permafrost (active layer, approximately 0.5 m thickness at the study site) is subjected to freezing and thawing cycles during the year with an extreme surface temperature from about 25°C to -45°C. In geological timescales cryogenic processes lead



**Fig. 4.** Principal component (PC) diagram of ester-linked phospholipid fatty acids (A), as well as unsaponifiable phospholipids and phosphoether lipids (B). The numerals 1–9 designate the different sampling depths (R, polygon rim; C, polygon centre).

**Table 2.** Concentrations of selected phospholipid biomarker (PLFA and PLEL).

Sample ID	Depth (cm)	PLFA + PLEL (nmol g <sup>-1</sup> )	Ester-linked PLFA			Non-ester-linked PLFA				PLEL		
			Total (nmol g <sup>-1</sup> )	16:1Δcis8 (nmol g <sup>-1</sup> )	18:1Δcis10 (nmol g <sup>-1</sup> )	Straight/iso + anteiso	Total (nmol g <sup>-1</sup> )	UNSFAnor (nmol g <sup>-1</sup> )	UNSFAnt (nmol g <sup>-1</sup> )	UNOH-α (nmol g <sup>-1</sup> )	Total (nmol g <sup>-1</sup> )	(%)
<i>Polygon centre (Typic Historthel)</i>												
C1	0–5	26.3	24.5	0.1	0.0	4.7	1.5	1.3	0.0	0.0	0.3	1.0
C2	5–10	851.6	830.6	4.4	0.0	4.6	17.0	6.7	0.5	3.4	4.0	0.5
C3	10–15	250.3	231.6	1.0	0.0	4.3	17.5	9.3	0.6	1.9	1.2	0.5
C4	15–20	83.5	65.9	0.3	0.0	3.4	17.5	3.0	0.8	6.3	0.2	0.2
C5	20–23	39.0	25.8	0.1	0.0	3.1	12.2	5.4	0.6	2.2	1.0	2.6
C6	23–30	23.4	16.9	0.1	0.0	2.6	5.9	3.1	0.0	1.5	0.6	2.4
C7	30–35	54.0	41.3	0.2	0.0	2.7	11.3	4.7	0.2	1.7	1.4	2.6
C8	35–40	53.0	38.7	0.1	0.0	2.5	12.4	2.7	0.5	5.0	1.8	3.4
C9	40–45	15.2	8.0	0.0	0.0	2.3	6.8	3.9	0.2	0.7	0.4	2.7
<i>Polygon rim (Glacic Aquiturbel)</i>												
R1	0–5	40.7	33.4	0.2	0.2	7.2	7.2	0.1	2.2	0.3	0.1	0.1
R2	5–12	20.5	17.6	0.1	0.1	5.7	2.9	0.0	1.7	0.1	0.03	0.2
R3	12–20	24.7	18.7	0.1	0.0	3.7	5.8	0.1	2.5	0.4	0.3	1.1
R4	20–27	105.5	82.4	0.6	0.0	2.6	19.3	0.0	3.8	1.7	3.8	3.6
R5	27–35	42.3	32.3	0.2	0.0	2.3	9.0	0.1	3.5	0.5	1.0	2.4
R6	35–42	60.9	50.4	0.5	0.0	2.5	10.3	0.5	3.5	0.6	0.3	0.4
R7	42–49	53.6	38.2	0.3	0.0	2.1	13.7	0.0	2.9	1.1	1.7	3.1

Subgroups of the non-ester-linked phospholipid fatty acids (PLFAs) were the unsubstituted (UNSFa) and hydroxy-substituted fatty acids (UNOH). Subgroups of UNSFA were named according to their functional groups: '-ant' (anteiso-branching), '-nor' (normal straight chain), '-uns' (unsaturations). UNOH subgroups were named according to the position of the hydroxy group in the fatty acid molecule ('a' or 'mid' position). 'ant/iso' describes the molar ratio of anteiso- to iso-branched ester-linked PLFAs and 'unsat/sat' the molar ratio of unsaturated to saturated ester-linked PLFAs.

Effects of microbial communities on CH<sub>4</sub> fluxes in permafrost 1587

to the formation of patterned grounds like the low-centred ice-wedge polygons of the investigation area in the Lena Delta. During the summer period soils within these polygons are also showing a large temperature gradient along their depths profiles, which is one of the main environmental factors influencing the microbial community in permafrost soils. The presented results revealed differences between the microrelief elements of the investigated polygon (elevated rim and depressed centre) in CH<sub>4</sub> fluxes, the microbial community structure and soil characteristics on the ecosystem scale (centimetres to metres).

Activities of methanogens and methanotrophs differed significantly in their rates and distribution patterns among the two investigated permafrost profiles. While the CH<sub>4</sub> production and oxidation in the polygon rim showed the typical activity patterns as known from other hydromorphic soils (Krumholz *et al.*, 1995), which means no or less activity in the dry and oxic upper horizons and increasing rates in the anoxic bottom layers, this is not the case in the polygon centre. Here the highest CH<sub>4</sub> production occurred in the upper soil horizons with a redox potential of about -50 mV, as shown in former studies at the same investigation site (Fiedler *et al.*, 2004). This is not the appropriate redox regime for CH<sub>4</sub> production. However, it was shown that a complex community composed by aerobic and facultative anaerobic microorganisms together with a certain soil matrix enables CH<sub>4</sub> production under oxic conditions (Wagner *et al.*, 1999). Integrated analyses of phospholipid biomarker revealed soil layers with a good relationship between the concentrations of archaeal PLELs as well as total phospholipid biomarker (indicator for microbial biomass) and CH<sub>4</sub> production under *in situ* conditions but there were other zones in the profiles without any correlation between both parameters (Fig. 2). Nevertheless, a stronger relationship was observed when archaeal PLEL concentration was compared with potential CH<sub>4</sub> production, as shown for the polygon centre. This finding indicates a substrate limitation for methanogenesis although organic carbon is highly accumulated in permafrost soils. Subsequent organic matter analyses revealed a decrease of BWEOC along with an increasing HIX with increasing soil depth. Accordingly, WEOC showed the highest values in the soil horizons of highest methanogenesis and archaeal PLEL concentration (Tables 1 and 2).

CH<sub>4</sub> oxidation capacities followed the curve of CH<sub>4</sub> production in the polygon rim, whereas in the centre CH<sub>4</sub> oxidation capacities were relatively high within the whole profile with exception of the bottom layer. The signature PLFA 18:1Δcis10 for the two methanotrophic genera *Methylosinus* and *Methylcystis* of the α-Proteobacteria was detected only in the polygon rim at 0–12 cm soil depth. In contrast, the PLFA 16:1Δcis8 indicative for the genera *Methylomonas*, *Methylomicrobium*, *Methylosa-*

*rcina* and *Methylosphaera* (Bowman *et al.*, 1993; 1997; Wise *et al.*, 2001) was in accordance with the CH<sub>4</sub> oxidation capacities in both soils. *In situ* labelling of corresponding samples with <sup>13</sup>C-enriched CH<sub>4</sub> supported our findings and revealed a significantly higher incorporation of labelled carbon into PLFAs belonging to type I methanotrophs (U. Zimmermann and A. Gattinger, unpublished results), all of them belonging to the group of γ-Proteobacteria. Furthermore, cell numbers of γ-Proteobacteria determined by FISH were closely correlated with the CH<sub>4</sub> oxidation profile in the polygon centre (Kobabe *et al.*, 2004). The activity of methanotrophic bacteria in the bottom layer of permafrost soils can be explained by high substrate affinity of type I methanotrophs (Hanson and Hanson, 1996) and by the plant-mediated transport of O<sub>2</sub> into the rhizosphere (Kutzbach *et al.*, 2004).

The high variability of environmental conditions within the polygon is reflected by the large differences in CH<sub>4</sub> emission from the different areas of this microrelief obtained by long-term studies since 1998. For example, in 1999 the mean flux rate of the polygon centre measured from the end of May to the beginning of September was 53.2 mg CH<sub>4</sub> m<sup>-2</sup> day<sup>-1</sup>, while the dryer rim part showed a mean value of 4.7 mg CH<sub>4</sub> m<sup>-2</sup> day<sup>-1</sup> (Wagner *et al.*, 2003a). The reason for this large spatial variability in CH<sub>4</sub> emission can be explained by the activity patterns of methanogens and methanotrophs, which are interacting with complex microbial communities. These showed differences in biomasses and structures between polygon rim and centre as revealed in the presented study by detailed phospholipid profiling. UNSFA-nor, UNSFA-ant and UNOH-α were identified as three of most responsible PLFAs for separation into the two major groups 'rim samples' and 'centre samples' according to PCA. UNSFA-nor occur, for example, in high concentrations in fermentative bacteria such as *Clostridia* (Gattinger *et al.*, 2002b) and in moderate concentrations in methanogens isolated from the investigation site as shown by <sup>13</sup>C-acetate labelling experiments (D. Wagner and A. Gattinger, unpublished results). UNSFA-ant were found in high concentrations in *Cytophaga* sp., whereas UNOH-α were determined in *Alcaligenes* sp. and *Flavobacterium* sp. (Zelles, 1999).

Although permafrost environments are characterized by extreme temperature conditions, the CH<sub>4</sub> emissions from these ecosystems (219–329 kg C ha<sup>-1</sup> a<sup>-1</sup>; calculated from Wagner *et al.*, 2003a) are in the same range compared with boreal (190–480 kg C ha<sup>-1</sup> a<sup>-1</sup>; Martikainen *et al.*, 1995) or temperate fens (11–293 kg C ha<sup>-1</sup> a<sup>-1</sup>; Augustin *et al.*, 1996). The maximal values for microbial biomass (total phospholipid biomarker concentrations) of 105.5 and 851.6 nmol g<sup>-1</sup> dw for the polygon rim and centre, respectively, are significantly higher than in arable soils (35.2–59.4 nmol g<sup>-1</sup>, Zelles, 1999; Gattinger *et al.*,

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2002a), rice paddies ( $44.7\text{--}90.9 \text{ nmol g}^{-1}$  dw, Bai *et al.*, 2000) and boreal Swedish peatlands ( $0.2\text{--}7.0 \text{ nmol g}^{-1}$  wet peat, Sundh *et al.*, 1997). The maximum value of  $851.6 \text{ nmol g}^{-1}$  determined in the polygon centre is within the range of a landfill cover soil studied by Börjesson and colleagues (2004).

The function of the cell membranes especially at low temperatures is highly dependent on the fluidity of the membrane (Ratledge and Wilkinson, 1988). There are several mechanisms known for the adaptation of the membrane fluidity under cold conditions. These are increases of the proportion of anteiso-branched to iso-branched fatty acids or of unsaturated to saturated fatty acids, because anteiso-branched fatty acids and unsaturated fatty acids have significantly lower melting points than their iso-branched and saturated analogues (Kaneda, 1991). At the study site the mean anteiso/iso ratio was 1.2 for the polygon centre and the rim respectively. Furthermore, the mean unsaturated/saturated ratio was 1.4 for the polygon centre and 1.6 for the rim. These ratios were significantly higher than those from ester-linked PLFAs determined for temperate soil microbial communities, which showed anteiso/iso ratios from 0.3 to 0.9 and unsaturated/saturated ratios from 0.1 to 0.5 (calculated from data of Zelles and Bai, 1994).

These findings along with the determined  $\text{CH}_4$  production and oxidation activities, which were independent of the temperature gradient in the active layer, show an adaptation of the microbial community to the low permafrost temperatures. This is also in accordance with the determination of high cell numbers of  $1.2 \times 10^8$  cells per gram of soil in the boundary layer to the permafrost in the polygon centre, which had a relatively constant temperature regime of  $1^\circ\text{C}$  (Kobabe *et al.*, 2004). In the same study only a minor part of the Eubacteria (EUB)-positive staining cells could be identified in the bottom layers by the common FISH probes, which shows on the one hand that a large part of species did not fit into the phylogenetic groups detected with used FISH probes and on the other hand it indicates probably a large number of unknown organisms in permafrost soils.

Although the fungal PLFA 18:2 $\Delta$ cis9,12 occurs also in a few bacterial species (see Zelles, 1997), one can assume the presence of fungi (Domain Eukarya) for the investigated soils. Hence it can be concluded that microbial communities in the active layer of an Arctic polygon tundra are composed by members of all three domains of life (Archaea, Bacteria and Eukarya) yielding a total biomass comparable to temperate soil ecosystems (Zelles, 1999; Bai *et al.*, 2000; Göttinger *et al.*, 2002a). At the same time the composition of the microbial communities and the activities of methanogens and methanotrophs are mainly influenced by the microrelief formed by cryogenic processes, which leads to different microenvironments.

The permafrost environment forces the adaptation of the microbial communities to low temperature conditions with a significant proportion of unknown species. Although the total amount of organic carbon in the depressed centre is significantly higher compared with the elevated rim, the methanogenesis is substrate limited because of a decreasing bioavailability of organic carbon within the soil profile. This is an important finding for modelling and calculating trace gas fluxes from permafrost environments, because the known models consider only the total carbon amount. Further integrative analyses are planned for detailed functioning analysis and forecasting of the development of permafrost environments under changing climate conditions.

## Experimental procedure

### Study site

Within the framework of the Russian–German cooperation ‘System Laptev Sea 2000’ an expedition to Northern Siberia was carried out in summer 2001 (Pfeiffer and Grigoriev, 2002). The study site Samoylov Island ( $N 72^\circ 22'$ ,  $E 126^\circ 28'$ ) lies within the active and youngest part (about 8500 years) of the Lena Delta, which is one of the largest deltas in the world with an area of  $32\,000 \text{ km}^2$  (Are and Reimnitz, 2000). It is located at the Laptev Sea coast between the Taimyr Peninsula and the New Siberian Islands in the zone of continuous permafrost. The Lena Delta is characterized by an arctic continental climate with low mean annual air temperature of  $-14.7^\circ\text{C}$  ( $T_{\min} = -48^\circ\text{C}$ ,  $T_{\max} = 18^\circ\text{C}$ ) and a low mean annual precipitation of 190 mm.

Soil and vegetation characteristics vary in rapid succession at the investigation site due to the patterned ground of low-centred ice-wedge polygons, which were formed by the annual freezing–thawing cycles. Accordingly, one investigation profile was located in the depressed polygonal centre and the other one at the elevated polygonal rim. The distance between the two investigated soils was about 10 m. The soil surface of the polygon depression was about 0.5 m below the surface of the elevated rim part. Further details of the study site were described previously by Wagner and colleagues (2003a).

### Soil properties

Vertical profiles of soil  $\text{CH}_4$  concentrations were obtained from both the elevated rim and the depression centre of the polygon by extracting  $\text{CH}_4$  from soil pore water by injection of 5 ml of water into saturated NaCl solution, shaking the solution and subsequently analysing the  $\text{CH}_4$  headspace concentration with gas chromatography. Soil temperature measurements (Greisinger GTH 100/2 equipped with Ni-Cr-Ni temperature sensor) were carried out during the experiments of  $\text{CH}_4$  production and oxidation under *in situ* conditions (5 cm increments from 0 to 40 cm soil depth).

The investigated soils were classified according to the US Soil Taxonomy (Soil Survey Staff, 1998). Soil properties were described during sampling (horizontal stepwise) according to

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Schoeneberger and colleagues (2002) and soil chemical and physical analyses were performed according to Schlichting and colleagues (1995). Samples were filled into 250 ml Nalgene boxes and transported in frozen conditions to Germany. Further details of the sample procedure were described elsewhere (Kobabe *et al.*, 2004).

#### CH<sub>4</sub> production and oxidation

The CH<sub>4</sub> production and oxidation capacity of the soils were analysed in summer (July to August) 2001. The CH<sub>4</sub> production was studied considering the *in situ* soil temperature gradient and different methanogenic substrates like H<sub>2</sub> and acetate (CH<sub>4</sub> production potential). Fresh soil material (20 g) from different soil horizons was weighed into 100 ml glass jars and closed with a screw cap containing a septum. The samples were evacuated and flushed with ultrapure N<sub>2</sub> (*in situ* CH<sub>4</sub> production). In the case of analysing the CH<sub>4</sub> production potential fresh soil material was supplied with 6 ml of acetate solution (10 mM) or with sterile and anoxic tap water in combination with H<sub>2</sub>/CO<sub>2</sub> (80:20 v/v, pressurized 150 kPa) as methanogenic substrates. The CH<sub>4</sub> oxidation capacity was studied considering the *in situ* CH<sub>4</sub> concentration and the natural soil temperature gradient. Fresh soil material (5 g, well homogenized) from different soil horizons was weighed into 50 ml glass jars and closed with a screw cap with septum. The samples were supplied with about 2000 p.p.m. CH<sub>4</sub> (corresponding to about 800 µmol CH<sub>4</sub> l<sup>-1</sup> pore water) in synthetic air. The prepared soil samples were restored for incubation in the same layers of the soil profile from which the samples had been taken. Three replicates were used for each layer. Gas samples were taken every 24 h for CH<sub>4</sub> production and every 12 h for CH<sub>4</sub> oxidation out of the jars headspace with a gastight syringe. CH<sub>4</sub> production and oxidation rates were calculated from the linear increase or decrease in CH<sub>4</sub> concentration analysed by gas chromatography.

#### CH<sub>4</sub> analysis

CH<sub>4</sub> concentrations were determined with a gas chromatograph (Chrompack GC 9003) in the field laboratory. The instrument was equipped with a Poraplot Q (100/120 mesh, 20 m) capillary column and a flame ionization detector (FID). Details of CH<sub>4</sub> analysis were described previously (Wagner *et al.*, 2003b).

#### Total and water-extractable organic carbon

Total organic carbon was analysed with an element analyser (Elementar Vario EL) using dried and homogenized soil samples. Before analysis the samples were treated with HCl (10%) at 80°C for carbonate removal.

The WEOC was quantified with a batch extraction method. Frozen soil samples were extracted with a 10 mM CaCl<sub>2</sub> solution using a soil:extractant ratio (w/w) of 1:10 and shaking for 10 min in an overhead shaker. Subsequently, the suspensions were centrifuged for 15 min (4000 r.p.m.) and the supernatants were filtered through 0.45 µm polycarbonate filters (Millipore, Eschborn, Germany). Filtered solutions were

quantified for dissolved organic carbon using catalytic high temperature combustion (680°C) with a Shimadzu® TOC 5050A analyser. Non-organic carbon was removed by acidification and purging the samples with pure O<sub>2</sub> for 2 min before measurement. The WEOC concentrations were referred to weighted soil mass (dry matter) and expressed as µg C g<sup>-1</sup> dry matter.

Water-extractable organic carbon quality was quantified using optical measurements (UV absorption and fluorescence emission intensity). The fluorescence emission intensity was measured between 300 and 480 nm with an excitation wavelength of 254 nm (Cary Eclipse F-4500, Varian®). Before measurement, soil extracts were adjusted to pH 2, due to the influence on soil pH on fluorescence of organic molecules (Zsolnay *et al.*, 1999). Based on the fact that highly substituted aromatic structures and condensed unsaturated systems fluoresce in the longer wavelength and fresh, non-humified organic matter fluoresce in the shorter wavelength (Senesi *et al.*, 1989), the HIX was calculated by dividing the upper quartile (435–480 nm) of the whole spectrum through the lower quartile (300–345 nm). The higher the (dimensionless) HIX, the more dissolved organic carbon in the samples is humified (Zsolnay, 2003).

Bioavailable water-extractable organic carbon was quantified mixing 5 ml of WEOC extract with 2 ml of nutrient solution (1 ml of NH<sub>4</sub>NO<sub>3</sub> + 1 ml of K<sub>2</sub>HPO<sub>4</sub>, each at a concentration of 1 g l<sup>-1</sup>) in Teflon vessels. After adding 30 µl of soil inherent inoculum (reference culture, obtained from the supernatant of a suspension of 50 g of pooled sample from rim and centre soil – each horizon in equal amounts – with 50 ml of drinking water) the closed vessels were incubated in the dark at room temperature for 7 days. Bioavailable water-extractable organic carbon was calculated by subtraction of WEOC<sub>day 0</sub> – WEOC<sub>day 7</sub> and expressed in percentage of the initial WEOC<sub>day 0</sub>. For further descriptions as well as for advantages and disadvantages of this method see Marschner and Kalbitz (2003)

#### Lipid extraction of soil samples

Lipids were extracted from a fresh soil sample equivalent to a dry weight of 50 g, according to the Bligh-Dyer method as described elsewhere (Zelles and Bai, 1993). The resulting lipid material was fractionated into neutral lipids, glycolipids and phospholipids on a silica-bonded phase column (SPE-SI; Bond Elute, Analytical Chem International, CA, USA) by elution with chloroform, acetone and methanol respectively.

#### Determination of PLFAs and PLEs

Both assays are based on the determination of phospholipid side-chains. An aliquot of the phospholipid fraction equivalent to 12.5 g of soil dw was taken for PLFA analysis. After mild alkaline hydrolysis, the lipid extract was separated into OH-substituted ester-linked PLFAs, non-OH-substituted ester-linked PLFAs and unsaponifiable lipids following procedures described elsewhere (Zelles and Bai, 1993).

The fraction of unsubstituted ester-linked PLFAs was reduced to dryness under nitrogen and dissolved in 100 µl of hexane supplemented with nonadecanoic methyl ester as

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internal standard. The analyses of the fatty acid methyl ester (FAME) extracts were performed by GC/MS as described previously (Lipski and Altendorf, 1997). The position of double bonds of monounsaturated fatty acids was determined by analysing the dimethyl disulfide (DMDS) adducts (Nichols *et al.*, 1986). Quantification of 16:1Δcis8 and 18:1Δcis10 (signature PLFAs for methanotrophic bacteria; Bowman *et al.*, 1993) was based on the abundance of characteristic ions of their DMDS adducts.

The fraction of unsaponifiable lipids was cleaved during acidic alkaline hydrolysis and the resulting non-ester-linked PLFAs were separated into OH-substituted non-ester-linked PLFAs (UNOH) and non-OH-substituted non-ester-linked PLFAs (UNSFA). Separation of the non-ester-linked PLFAs, derivatization and measurement were performed according to Göttinger and colleagues (2002b). Subgroups of UNSFA were named according to their functional groups: ‘-ant’ (anteiso-branching), ‘-nor’ (normal straight chain), ‘-uns’ (unsaturations). The positions of double bonds were given from the carboxyl group of the fatty acid molecule according to the recommendations of the IUPAC-IUB Commission on biochemical nomenclature (IUPAC-IUB Commission on biochemical nomenclature, 1977). Another aliquot of the phospholipid fraction equivalent to 25.0 g of soil dw was used for PLEL analysis according to Göttinger and colleagues (2003). After the formation of ether core lipids, ether-linked isoprenoids were released following cleavage of ether bonds with HI and reductive dehalogenation with Zn in glacial acetic acid. The resulting isoprenoid hydrocarbons were dissolved in 100 µl of internal standard solution (nonadecanoic methyl ester) and subjected to GC/MS analysis at operating conditions described elsewhere (Göttinger *et al.*, 2003). PLFA/PLEL concentrations are expressed in nmol g<sup>-1</sup> dw.

#### Statistical analysis

Statistical analyses were carried out using Systat 10. Concentrations of the individual PLFAs and PLELs were subjected to PCA to elucidate major variation patterns. Functional subgroups of UNSFA and UNOH were included (see Zelles, 1999) in the PCA data set to ease interpretation of the PCA result as both fractions were compiled by 20–40 different single compounds (data not shown). There was no significant influence on the PCA results, if single compounds of UNSFA and UNOH or their functional subgroups were used.

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## 5.3

# Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints



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#### Keywords

archaea; methanogenic diversity; 16S rRNA gene; DGGE; methane; permafrost soils.

#### Abstract

Permafrost environments in the Arctic are characterized by extreme environmental conditions that demand a specific resistance from microorganisms to enable them to survive. In order to understand the carbon dynamics in the climate-sensitive Arctic permafrost environments, the activity and diversity of methanogenic communities were studied in three different permafrost soils of the Siberian Laptev Sea coast. The effect of temperature and the availability of methanogenic substrates on CH<sub>4</sub> production was analysed. In addition, the diversity of methanogens was analysed by PCR with specific methanogenic primers and by denaturing gradient gel electrophoresis (DGGE) followed by sequencing of DGGE bands reamplified from the gel. Our results demonstrated methanogenesis with a distinct vertical profile in each investigated permafrost soil. The soils on Samoylov Island showed at least two optima of CH<sub>4</sub> production activity, which indicated a shift in the methanogenic community from mesophilic to psychrotolerant methanogens with increasing soil depth. Furthermore, it was shown that CH<sub>4</sub> production in permafrost soils is substrate-limited, although these soils are characterized by the accumulation of organic matter. Sequence analyses revealed a distinct diversity of methanogenic archaea affiliated to *Methanomicrobiaceae*, *Methanosarcinaceae* and *Methanosaetaceae*. However, a relationship between the activity and diversity of methanogens in permafrost soils could not be shown.

## Introduction

Arctic tundra wetlands are an important source of the climate-relevant greenhouse gas methane (CH<sub>4</sub>). The estimated methane emissions from these environments varies between 20 and 40 Tg year<sup>-1</sup> CH<sub>4</sub>, which corresponds to up to 8% of the global warming (Cao *et al.*, 1996; Christensen *et al.*, 1999). The degradation of organic matter is slow, and large amounts of organic carbon have accumulated in these environments as a result of the extreme climate conditions with long winters and short summers (Gorham, 1991), and the wet conditions in the soils during the vegetation period. Arctic wetlands could therefore be significant for the development of the Earth's climate, because the Arctic is observed to heat up more rapidly and to a greater extent than the rest of the world (Hansen *et al.*, 2005). In particular, the melting of permafrost and the associated release of climate-relevant trace gases driven by intensified microbial turnover of

organic carbon represent a potential environmental hazard (IPCC, 2001). However, the control mechanisms of methane production, oxidation and emission from tundra environments are still not completely understood.

Permafrost relates to permanently frozen ground with a shallow surface layer of several centimetres (the active layer) that thaws only during the short summer period. The seasonal freezing and thawing of the active layer, with extreme soil temperatures varying from about +18 °C to -35 °C, leads to distinct geochemical gradients in the soils (Fiedler *et al.*, 2004). During the short arctic summer, permafrost soils also show a large temperature gradient along their depth profiles, and this is one of the main environmental factors that influence the microbial communities in these extreme habitats (Kotsyurbenko *et al.*, 1993; Wagner *et al.*, 2003). Water is another important factor for microbial life in these environments. The seasonal thawing of the upper permafrost promotes water saturation of the

soils, leading to anaerobic degradation of complex organic matter to simple compounds, such as acetate, H<sub>2</sub>, CO<sub>2</sub>, formate and methanol, by fermentative bacteria. These compounds serve as substrates for methanogenic archaea, which are responsible for the production of CH<sub>4</sub> (Garcia *et al.*, 2000).

Methanogenic archaea, which belong to the kingdom *Euryarchaeota*, are ubiquitous in anoxic environments. They can be found both in moderate habitats such as rice paddies (Grosskopf *et al.*, 1998a), lakes (Jurgens *et al.*, 2000; Keough *et al.*, 2003) and freshwater sediments (Chan *et al.*, 2005), as well as in the gastrointestinal tract of animals (Lin *et al.*, 1997) and in extreme habitats such as hydrothermal vents (Jeanthon *et al.*, 1999), hypersaline habitats (Mathrani & Boone, 1995) and permafrost soils and sediments (Kobabe *et al.*, 2004).

Several studies have revealed the presence of methanogens in high-latitude peatlands by finding sequences of 16S rRNA gene and methyl coenzyme M reductase (*mcrA*) genes affiliated with *Methanosarcinaceae*, *Methanoacetaceae*, *Methanobacteriaceae* and *Methanomicrobiales* (Galand *et al.*, 2002; Basiliko *et al.*, 2003; Galand *et al.*, 2003; Kotsyurbenko *et al.*, 2004; Høj *et al.*, 2005). It has recently been shown using FISH and phospholipid analyses that the active layer of Siberian permafrost is colonized by high numbers of bacteria and archaea with a total biomass comparable to that of temperate soil ecosystems (Kobabe *et al.*, 2004; Wagner *et al.*, 2005).

The present investigation is part of a long-term study on carbon dynamics and microbial communities in permafrost-affected environments in the Lena Delta, Siberia (Hubberten *et al.*, 2006). The overall purpose of this study was a basic characterization of the methanogenic communities in different extreme habitats of the Laptev Sea coast using both physiological and molecular ecological methods. DNA was extracted from the active layer of three vertical permafrost profiles and analysed by PCR with primers specific for 16S rRNA genes of methanogenic archaea and by denaturing gradient gel electrophoresis (DGGE) followed by sequencing of DNA bands reamplified from the gels. In addition, the potential methane production was analysed under various temperature and substrate conditions.

## Materials and methods

### Study sites and sample collection

Soil samples were collected at various sites on the Laptev Sea coast, northeast Siberia during two Russian–German expeditions in 2002 and 2003. The investigation sites were located in the Lena River Delta on Samoylov Island (72°22'N, 126°28'E) and in the Lena–Anabar lowland on the Nuchcha Dzhiele river near Cape Mamontovy Klyk

(73°36'N, 117°20'E). Both study sites are located in the zone of continuous permafrost and are characterized by an Arctic continental climate with a mean annual air temperature of  $-14.7^{\circ}\text{C}$  ( $T_{\min} = -48^{\circ}\text{C}$ ,  $T_{\max} = 18^{\circ}\text{C}$ ) and a mean annual precipitation of about 190 mm. Further details of the study sites can be found in Schwamborn *et al.* (2002) and Wagner *et al.* (2003).

Soil and vegetation characteristics show great variation over small distances owing to the geomorphological situation of the study sites (Fiedler *et al.*, 2004; Kutzbach *et al.*, 2004). Two sites were chosen for sampling on Samoylov Island, a floodplain (FP) and a polygon centre (PC). The floodplain was characterized by recent fluvial sedimentation, whereas the polygon centre was characterized by peat accumulation with interspersed sand layers. The vegetation at the floodplain site was dominated by *Arctophila fulva*. In the polygon centre, typical plants were *Sphagnum* mosses, *Carex aquatilis* and lichens. The sampling site at Cape Mamontovy Klyk (MAK) was located in a small low-centre polygon plain. The vegetation here differed from that of the polygon centre on Samoylov Island and was dominated by *Eriophorum* spp., *Carex aquatilis*, some *Poaceae* and mosses.

For soil sampling, vertical profiles were arranged and samples were taken from defined soil horizons for physico-chemical (e.g. CH<sub>4</sub> concentration, dissolved organic carbon and total organic carbon contents) and microbiological (e.g. potential CH<sub>4</sub> production, DNA-based analyses) analyses. The samples for microbiological analyses were placed in 250-mL sterile Nalgene boxes, which were immediately frozen at  $-22^{\circ}\text{C}$ . For detailed investigations, horizons with a thickness of more than 10 cm were divided and subsamples were taken. Continuous cooling at  $-22^{\circ}\text{C}$  was guaranteed for the sample transport from the Lena Delta (Siberia) to Potsdam (Germany). Samples were thawed at  $4^{\circ}\text{C}$  and used directly for the analyses, or subsamples were separated and refrozen for later analyses at  $-22^{\circ}\text{C}$ .

### Soil properties

The investigated soils were classified according to US Soil Taxonomy (Soil Survey Staff, 1998). The depth of the permafrost table was measured by driving a steel rod into the unfrozen soil until frozen ground was encountered. The water table was measured in perforated plastic pipes that were installed in the active layer. Soil temperature measurements (a Greisinger GTH 100/2 equipped with a Ni–Cr–Ni temperature sensor) were carried out in each horizon before soil sampling.

Vertical profiles of soil CH<sub>4</sub> concentrations were obtained by extracting CH<sub>4</sub> from fresh soil samples by adding 10 g of soil to saturated NaCl solution, shaking the solution, and subsequently analysing the CH<sub>4</sub> headspace concentration with gas chromatography.

Dissolved organic carbon (DOC) was extracted from various horizons of the soil profiles. Fresh soil material (9 g) was taken from each horizon, weighed into glass jars (50 mL) and mixed with 45 mL of distilled water. The bottles were closed and shaken for 1 h in the dark. Afterwards, the suspension was filtered (0.45-µm mesh, Gelman Science) and the clear solution was inactivated by the addition of sodium azide. The DOC analysis was carried out with an Elementar High-TOC-II. Total organic carbon (TOC) and total nitrogen (TN) were analysed with an element analyser (Elementar Vario EL) using dried and homogenized soil samples. Prior to analysis the samples were treated with HCl (10%) at 80 °C for carbonate removal.

### CH<sub>4</sub> production rates

The influence of temperature, as well as of different substrates (no substrate, methanol or H<sub>2</sub>/CO<sub>2</sub>), on microbial CH<sub>4</sub> production was determined for each horizon. The substrates were chosen according to previous results obtained for the same study site, which showed that hydrogen is more important than acetate for methanogenesis in permafrost soils (Wagner *et al.*, 2005), while no information is available on the importance of methanol as a methanogenic substrate in permafrost environments. Under anoxic conditions, 30-mL glass bottles were filled with 10 g of soil material, and 3 mL of sterile water was added. All bottles were sealed with sterile butyl rubber stoppers. In the case of methanol as additional substrate, 0.1 mL of 1 M methanol stock solution was added to reach a final methanol concentration of about 30 mM. Afterwards, all jars were flushed with N<sub>2</sub>/CO<sub>2</sub> (80:20 v/v). For growth with hydrogen, samples were flushed a second time with H<sub>2</sub>/CO<sub>2</sub> (80:20 v/v, pressurized to 150 kPa). Three replicates were used for the different experiments. The incubation temperatures were 5 °C and 18 °C. CH<sub>4</sub> production was measured daily over a period of one week by sampling the headspace using a Hamilton gastight syringe. Gas analysis were performed with an Agilent 6890 gas chromatograph equipped with a Carbonplot capillary column (φ 0.52 mm, 30 m) and a flame ionization detector (FID). Helium was used as carrier gas. The injector, oven and detector temperatures were set at 45, 45 and 250 °C, respectively. CH<sub>4</sub> production rates were calculated from the linear increase in CH<sub>4</sub> concentration. Samples were dried after incubation at 55 °C, and the methane production was calculated to the dry weight.

### DNA extraction and PCR amplification

DNA was extracted directly from 0.75 g of soil material using an UltraClean™ Soil DNA Isolation Kit (Mo Bio Laboratories Inc.), following the manufacturer's instructions. The quality and quantity of DNA were controlled on 0.8% agarose gels with SYBR Gold staining.

16S rRNA gene fragments with a length of approximately 350 bp were amplified using PCR with the primer pair GC\_357F-691R specific for methanogens (Watanabe *et al.*, 2004). The 50-µL PCR mixture contained 1 × PCR reaction buffer, 0.25 mM of each dNTP, 2 mM MgCl<sub>2</sub>, 0.4 µM of each primer, 2.5 U HotStarTaq DNA Polymerase (Qiagen) and 1–3 µL of DNA template, depending on the quality and quantity of extracted DNA. In some cases, extracted DNA from permafrost soils was diluted 10-fold. PCR was performed using an iCycler Thermal Cycler (Bio-Rad). The amplification conditions consisted of an initial activation step for the HotStarTaq at 94 °C for 10 min, followed by 35 cycles of 94 °C for 60 s, 53 °C for 60 s and 72 °C for 2 min, with a final elongation step of 8 min at 72 °C. PCR products were checked on 2% agarose gels stained with SYBR Gold (Molecular Probes).

### Denaturing gradient gel electrophoresis and sequencing

All samples were separated on 8% polyacrylamide gels in 1 × TAE buffer using a D-Code System (Bio-Rad). The denaturing gradient ranged from 30 to 60% (100% denaturant consisted of 7 M urea and 40% (v/v) deionized formamide). The gels were run at 60 °C, at a constant voltage of 100 V for 14 h. After electrophoresis, the gels were stained for 30 min with SYBR Gold (1 : 10 000 dilution) and visualized under UV light using a GeneFlash system (Syn-gene).

DNA bands that appeared sharp and clear in the gel were cut out with a sterile scalpel and were transferred to sterile 0.5-mL Eppendorf tubes. DNA was eluted overnight in 30 µL of sterile milliQ water at 4 °C. Reamplified products with the expected migration in a new DGGE gel were reamplified again without GC clamp. After purification, using a QIAquick PCR Purification Kit (Qiagen), the DNA bands were sequenced. Sequencing was done by AGOWA GmbH (Berlin, Germany) with forward and reverse primers.

### Phylogenetic analysis

Sequences were compared with those in the GenBank database using the BLAST ([www.ncbi.nlm.nih.gov/blast](http://www.ncbi.nlm.nih.gov/blast)) and FASTA3 ([www.ebi.ac.uk](http://www.ebi.ac.uk)) tools in order to find and include in the analysis all closest relatives. The phylogenetic analysis of partial 16S rRNA gene sequences was performed using the ARB software package ([www.arb-home.de](http://www.arb-home.de); Ludwig *et al.*, 2004) and RAXML-IV (Stamatakis *et al.*, 2005). The ARB\_EDIT tool of the ARB was used for automatic sequence alignment, and the sequences were then corrected manually. A 50% invariance criterion for the inclusion of individual nucleotide sequence positions in the analysis was used to avoid possible treeing artifacts during construction of the 'backbone' trees. 'Backbone' trees were inferred using an

algorithm of the RAXML-IV program with 685, 625 and 602 informative nucleotide positions for *Methanosaetaceae*, *Methanosaetaeaceae* and *Methanomicrobiales*/Rice cluster II trees, respectively. Next, 'backbone' trees were exported back to ARB and the partial sequences of the DGGE bands were added to the trees using the parsimony addition tool of the ARB program package. The partial 16S rRNA gene sequences of the DGGE bands obtained in this study are available in the EMBL/GenBank/DDBJ database under accession numbers AM259179–AM259207.

## Results

### Soil environmental conditions

The physicochemical soil properties of the investigated sites showed a large vertical gradient and high small-scale variability in dependence of microrelief of the different permafrost soils (Table 1).

The soil of the polygon centre on Mamontovy Klyk was classified as a *Typic Aquiturbel*. The water level reached about 1 cm below the soil surface and the perennially frozen ground started at 44 cm. The comparable centre soil on Samoylov Island was a *Typic Historthel*, with a water table

near the soil surface and the permafrost beginning at 33 cm depth. Both soils are characterized by peat accumulation, with extremely high contents of organic carbon in the upper soil layers (> 18%) which decrease with increasing depth of the active layer.

The floodplain soil on Samoylov Island was classified as a *Typic Aquorthel*. The water table was near the soil surface and the permafrost started at 54 cm depth. In contrast to the other two permafrost soils, the floodplain soil on Samoylov Island was characterized by a silty soil texture with organic carbon contents between 0.8 and 3.1%.

The DOC concentration varied between 4.7 and 12.8 mg L<sup>-1</sup> on Samoylov Island polygon centre and between 2.7 and 7.8 mg L<sup>-1</sup> on Samoylov Island floodplain. The CH<sub>4</sub> concentration increased with increasing soil depth and showed values from 0.15 to 541.71 µmol g<sup>-1</sup> and from 0.002 to 0.411 µmol g<sup>-1</sup> in the polygon centre and floodplain, respectively.

In general, the active layer of permafrost was characterized by a strong temperature gradient from top to bottom, which ranged from 6 to 1 °C in the polygon centre on Mamontovy Klyk, and from 17.8 to 0.8 °C and from 7.5 to 0.4 °C, respectively, for the two investigated soils on Samoylov Island.

**Table 1.** Selected soil properties of the investigated permafrost soils

Sample ID	Horizon	Depth (cm)	T (°C)	CH <sub>4</sub> conc. (µmol g <sup>-1</sup> )	DOC (mg L <sup>-1</sup> )	TOC (%)	TN (%)
Polygon centre ( <i>Typic Aquiturbel</i> ), Mamontovy Klyk							
221	Oi1	0–6	6			39.4	1.42
222	Oi2	6–12	5			28.1	1.23
223	Bjjg1	12–17	4	ND	ND	11.2	0.73
224	Bjjg2	17–22	3			14.1	0.90
225	Bjjg3	22–29	2			7.6	0.52
226	Bjjg4	29–36	2			5.5	0.40
227	Bjjg5	36–44	1			4.5	0.27
Floodplain ( <i>Typic Aquorthel</i> ), Samoylov Island							
6941	Ai	0–5	17.8	0.004	4.5	3.1	0.4
6942	Ajj	5–9	14.2	0.004	3.8	1.1	0.2
6943	Bg1	9–18	8.8	0.002	2.7	2.2	0.3
6944	Bg2	18–20		ND	4.4	3.0	ND
6945	Bg3	20–35	4.0	0.035	7.8	2.5	0.4
6946	Bg4	35–40	1.9	0.114	4.9	2.0	0.3
6947	Bg5	40–52	0.8	0.411	4.8	0.8	0.2
Polygon centre ( <i>Typic Historthel</i> ), Samoylov Island							
6968	Oi1	0–5	7.5	0.15	11.7	18.3	0.51
6969	Oi2	5–10	5.8	13.19	8.8	13.8	0.43
6970	Ajj1	10–15	4.0	24.37	4.7	13.7	0.36
6971	Ajj2	15–20	2.7	70.50	9.5	9.3	0.23
6972	Bg1	20–23	1.2	ND	ND	7.0	0.19
6973	Bg2	23–30	0.4	163.24	11.9	4.7	0.16
6974	Bg2	30–35	< 0.4	328.87	12.8	3.6	0.15
6975	Bg3	35–40	< 0.4	541.71	ND	4.3	0.18
6976	Bg3	40–45	< 0.4	ND	ND	4.9	0.22

Horizon nomenclature and soil classification according to Soil Survey Staff (1998).

T, *in situ* temperature; DOC, dissolved organic carbon; TOC, total organic carbon; TN, total nitrogen; ND, not determined.

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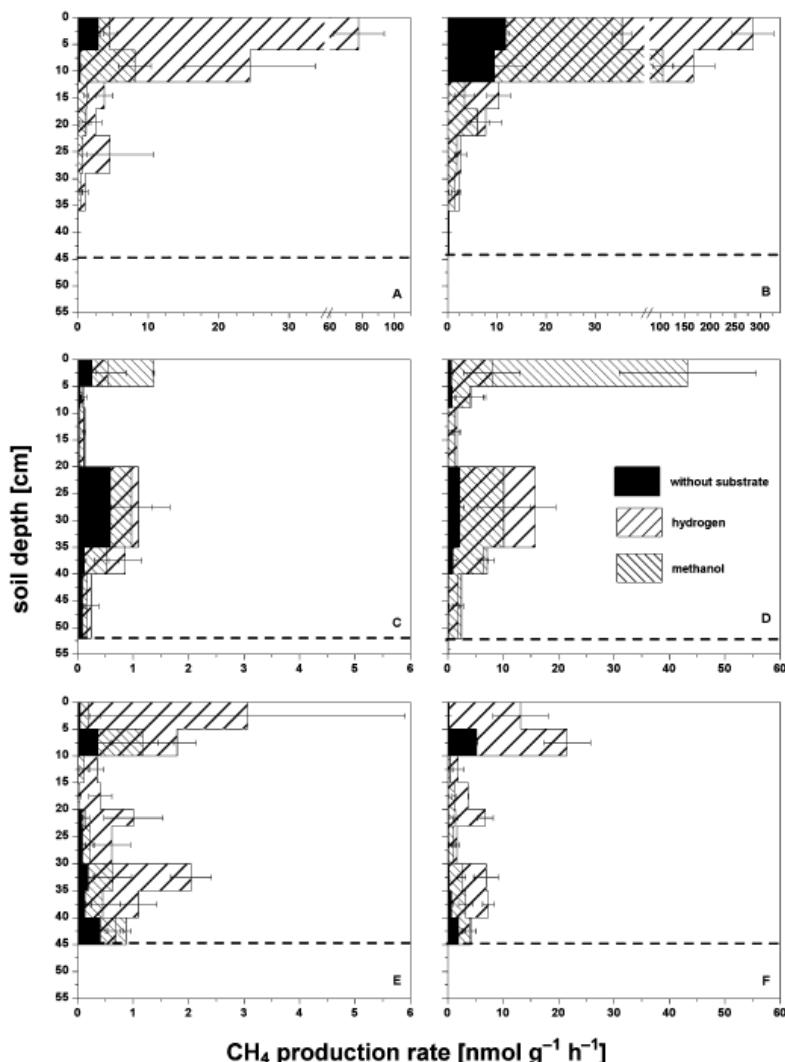
### Temperature and substrate effect on methanogenesis

The CH<sub>4</sub> production of the three different soils showed significant differences in the rate of activity and vertical distribution (Fig. 1). In general, the activity in each profile was higher with hydrogen or methanol as additional substrate than it was without any substrate. Furthermore, the CH<sub>4</sub> production was much higher at 18 °C than at 5 °C.

The highest CH<sub>4</sub> production rate within the centre soil (*Typic Aquiturbel*) on Mamontovy Klyk was found with hydrogen as substrate, followed by methanol as substrate. Without any substrate addition, only a limited activity was detectable (Fig. 1a and b). The activity was highest in the two upper horizons, and decreased with increasing soil depth.

The activity pattern of the other two studied sites on Samoylov Island was different from that for Mamontovy Klyk. The floodplain soil (*Typic Aquorthel*) showed two maxima of CH<sub>4</sub> production, one in the upper soil horizon and a second in the zone with the highest root density at a depth between 20 and 35 cm (Fig. 1c and d). Here, the highest activity was measured in the upper soil horizon with methanol, while the CH<sub>4</sub> production rates in all other horizons were higher with hydrogen as substrate.

The soil (*Typic Historthel*) of the polygon centre on Samoylov Island was characterized by the highest CH<sub>4</sub> production taking place in the upper soil horizons. This was also observed for the comparable soil on Mamontovy Klyk (Fig. 1e and f). However, in contrast to the latter soil, high activity also occurred in the polygon centre on Samoylov Island in the bottom zone of the active layer close to the



**Fig. 1.** Vertical profiles of CH<sub>4</sub> production for the three study sites at 5 °C (left column) and 18 °C (right column) without any substrate as well as with hydrogen and methanol as additional methanogenic substrates. (a and b) *Typic Aquiturbel* (polygon centre on Mamontovy Klyk); (c and d) *Typic Aquorthel* (floodplain on Samoylov Island); and (e and f) *Typic Historthel* (polygon centre on Samoylov Island). Dashed lines indicate the permafrost tables.

permafrost table with a temperature near to the freezing point of water. With the exception of the bottom horizon, where the highest CH<sub>4</sub> production occurred with methanol, the preferred substrate in all other horizons was hydrogen.

The effect of increasing temperature was different for the three sites, as well as in the vertical profile of each soil. Compared with the CH<sub>4</sub> production at 5 °C on Mamontov Klyk, CH<sub>4</sub> production at 18 °C was about three times higher, that in the floodplain soil on Samoylov Island was at least 10 times higher, and that in the polygon-centre soil on Samoylov Island was at least two times higher. In general, the methane production activity in the upper part of the active layer of all soils rose after the increase of temperature more strongly than it did within the bottom part of the profiles near the permafrost table.

### DGGE analysis of permafrost soil samples

Three permafrost sites on the Laptev Sea coast were compared with regard to variation in the community structure of methanogenic archaea from the top to the bottom of the investigated soil profiles. DGGE profiles showed up to nine well-defined bands per depth, and a shift within the vertical profiles of Samoylov Island polygon centre (*Typic Historthel*) and Mamontov Klyk polygon centre (*Typic Aquiturbel*). In the polygon centre on Samoylov Island (*Typic Historthel*), the number of DNA bands increased to a depth of 23 cm (zone of highest root density) and then decreased again (Fig. 2a). The number of bands in the polygon-centre soil on Mamontov Klyk was constant to a depth of 22 cm, with about four DNA bands in each lane (Fig. 2b). Most DNA bands were observed in the middle of the profile (22–29 cm soil depth) and this number decreased with increasing soil

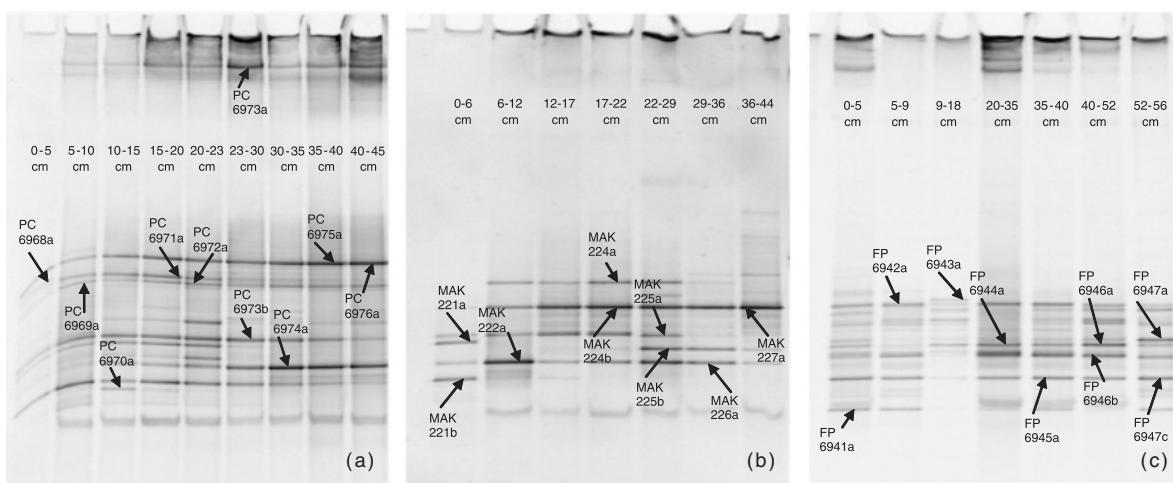
depth, as was also observed for the soil of the polygon centre on Samoylov Island (Fig. 2a). Interestingly, the floodplain on Samoylov Island showed a completely different pattern. Here, the number of bands did not decrease with increasing depth (Fig. 2c). Even the soil horizon close to the permafrost table showed a diversity of methanogens comparable with the highest diversity in the middle of the two other profiles.

Besides the number of bands, the distribution pattern showed distinct differences, particularly within the vertical profiles of the polygon-centre soils on Samoylov Island (soil depth 20–23 cm compared with the bottom of the active layer) and Mamontov Klyk (the first horizon in comparison with the bottom of the active layer).

Some DGGE bands were found only in certain horizons, such as PC 6970a (*Methanosarcinaceae*), PC 6943a, MAK 221a and MAK 221b (all *Methanomicrobiaceae*). Beside these unique bands, some other bands that did not occur throughout the whole soil profile could also be seen. For example, DGGE bands corresponding to MAK 224a (*Methanomicrobiaceae*) were found only in the middle of the soil profile at a depth of 6–29 cm, and bands corresponding to MAK 225b (*Methanosarcinaceae*) were found only in the deeper regions of the soil profile.

### Phylogenetic analysis of permafrost sequences

A total of 36 DGGE bands from three soil profiles were sequenced. Eight sequences were excluded from further analysis because of their short (< 200 nucleotides) length. All sequences can be differentiated at the genus level. Twenty-eight sequences of 16S rRNA gene fragments obtained from the investigated permafrost environments fell within known euryarchaeotal lineages belonging to the



**Fig. 2.** DGGE profiles of 16S rRNA genes amplified from permafrost community DNA obtained from various horizons (thickness in centimetres from top to bottom) of the active layer. (a) *Typic Historthel* (polygon centre on Samoylov Island); (b) *Typic Aquiturbel* (polygon centre on Mamontov Klyk); and (c) *Typic Aquorthel* (floodplain on Samoylov Island). Selected bands marked with arrows and sample IDs were used for sequence analyses.

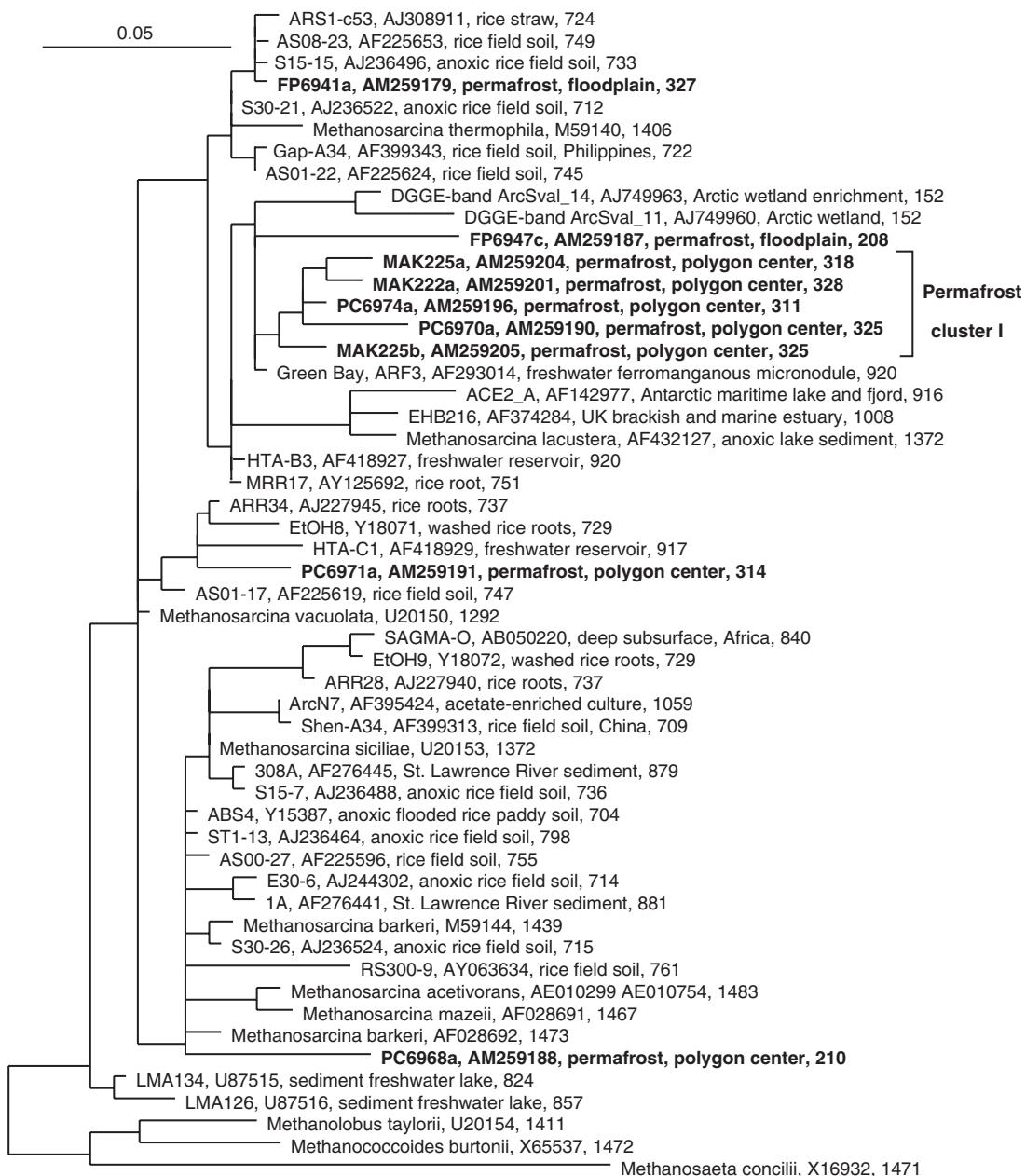
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major methanogenic groups *Methanosarcinaceae* (11 sequences, 98–99% homology over 312–316 nucleotides; including three, which were 100% identical), *Methanosaeta* (two sequences with 97% homology over 308 nucleotides), and *Methanomicrobiales* (12 sequences, 91–99% homology

over 300–321 nucleotides), and three sequences fell within (97% similarity) an as yet uncultivated archaea lineage named Rice cluster II (Fig. 3a and b).

Sequences affiliated to *Methanosarcinaceae* and *Methanomicrobiales* were found in all studied soil profiles, whereas



**Fig. 3.** Phylogenetic trees illustrating the affiliation of methanogenic 16S rRNA gene sequences reamplified from DGGE bands. The sequences recovered from permafrost belong to *Methanosarcinaceae* (a), and *Methanomicrobiales* together with Rice cluster II (b). The 'backbone' trees are based on maximum likelihood analysis of the dataset made with RAxML-IV, and partial sequences of the permafrost DGGE bands (shown in bold) were added to these trees using the parsimony addition tool of the ARB program package. The scale bar represents 0.05 changes per nucleotide. Identification of the bands is shown in Fig. 2. Clone name, accession number, environment and length of each sequence are indicated.

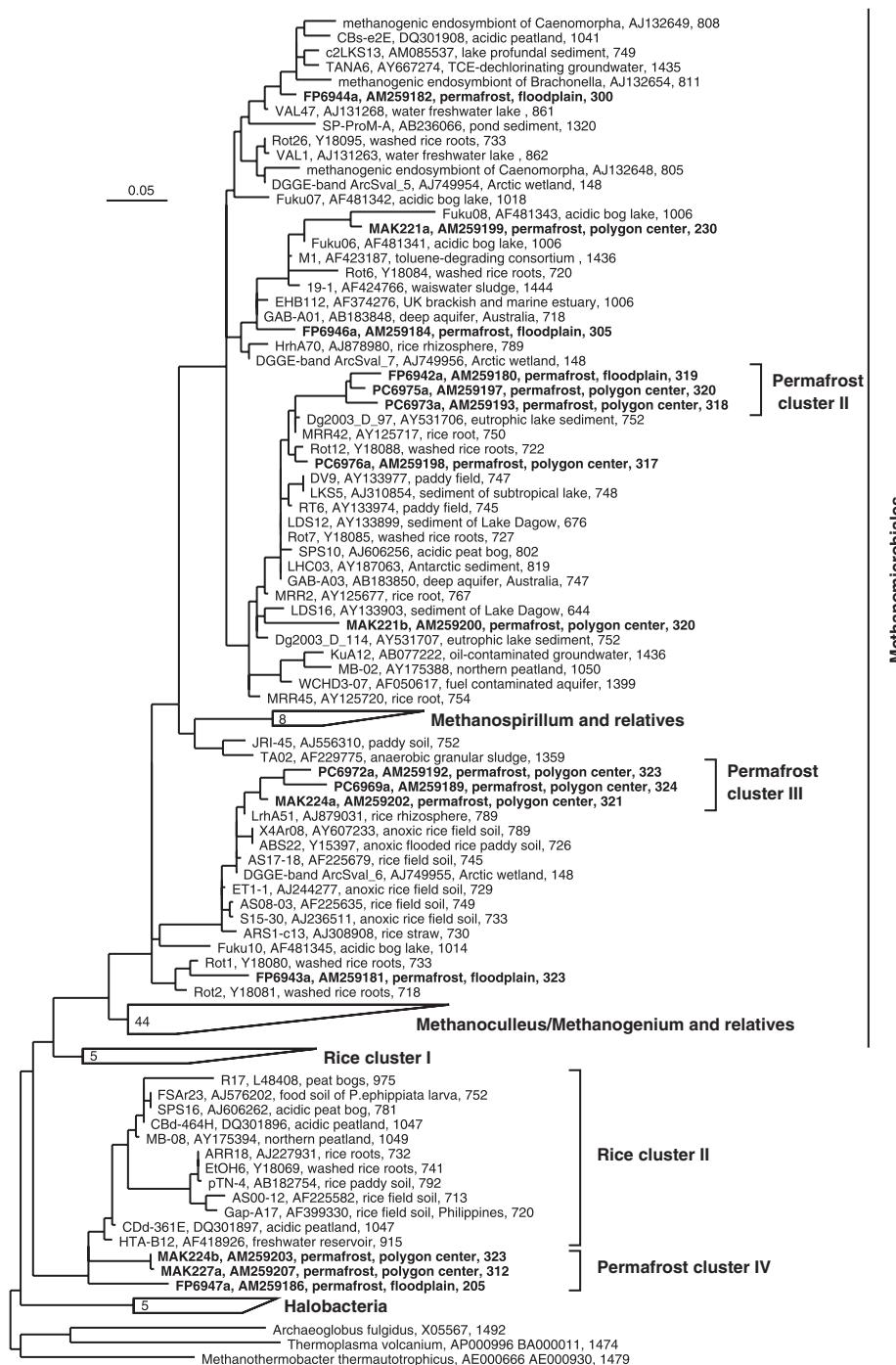


Fig. 3. Continued.

sequences associated with *Methanosaeta* were found only in the floodplain and in the polygon centre on Samoylov Island, but not in the polygon centre of Cape Mamontovy Klyk.

## Discussion

Our results showed differences in the CH<sub>4</sub> production activities and the biodiversity patterns of methanogenic

archaea in the investigated permafrost soils. Activities of methanogenic archaea differed significantly in their rates and distributions among the different soils. While the CH<sub>4</sub> production rates in the active layer on Mamontov Klyk decreased with increasing soil depth, the two other sites on Samoylov Island showed at least two activity optima. The highest activity occurred in the upper soil horizons, which are characterized by *in situ* temperatures of up to 17.8 °C. The second optimum of methane production was found in the middle or bottom part of the active layer in both soils on Samoylov Island. In the floodplain soil, the highest activity was detected in a horizon constantly exposed to temperatures below 4 °C, which indicates the dominance of methanogens that must be well adapted to the cold conditions observed close to the permafrost table. Here, the second activity optimum correlates with the zone of the highest root density and amount of DOC. It is well known that plants can supply root exudates consisting of low-molecular-weight organic compounds, which can serve as a substrate for methanogens (Chanton *et al.*, 1995; Ström *et al.*, 2003). However, the extraordinarily high CH<sub>4</sub> production rates in the upper layer of Mamontov Klyk correlates with the high amount of organic carbon in these horizons.

The addition of different substrates led to an increase in the potential CH<sub>4</sub> production in all horizons of all sites. This effect was not confined to horizons with a low content of organic carbon, but could also be observed in horizons with a high amount of organic carbon. Wagner *et al.* (2005) reported that the humification of soil organic matter increased with increasing soil depth. This was shown to be reciprocally correlated with the amount of bioavailable organic carbon. A reduced quantity and quality of organic matter in permafrost soils could lead to a substrate-limited methanogenesis.

The potential CH<sub>4</sub> production at 5 °C was distinctly different from that at 18 °C. A higher incubation temperature resulted in a marked increase of the methanogenic activity in almost all investigated soil horizons. It is noteworthy that the effect of higher temperature on the activity was larger in the upper soil horizons with higher *in situ* temperatures than in the bottom of the active layer with lower *in situ* temperatures. Hence, taking into consideration the physiological studies, we can conclude that the activity of methanogenic archaea in permafrost soils depends on the quality of soil organic carbon, and our results show that methanogens in deep active-layer zones might be better adapted to low temperatures.

Only a few psychrophilic strains of methanogenic archaea have been described so far (Simankova *et al.*, 2003; Cavicchioli, 2006). However, our results indicate a shift in the methanogenic community from mesophilic to psychrotolerant or psychrophilic methanogens with increasing soil depth. Similar results have been obtained from permafrost

soils on Samoylov Island in the context of the methane-oxidizing community (Liebner & Wagner, 2006). An important requirement for microorganisms to adapt to cold environmental conditions is constantly low *in situ* temperatures over a long period of time (Morita, 2000). This is the case in the bottom zone of the active layer close to the perennially frozen ground. A prerequisite for prokaryotes to adapt to low temperatures is that their cell membranes should maintain fluidity. This effect was shown in a related study, carried out for the centre profile on Samoylov Island, which revealed an increase of branched-chain fatty acids in relation to the amount of straight-chain fatty acids with increasing active-layer depth (Wagner *et al.*, 2005).

The DGGE pattern of the investigated permafrost soils showed differences within the depth profile and between the different sites. The number of DNA bands at the floodplain site on Samoylov Island remained fairly constant through the whole profile. While the temperature drastically decreased with soil depth, the carbon (DOC and TOC) and nitrogen concentrations in the profile remained relatively constant. These geochemical profiles can be explained by the fact that the floodplain is periodically flooded by the Lena River. Thus the vegetation is regularly buried by the accumulation of new sediments, which causes the even distribution of organic matter in the profile. Galand *et al.* (2003) reported that vegetation characterizing microsites in a studied boreal fen influences the microbial communities in layers with significant methane production. The similarity of the community pattern for the whole soil profile of the floodplain can probably be attributed to the regular sedimentation at this site, but a significant relationship between this pattern and the methane production as reported by Galand *et al.* (2003) was not determined. This is in accordance with studies in Arctic wetlands in Spitzbergen, which found that methane fluxes depend more on the temperature and thaw depth than on the archaeal community structure (Høj *et al.*, 2005).

In contrast to the profile for the floodplain site, the polygon-centre profiles for Mamontov Klyk and on Samoylov Island showed a variety of diversity patterns. These soils were characterized by humus accumulation in the upper part of the active layer, with decreasing organic matter content in the underlying mineral soils. However, the number of bands increased until the zone with the highest root density, but started to decrease in the deeper zones of the active layer. The presence of root exudates (Chanton *et al.*, 1995; Ström *et al.*, 2003), as discussed earlier with regard to the methane production activity, seems also to affect the diversity of the methanogenic archaea in permafrost soils. Among the differences in the number of detected DNA bands within the various horizons of the vertical profiles, different band patterns indicated differences in the community structure of methanogens, particularly in the

polygon-centre profiles on Mamontovy Klyk and on Samoylov Island. These differences refer to the bottom zone of the active layer compared with horizons, which lie further above in the respective profiles. A depth-related change of the methanogenic community was also observed in northern peatlands (Galand *et al.*, 2005).

The results of the DGGE analysis indicate changes of the methanogenic community within the vertical soil profiles. Some DGGE bands appeared throughout the whole profile, while others were specific for distinct active-layer depths. Moreover, the band pattern showed distinct differences between specific horizons. On one hand this indicates the presence of methanogenic archaea that can exist under different environmental conditions (temperature, substrate, geochemistry), which are changing within the depth of the active layer. On the other hand, it indicates the presence of methanogens that can exist only under defined environmental conditions. Some sequences, for example those affiliated to *Methanosarcinaceae* (PC 6974a, MAK 225a), were detected only in the cold zones ( $< 3^{\circ}\text{C}$ ) of the active layer.

Our results indicate the presence of hydrogenotrophic, acetotrophic and methylotrophic methanogens in the investigated permafrost soils. Sequences were affiliated with the families of *Methanomicrobiaceae*, *Methanosarcinaceae* and *Methanosaetaceae*, while members of the family *Methanobacteriaceae*, as shown in other studies on archaeal diversity in northern peatlands (Høj *et al.*, 2005; Juottonen *et al.*, 2005), could not be detected. One reason could be the inhomogeneous distribution of microorganisms in soil depending on the distribution of usable organic carbon (Wachinger *et al.*, 2000). Species of *Methanomicrobiaceae* can grow only with hydrogen, formate and alcohols (except methanol), *Methanosarcinaceae* can grow with all methanogenic substrates except formate, and members of *Methanosaetaceae* grow exclusively with acetate as energy source (Hedderich & Whitman, 2005). An important finding is the detection of hydrogenotrophic methanogens in permafrost environments, because several studies have shown that acetate is more important as a substrate in cold than in temperate environments (Chin & Conrad, 1995; Wagner & Pfeiffer, 1997). However, a related study at the polygon-centre site on Samoylov Island showed that the potential methane production in all horizons was lower with acetate as substrate compared with the activity after hydrogen amendment (Wagner *et al.*, 2005). Acetate is likely to be available only to habitats with a significant portion of polysaccharides, which is not the case in Arctic peatlands (Kotsyurbenko *et al.*, 2004). Only representatives of the genera *Methanosarcina* and *Methanosaeta* are able to use acetate as a substrate. In particular, *Methanosarcina* species prefer methanol as carbon and energy source, although methanogenesis via acetate and hydrogen represents the

main pathway of methane production in most environments (Conrad, 2005). The significance of methanol, which is derived from pectin or lignin (Schink & Zeikus, 1982), for methanogenesis in permafrost environments was verified by this study for the floodplain site on Samoylov Island. However, unknown methanogenic archaea could make a contribution to hydrogenotrophic methanogenesis at low temperatures. An indication for this assumption is the presence of sequences affiliated with the order *Methanomicrobiales* that could be detected in deeper layers of all studied sites. One of the few known psychrophilic H<sub>2</sub>using methanogens that belongs to this group of methanogens is *Methanogenium frigidum* (Franzmann *et al.*, 1997), which was isolated from an Antarctic sediment.

Detailed phylogenetic analysis showed that two DGGE bands belonging to *Methanosaetaceae* branched very close to each other (data not shown). Both were extracted from relatively deep and cold (0.4–0.8 °C) soil horizons on Samoylov Island, but had different CH<sub>4</sub> production rates. The closest relatives of these sequences have been detected in environments with different physicochemical characteristics, such as rice-field soils (Lueders & Friedrich, 2000; Ramakrishnan *et al.*, 2001), lake sediments (Banning *et al.*, 2005), and an acidic bog lake (Chan *et al.*, 2002).

Four of the *Methanosarcina*-like permafrost sequences (FP6941a, FP6947c, PC6971a and PC6968a) were clustered with cultivated methanogens (e.g. *Methanosarcina barkeri*) and among numerous environmental sequences with the closest relatives from rice-field soils (Lueders & Friedrich, 2000), freshwater environments (Stein *et al.*, 2002) and Arctic wetland (Høj *et al.*, 2005; Fig. 3a). The remaining seven sequences (two of them, FP6945a and MAK226a, were 100% identical to MAK225b) form a cluster with the closest relative sequences ARF3 from Green Bay, recovered from a ferromanganese micronodule (Stein *et al.*, 2001), FP6947c, and sequences ArcSval\_11 and ArcSval\_14 from Arctic wetland (Høj *et al.*, 2005). Sequences in this *Permafrost cluster I* were recovered mainly from cold layers ( $< 4^{\circ}\text{C}$ ) of the medium-depth horizons (6–36 cm) of all the studied sites.

Similar results were obtained for the *Methanomicrobiales*-like permafrost sequences. Six of them were distributed among numerous environmental sequences with closest relatives recovered from rice roots (Lehmann-Richter *et al.*, 1999), PC6976a and FP6943a; acidic bog lake (Chan *et al.*, 2002), MAK221a; freshwater lake (Jurgens *et al.*, 2000), FP6944a; geothermal aquifer GAB-A01 (Kimura *et al.*, 2005), FP6946a; and LDS16 from Lake Dagow sediment (Glissman *et al.*, 2004), MAK221b (Fig. 3b). *Permafrost cluster II*, which consists of three sequences from the floodplain and the polygon centre of Samoylov Island, is closely (97–98%) related to sequences Dg2003\_D\_97 from Lake Stechlin sediment (Chan *et al.*, 2005) and MRR42 from rice roots. Sequences were obtained from soil horizons of

various depths (from 5 to 40 cm) and various temperatures (from < 0.4 °C to 14.2 °C). Three sequences (PC6972a, PC6969a and MAK224a) from polygon-centre soils (depth 5–23 cm, temperatures from 1.2 to 5.8 °C) form *Permafrost cluster III*, related (with about 98% similarity) to sequences recovered recently from rice rhizosphere (Lu & Conrad, 2005). Three other permafrost sequences (MAK224b, MAK227a and FP6947a) recovered from polygon-centre and floodplain soils (depth 17–56 cm) form *Permafrost cluster IV*, related (with about 97% similarity) to Rice cluster II (Grosskopf *et al.*, 1998b). The first representative of Rice cluster II – clone R17 – was found in a peat bog (Hales *et al.*, 1996), but now this cluster consists of about 20 environmental sequences recovered from a broad range of environments (Ramakrishnan *et al.*, 2001; Stein *et al.*, 2002).

The sequences that can be assigned to specific permafrost clusters, might possibly include methanogenic archaea, which are adapted to their extreme habitat by special physiological characteristics. This assumption is supported by the fact that pure cultures of *Methanosarcina*-like species isolated from permafrost soils of the same study site are more persistent to unfavourable environmental conditions (e.g. subzero temperatures, high salinity, dryness) than those from non-permafrost environments (D. Morozova and D. Wagner, pers. comm.). However, further studies that address the activity, diversity and physiological characteristics of methanogenic archaea in permafrost environments should be undertaken.

In conclusion, this study provides the first results concerning the methanogenic communities in three different permafrost soils of the Laptev Sea coast. It has demonstrated methanogenesis with a distinct vertical profile in each studied soil. The results show that CH<sub>4</sub> production is regulated more by the quality of soil organic carbon than by the *in situ* temperature. We can also say that methanol is an important substrate in these habitats, as indicated by activity tests and by the presence of methylotrophic methanogens. The phylogenetic analysis revealed a distinct diversity of methanogens in the active layer of all study sites, with species belonging to the families *Methanomicrobiaceae*, *Methanosaetaceae* and *Methanococcaceae*. There were no restrictions of the detected families to specific depths or sites. Only sequences of *Methanosaetaceae* could not be detected in the polygon-centre soil of Mamontovy Klyk. Out of the 28 sequences, 16 sequences form four specific permafrost clusters. We hypothesize, albeit somewhat speculatively, that these clusters are formed by methanogenic archaea characterized by specific adaptation processes to the harsh permafrost conditions. However, a relationship between the activity and the diversity of methanogens in permafrost soils could not be shown. Molecular ecological analysis of the microbial permafrost communities in combination with process studies on CH<sub>4</sub> production, oxidation

and emission will be able to improve our understanding of the future carbon dynamics in climate-sensitive permafrost environments.

## Acknowledgements

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## 6 Methanogenic Archaea as Model Organisms for Life in Extreme Habitats and Their Astrobiological Relevance

### 6.1 Microbial Life in Terrestrial Permafrost: Methanogenesis and Nitrification in Gelisols as Potentials for Exobiological Processes

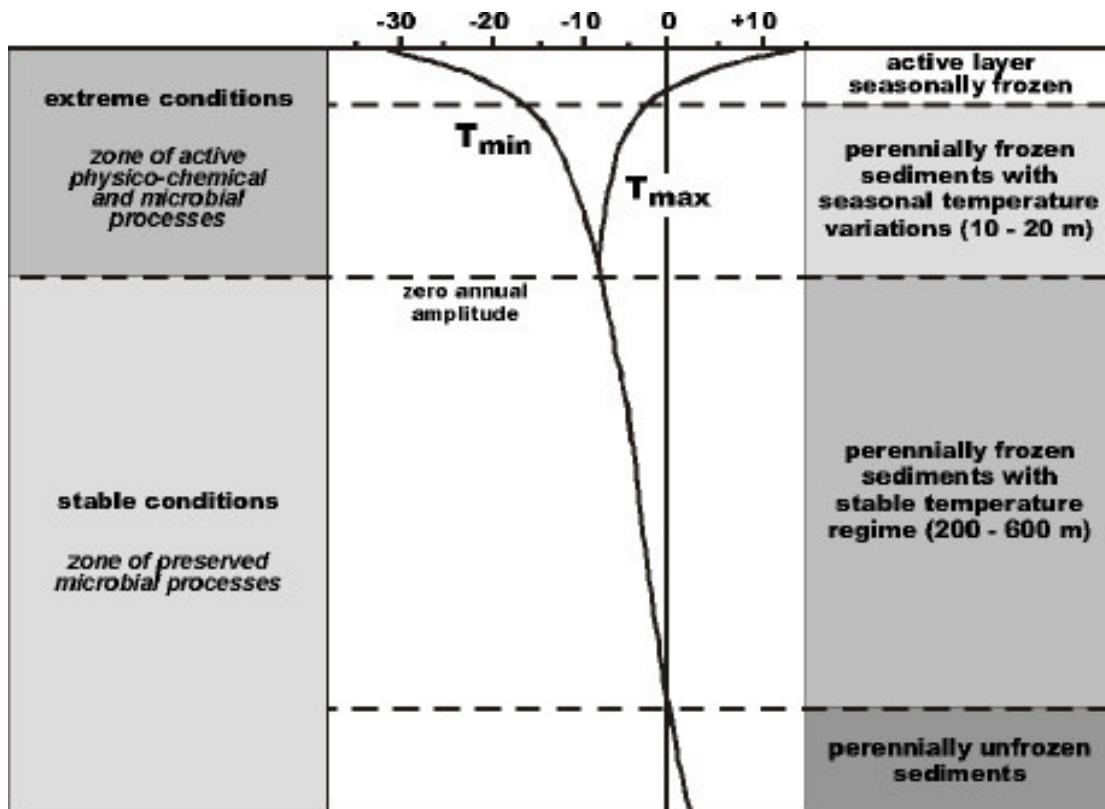
Dirk Wagner, Eva Speck, Eberhard Bock and Eva-Maria Pfeiffer

The comparability of environmental and climatic conditions of the early Mars and Earth is of special interest for the actual research in astrobiology. Martian surface and terrestrial permafrost areas show similar morphological structures, which suggests that their development is based on comparable processes. Soil microbial investigations of adaptation strategies of microorganisms from terrestrial permafrost in combination with environmental, geochemical and physical analyses give insights into early stages of life on Earth. The extreme conditions in terrestrial permafrost soils can help to understand the evolution of life on early Mars and help searching for possible niches of life on present Mars or in other extraterrestrial permafrost habitats [1, 2].

#### 9.1 Permafrost Soils and Active Layer

In polar regions huge layers of frozen ground are formed - termed permafrost - which are defined as the thermal condition, in which soils and sediments remain at or below 0 °C for two or more years in succession. Terrestrial permafrost, which underlays more than 20% of the world's land area, is above all controlled by climatic factors and characterized by extreme terrain condition and landforms. On Earth the permafrost thickness can reach several hundreds of meters, e.g., in East Siberia (Central Yakutia) about 600-800 m. During the relatively short period of arctic/antarctic summer only the surface zone of permafrost sediments thaws. This uppermost part of the permafrost (active layer) includes the so called Gelisols [3], which contains permafrost in the upper 100 cm soil depth. Gelisols are characterized by gelic material that have the evidence of cryoturbation and ice segregation. Permafrost soils may be cemented by ice which is typical for the Arctic regions, or, in the case of insufficient interstitial water, may be dry like the Antarctic polar deserts.

Permafrost can be divided into three temperature regimes (Fig. 9.1), which characterize the extreme living conditions: (i) The surface near upper active layer (0.2-2.0 m thickness) is subjected to seasonal freezing and thawing with an extreme temperature regime from about +15 °C to -35 °C, (ii) the correlated upper, perennially frozen permafrost sediments (10-20 m thickness) with smaller seasonal temperature variation of about 0 °C to -15 °C above the zero annual amplitude and (iii) the deeper permafrost sediments which are characterized by a stable temperature regime of about -5 °C to -10 °C [4].



**Fig. 9.1** Scheme of temperature amplitude in permafrost sediments (according to French [4], modified)

The main Gelisol-forming processes in permafrost landscapes are cryopedogenesis, which include freezing and thawing, frost stirring, mounding, fissuring and solifluction. The repeating cycles of freezing and thawing leads to cryoturbation features (frost churning) that includes irregular, broken or involuted horizons and an enrichment of organic matter and other inorganic compounds, especially along the top of the permafrost table. As a result of cryopedogenesis many Gelisols are influenced by a strong micro-relief (patterned ground, Fig. 9.2). The type of patterned ground has effects on soil formation and soil properties.

Ice wedge polygons for example of the Siberian lowlands (Fig. 9.3-A) which are typical for high arctic, are characterized by two different soil conditions: The Gelisols of the polygon center (*Historthels*) are water saturated and have a large amount of organic matter due to the accumulation under anaerobic conditions (Fig. 9.3-B). The Gelisols of the polygon border (*Aquiturbels*) show evidence of cryoturbation in more or less all horizons of the active layer (Fig. 9.3-C). These soils drain into the polygon center, which leads to dryer conditions in the upper layer of the border.

These examples demonstrate that the Gelisols of the active layer and upper permafrost sediments are the zone with active physico-chemical processes under extreme conditions. Therefore, microbial life in permafrost soils and sediments is influenced by extreme gradients of temperature, moisture and chemical properties. However, deeper permafrost layers characterize living conditions, which have been stable for long periods of time and microbial life is preserved (see Chap. 8, Gilichinsky).

## 9.2 Microbial Life under Extreme Conditions

Over 80% of the Earth's biosphere - including the polar regions - is permanently cold. Most natural environments have a temperature regime colder than 5 °C. Temperature is one of the most important parameters regulating the activity of microorganisms because it controls all metabolic activity of living cells [5]. The temperature in the upper zone of the cryolithosphere (active layer and upper permafrost sediments) ranged between –50 °C to +30 °C [6]. Especially permafrost soils are characterized by extreme variation in temperature. Previously the potential of growth as well as the molecular, physiological and ecological aspects of microbial life at low temperatures were investigated [7, 8]. Many microorganisms are able to survive in cold permafrost sediments, but this adaptation can be a tolerance or a preference. According to Morita [9] bacteria can be described by their temperature range of growth: psychrophiles ( $T_{\min} < 0$  °C,  $T_{\text{opt}} \leq 15$  °C,  $T_{\max} \leq 20$  °C), psychrotrophs ( $T_{\min} \leq 0$  °C,  $T_{\text{opt}} \geq 15$  °C,  $T_{\max} \leq 35$  °C) and mesophiles ( $T_{\text{opt}} 25\text{-}40$  °C). The minimum temperature for growth of bacteria was recently reported with –20 °C [10], whereas the minimum temperature for enzyme activity was –25 °C [11].

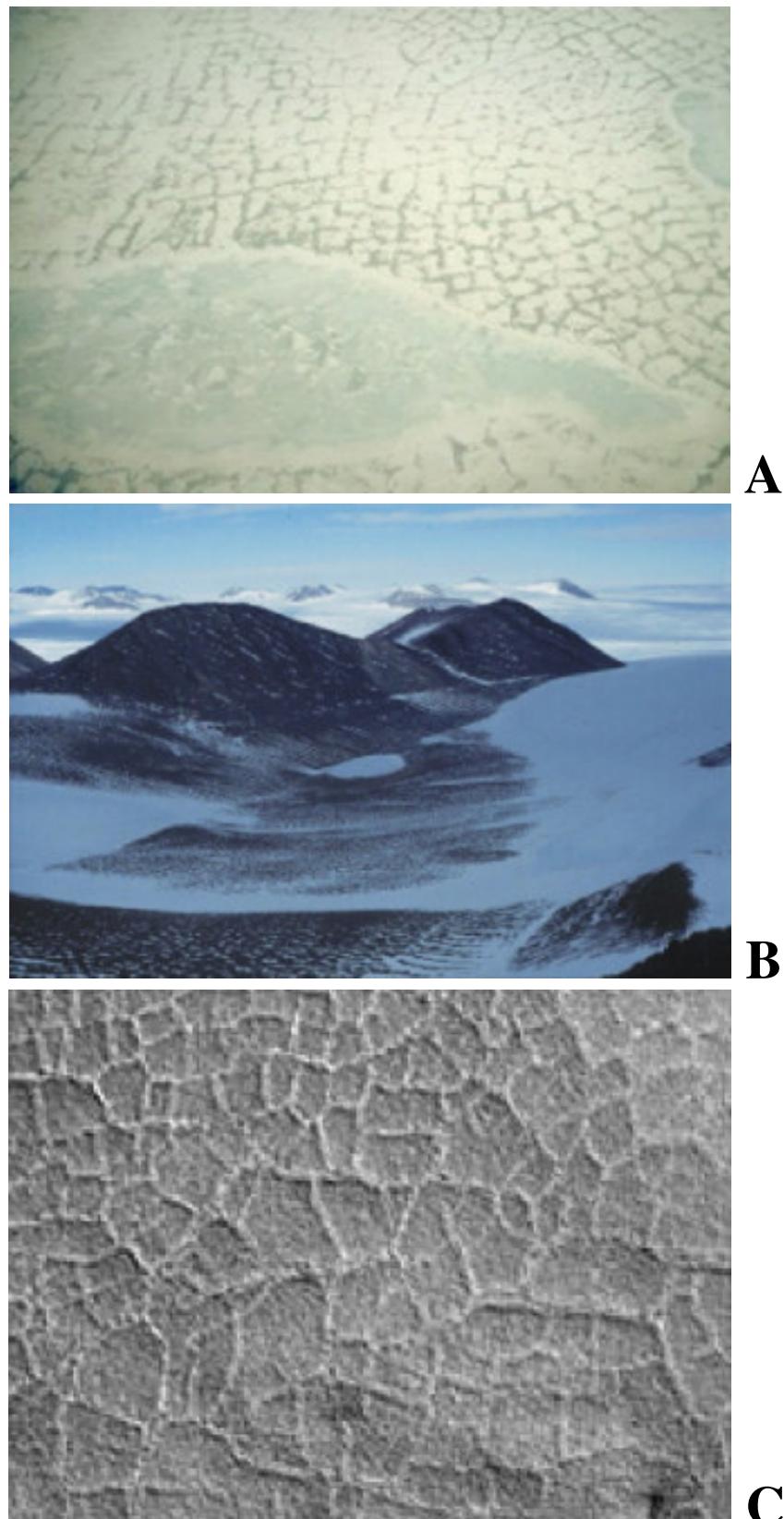
The seasonal variation of soil temperature influences also the availability of pore water. The presence of unfrozen water is an essential biophysical requirement for the survival and activity of microorganisms in permafrost. Temperature below zero stands for an increasing loss of water. At the same time freezing of water leads to an increase of salt content in the remaining pore solution. However, in clayey permafrost soils liquid water was analyzed at temperatures up to –60 °C [12]. The most important feature of this water is the possible transfer of ions and nutrients [13]. Furthermore, McGrath et al. [14] showed that the intercellular water in fossil bacteria from permafrost soils was not crystallized as ice even at an extreme temperature of –150 °C.

For studying microbial life under extreme conditions it is also necessary to consider whether and where these conditions are changing or stable in a permafrost profile. The seasonal variation in soil temperature, particularly freeze-thaw cycles in the active layer, results in drastic changes of other environmental conditions like salinity, soil pressure, changing oxygen conditions (anoxic, microaerophilic, oxic) and nutrient availability. Therefore, besides the physico-chemical conditions of permafrost, the physiological properties of microorganisms are relevant for the adaptation to extreme conditions. On account of this potential, they developed strategies to resist salt stress, physical damage by ice crystals and background radiation [15]. Survival could be also possible by anabiosis (dormant stage of life) or by reduced metabolic activity in unfrozen waterfilms (see Chap. 8, Gilichinsky).

## 9.3 Microbial Key Processes

Terrestrial permafrost is colonized by high numbers of chemoorganotrophic bacteria as well as microbes like methanogenic archaea and nitrifying bacteria [16–18], which are highly specialized organisms. They are characterized by litho-autotrophic growth

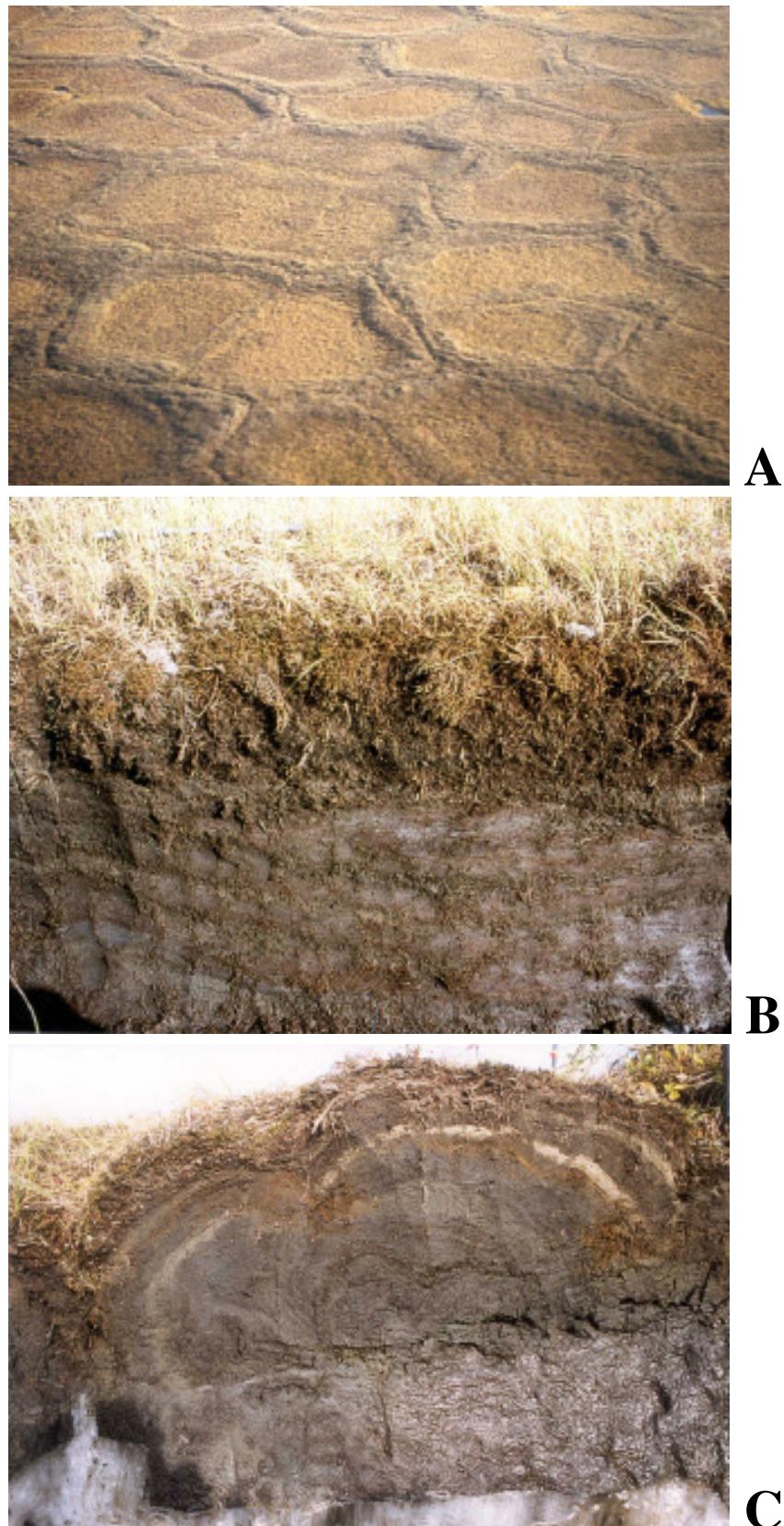
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**Fig. 9.2** Permafrost structures. A: Lena Delta, Russian Arctic (April 1999, photo W. Schneider, AWI); B: Haskard Highlands, Antarctica (December 1994, photo W.-D. Hermichen, AWI); C: Mars, Northern Hemisphere (May 1999, photo NASA).

## 9 Microbial Life in Terrestrial Permafrost: Methanogenesis and Nitrification

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**Fig. 9.3** Landscape and soils of polygon tundra, Lena Delta/Siberia. A: Low-centered polygons; B: *Typic Historthel* of the polygon center; C: *Glacic Aquiturbel* of the polygon border (photos L. Kutzbach, AWI).

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gaining energy by the oxidation of inorganic substances. Carbon dioxide can be used as the only carbon source. Lithoautotrophic growth is an important presumption for long-term survival [19] of microbes in extreme environments like terrestrial permafrost or maybe on other planets of our Solar System.

### 9.3.1 Methanogenesis

Responsible for the biogenic methane production (methanogenesis) is a small group of microorganisms called methanogenic archaea [20]. Methanogenesis represents the terminal step in carbon flow in many anaerobic habitats, including permafrost soils, marshes and swamps, marine and freshwater sediments, flooded rice paddies and geo-thermal habitats. Although methanogens are widely spread in nature they show an extremely specialized metabolism. They are able to converse only a limited number of substrates (e.g., hydrogen, acetate, formate, methanol, methyl- amines) to methane. In permafrost soils two main pathways of energy-metabolism dominate: (i) the reduction of CO<sub>2</sub> to CH<sub>4</sub> using H<sub>2</sub> as a reductant and (ii) the fermentation of acetate to CH<sub>4</sub> and CO<sub>2</sub>. In the case of CO<sub>2</sub>-reduction organic carbon is not necessary for growth of methanogenic archaea [21].

At present, 68 species of methanogenic archaea are known including common genera like *Methanosarcina*, *Methanobacterium* and *Methanococcus*. Phylogenetically, they are classified as ARCHAEA [22], a group of microbes that are distinguished from BACTERIA by some specific characteristics (e.g., cell wall composition, coenzymes). They show a high adaptability at extreme environmental conditions like temperature, salinity and oxygen. Besides the mesophilic species, also thermophilic methanogens are known (see Chap. 11, Stetter). In newer times, more attention has been paid on the search for psychrophilic strains since many of methanogenic habitats belong to cold climates [23]. A lot of methanogens (e.g., *Methanogenium cariaci*, *Methanosarcina thermophila*) are able to adapt to high salinity by the accumulation of compatible solutes to equalize the external and internal osmolarity [24]. Although, they are regarded as strictly anaerobic organisms without the ability to form spores or other resting stages, they are found in millions of years old permafrost sediments [25] as well as in other extreme habitats like aerobic desert soils [26] and hot springs [27].

Because of the specific adaptations of methanogenic archaea to conditions like on early Earth (e.g., no oxygen, no or less organic compounds), they are considered to be one of the initial organisms from the beginning of life on Earth.

### 9.3.2 Nitrification

Nitrifying bacteria play a main role in the global nitrogen cycle by the transformation of reduced nitrogen compounds. Two groups of distinct organisms - the ammonia and nitrite oxidizers - are responsible for the oxidation of ammonia to nitrite and further to nitrate [28]. The genera of ammonia oxidizers have the prefix nitroso- whereas the nitrite oxidizers start with nitro-. The best known nitrifiers are *Nitrosomonas* and *Nitrobacter*. Up to now, 5 genera of ammonia oxidizers (with 16 species) and 4 genera of nitrite oxidizers (with 8 species) have been described [29, 30]. Phylogenetically,

ammonia and nitrite oxidizers are affiliated to different subclasses of the *Proteobacteria* with the exception of *Nitrospira* (and maybe *Nitrospina*), which belong to a separate phylum [31, 32]. Although *Nitrosomonas* and *Nitrobacter* are usually the most isolated nitrifiers, they are not obligately the most abundant ones in a given habitat. For example, organisms of the genus *Nitrospira* seem to have a higher ecological importance than previously assumed since they were recognized as dominant nitrite oxidizers in several aquatic habitats (reviewed by Speck and Bock [33]). These bacteria are the phylogenetic most ancient nitrifiers since they belong to a deep branching phylum. Here, a high diversity of new species was detected recently.

Nitrifiers exist in most aerobic environments where organic matter is mineralized (soils, compost, fresh- and seawater, waste water). In general, cell growth is slow with regard to the poor energy sources but can be adapted to changing environmental conditions. Especially for *Nitrobacter*, mixotrophic and heterotrophic growth with organic compounds is an alternative to the oxidation of nitrite. Nitrifiers are also active in low oxygen and anaerobic environments like sewage disposal systems and marine sediments where they are able to act as denitrifiers [34]. Although they form no endospores, they can survive long periods of starvation and dryness. Therefore, nitrifying bacteria were also detected in e.g., antarctic soils [35], natural stones [36], heating systems [32] as well as in subsurface sediments in a depth of 260 m [37]. Especially ammonia oxidizers form dense cell clusters, where cells are embedded in a dense layer of EPS (extracellular polymeric substances). These microcolonies may protect the cells against stress factors like dryness. Another protecting mechanism is the production or accumulation of compatible solutes (e.g., trehalose, glycine betaine or sucrose, see Chap. 12, Kunte et al.). Due to salt stress and dryness an increasing amount of compatible solutes was found in cells of *Nitrobacter* [38].

## 9.4 Methods for Analogue Studies of Microbial Processes in Terrestrial and Extraterrestrial Habitats

Increased attention has been paid on the processes of methanogenesis and nitrification in the last decades, because the involved bacteria [39] influence the global climate by the generation and transfer of climate-relevant trace gases like methane and gaseous nitrogenous oxides ( $\text{NO}$ ,  $\text{N}_2\text{O}$ ,  $\text{NO}_2$ ). Recently, these microorganisms are also important in the area of astrobiology research because of their adaptation ability to extreme location-conditions and consequently for the search of extraterrestrial lives of particular interest.

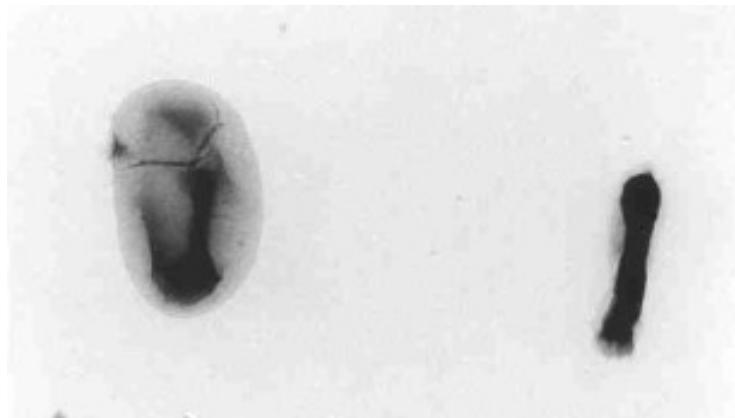
In order to understand the described microbial key-processes in permafrost soils, it is necessary to know the microbial community which is involved in methanogenesis and nitrification. The most important members of this community are those archaea/bacteria, which are metabolic active under such extreme conditions in the soils. To learn about these microbes and their adaptation strategies, they must be isolated and characterized. For quantitative aspects, bacterial cell numbers and microbial biomass have to be determined using classical microbiological and modern molecular biological techniques.

### 9.4.1 Methanogenic and Nitrifying Populations

A polyphasic approach is needed to reveal the diversity, population dynamics and ecological significance of bacteria in permafrost soils and sediments. Enrichment and isolation of microorganisms is necessary for taxonomical and ecophysiological characterization of microbial populations in order to understand their adaptation strategies and potential to extreme environmental conditions.

Traditionally, cell numbers of methanogenic archaea and nitrifying bacteria were quantified by the most probable number (MPN) technique in selective chemolithoautotrophic media [28, 40]. The highest dilution serves as initial inoculum for cultivation studies like identification and characterization of the relevant bacteria. Viable methanogens and nitrifiers were detected in the Kolyma-Indigirka Lowland in northeast Siberia by Russian and German scientists [41]. The bacteria occurred in high cell numbers in the upper layers and in decreasing numbers in more ancient deposits. MPN counts of methanogenic archaea varied between  $2.0 \times 10^2$  and  $2.5 \times 10^7$  cells g<sup>-1</sup>. Soina et al. [42] detected mesophilic nitrifying bacteria with  $2.5 \times 10^2$  cells g<sup>-1</sup> soil in a depth of 28 m. Lebedeva and Soina [17] found nitrifying bacteria in geological horizons up to 3 millions of years in a depth of 60 m. With increasing age of the sediments, psychrotrophic nitrifiers were found to be replaced by psychrophilic ones, although the permafrost communities are dominated by psychrotrophs [43]. Nevertheless, psychrophilic bacteria have a significant part in the microbial community in cold environments like permafrost soils [44]. The investigations of the methanogenic community on Taimyr Peninsula [45] and in the Lena Delta [46] gave hints for the adaptation to the low *in situ* temperatures. However, isolation of psychrophilic methanogens and nitrifiers from permafrost soils seems to be more complicated than in other physiological defined groups like acetogenic [47] and methane-oxidizing bacteria [48] as well as clostridia [49]. Cultivation at 5 °C of the slow growing microbes is hindered by prolonged lag-periods, which amounted to 2- 14 months in the case of nitrite oxidizers [50]. Therefore, the organisms had to be incubated at higher temperatures of e.g., 17 °C. So far, there was only one methanogenic archaea isolated from Ace Lake/Antarctica, which showed psychrophilic growth characteristics [51].

In order to obtain pure cultures for physiological characterization (e.g., determination of the temperature optima) isolation of typical bacteria is required. This can be done by serial dilution in liquid growth media or deep-agar tubes (Agar-shakes), plating on agar plates under aerobic or anaerobic conditions and is also possible by percoll density gradient centrifugation [52]. However, separation of aggregated cells is problematically and requires further treatment. Identification of isolates and enriched organisms was performed by traditional light and electron microscopy with genus-specific morphology and ultrastructure as criteria. Classified by their spiral cell shape, the ammonia oxidizers isolated from soil samples taken during the expedition “Berinia” in 1991 and 1992 were identified as members of the genus *Nitrosospira* (or *Nitrosovibrio*). Among nitrite oxidizers, *Nitrobacter* was identified by its pleiomorphic morphology and a polar cap of intra-cytoplasmic membranes [50]. In surface samples which were taken during the expedition “Lena 1999” [46], the coexistence of *Nitrobacter* and *Nitrospira* in enrichment cultures was demonstrated by their typical morphology (Fig 9.4) of pleiomorphic short rods (with a diameter of 0.8 µm) respectively spiral rods (with a diameter of 0.2 µm).



**Fig. 9.4** Enriched bacteria in nitrite oxidizing medium from the active layer of a permafrost soil (Samoylov/Lena Delta) with a morphology similar to *Nitrospira* respectively *Nitrobacter*. Negative staining was performed with uranyl acetate. Magnification 20300x.

Since the isolated organisms may not be the ecologically relevant ones, the development of new detection strategies was necessary to monitor the enrichment procedure. Modern microscopic techniques like CLSM (confocal laser scanning microscopy) in combination with fluorescent dyes enable specific or unspecific labeling of viable cells. In Fig. 9.5 an unspecific labeling of bacteria probably belonging to the genus *Nitrospira* is presented. Here, the organisms were affiliated by the formation of characteristic cell clusters. Like many ammonia oxidizers the *Nitrospira*-like bacteria were aggregated to micro-colonies.

New molecular techniques were developed for the detection of ecological relevant bacteria without cultivation [53, 54]. Fluorescence *in situ* hybridization (FISH) using population-specific gene probes targeting 16S rRNA enables direct microscopic enumeration of single cells (Fig. 9.6). Demanding low amounts of cell material, such methods are well suited for methanogenic archaea and nitrifying bacteria. Molecular 16S rDNA sequence analysis is required for phylogenetic affiliation of new isolates.

An immunological approach for the identification of nitrite as well as ammonia oxidizers was developed by Bartosch et al. [55] and Pinck et al. [56]. They used monoclonal and polyclonal antibodies, respectively, recognizing the key-enzymes of these functional groups of bacteria as phylogenetic marker. Nitrite oxidizers enriched from permafrost sediments were identified immunologically as members of the genus *Nitrobacter* [55]. These nitrifiers originated from sediments with an age of 40 000 years. Further on, in the active layer of a permafrost soil from Samoylov Island/Lena Delta nitrite oxidizers of the genus *Nitrospira* were detected by Hartwig [57]. Depending on the substrate concentration, *Nitrospira* together with *Nitrobacter* ( $0.2 \text{ g NaNO}_2 \text{ l}^{-1}$ ) or *Nitrobacter* alone ( $2 \text{ g NaNO}_2 \text{ l}^{-1}$ ) could be enriched. Both genera of nitrite oxidizers could be distinguished in Western blot analysis by different molecular masses of the  $\beta$ -subunit of their nitrite oxidizing systems (Fig. 9.7). This protein of *Nitrobacter* has a molecular mass of 65 kDa, whereas in *Nitrospira* 46 kDa were determined [55].

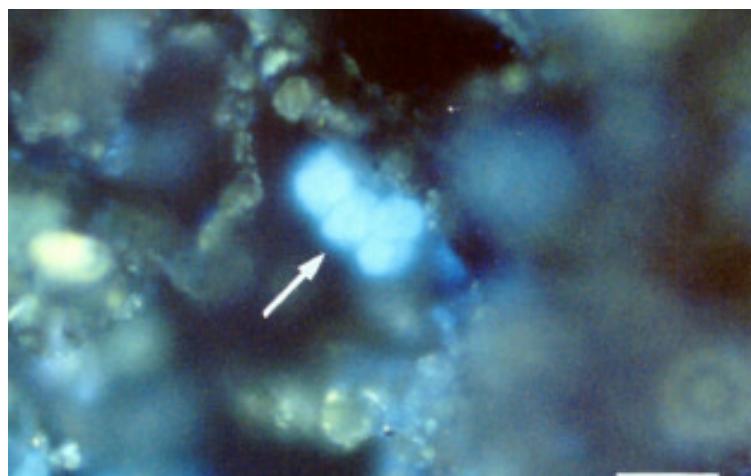
Phospholipid analysis in microbial ecology is a further method to study the biomass, population structure, metabolic status and activity of natural communities [59]. Specific groups of microorganisms (like the nitrite oxidizers) contain characteristic phospholipid ester-linked fatty acids (PLFA), whereas methanogenic archaea are characterized by ether-linked glycerolipids [60]. Lipid biomarkers are important for the detec-

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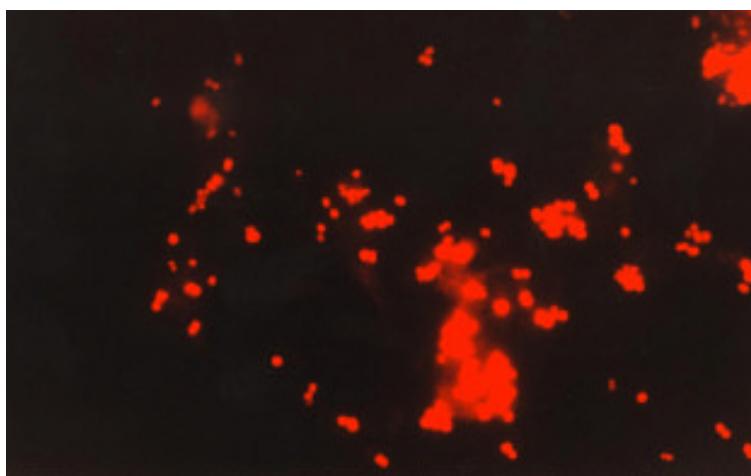
tion of single taxons. Such a characteristic new fatty acid (11-methyl-palmitate) was recently found in *Nitrospira moscoviensis* [61].

#### 9.4.2 *In situ* Activity

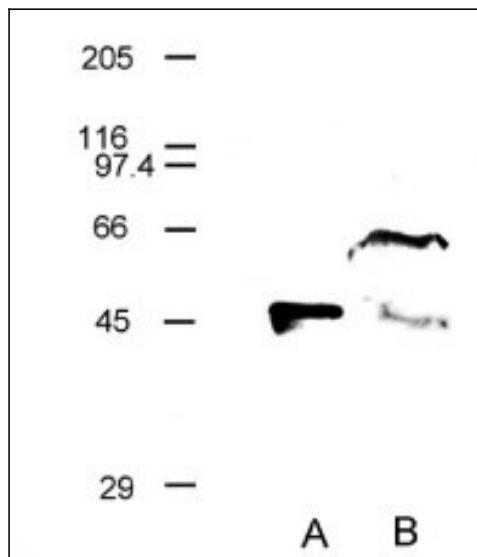
The activity of microorganisms depends not only on their own physiological capability but is influenced also by habitat-qualities like the grain size or the availability of nutrients. That is why, besides the characterization of the microflora, their activity in



**Fig. 9.5** With DAPI (4,6-diamidino-2-phenylindol) stained micro-colony of *Nitrospira*-like bacteria (arrow), enriched from the active layer of a permafrost soil (Samoylov/Lena Delta). Bar = 25 µm (photo C. Hartwig, University of Hamburg).



**Fig. 9.6** Confocal microscopy of archaea from the family *Methanomicrobiales* enriched from permafrost soils (Lena Delta/Siberia). The culture was grown with H<sub>2</sub>/CO<sub>2</sub> (80:20, v:v) at 10°C. The hybridization was carried out with the oligonucleotide probe MG1200 [54] (photo S. Kobabe, AWI).



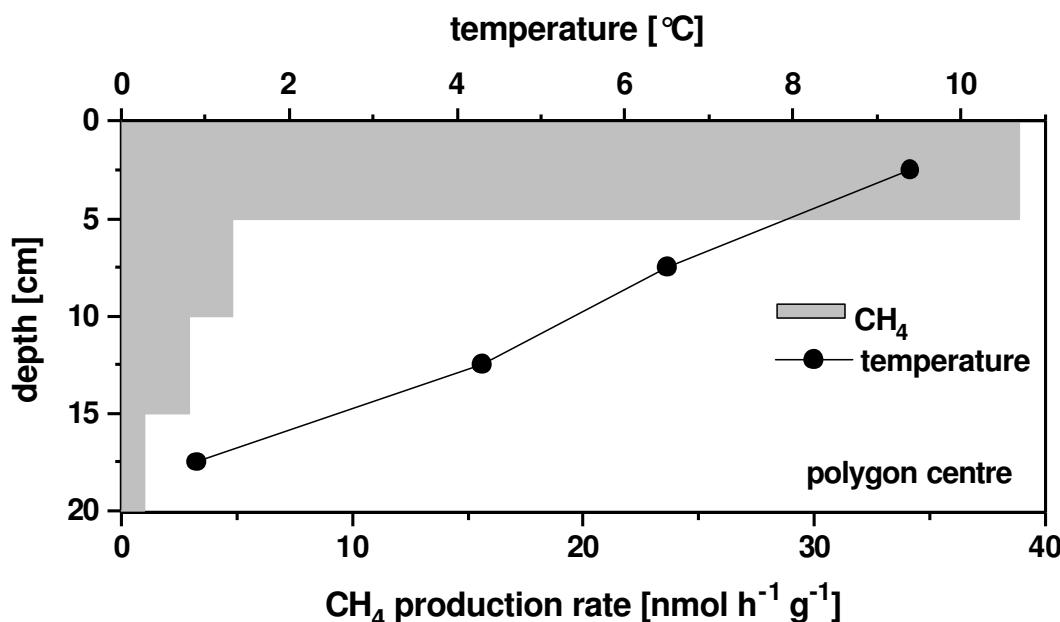
**Fig. 9.7** Immunoblot of an enrichment culture derived from permafrost soil using monoclonal antibodies recognizing the  $\beta$ -subunit of the nitrite-oxidizing system. The values on the left are molecular masses in kDa. A: pure culture of *Nitrospira moscoviensis*, B: enrichment culture using 0.2 g NaNO<sub>2</sub> l<sup>-1</sup> (modified from Bartosch et al. [58]).

the natural habitat is of importance for the understanding of life under extreme conditions. There are different methods for analyzing *in situ* activities, i.e. determination of concentration gradients [62], flux measurements [63] and assay of activity in soil samples [64]. A new technique for the determination of nitrification rates *in situ* is the introduction of microelectrodes (e.g., for ammonia and nitrate). These sensors make it possible to monitor metabolic reactions in the nitrogen cycle [65].

The activity of methanogenic archaea can be followed by the measurement of the metabolic end product CH<sub>4</sub> over a period of time. Methane generation takes place only under anaerobic conditions in the permafrost soils and sediments, for example in the water saturated soils of the polygon center. *In situ* rates of methanogenesis can only be obtained if the anaerobic food chain is not affected by the experimental procedure because methanogenesis depends on the substrates produced by other microorganisms. The *in situ* methane production can be investigated by incubation of soil samples from permafrost sites. Figure 9.8 shows the *in situ* methane production in dependence from the natural temperature gradient of a permafrost soil. For this investigation, fresh soil material was used. The prepared soil samples were re-installed in the same layers of the soil profile from which the samples had been taken [46].

The influence of soil texture on the activity of microorganisms can be examined by incubation experiments with model soils of a different grain size [66]. Changing temperature and pressure conditions as well as the impact of different substrates on microbial activity can be studied in special simulation experiments with undisturbed soil samples (see 9.4.4).

To estimate the nitrifying activity in permafrost sediments, the potential activity of soil bacteria was determined under optimal laboratory conditions. For that purpose, soil samples were taken from drill cores and transferred to the laboratory under sterile



**Fig. 9.8** Vertical profile of in situ methane production and soil temperature for a permafrost soil of the polygon center.

conditions. In the active layer and in sediments with an age of 40 000 years the nitrifying activity was higher at 28 °C in comparison to 17 °C, whereas in more ancient deposits (0.6-1.8 million of years and 2.5-3 million of years) the bacteria preferred lower temperatures of 17 °C (Lebedeva, pers. comm.).

Further investigations about nitrifying bacteria in permafrost sediments included measurements of ammonia, nitrite and nitrate as substrates respectively products of nitrification. Janssen [50] determined the concentrations of these nitrogen compounds in the soil samples by high-performance-liquid chromatography. The profiles showed that nitrite and nitrate were always found in the ppm range in sediments up to 150 000 years and occasionally in deeper layers. Ammonia concentrations amounted up to 100 ppm with increasing amounts in sediments with an age of 1-5 millions years. Nitrite and nitrate correlated with the presence of nitrifying bacteria although nitrifiers were also detected in samples without these nitrogen salts. The detection of the chemical unstable metabolic intermediate nitrite in correlation with the presence of viable ammonia oxidizers gave first evidence of modern microbial activity in permanently frozen sediments.

#### 9.4.3 Isotopic Analysis: Carbon Fractionation via Microbial Processes in Permafrost

It is well known that microbial processes tend to fractionate the C-isotopes of organic matter in soils and sediments by favoring the lighter <sup>12</sup>C-carbon over the heavier <sup>13</sup>C-compounds. Methanogenesis for example leads to the strongest C-discrimination in nature with the result that soil organic matter will be enriched with <sup>13</sup>C-carbon (e.g.,

$\delta^{13}\text{C}$ -values of about  $-16\text{\textperthousand}$  to  $-22\text{\textperthousand}$ ) while the product of anaerobic decay - methane - will be depleted with  $^{13}\text{C}$  (values of about  $-60\text{\textperthousand}$  [67]). In anaerobic zones of permafrost soils with methane production the soil organic matter showed an absolute enrichment of  $^{13}\text{C}$ -carbon of about  $3.7\text{\textperthousand}$  to  $8.3\text{\textperthousand}$  [45, 68]. Therefore, isotope-related analysis in combination with the microbial studies may be a powerful tool to search for traces of microbial life in extraterrestrial habitats, even if the applicability for extraterrestrial environments could not be examined until now sufficiently [69].

#### 9.4.4 Simulation Experiments

The influence of environmental conditions on the activity and survival of microorganisms could be investigated by simulation experiments with bacterial cultures and with undisturbed soil material.

Thawing and freezing processes influence not only the soil temperature regime but also the availability of liquid water, the pressure conditions and the salinity of pore water. They produce also granular, platy and vesicular soil structure in the surface near horizons and a massive structure in the subsurface zones. Undisturbed soil samples (soil cores of different size) save the structure, pore system and stratification of the natural soil, which influence the interaction between microbes and soil matrix. Simulation of freeze-thaw cycles can help to understand: how will the microbial population be influenced by the natural permafrost system and by the interaction of the combined parameters?

The viability of the permafrost microflora under the environmental conditions of Martian atmosphere can be investigated by simulation experiments in special ice laboratories (Alfred Wegener Institute for Polar and Marine Research, AWI) and in a special Martian simulator (German Aerospace Center, DLR). Natural soil material and pure cultures of bacteria isolated from terrestrial permafrost habitats can be exposed to extreme cold temperatures ( $-60\text{ }^{\circ}\text{C}$ ), lower pressure (560 Pa), higher background radiation (UV 200 nm), drier soil moisture conditions and varying ice contents in comparison with well known terrestrial permafrost.

### 9.5 Conclusion and Future Perspectives

Microbial life in permafrost soils depends on available water (see Chap. 5, Brack). If inorganic compounds like hydrogen as well as ammonia or nitrite are present as substrates, conditions are favorable for the growth of methanogenic archaea and nitrifying bacteria. Since cell synthesis is carried out by the assimilation of carbon dioxide there is no further need for organic material. This mineralic basis resembles the situation on mars (e.g., C, H, O, N, P, K, Ca, Mg and S, reviewed by Horneck [70]). Lithoautotrophic bacteria are well investigated and ubiquitous distributed organisms on Earth. They survived even in terrestrial permafrost for several millions of years [41]. Here, they demonstrate the residue of the autochthon population within the paleosoils which was enclosed during deposition of fresh sediments. The frozen microorganisms in the deeper permafrost sediments are thought to have not evolved significantly during the

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past several million years because it was not necessary to adapt to their environment [16]. In contrast, the microbes living in the active layer and the transition permafrost sediments are influenced by extreme changes of live-decisive environmental conditions. Preserving their viability in such an extreme environment they had to develop different strategies to resist desiccation, freezing processes and nutrient-lack. The isolation and characterization of methanogenic archaea and nitrifying bacteria from permafrost soils should clarify the possible growth characteristic (psychrophile and psychrotroph) and ecological significance of these microbes.

The data obtained from future research on living conditions and adaptation strategies of microorganisms in terrestrial permafrost soils should be compared with the postulated environmental conditions on early Mars [1, 71]. They were characterized by liquid water, a moderate climate and a postulated biosphere which had been dominated by anaerobic processes and diversification of anaerobic organisms. Furthermore, the comparative system studies will serve for understanding the modern Mars cryosphere and other extraterrestrial permafrost habitats. This knowledge represents an essential basis for the understanding of the origin of life and the environmental development on extreme habitats.

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## 6.3

# Stress response of methanogenic archaea from Siberian permafrost compared to methanogens from non-permafrost habitats

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### Keywords

methanogenic archaea, permafrost, low temperature, stress response, life on Mars

### Abstract

We examined the survival potential of methanogenic archaea exposed to different environmental stress conditions such as low temperature (down to -78.5 °C), high salinity (up to 6 M NaCl), starvation (up to 3 months), long-term freezing (up to 2 years), desiccation (up to 25 days) and oxygen exposure (up to 72 hours). The experiments were conducted with methanogenic archaea from Siberian permafrost and were complemented by experiments on well-studied methanogens from non-permafrost habitats. Our results indicate a high survival potential of a methanogenic archaeon from permafrost when exposed to the extreme conditions tested. In contrast, these stress conditions were lethal for methanogenic archaea isolated from non-permafrost habitats. A better adaptation to stress was observed at a low temperature (4 °C) compared to a higher one (28 °C). Given the unique metabolism of methanogenic archaea in general and the long-term survival and high tolerance to extreme conditions of methanogens investigated in this study, methanogenic archaea from permafrost should be considered as primary candidates for possible subsurface Martian life.

## Introduction

Permafrost on Earth, which covers around 24 % of the land surface, is a significant natural source of methane (Fung *et al.*, 1991; Wagner *et al.*, 2003, Smith *et al.* 2004). The processes responsible for the formation of methane in permafrost soils are primarily of biological origin, carried out by methanogenic archaea, a small group of strictly anaerobic chemolithotrophic organisms. They can grow with hydrogen as an energy source and carbon dioxide as the only carbon source. In addition to this specific metabolism methanogens are able to convert only a limited number of organic substrates (acetate, formate, methanol, methylamines) to methane (Conrad, 2005). Methanogenic archaea are widespread in nature and highly

<sup>#</sup> since the submission of the thesis the paper is published in *FEMS Microbiology Ecology* 61, 16-25 (2007)

abundant in extreme environments tolerating low/high temperatures (permafrost, hot springs), extreme salinity (saltern ponds) and low/high pH (solfataras, soda lakes). In addition to mesophilic species, thermophilic and hyperthermophilic methanogens have also been identified (Stetter *et al.*, 1990; Garcia *et al.*, 2000). Recently, more attention has been paid to the isolation of psychrophilic strains since a number of methanogenic habitats are located in cold climates (Gounot, 1999). So far, only a few strains (e.g. *Methanococcoides burtonii*, *Methanogenium frigidum*, *Methanosarcina* spec.) have been isolated from cold habitats (Franzmann *et al.*, 1992; Franzmann *et al.*, 1997, Simankova *et al.*, 2003). Although the metabolism of methanogenic archaea was studied in different environments (Shuisong & Boone, 1998; Garcia *et al.*, 2000; Eicher, 2001; Lange & Ahring, 2001), only few studies focussed on the ecology of the methanogenic archaea exposed to the permafrost's harsh environmental conditions like sub-zero temperatures, low water activity and low nutrient availability (Vishnivetskaya *et al.*, 2000; Høj *et al.*, 2005, Ponder *et al.*, 2005, Ganzert *et al.*, 2006).

Furthermore, permafrost is in the main focus of extraterrestrial research in astrobiology, because it is a common phenomenon in our solar system. Evidence of cryotic systems on present-day Mars (patterned ground, glaciers and thermokarst) has been found by *Mars Express*. The possibility of extant or extinct life on Mars has been fueled by the recent U.S. Mars Exploration Rover Opportunity discovery that liquid water most likely exists on Mars (Christensen *et al.*, 2004; Klingelhofer *et al.*, 2004) and findings from the Planetary Fourier Spectrometer onboard of *Mars Express*, as well as ground-based observations, indicating that methane currently exists in the Martian atmosphere (Formisano *et al.*, 2004). Considering the short lifetime of methane, this trace gas could only originate from active volcanism – which has not yet been observed on Mars – or from biological sources. There is evidence that prior to 3.8 Ga ago, when terrestrial life arose, environmental conditions on Mars were most likely similar to those on early Earth (Carr, 1989; Durham *et al.*, 1989; Wharton *et al.*, 1989; McKay & Davis, 1991; McKay *et al.*, 1992; Carr, 1996). If life had also emerged on Mars, it either subsequently adapted to the drastically changed environment or it became extinct. One possibility for survival of Martian microorganisms could be in lithoautotrophic subsurface ecosystems such as deep sediments near the polar ice caps and in permafrost regions. In the light of this assumption, methanogens from terrestrial permafrost habitats could be considered as analogues for probable extraterrestrial organisms.

The objective of this study was to characterize the potential stress response of methanogenic archaea from Siberian permafrost exposed to different extreme environmental conditions. In particular, high salinity, low temperature, starvation, desiccation and exposure to oxygen were studied. Particular emphasis was placed on *Methanosarcina* spec. SMA-21 isolated from the active layer of permafrost. Previous studies had shown that this archaeon exhibit a high survival potential under simulated Martian conditions (Morozova *et al.*, 2006). In comparison, two methanogens from non-permafrost habitats have been used. The study will contribute to an improved understanding of extraterrestrial life, if present, especially with regards to possible protected niches on present-day Mars.

## Materials and methods

### Microbial cultures

Permafrost samples were obtained from Samoylov Island (N 72°22', E 126°28'), located within the central part of the Lena Delta, Siberia. A detailed description of the geomorphologic situation of the island and the whole delta was given previously (Schwamborn *et al.*, 2002; Wagner *et al.*, 2003). To enrich and isolate methanogenic archaea the bicarbonate-buffered,

oxygen-free OCM culture medium was used, prepared according to Boone *et al.* (1989). The cultures were grown under an atmosphere of H<sub>2</sub>/CO<sub>2</sub> (80/20, v/v) as substrate. Cultures were incubated at 4 °, 10 ° and 28 °C.

*Methanosarcina* spec. SMA-21 (isolated from permafrost sediments sampled in summer 2002 from Siberian permafrost, Russia) grew well at 28 °C and more slowly at low temperatures (4 °C and 10 °C). The strain appeared as irregular cocci, 1-2 µm in diameter. Large cell aggregates were regularly observed. *Methanobacterium* spec. MC-20 from non-permafrost sediments from Mangalia, Romania was isolated at 28 °C. The cells were rod-shaped, 1-2 µm in width and a maximum of 8 µm in length. *Methanosarcina barkeri* DSM 8687, originating from a peat bog in Northern Germany (Maestrojuan *et al.*, 1992), was obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany).

### Salt stress experiments at different temperatures

The effect of salt shock on methanogenic archaea was studied using the *Methanosarcina* spec. SMA-21, as well as *Methanobacterium* spec. MC-20 and *Methanosarcina barkeri*, which were used as reference organisms. An aliquot of 5 ml of each culture grown to a cell density of 10<sup>8</sup> cells ml<sup>-1</sup> was supplemented with anaerobic salt solution and incubated at 4 °C and 28 °C for up to 3 months. The selected NaCl end concentrations were 0 M, 0.1 M, 0.2 M, 0.3 M, 0.4 M, 1.0 M, 3.0 M and 6 M (saturated). Sterilized cultures (2 h at 121 °C) supplemented with 0.4 M and saturated salt solution were used as negative controls. The cell numbers and activities were measured as described below.

After having been stored in concentrated salt solution for just over 3 months, the 5 ml aliquot of each culture was placed into the fresh OCM medium and supplemented with the appropriate substrates (H<sub>2</sub>/CO<sub>2</sub> for *Methanosarcina* SMA-21 and *Methanobacterium* MC-20; methanol for *Methanosarcina barkeri*). Survival was calculated according to cell count and activity measurements. All the experiments were done in triplicate.

### Freezing experiments

Cultures of *Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri* grown to a cell density of 10<sup>8</sup> cells ml<sup>-1</sup> were divided into two portions; one portion was immediately frozen at -78.5 °C, and the other one was cold shocked at 10 °C for 2 h before being frozen at -78.5 °C. For each portion, an aliquot of 1 ml was removed just before freezing. After storage at -78.5 °C for 24 h, the frozen cells were thawed at room temperature. Cell numbers were calculated before and after the freezing as described below. After thawing, aliquots were placed under anaerobic conditions in 25 ml glass flasks, supplemented with 10 ml of fresh OCM medium and appropriate substrates (H<sub>2</sub>/CO<sub>2</sub> for *Methanosarcina* SMA-21 and *Methanobacterium* MC-20; methanol for *Methanosarcina barkeri*). The flasks were sealed with a screw cap containing a septum and incubated at 28 °C. The activities were measured as described below.

In addition, *Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri* grown at 28 °C to a cell density of 10<sup>8</sup> cells ml<sup>-1</sup> were slowly frozen (0.2 °C min<sup>-1</sup>) to -20 °C. Initial methane production rates were measured before freezing and compared to those obtained after thawing for samples held at -20 °C for a period of 1 to 2 years. Once the samples were thawed, an aliquot of 5 ml of each culture was placed into the fresh OCM medium and supplemented with the appropriate substrates. Survival was calculated as described below. All the experiments were done in triplicate.

### Starvation experiments at different temperatures

Cultures of *Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri* grown to a cell density of  $10^8$  cells ml $^{-1}$  were harvested by centrifugation (10 min at 15000 g), washed twice, resuspended in PBS (Phosphate Buffered Saline), and divided into six portions. For each portion, an aliquot of 1 ml was placed in a 25 ml glass flask, supplemented with 10 ml of a MM mineral medium without any carbon source (Boone *et al.*, 1989) and stored for 1, 2 and 3 months at 4 °C and 28 °C. Having been stored without substrates, the 1 ml aliquots of each culture were then placed into the fresh OCM medium, supplemented with the appropriate substrates and incubated at 28 °C. Survival was calculated as described below. All the experiments were done in triplicate.

### Desiccation experiments

The effect of desiccation on methanogenic archaea was studied using the strains of *Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri*. An aliquot of each culture grown to a cell density of  $10^8$  cells ml $^{-1}$  was placed onto microscope cover slips (1 ml per cover slip) and allowed to dry completely. For some experiments glass beads (1.0 g, 1 mm diameter) were added to cell suspension. Cover slips were stored anaerobically at 28 °C for 2, 5, 7 and 25 days. Cells were rehydrated by placing the cover slip in 2 ml of the appropriate growth medium for 30 min at room temperature. The resulting cell suspensions were placed under anaerobic conditions into 25 ml glass flasks, supplemented with 10 ml of the fresh OCM medium and appropriate substrates. The flasks were sealed with a screw cap containing a septum and incubated at 28 °C. Survival was determined as described below. All the experiments were done in triplicate.

### Oxygen exposure experiments

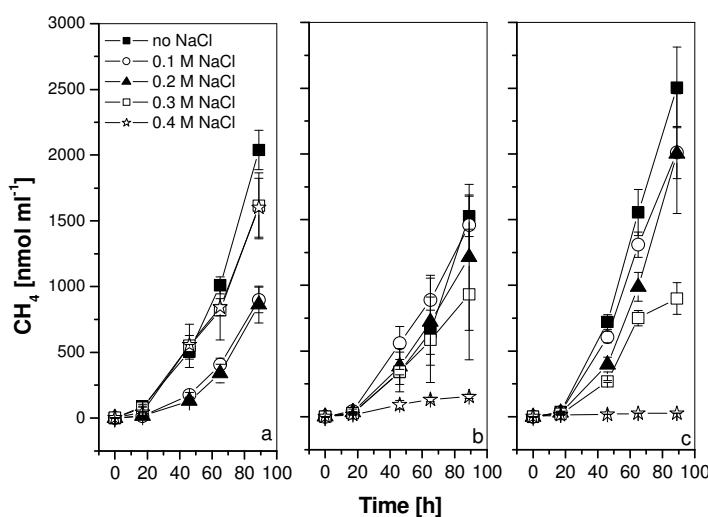
The oxygen sensitivity of methanogenic archaea was investigated using the permafrost strain *Methanosarcina* SMA-21 and non-permafrost strain *Methanobacterium* MC-20. An aliquot of the culture grown to a cell density of  $10^8$  cells ml $^{-1}$  was placed onto microscope cover slips (1 ml per cover slip) and exposed to aerobic conditions. The cover slips were stored at room temperature for 1, 3, 24, and 72 hours. Then cell suspensions were placed under anaerobic conditions into 25 ml glass flasks, supplemented with 10 ml of the fresh OCM medium and H<sub>2</sub>/CO<sub>2</sub>. The flasks were sealed with a screw cap containing a septum and incubated at 28 °C. The activities and cell numbers before and after oxygenation were detected as described below. The oxygen sensitivity of *Methanosarcina barkeri* was investigated previously (Zhilina, 1972; Kiener and Leisinger, 1983; Fetzer *et al.*, 1993).

### Methane analysis

The activity of the methanogenic archaea was calculated based on the linear increase of the CH<sub>4</sub> concentration in the headspace. The methane concentration was measured by gas chromatography. The gas chromatograph (Agilent 6890, Fa. Agilent Technologies) was equipped with a Carbonplot capillary column ( $\varnothing$  0.53 mm, 30 m length) and a flame ionisation detector (FID). Both the oven and the injector temperature were 45 °C. The temperature of the detector was 250 °C. Helium served as the carrier gas. All the gas sample analyses were done following calibration with standards of the respective gases.

### Cell counts determination

Cell numbers were calculated by Thoma cell counts and by fluorescence *in situ* hybridization (FISH) using the universal oligonucleotid probe for Archaea (ARC915 Cy3). For microscopic performance a Zeiss Axioskop 2 equipped with filters 02 (DAPI), 10 (FLUOS, DTAf) and 20 (Cy3), a mercury-arc lamp and an AxioCam digital camera for recording visualization of cells was used. The counting was done manually. For each hybridisation approach and sample at least 800 DAPI stained cells were counted on 30 randomly chosen counting squares. Microscopic performance was carried out using a magnification of 63 x 100 giving an area of  $3.9204 \times 10^{-2} \text{ mm}^2$  per counting square.



**Fig. 1.** Methane production of the permafrost strain *Methanosarcina* SMA-21 (a), and the reference organisms *Methanobacterium* MC-20 (b) and *Methanosarcina* barkeri (c), incubated with varying salt concentrations at 28°C (means  $\pm$  standard error, n=3).

### Statistical analyses

Significant differences between the three replicates used in the different stress experiments were analyzed using the Student's t-test (Wardlaw, 1985).

## Results

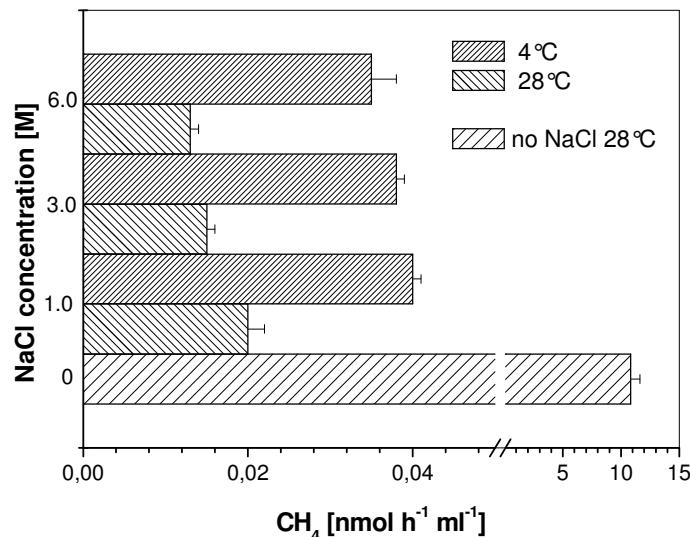
### Effect of salt stress on methanogenic archaea

Salt tolerance was assessed in the permafrost strain *Methanosarcina* SMA-21 and the non-permafrost organisms *Methanobacterium* MC-20 and *Methanosarcina* barkeri using NaCl salt solutions at different concentrations as an osmolite. High methane production of *Methanosarcina* SMA-21 was observed at all salt concentrations. The methane production of *Methanobacterium* MC-20 and the *Methanosarcina* barkeri was significantly different when exposed to different concentrations (Fig. 1).

The highest activity of *Methanosarcina* SMA-21 was detected in samples incubated with 0.3 and 0.4 M NaCl ( $18.14 \pm 2.81$  and  $17.98 \pm 2.51$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>, respectively), which was similar to the activity in the samples which had no salt added. The methane production rate at low salt concentrations (0.1 M and 0.2 M) was about a half as high as in the samples with no additional salt (Fig. 1). In contrast, increasing salt concentrations lead to a gradual decrease in the methane production of the *Methanobacterium* MC-20 and *Methanosarcina*

*barkeri*. Thus, the methane production rate of the non-permafrost organism *Methanobacterium* MC-20, incubated with 0.4 M NaCl ( $1.72 \pm 0.18$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>), was one order of magnitude lower compared to the samples with no additional salt, which had a methane production of  $17.13 \pm 1.72$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>. The methane production rates of *Methanosarcina barkeri*, incubated with 0.4 M NaCl, decreased from  $29.8 \pm 2.3$  to  $0.85 \pm 0.16$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>.

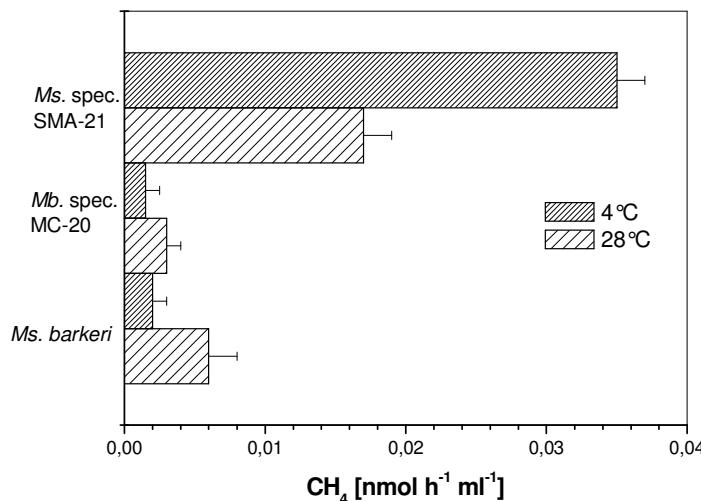
Higher methane production at low incubation temperature was observed in all *Methanosarcina* SMA-21 samples at all salt concentrations tested (Fig. 2). Moreover, a significant activity of *Methanosarcina* SMA-21 was observed even in cultures incubated in the saturated NaCl solution at 4 °C and 28 °C (Fig. 2). Based on the cell counts and on the methane production measured at different incubation temperatures, the methane production rates per cell and per hour were calculated. At 4 °C the methane production rates detected per methanogenic cell ( $0.1 \pm 0.0 \times 10^{-7}$  nmol CH<sub>4</sub> h<sup>-1</sup> cell<sup>-1</sup>) were five times higher than the methanogenic activity at 28 °C ( $0.027 \pm 0.0 \times 10^{-7}$  nmol CH<sub>4</sub> h<sup>-1</sup> cell<sup>-1</sup>).



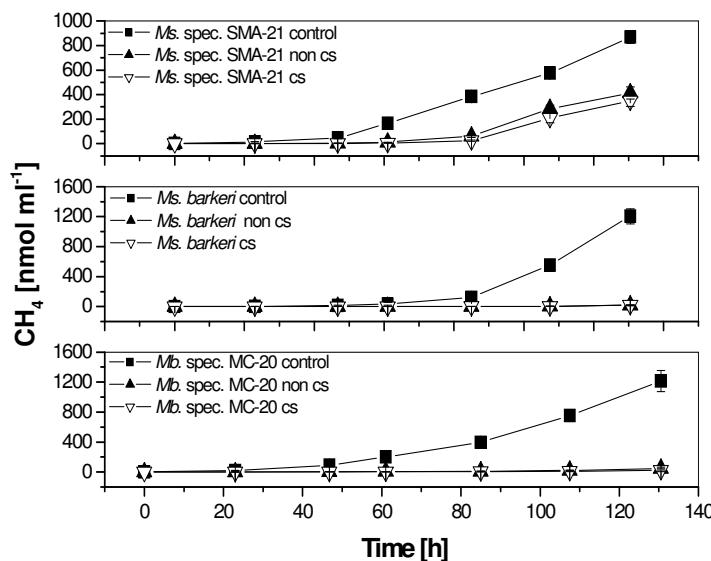
**Fig. 2.** Methane production rates of the *Methanosarcina* SMA-21 incubated in a concentrated salt solution (1-6 M NaCl) at 4 °C and 28 °C (means  $\pm$  standard error, n=3).

In contrast, the methane production rates of *Methanosarcina barkeri* and *Methanobacterium* MC-20 under salt saturated conditions were not very significant at 28 °C ( $0.01 \pm 0.002$  and  $0.003 \pm 0.0001$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> respectively), but they were still higher than those at 4 °C ( $0.002 \pm 0.0001$  and  $0.0014 \pm 0.0001$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> respectively) [Fig. 3]. Any viable cells were detected. No methane production was observed in the sterilized cultures.

When the cells were transferred to a fresh OCM medium after incubation under salt saturated conditions for a period of 3 months, methane production rates of *Methanosarcina* SMA-21 observed after one week were similar to those under standard growth conditions. Thus, the methane production rates per cell calculated for the recovering samples ( $1.4 \pm 0.04 \times 10^{-7}$  nmol CH<sub>4</sub> h<sup>-1</sup> cell<sup>-1</sup>) were comparably to the samples which had no salt added ( $1.9 \pm 0.06 \times 10^{-7}$  nmol CH<sub>4</sub> h<sup>-1</sup> cell<sup>-1</sup>). Conversely, no methane production was detectable after re-incubation of *Methanobacterium* MC-20 and *Methanosarcina barkeri* (data not shown).



**Fig. 3.** Methane production rates of *Methanosarcina barkeri*, *Methanobacterium MC-20* and *Methanosarcina SMA-21* incubated in a saturated salt solution at two different temperatures.



**Fig. 4.** Methane production of *Methanosarcina SMA-21*, *Methanosarcina barkeri* and *Methanobacterium MC-20* after freezing for 24 h at -78.5°C for cold shocked (cs) and non-cold-shocked (non cs) cultures in comparison to untreated control samples (means ± standard error, n=3).

### Freezing tolerance

The methanogenic strains *Methanosarcina SMA-21*, *Methanobacterium MC-20* and *Methanosarcina barkeri* showed significant differences in their ability to survive freezing at -78.5 °C for 24 h. Highest survival was seen in the *Methanosarcina SMA-21*. The average cell numbers of this archaeon decreased from  $4.37 \pm 1.4 \times 10^8$  cells ml<sup>-1</sup> at the beginning of the experiment to  $3.89 \pm 0.6 \times 10^8$  cells ml<sup>-1</sup> at the end of freezing, giving a survival rate of 89.5 %. In comparison, only 1 % of *Methanobacterium MC-20* and 0.8 % of *Methanosarcina barkeri* survived incubation at -78.5 °C. The decrease in cell numbers correlated well with the methane production rates of the cultures. The activity of *Methanosarcina SMA-21* measured

before freezing ( $10.87 \pm 1.22$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>) was only two times higher than the activity after the experiment ( $5.57 \pm 0.67$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>), while the methane production rates of the reference organisms *Methanobacterium* MC-20 and *Methanosarcina barkeri* decreased drastically after the experiment (Fig. 4). In particular, the methane production rates of the *Methanobacterium* MC-20 after freezing ( $0.21 \pm 0.07$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>) were two orders of magnitude lower than those before the experiment ( $19.53 \pm 1.59$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>), while the activity of the *Methanosarcina barkeri* was three orders of magnitude lower.

The potential of *Methanosarcina barkeri* to survive freezing at -78.5 °C was slightly higher when the culture was exposed to a temperature of 10 °C for 2 h prior to freezing (pre-cooling). The cultures transferred to 10 °C had a survival rate of 1.4 % and a methane production rate of  $0.06 \pm 0.01$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>. In contrast, a positive effect of pre-incubation at 10 °C on the ability to survive freezing at -78.5 °C was not seen for the *Methanosarcina* SMA-21 or the *Methanobacterium* MC-20 strains.

Most striking is that the *Methanosarcina* SMA-21 showed high survival rates and methane production of  $9.01 \pm 0.5$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> after two-years freezing at -20 °C. The measured methane production rates prior to freezing were  $10.58 \pm 0.8$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>. No methane production was detected in either reference organism after just one year of exposure to -20 °C.

### Temperature dependent starvation tolerance

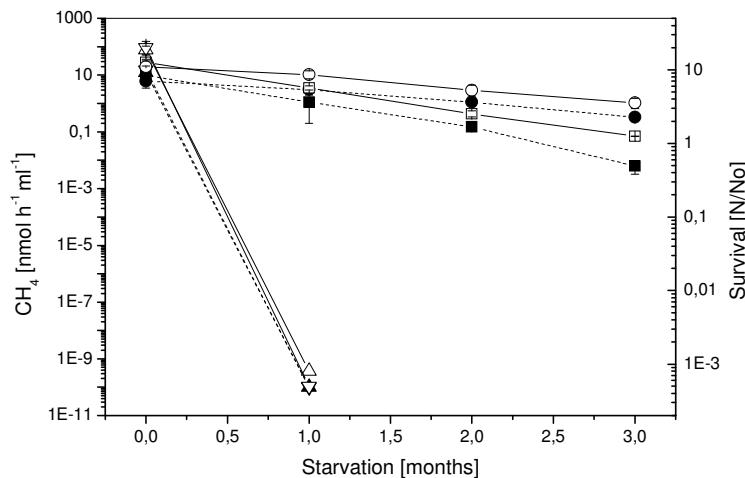
*Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri* were tested for their ability to survive substrate limiting conditions at different incubation temperatures. The *Methanosarcina* SMA-21 showed a high survival potential following the starvation experiment. Significant methane production of *Methanosarcina* SMA-21 was observed even in the cultures that had starved for 3 months ( $1.25 \pm 0.01$  and  $3.55 \pm 0.56$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> for cultures at 28 °C and 4 °C, respectively). Methane production rates were higher at 4 °C than these at 28 °C (Fig. 5). The different activities at different incubation temperatures correlated well with the viable cell numbers of this methanogenic archaeon. The average cell numbers of *Methanosarcina* SMA-21 after 3 months of starvation decreased from  $6.1 \pm 2.6 \times 10^8$  to  $3.28 \pm 1.9 \times 10^7$  cells ml<sup>-1</sup> at 4 °C and from  $9.2 \pm 2.8 \times 10^8$  to  $6.2 \pm 2.3 \times 10^5$  at 28 °C. Thus, survival potential of *Methanosarcina* SMA-21 at 4 °C was ten times higher than at 28 °C (Fig. 5).

In contrast, there was no survival of any cells of *Methanobacterium* MC-20 and *Methanosarcina barkeri* after 1 month of starvation, regardless of the incubation temperature. This was in accordance with the lack of any methane formation after re-incubation of *Methanobacterium* MC-20 and *Methanosarcina barkeri* (Fig. 5).

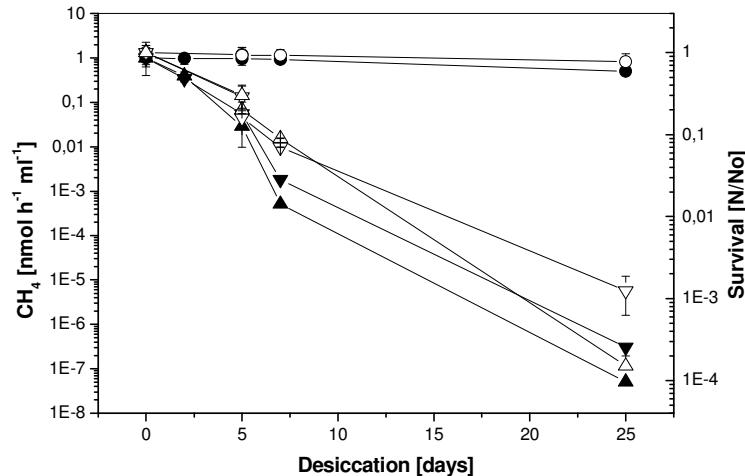
### Desiccation tolerance

The survival of the strains after desiccation was evaluated for up to 25 days of treatment. In general, the presence of glass beads strongly reduced the inhibitory effect of desiccation on survival. Survival and methane production rates for all the methanogenic strains (*Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri*) were higher with glass beads than without. However, *Methanosarcina* SMA-21 from permafrost showed significant differences in their desiccation resistance than did the reference organisms from non-permafrost habitats. The *Methanosarcina* SMA-21 was found to resist 25 days of desiccation without loss of activity and cultivability (Fig. 6). The average cell numbers of the *Methanosarcina* SMA-21 decreased from  $2.3 \pm 0.8 \times 10^8$  to  $1.8 \pm 0.4 \times 10^8$  cells ml<sup>-1</sup>, equivalent to a cell survival rate of 77.5%. The methane production rates decreased

slightly from  $10.46 \pm 2.34$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> to  $5.23 \pm 1.7$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>. Survival and methane production rates of both non-permafrost strains (*Methanobacterium* MC-20 and *Methanosarcina barkeri*) were drastically reduced after desiccation (Fig. 6). When tested for this ability, cells of the reference cultures were no longer able to grow after desiccation of 25 days.



**Fig. 5.** Methane production rates (dark symbols) and cell survival rates (open symbols) of starved cells of *Methanosarcina barkeri* (up-looking triangles), *Methanobacterium* MC-20 (upside down-looking triangles) and *Methanosarcina* SMA-21 at 4 °C (circles) and 28 °C (squares) [means ± standard error, n=3].

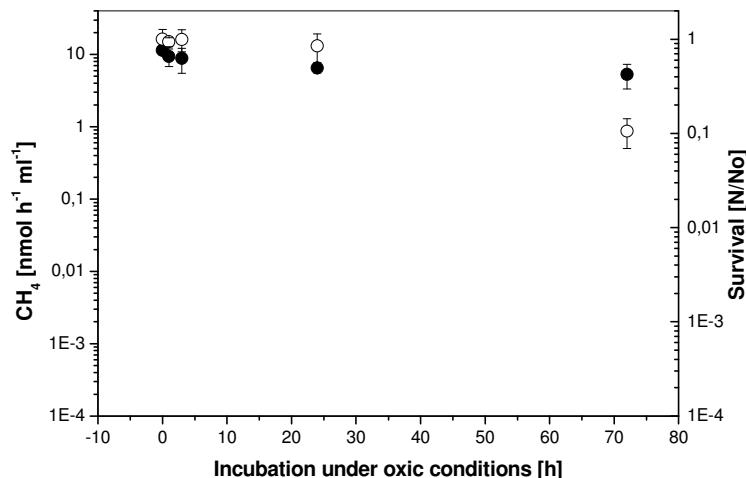


**Fig. 6.** Methane production rates (dark symbols) and cell survival rates (open symbols) of desiccated cells of *Methanosarcina barkeri* (up-looking triangles), *Methanobacterium* MC-20 (upside down-looking triangles) and *Methanosarcina* SMA-21 (circles) [means ± standard error, n=3].

### Oxygen sensitivity

The oxygen sensitivity of the *Methanosarcina* SMA-21 permafrost strain was examined by

determining cell viability and methane production following oxygenation. There was good agreement between these parameters. As shown in Fig. 7, exposure to oxygen for 1 to 3 h resulted in no significant effect on the cultivability and activity of the permafrost microorganism. The viability of *Methanosarcina* SMA-21 appeared to be only slightly affected by exposure to oxygen for 24 h, with a survival rate of 85 %. The calculated methanogenic activity decreased slightly from  $11.47 \pm 1.23 \text{ nmol CH}_4 \text{ h}^{-1} \text{ ml}^{-1}$  to  $6.46 \pm 0.9 \text{ nmol CH}_4 \text{ h}^{-1} \text{ ml}^{-1}$ . However, the survival potential of the permafrost strain was reduced after prolonged oxygen exposure. After 72 h of exposure, 90 % of the *Methanosarcina* SMA-21 cells had died. In contrast, 100 % of the *Methanobacterium* MC-20 cells had died after 24 h exposure to oxygen. No methane production was detected. These results were compared with a previous study investigating the survival potential of the *Methanosarcina barkeri* (Kiener and Leisinger, 1983). For this strain, exposure to oxygen for 10 to 30 h had no effect on cell numbers. A decrease in cell numbers and methane production was observed only after 48 h exposure to oxygen.



**Fig. 7.** Oxygen sensitivity (methane production rates, dark symbols and cell survival rates, open symbols) of *Methanosarcina* SMA-21 (means  $\pm$  standard error, n=3).

## Discussion

Different strains of methanogens, which include representatives from permafrost and non-permafrost habitats, exhibit marked differences in their stress tolerance. The methanogenic archaeon *Methanosarcina* SMA-21 from permafrost showed high resistance to high salinities, extremely low temperatures, desiccation, the presence of oxygen, and starvation. The stress tolerance of *Methanosarcina* SMA-21 was even higher at low incubation temperatures. In contrast, the reference organisms from non-permafrost habitats were sensitive to the extreme conditions tested. High cell numbers of reference strains were killed without lag upon exposure to these stress factors.

Terrestrial permafrost provides an opportunity to obtain microorganisms that have exhibited long-term exposure to cold temperatures, freeze-thaw cycles, starvation, aridity, and high levels of long-lasting back-ground radiation resulting from accumulation over geological time scales. In spite of the unfavorable living conditions, permafrost is colonized by high numbers of viable microorganisms ( $10^2$ - $10^8$  cells per g<sup>-1</sup>), including fungi, yeasts, algae and bacteria as well as highly specialized organisms like methanogenic archaea

(Vishnivetskaya *et al.*, 2000, Kobabe *et al.*, 2004, Wagner *et al.*, 2005). Seasonal variations in soil temperatures, particularly freeze-thaw cycles in the active layer, result in drastic changes in other environmental conditions such as water availability, salinity, soil pressure, desiccation, changing oxygen conditions, and the availability of nutrients. The permafrost microbial community, described as a “*community of survivors*” (Friedmann, 1994), has to resist this combination of extreme conditions as well as their extreme fluctuations. The high survival rates of methanogenic archaeon from permafrost compared to non-permafrost strains under the investigated stress conditions suggest that these microorganisms have developed ways to cope with stress which have to include repair of damaged DNA and cell membranes, and the maintenance of other vital functions needed to sustain cell viability. Our results indicate a higher resistance of the methanogenic archaeon *Methanosarcina* SMA-21 to increased salinity and lack of nutrients at low temperatures. An incubation temperature of +4 °C correlates well with *in situ* temperatures of the active layer of permafrost, fluctuating in summer months from 0 °C to about +10 °C. It remains to be determined if freeze protection mechanisms overlap with tolerance mechanisms, which protect against various other stresses such as desiccation, starvation or high salt concentration (Berry & Foegeding, 1997; Macario *et al.*, 1999; Cleland *et al.*, 2004; Georlette *et al.*, 2004).

*Methanosarcina* SMA-21 from Siberian permafrost was shown to be well adapted to a wide range of salt concentrations. Higher methane production rates, which were determined for *Methanosarcina* SMA-21 incubated with 0.3-0.4 M NaCl compared to the 0.1-0.2 M salt concentrations, indicate better adaptation to a rapid increase in osmolarity, which occurs while freezing of the active layer of permafrost. Again, the ability to resist the stress factor, in this case high salinity, was enhanced by low incubation temperatures. Furthermore, *Methanosarcina* SMA-21 cells remained to stay viable after three months incubation under salt saturated conditions. In contrast, increasing salinity leads to reduced activity of the reference organisms *Methanobacterium* MC-20 and *Methanosarcina barkeri* originated from non-permafrost sediments. The methane production of *Methanosarcina barkeri* and *Methanobacterium* MC-20 was marginal under salt saturated conditions and did not appear to be favourably influenced by low temperatures (Fig. 3). Also no viable cells of these strains could be detected after prolonged salt stress.

The salt tolerance could be associated with cold tolerance, a possibility which was also postulated by Vishnivetskaya (2000) and Gilichinsky (2003) and which is confirmed by the present results. *Methanosarcina* SMA-21 showed excellent survival of more than 70 % of the cells following freezing at -78.5 °C. Pre-conditioning to cold temperatures (cold shock), known to increase the resistance to freezing of many microorganisms due to the expression of cold-responsive genes and cryoprotectant molecules (Kim & Dunn, 1997; Wouters *et al.*, 2001; Georlette, 2004; Weinberg *et al.*, 2005), does not increase the freezing tolerance of *Methanosarcina* SMA-21. This correlates well with the resistance of this strain to a two-year exposure at -20°C without any pre-conditioning. Both results suggest that this strain is already adapted to sub-zero environments. Generally, all the cell components must be adapted to the cold to enable an overall level of cellular protection that is sufficient for survival and growth (Cavicchioli, 2006).

Starvation tolerance experiments with two different incubation temperatures were conducted to evaluate the ability of methanogenic archaea from permafrost and non-permafrost habitats to survive prolonged periods of nutrient limitation associated with the freezing of the active layer in permafrost habitats. Starvation stress was very efficient in reducing the survival potential of the reference strains. While the non-permafrost archaea (*Methanosarcina barkeri* and *Methanobacterium* MC-20) ceased to exist after one month of starvation, *Methanosarcina* SMA-21 maintained high survival rates even after being starved for 3 months. The slow metabolism rates of organisms in cold environments could be

important for successful adaptation to starvation conditions since this adaptation requires protein synthesis, the most energy demanding process in the cell (Thomsson *et al.*, 2005).

Prolonged desiccation stress was lethal for non-permafrost strains, whereas *Methanosarcina* SMA-21 survived for at least 25 days. Surprisingly, this methanogenic archaeon was able to produce methane immediately following rehydration, which indicates efficient repair mechanisms. The experiment was done at room temperature; colder temperatures might slow the rate of desiccation damage and lead to even longer survival periods. The survival and potential methane production of *Methanosarcina* SMA-21 was even higher in the presence of glass beads, which probably provided partial mechanical protection of methanogens against desiccation. This observation was the same, regardless if the methanogens were obtained from a DSMZ culture collection or freshly isolated from permafrost.

Exposure to oxygen, the last stress factor tested, occasionally occurs during late summer when the uppermost permafrost thaws and water table of the active layers decreased. Metabolic activity of methanogenic archaea within aerated soil slurries has been previously observed (Wagner *et al.*, 1999). Even without a protective soil matrix, the permafrost strain *Methanosarcina* SMA-21 still exhibits a marked oxygen resistance. The organisms survived for hours the presence of oxygen without any decrease in the cell numbers or methane production rates. Moreover, a significant percentage (10 %) of the population of *Methanosarcina* SMA-21 survived up to 72 hours of oxygenation. This is a very interesting result since methanogenic archaea are strictly anaerobic organisms, which are not known to have resting stages. These survival rates are high compared to those from earlier studies for *Methanosarcina barkeri* and other methanogenic archaea from ecosystems periodically subjected to oxygen stress (Kiener & Leisinger, 1983). Protection from oxygen may occur at the cellular level (e.g. superoxide dimutase, catalase and other SOD protective enzymes) or at the level of cell aggregates (Kiener & Leisinger, 1983; Brioukhanov *et al.*, 2006; Zhang *et al.*, 2006). The arrangements of cells aggregates, which have been regularly observed in *Methanosarcina barkeri* and *Methanosarcina* SMA-21 might lead to the protection of the cells in the interior and thereby secure survival during extended periods of oxygen stress. This assumption is in agreement with the data of Kobabe *et al.* (2004), who found aggregates of methanogenic archaea in the dried upper layers of soils in polygon depressions.

In summary, the high survival rates and activity of a *Methanosarcina* SMA-21 from Siberian permafrost under different stress conditions suggest that these organisms possess natural adaptation mechanisms to sub-zero temperatures, increased salinity, starvation, desiccation and oxygen stress and have efficient repair mechanisms that allow them to live under extreme fluctuating conditions of terrestrial permafrost, in contrast to other methanogens isolated from non-permafrost habitats, which probably lack such mechanisms. Most striking was the difference in survival potential between *Methanosarcina barkeri* and *Methanosarcina* SMA-21, two representatives of the same genus. Therefore, it is of great importance to sequence the genome of *Methanosarcina* SMA-21, as one of the representatives of a permafrost community. The characterization of the physiological traits potentially important to cryo-adaptation is necessary to begin understanding the adaptations at the genome level.

From the astrobiological point of view, the physiological potential and the metabolic specificity of *Methanosarcina* SMA-21 from permafrost provide very useful insight for the investigation of potential life in extremely cold environments on other planets of our solar system. We might conclude that the permafrost habitats on Earth represent an excellent analogue for studying putative life on Mars. Recent analyses of *Mars Express* HRSC (High Resolution Stereo Camera) images of many regions of the planet showed that the morphology of Martian polygonal features is very similar to the morphology of terrestrial ice-

wedge polygons and is most likely the result of comparable processes (Kuzmin, 2005). Although the experimental conditions presented here did not simulate all extreme permafrost environmental conditions, major stresses were simulated, which organisms in terrestrial permafrost and in Martian permafrost might be exposed to. The observation of high survival rates of permafrost methanogen under defined stress conditions as well as under simulated Martian conditions (Morozova *et al.*, 2006) supports the possibility that microorganisms similar to methanogens from Siberian permafrost could also exist in Martian permafrost habitats. Methanogenic archaea from terrestrial permafrost may therefore serve as useful models for further exploration of extraterrestrial life.

### Acknowledgements

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## 6.4 Survival of Methanogenic Archaea from Siberian Permafrost under Simulated Martian Thermal Conditions

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**Abstract** Methanogenic archaea from Siberian permafrost complementary to the already well-studied methanogens from non-permafrost habitats were exposed to simulated Martian conditions. After 22 days of exposure to thermo-physical conditions at Martian low- and mid-latitudes up to 90% of methanogenic archaea from Siberian permafrost survived in pure cultures as well as in environmental samples. In contrast, only 0.3%–5.8% of reference organisms from non-permafrost habitats survived at these conditions. This suggests that methanogens from terrestrial permafrost seem to be remarkably resistant to Martian conditions. Our data also suggest that in scenario of subsurface lithoautotrophic life on Mars, methanogenic archaea from Siberian permafrost could be used as appropriate candidates for the microbial life on Mars.

**Keywords** methanogenic archaea · permafrost · astrobiology · life on Mars · Mars simulation experiments

### Introduction

Of all the planets explored by spacecrafts in the last four decades, Mars is considered as one of the most similar planets to Earth, even though it is characterized by extreme cold and dry conditions today. This view has been supported by the current ESA mission *Mars Express*, which identified several different forms of water on Mars and methane in the Martian atmosphere (Formisano 2004). Because of the expected short lifetime of methane, this trace gas could only originate from active volcanism – which was not yet observed on Mars – or from biological sources. Data obtained by the *Mars Express* showed that water vapor and methane gas are concentrated in the same regions of the Martian atmosphere (European Space Agency 2004). This finding may have important implications for the possibility of microbial life on Mars (Moran et al. 2005). Furthermore, there is evidence that prior to 3.8 Ga ago, the environmental conditions on Mars may have been similar to those on early Earth (Carr 1989; Durham et al. 1989; Wharton

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et al. 1989; McKay and Davis 1991; McKay et al. 1992; Carr 1996). At this time microbial life had already started on Earth and Archaea are thought to have been among the earliest living organisms. If life had also emerged on Mars, it either adapted to the drastically changed environments or it became extinct. One possibility for survival of Martian microorganisms could be lithoautotrophic subsurface ecosystems such as deep sediments near polar ice caps and in permafrost regions, where liquid-like (unfrozen) adsorption water can play a key-role for transport of nutrients and waste products of biological processes (Möhlmann 2005). Evidence of permafrost occurrence on present Mars (patterned ground, glacier or thermokarst) has been found by *Mars Express*. Comparable environments exist in polar regions on Earth, for example Antarctic ice cores (Abyzov et al. 1998, 1999), Greenland glacial ice (Tung et al. 2005) and Siberian permafrost (Gilichinsky et al. 1993), where microorganisms existed for several million years independent of photosynthetic energy production (Gilichinsky and Wagener 1994; Vorobyova et al. 1998; Rivkina et al. 1998, Wagner et al. 2001).

Terrestrial permafrost, which covers around 24% of the Earth's surface, is a significant natural source of methane (Fung et al. 1991; Wagner et al. 2003, Smith et al. 2004). The processes responsible for the formation of methane in permafrost soils are primarily biological, carried out by methanogenic archaea, a small group of strictly anaerobic chemolithotrophic organisms, which can grow using hydrogen as an energy source and carbon dioxide as the only carbon source. They are widespread in nature and highly abundant in extreme environments, tolerating low/high temperatures (permafrost, hot springs), extreme salinity (saltern ponds) or low/high pH (solfataras, soda lakes). Beside mesophilic species, also thermophilic and hyperthermophilic methanogens are known (Stetter et al. 1990; Garcia et al. 2000). Recently, more attention has been paid to the isolation of psychrophilic strains, since many habitats in which methanogens are found belong to cold climates (Gounot 1999). So far, only a few strains (e.g., *Methanococcoides burtonii*, *Methanogenium frigidum*, *Methanosarcina* spec.) have been isolated from cold habitats (Franzmann et al. 1992, 1997; Simankova et al. 2003). Although the metabolism of methanogenic archaea has been studied in different environments (Ni and Boone 1998; Garcia et al. 2000; Eicher 2001; Lange and Ahring 2001), only a few studies have focussed on the ecology of the methanogenic archaea in permafrost ecosystems (Vishnivetskaya et al. 2000; Høj et al. 2005). Studies have shown that methanogenic archaea from Siberian permafrost are well adapted to osmotic stress and are also highly resistant to inactivation by desiccation, radiation, extremely low temperatures (Morozova and Wagner, data under processing) and high oxygen partial pressure (Wagner et al. 1999).

Few investigations have been performed under conditions applicable to Mars, particularly under water-stressed conditions (Sears et al. 2002). The present study focuses on the ability of methanogenic archaea to survive under simulated Martian thermal conditions. For this purpose, permafrost samples and pure cultures of methanogens were used. Their resistance renders these organisms eminently suitable for this purpose.

## Description of the Mars Simulation Experiment

### Biological samples

#### *Permafrost samples and preparation*

Permafrost samples were obtained from the Lena Delta, Siberia. The investigation site Samoylov Island (72°22'N, 126°28'E) is located within the central part of the Lena Delta,

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which is one of the largest deltas in the world with an area of about 32,000 km<sup>2</sup>. A detailed description of the geomorphologic situation of the island and the whole delta was given previously (Schwamborn et al. 2002). The Lena Delta is located within the continuous permafrost zone. It is characterized by an arctic continental climate with low annual air temperature of  $-14.7^{\circ}\text{C}$  ( $T_{\min}=-48^{\circ}\text{C}$ ,  $T_{\max}=18^{\circ}\text{C}$ ) and a low mean annual precipitation of 190 mm. The island is dominated by the typical permafrost pattern of low-centred polygons which cover at least 70% of the island's area. The soils in the Lena Delta are entirely frozen, leaving only 20–50 cm upper part, so-called 'active layer', remaining unfrozen during the summer months.

During the expedition 'Lena 2004' soil samples were collected from the active layer of two soil profiles. These profiles represent major characteristic geomorphic units of the island. They are different in regard to soil genesis and soil properties. One of these profiles was located at the depression of a low-centred polygon ( $72^{\circ}22'\text{N}$ ,  $126^{\circ}28'\text{E}$ ) in the eastern part of the island. The prevalent soil type of the polygon depressions was a *Typic Historthel*, classified according to the US Soil Taxonomy (Soil Survey Staff 1998). The samples from the polygon depression were characterized by a high content of organic matter and high porosity.

The second profile was located on a flood plain in the northern part of the island. At this location, annual flooding leads to a continuing accumulation of fluvial sediments. The substrate was dominated by sandy and silty fluvial material. The prevalent soil type of the flood plain was a *Typic Aquorthel* (Soil Survey Staff 1998). Additional soil characteristics, analysed according to Schlichting et al. (1995), are summarized in Table I. Soil samples were filled in gas-tight plastic jars (Nalgene) and transported to Germany in frozen condition. Approximately 10 g of each soil sample was used for dry weight determination. All results were expressed per gram of dry soil.

*Microbial cultures*

For enrichment and isolation of methanogenic archaea the bicarbonate-buffered, oxygen-free OCM culture medium was used, prepared according to Boone et al. (1989). The

**TABLE I** Selected soil properties of a polygon centre and a flood plain soil on Samoylov Island, Lena Delta

Depth (cm)	H <sub>2</sub> O content (%)	<i>C</i> <sub>org</sub> (%)	N (%)	Grain size fraction (%)		
				Clay	Silt	Sand
<b>Centre</b>						
0–5	85.7	15.5	0.7	2.4	18.6	79.0
5–10	77.3	15.1	0.4	2.8	24.0	73.3
10–15	80.6	16.1	0.4	2.6	18.6	78.8
15–20	73.4	7.3	0.2	7.9	15.4	76.6
20–25	58.9	2.2	0.2	6.1	18.2	75.7
25–30	68.5	4.7	0.2	5.0	25.9	69.2
<b>Flood plain</b>						
0–5	30.1	3.1	0.4	11.1	64.8	24.2
5–9	31.9	1.1	0.2	20.2	61.4	18.4
9–18	28.3	2.2	0.3	18.3	63.5	18.2
18–35	35.4	2.8	0.4	20.2	62.7	17.1
35–40	32.4	2.4	0.3	20.4	55.6	24.0
40–52	31.8	1.7	0.2	17.6	67.7	14.7

medium was anaerobically dispensed into vials and 10 g of permafrost sample from anoxic horizons of the floodplain were added. The head space was filled with an N<sub>2</sub>/CO<sub>2</sub> mixture (80:20, v/v). Methanol (20 mM) or H<sub>2</sub>/CO<sub>2</sub> (80:20, v/v) were used as substrates. Inoculated vials were incubated at 10 °C. For the isolation of methanogenic archaea, serial dilutions (1:10) were carried out and cultures were incubated at 28 °C. Growth of contaminants was inhibited by different antibiotics (5 g ml<sup>-1</sup> erytromycin or phosphomycin). Purity was checked microscopically and by lacking growth on medium containing 5 mM glucose, 5 mM pyruvate, 5 mM fumarate and 0.1% yeast extract.

All strains grew well at 28 °C and slowly at low temperatures (4 and 10 °C). The isolated strains showed different morphologies. *Methanosarcina* spec. SMA-21 cells were irregular cocci and 1–2 μm in diameter. Large cell aggregates were regularly observed. Cells of the strain SMA-16 were small irregular diplococci, 0.5–1 μm in diameter. Strain SMA-23 appeared as rod-shaped cells, ca. 1–2 μm in width and max. 10 μm in length, often forming long cell chains.

#### Reference organisms

*Methanobacterium* spec. MC-20 was isolated from a non-permafrost sediments from Mangalia, Romania at an incubation temperature of 28 °C. The cells were rod-shaped, 1–2 μm in width and max. 8 μm in length. *Methanosarcina barkeri* DSM 8687 was originated from peat bog in northern Germany (Sherer et al. 1983) and *Methanogenium frigidum* DSM 16458 (Franzmann et al. 1997) was originated from the water column of the Ace Lake, Antarctica. Both cultures were obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany).

#### The experimental set-up

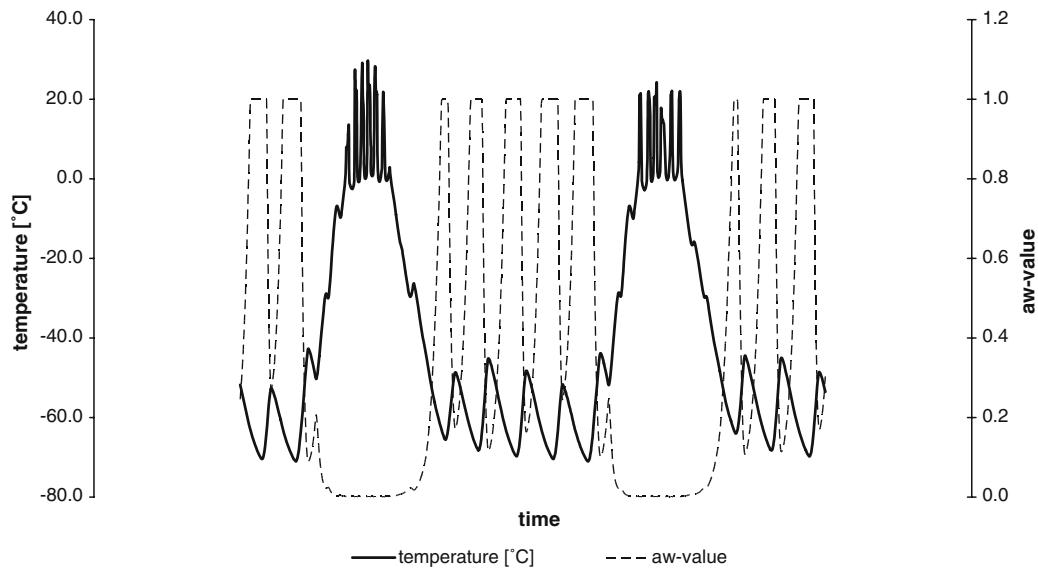
##### *Mars simulator*

Simulation of the thermal conditions, typical for Martian mid- and low-latitudes, was achieved in the laboratory for humidity related studies (HUMIDITY-Lab) of the German Aerospace Center (DLR), Institute of Planetary Research in Berlin. The ‘Cold chamber’ provided a combination of diurnal temperature fluctuations in the range from −75 to +20 °C and humidity fluctuations between  $a_w$ -values of 0.1 and 0.9 in a Mars-like atmosphere dominated by carbon dioxide (95.3%). The humidity corresponds to a water vapor pressure of about 10<sup>−3</sup> mbar (0.1 Pa) that equals to the average water vapor pressure on Mars (corresponding to 10 pr 4 μm). The simulation experiment was carried out in a 6 mbar Mars-like atmosphere for a period of 22 days (Figure 1). The average  $a_w$ -value was 0.52.

##### *Martian simulation experiments with permafrost soils*

To determine the influence of simulated Martian conditions on survival potential of methanogenic archaea in soil samples, fresh soil material (1 g) from the polygon depression (*Typic Historthel*, Oi horizon, 5–10 cm depth) and the floodplain (*Typic Aquorthel*, A horizon, 0–5 cm depth) was weighed into 12.5 ml plastic boxes (A/S NUNG, Denmark) under anoxic conditions. Three replicates were used for each soil type. Before and after the experiment the cell numbers were calculated as described in “Cell counts determination”. After exposure to Martian conditions the soil samples were anaerobically incubated into

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**Figure 1** Diurnal profile of simulated Martian temperature (bold line) and humidity ( $a_w$ ), – dashed line – in the Mars simulator (2 days are shown).

25 ml glass flasks, 5 ml of sterile deionized water was added and the flasks were closed with a screw cap containing a septum and incubated at 10 °C. The activity of methanogenic archaea was measured before and after the experiment as described in “[Methane analysis](#).”

#### *Martian simulation experiments with pure methanogenic cultures*

Six strains of methanogenic archaea were used in the simulation experiment. Strains *Methanosarcina* spec. SMA-21, SMA-16 and *Methanobacterium* spec. MC-20 were grown on bicarbonate-buffered, oxygen-free OCM culture medium (Boone et al. 1989) under an atmosphere consisting of H<sub>2</sub>/CO<sub>2</sub> (80:20, v/v, pressurized 150 kPa). Strain SMA-23 and *Methanosarcina barkeri* were grown on oxygen-free MS culture medium (DSMZ No. 120) supplemented with 20 mM methanol as a substrate. *Methanogenium frigidum* was grown on oxygen-free EM culture medium (DSMZ No. 141) under an atmosphere of H<sub>2</sub>/CO<sub>2</sub> (80:20, v:v, pressurized 150 kPa) at 15 °C. All strains except *Methanogenium frigidum* were incubated at 28 °C for about two weeks. Cells were harvested by centrifugation and 50 mg of the cell pellet was inoculated into 1,500-μl glass jars (A-Z Analytik Zubehör GmbH). Three replicates of each culture were used. Cell density of the cultures was between 2.3 and  $8.1 \times 10^7$  cells ml<sup>-1</sup>. Before and after the experiment cell numbers were calculated as described in “[Cell counts determination](#).” After the exposure to Martian conditions the cell pellets were placed under anaerobic conditions into 25 ml glass flasks, supplemented with 10 ml fresh OCM medium and H<sub>2</sub> as a substrate. The flasks were closed with a screw cap containing a septum and incubated at 28 °C (*Methanogenium frigidum* at 15 °C). The activity was measured before and after the experiment as described in “[Methane analysis](#).”

#### *Methane analysis*

The activity of methanogenic archaea was calculated based on the lineal increase of CH<sub>4</sub> concentration in the headspace. Methane concentration was measured by gas chromatography.

The gas chromatograph (Agilent 6890, Fa. Agilent Technologies) was equipped with a Carbonplot capillary column ( $\varnothing$  0.53 mm, 30 m length) and a flame ionization detector (FID). Oven as well as injector temperature was 45 °C. The temperature of the detector was 250 °C. Helium served as carrier gas. All gas sample analyses were done after calibration with standards of the respective gases.

#### *Cell counts determination*

Cell numbers were calculated by Thoma cell counts and by fluorescence *in situ* hybridization (FISH) using the universal oligonucleotid probe for Archaea (ARC915 Cy3). For microscopic performance a Zeiss Axioskop 2 equipped with filters 02 (DAPI), 10 (FLUOS, DTAF) and 20 (Cy3), a mercury-arc lamp and an AxioCam digital camera for recording visualization of cells was used.

## Results

### Effect of Martian conditions on methanogenic archaea in permafrost soils

The survival rates of methanogenic archaea in permafrost soils after three weeks of exposure to the Martian thermal conditions was determined by both the methane production rates and the cell counts before and after the experiment. Methanogenic archaea of the floodplain showed high survival rates. The average cell numbers decreased from  $9.1 \times 10^6$  cells g<sup>-1</sup> at the beginning of the experiment to  $6.6 \times 10^6$  cells g<sup>-1</sup> after exposure to Martian conditions, which equals 72.2% cell survival. Average cell numbers of methanogenic archaea of the polygon depression decreased from  $6.7 \times 10^7$  to  $3.1 \times 10^7$  corresponding to a survival of 46.6% of the cells. The methane production rates of the flood plain soil samples slightly decreased after exposure to simulated Martian conditions from  $0.07 \pm 0.01$  nmol CH<sub>4</sub> h<sup>-1</sup> g<sup>-1</sup> to  $0.02 \pm 0.0004$  nmol CH<sub>4</sub> h<sup>-1</sup> g<sup>-1</sup> (Table II). The methane production rates of the methanogenic archaea observed in the polygon depression samples decreased from  $1.64 \pm 0.15$  nmol CH<sub>4</sub> h<sup>-1</sup> g<sup>-1</sup> to  $0.09 \pm 0.004$  nmol CH<sub>4</sub> h<sup>-1</sup> g<sup>-1</sup> after exposure to Martian conditions. The decrease of activity after the experiment was much higher in the polygon depression soils compared to the decrease of activity in soils of floodplain depression.

**TABLE II** Methane production rates and cell counts of methanogenic archaea in permafrost soil samples before and after exposure to Martian conditions

Soil samples	Cell counts 10 <sup>6</sup>	Survival rates (%)	CH <sub>4</sub> production (nmol h <sup>-1</sup> g <sup>-1</sup> )
Flood-plain (5–10 cm depth), control <sup>a</sup>	$9.1 \pm 4.2$	100	$0.07 \pm 0.01$
Flood-plain (5–10 cm depth), after experiment	$6.6 \pm 3.4$	72.2	$0.02 \pm 0.0004$
Centre (0–5 cm depth), control <sup>a</sup>	$66.5 \pm 16.9$	100	$1.64 \pm 0.15$
Centre (0–5 cm depth), after experiment	$31.1 \pm 9.8$	46.6	$0.09 \pm 0.004$

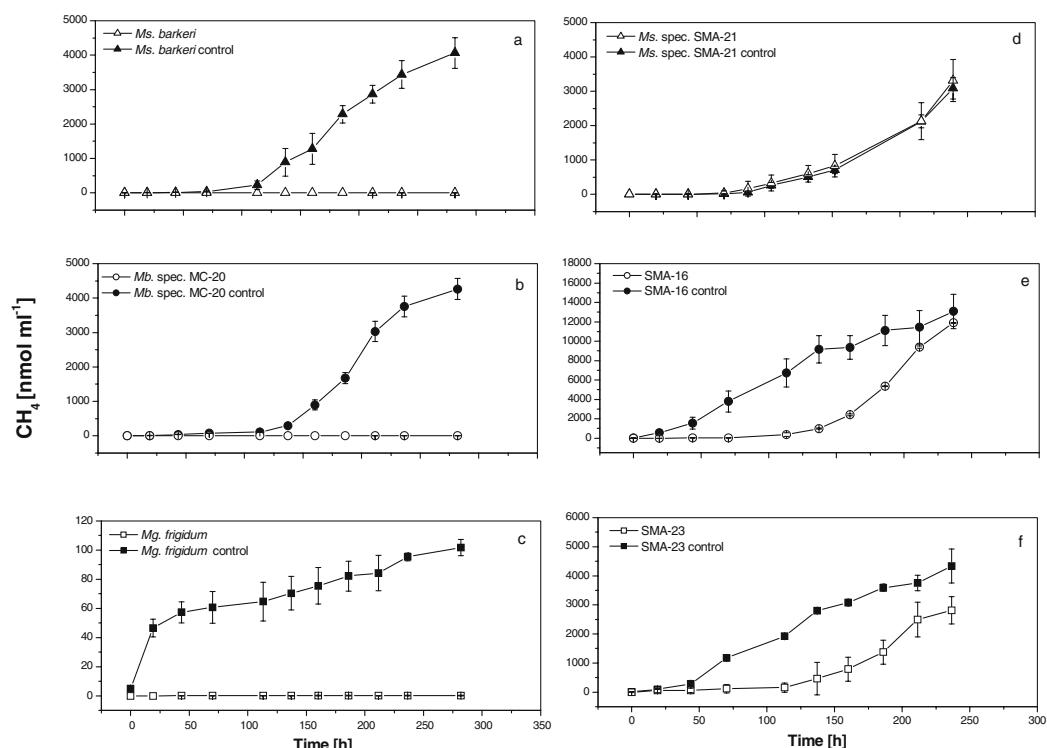
Mean  $\pm$  standard error, n=3.

<sup>a</sup> Soil samples, which were not exposed to the Martian thermal conditions.

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## Effect of Martian conditions on pure methanogenic cultures

The methanogenic strains from Siberian permafrost and the reference organisms from non-permafrost habitats showed significant differences in their survival potential under simulated Martian conditions. The average cell number of strain *Methanosarcina* spec. SMA-21 decreased from  $6.1 \times 10^7$  cells ml $^{-1}$  at the beginning of the experiment to  $5.5 \times 10^7$  cells ml $^{-1}$  at the end of the simulation, which equals a cell survival of 90.4%. Strains SMA-16 and SMA-23 showed 67.3% and 60.6% survival, respectively (Figure 2, Table III). In comparison, only 1.1% of strain *Methanobacterium* spec. MC-20, 5.8% of *Methanogenium frigidum* and 0.3% of *Methanosarcina barkeri* survived the simulation of Martian conditions (Table III). The decrease of cell numbers correlates well with the methane production rates of the cultures. Thus, activity of strains SMA-21, SMA-16 and SMA-23 measured before the exposure to simulated Martian conditions was similar to that after the simulation, whereas methane production of the reference organisms *Methanobacterium* spec. MC-20, *Methanogenium frigidum* and *Methanosarcina barkeri* drastically decreased after the experiment (Figure 2, Table III). The methane production rates of *Methanosarcina* spec. SMA-21 slightly decreased after exposure to simulated Martian conditions from  $48.61 \pm 6.57$  nmol CH $_4$  h $^{-1}$  ml $^{-1}$  to  $44.11 \pm 5.08$  nmol CH $_4$  h $^{-1}$  ml $^{-1}$  (Table III). The activities of two other permafrost isolates, SMA-16 and SMA-23 were also only marginally affected by the Martian experiment. The methane production rates of SMA-16 decreased from  $52.77 \pm 6.18$  nmol CH $_4$  h $^{-1}$  ml $^{-1}$  at the beginning of the experiment to  $45.37 \pm 0.03$  nmol CH $_4$  h $^{-1}$  ml $^{-1}$  after the exposure. The methane production rates of SMA-23



**Figure 2** Methane production activities of the reference organisms *Methanosarcina barkeri* (a), *Methanobacterium* spec. MC-20 (b), *Methanogenium frigidum* (c) and methanogens isolated from Siberian permafrost *Methanosarcina* spec. SMA-21 (d), SMA-16 (e), SMA-23 (f) before and after exposure to simulated Martian conditions (the error bars represent the standard deviation,  $n=3$ ).

**TABLE III** Methane production rates and cell counts of methanogenic archaea before and after exposure to Martian conditions

Cultures	Cell counts $10^7$	Survival rates %	$\text{CH}_4$ production nmol $\text{h}^{-1}$ $\text{ml}^{-1}$
<i>Methanosarcina</i> spec. SMA-21, control	6.1±0.6	100	48.61±6.57
<i>Methanosarcina</i> spec. SMA-21	5.5±0.8	90.4	44.11±5.08
SMA-16, control	6.2±1.1	100	52.77±6.18
SMA-16	4.2±0.9	67.3	45.37±0.03
SMA-23, control	7.8±1.4	100	22.13±1.94
SMA-23	4.7±1.2	60.6	13.92±3.87
<i>Methanobacterium</i> spec. MC-20, control	8.1±1.3	100	27.38±3.09
<i>Methanobacterium</i> spec. MC-20	0.09±0.01	1.1	0.03±0.001
<i>Methanogenium frigidum</i> , (DSM 16458) control	2.3±0.8	100	2.76±0.07
<i>Methanogenium frigidum</i> (DSM 16458)	0.1±0.04	5.8	0.003±0.005
<i>Methanosarcina barkeri</i> (DSM 8687), control	3.7±0.5	100	20.43±2.38
<i>Methanosarcina barkeri</i> (DSM 8687)	0.01±0.00	0.3	0.01±0.01

Mean ± standard error,  $n=3$ .

decreased from  $22.13\pm1.94$  nmol  $\text{CH}_4 \text{ h}^{-1} \text{ ml}^{-1}$  to  $13.92\pm3.87$  nmol  $\text{CH}_4 \text{ h}^{-1} \text{ ml}^{-1}$ . The activities of the reference organisms *Methanosarcina barkeri* and *Methanobacterium* spec. MC-20 after the simulation experiment were almost extinct (Figure 2, Table III). Methane production rates of *Methanogenium frigidum* significantly decreased from  $2.76\pm0.07$  nmol  $\text{CH}_4 \text{ h}^{-1} \text{ ml}^{-1}$  measured before the experiment to  $0.003\pm0.005$  nmol  $\text{CH}_4 \text{ h}^{-1} \text{ ml}^{-1}$  after the exposure.

## Discussion

Methanogenic archaea from Siberian permafrost showed unexpectedly high survival under simulated Martian thermal conditions. Three weeks of diurnal temperature and humidity cycles did not have significant effects on the viability of the methanogens in permafrost soil samples and in pure cultures. In contrast, the diurnal changes in humidity and temperature killed up to 99.7% of methanogenic archaea that originated from non-permafrost habitats. This indicates that methanogenic archaea from permafrost are more resistant to temperature shifts between  $-75^\circ\text{C}$  and  $20^\circ\text{C}$  as well as an  $a_w$ -value between 0.1 and 0.9 than well studied methanogens from other environments.

Terrestrial permafrost is characterized by extreme environmental conditions such as sub-zero temperatures, aridity and higher than normal levels of background radiation as a result of an accumulation over geological time scales. In spite of the unfavorable living conditions permafrost is colonized by a high number of viable microorganisms ( $10^2\text{--}10^8$  cells per  $\text{g}^{-1}$ ), including fungi, yeasts, algae, actinomycetes and bacteria as well as highly specialized organisms like methanogenic archaea (Kobabe et al. 2004; Wagner et al. 2005). Seasonal variation of soil temperatures, particularly freeze-thaw cycles in the active layer, results in drastic changes of other environmental conditions like salinity, soil pressure, changing oxygen conditions, availability of nutrients. The temperature variations also influence the availability of pore water, which is an essential bio-physical requirement for the survival of microorganisms in permafrost. The most important biological feature of this water is its possible role in the transfer of ions and nutrients (Ostromov and Siegert 1996).

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Comparing different permafrost samples it could be shown, that the extreme fluctuations in humidity and temperature conditions were more harmful for the methanogens in a polygon depression soil than those in a floodplain soil. One of the factors favoring the viability of methanogens under simulated Martian conditions might be the soil texture. Methanogenic archaea have a hydrophobic cell surface and a low electrophoretic mobility which support the attachment of these organisms to the surface of charged soil particles (Grotenhuis et al. 1992). The sorptive capacities of natural soil particles like clay and silt or soil organic matter provide a protective effect on methanogenic archaea (Heijnen et al. 1992; Wagner et al. 1999). Previous investigations already demonstrated that due to a protective role of the soil matrix and the existence of a complex microbial community composed of aerobic and facultative anaerobic microorganisms, methanogenic archaea exhibit a high survival potential against different stress factors like high oxygen partial pressure (Wagner et al. 1999). The different survival rates found in two permafrost soils might therefore result from differences in grain sizes or in the water adsorption capacity of these two soils (so-called tension or matrix potential). Thus, higher rates of survival and activity of methanogens after an exposure to Martian conditions in samples of the flood plain soil could be a consequence of high silt content which protects the methanogenic archaea against harsh conditions. Compared to the flood plain, the polygon depression was dominated by sandy material.

Also the strong aggregate formation of up to 100 cells of *Methanosarcina* spec. SMA-21 could be one of the mechanisms for the resistance of this archaeon. The outer cells of an aggregate may shield the inner cells from the damaging influence of low temperature, high salinity or intensive radiation. Probably, soil or rock grains could also serve as a shield against UV for these organisms and provide a habitat with stable temperatures. Since permafrost is expected to be extensively present on Mars, it is possible that methanogenic archaea could segregate in the subsurface niches and could survive under the harsh Martian thermal conditions.

The pure cultures of methanogens, which were not associated with a protective soil matrix, were also exposed to simulated Martian thermal conditions. These experiments showed that *Methanosarcina* spec. SMA-21 and two other permafrost strains, SMA-16 and SMA-23, exhibit a higher resistance than the reference organisms *Methanobacterium* spec. MC-20 and *Methanosarcina barkeri*. Most striking is that temperature shifts between -75 °C and 20 °C as well as humidity shifts with an  $a_w$ -value between 0.1 and 0.9 (averaged 0.52) have no influence on the activity and survival rates of strain *Methanosarcina* spec. SMA-21. The survival rate of *Methanogenium frigidum*, a psychrophilic methanogen isolated from Ace Lake in Antarctica (Franzmann et al. 1997), was higher than that of the other reference organisms. Nevertheless, the metabolic activity of this strain also drastically decreased after exposure. It could be hypothesized that this methanogenic archaeon is highly adapted to perennially cold environments but is affected by fluctuations of temperature and water activity.

The simulation experiment indicates high survival rates of methanogenic archaea from permafrost after exposure to simulated Martian thermal conditions. Without exception, every environment can only support life when water is present in liquid form, at least temporary. As has been shown by *Mars Odyssey* measurements, the present Martian surface is not as dry as has been postulated. In the upper meters of the Martian surface liquid water is present in the form of adsorbed water. The content of adsorption water in the upper millimeter to centimeter thick surface layer ranges from multiple layers of water molecules, when the atmosphere is saturated, to less than one single molecular layer when the atmosphere is dry (Möhlmann

et al. 2004). At larger depths, the content of adsorption water tends to become stable with about one to two mono-layers. The presence of adsorption water layers is restricted to the upper parts of the Martian surface. Adsorption is strongest during night and morning hours. The amount of adsorption water depends on the surface properties and on the humidity of the atmosphere. While the upper layers freeze at low temperatures, the lower one to two mono-layers remains unfrozen down to a temperature of about  $-133^{\circ}\text{C}$  (Möhlmann 2005). The temporary existence of adsorption water in the uppermost layers of the Martian surface enables potential organisms to accumulate liquid-like water during the time adsorption water is present at night and morning. The Mars simulation experiment with diurnal profiles of Martian temperature and humidity within 6 mbar CO<sub>2</sub>-atmosphere indicate the availability of adsorption water on Mars for biological processes. Comparable environments could be found in terrestrial permafrost, where adsorption water exists in a liquid-like state at temperatures down to  $-60^{\circ}\text{C}$  (Ananyan 1970).

The permafrost microbial community has been described as a “community of survivors” (Friedmann 1994), which has to resist the combination of extreme conditions and the extreme fluctuation of these conditions. High survival rates of methanogenic archaea under simulated Martian conditions indicate unknown physiological adaptations and suggest that these microorganisms have established ways to cope with stresses which has to include repair of the damaged DNA, repair of cell membranes and other vital functions to maintain the viability of cells. It remains to be determined that freeze protection mechanisms (i.e., trehalose accumulation, synthesis of molecular chaperones, adaptation of plasma membrane composition, synthesis of antioxidant proteins, accumulation of compatible solutes, expression of hydrophylins and other cryoprotectants) overlap with tolerance mechanisms protecting against various other stress types like desiccation, starvation or high salt concentration (Berry and Foegeding 1997; Macario et al. 1999; Cleland et al. 2004; Georlette et al. 2004).

Furthermore, it remains to be determined whether Martian and terrestrial permafrost have zones with similar physical and chemical conditions (Ostroumov, 1995). Due to the physiological potential and metabolic specificity of methanogenic archaea, no organic matter is needed for their growth. Kral et al. (2004) have demonstrated that certain methanogens can survive on Mars soil simulant (JSC Mars-1, collected from volcanoes on the Hawaii island) when they are supplied with CO<sub>2</sub>, molecular hydrogen and varying amounts of water.

The permafrost habitats on Earth represent an excellent analogue for studying putative life on Mars. Recent analyses of *Mars Express* high resolution stereo camera (HRSC) images of many regions of the planet showed that the morphology of the Martian polygonal features is very similar to the morphology of the terrestrial ice-wedge polygons and is most likely formed by comparable processes (Kuzmin 2005). The observation of high survival rates of methanogens under simulated Martian conditions supports the possibility that microorganisms similar to the isolates from Siberian permafrost could also exist in the Martian permafrost. Methanogenic archaea from terrestrial permafrost may therefore serve as useful models for further exploration of extraterrestrial life.

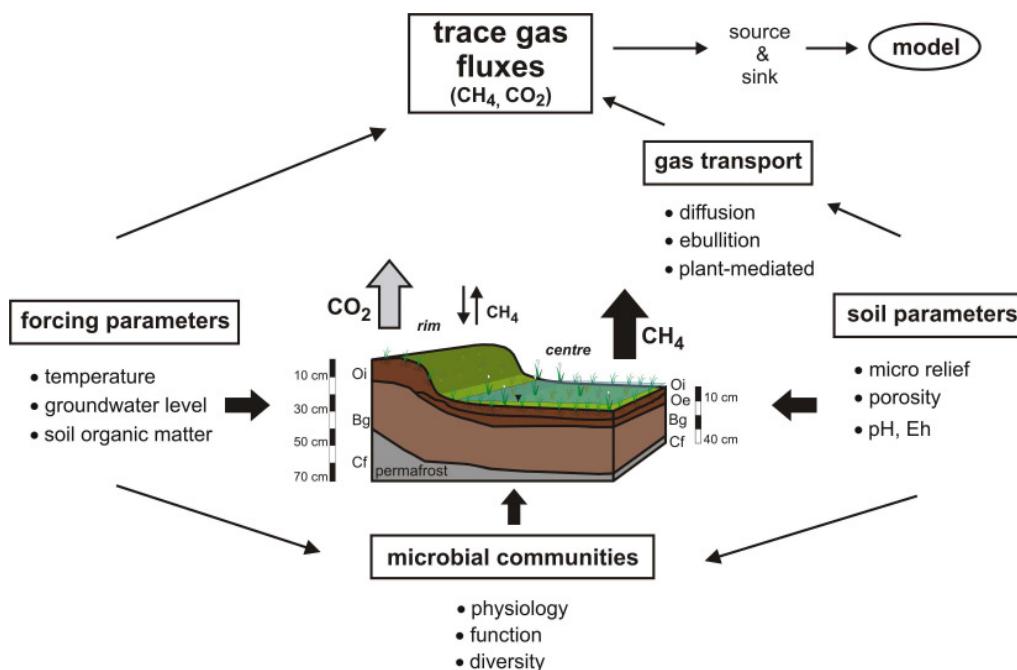
**Acknowledgments** The authors wish to thank the Russian–German team of the Expedition LENA 2004 for enjoyable field work under *extreme conditions*, in particular Waldemar Schneider for logistic and Günter ‘Molo’ Stoof for technical support (both Alfred Wegener Institute for Polar and Marine Research). Special thanks go to the team of the HUMIDITY-Lab Dr. Roland Wernecke & Partner and Andreas Lorek (German Aerospace Center) as well as to the whole group of simulators (especially Dr. Jelka Ondruschka, Ulrike Pogoda de la Vega, Prof. Dr. Sieglinde Ott, and Dr. Petra Rettberg) for successful cooperation. This research has been supported by Deutsche Forschungsgemeinschaft (German Research Foundation) priority program 1115 “Mars and the terrestrial planets” (WA 1554/1–2).

## 7 Synthesis

### 7.1 Introduction

The Arctic plays a key role in the Earth's climate system for two reasons. On the one hand, global warming is predicted to be most pronounced at high latitudes, and observational evidence over the past 25 years suggests that this warming is already under way (Serreze et al., 2000; Mack et al., 2004; Richter-Menge et al., 2006). On the other hand, one third of the global carbon pool is stored in ecosystems of the northern latitudes (Post et al., 1982; Gorham, 1991). Thus there is considerable socio-economic interest in predicting how the carbon balance of the northern ecosystems will respond to ongoing climate warming.

Global warming will have important implications for the functional diversity of microbial communities in these systems. It is likely that temperature increases in high latitudes may stimulate microbial activity and carbon decomposition in Arctic environments and are accelerating climate change through the increase of trace gas ( $\text{CH}_4$ ,  $\text{CO}_2$ ) release (Melillo et al., 2002; Zimov et al., 2006). Figure 7.1 summarises the process variables of trace gas fluxes from permafrost environments.



**Figure 7.1:** Schematic view of the process variables influencing the formation, transport and release of climate-relevant trace gases.

The microorganisms, which are the drivers of methane production and oxidation in Arctic wetlands, have remained obscure. Their function, population structure and reaction to environmental change is largely unknown, which means that also an important part of the process knowledge on methane fluxes in permafrost ecosystems, as shown in Figure 7.1, is far from completely understood. This hampers prediction of the effects of climate warming on arctic methane fluxes, in particular when these predictions are based on models that do not take into account the specific nature of microbial populations in permafrost soils and sediments. Understanding these microbial populations is therefore highly important for understanding the global climatic effects of a warming Arctic.

Under the umbrella of the Russian-German Cooperation SYSTEM LAPTEV SEA a multidisciplinary research concept was developed and applied on the Arctic methane cycle that connect trace gas flux measurements with studies on microbial processes and communities. During eight expeditions to the Lena Delta from 1998 to 2005 (Rachold 1999, 2000; Rachold and Grigoriev, 2001; Pfeiffer and Grigoriev, 2002; Grigoriev et al., 2003; Schirrmeyer et al., 2004; Wagner et al., 2006; Schirrmeyer and Wagner., in press), methane fluxes were measured, microbial processes under *in situ* conditions were studied, and samples from different permafrost ecosystems were taken for further molecular ecological analyses. The Russian-German research station Samoylov provided the basis for all the field investigations (Hubberten et al., 2006). In particular, the objectives of the present study were:

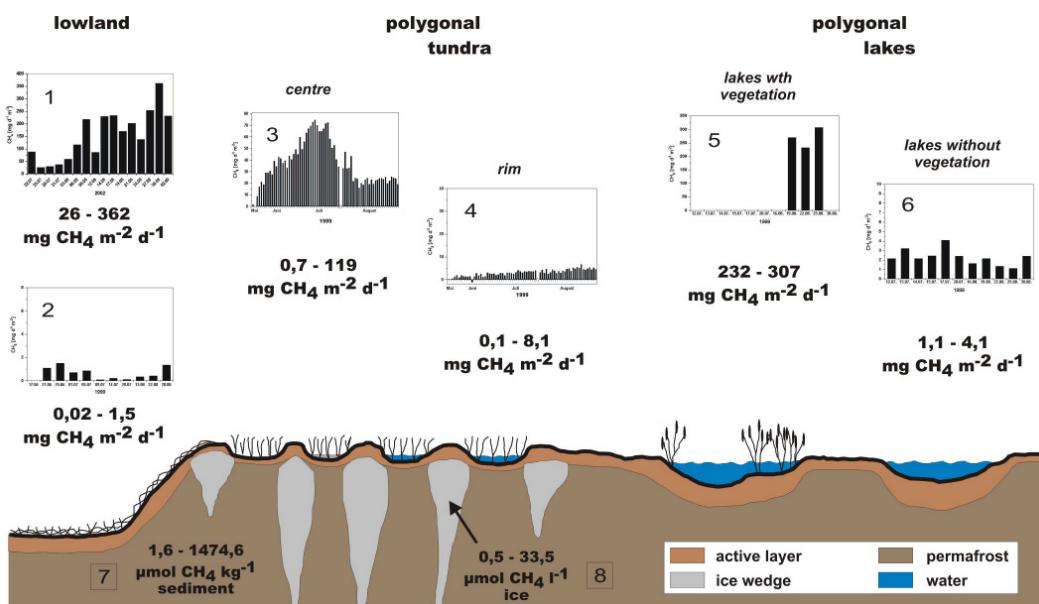
- to **measure** and **balance** methane fluxes from tundra environments of the Lena Delta
- to characterize **soil ecological parameters** determining microbial processes in permafrost ecosystems
- to gain more insights into the **control functions** of microorganisms involved in the Arctic methane cycle and their response to climate change
- to improve and increase the knowledge of the **abundance** and **biodiversity** of microbial communities involved in the carbon decomposition in permafrost environments and of their phylogenetic affiliations
- to determine **tolerance limits** of methanogenic archaea isolated from Siberian permafrost under extreme living conditions

## 7.2 Methane Release from Tundra Environments of the Lena Delta

The methane release from tundra environments was measured within the scope of a long-term study on trace gas fluxes ( $\text{CH}_4$ ,  $\text{CO}_2$ ) in the Lena Delta since 1998 until today and will be still continue in the future. Presently, a data record of the methane

emissions for the last eight years is evaluated in regard to long-term trends of trace gas fluxes in the Siberian Arctic (Wagner et al., in prep.).

Closed chamber measurements were used to study the methane fluxes on the plot scale and to link these results with soil ecological variables and microbial processes (see chapter 7.3). In 2002, these measurements were complemented by a micrometeorological eddy covariance measurement system, which was designed for high-resolution measurements on turbulent fluxes of methane, carbon dioxide, momentum, heat and water in the atmospheric boundary layer (Kutzbach, 2005). These additional analyses provide data for the balance on trace gas fluxes on the ecosystem scale and for the validation and improvement of process-based flux models.



**Figure 7.2:** Schematic overview of the spatial fluctuations of methane emissions from the different landscape units on Samoylov Island (study site and expedition): 1. northern floodplain, LENA 2002 and LENA-ANABAR 2003; 2. western floodplain, LENA 1999; 3./4. low-center ice-wedge polygons on the long-term study site, LENA 1999 until LENA 2006; 5./6. polygonal lakes, LENA 2000 and LENA 2001; 7. perennially frozen ground, LENA 2001 and LENA 2002; 8. Holocene and Pleistocene ice-wedges, LENA 2000.

The long-term study site is located on Samoylov Island in the polygonal tundra, which is the typical patterned ground in the Siberian Arctic. The microrelief elements (rim and depression) of these low-centered polygons and the respective soil and vegetation types appear in regular cyclic intervals of 10–30 m (see page 150, Fig. 9.3 A). Thus, soil conditions, vegetation characteristics, and consequently methane

fluxes in polygonal tundra are highly variable on the small scale (decimetres to metres) but rather homogenous on the large scale ( $10^2$  to  $10^4$  metres). Figure 7.2 illustrates the spatial fluctuations in methane emission from the different landscape units on Samoylov Island. In the following, each one data set of methane emission measured with closed chamber (1999) and micrometeorological eddy covariance (2003/2004) technique will be discussed. The two data records were exemplarily selected, because they cover the longest time series in the Lena Delta.

**Table 7.1:** Daily methane emission rates for the entire vegetation period from the polygonal study site on Samoylov Island ( $n = 5$ ).

micro site		methane emission ( $\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ )				
		June	July	August	September	October
depressed center	Mean	54.1	93.7	44	17.9	11.2
	Min	13.7	60.3	32.9	7	2.3
	Max	89.4	119.6	72.6	25.8	25.3
elevated rim	Mean	2.5	4.7	6.1	2.1	1.7
	Min	0.7	3.3	3.1	0.6	0.7
	Max	4.6	6.2	11.4	4	3.9

In 1999, the methane release from the polygonal site (plot scale) could be measured for the first time during the entire vegetation period from the end of May to the beginning of September (chapter 4.2). The methane fluxes of the depression differed strongly from those of the rim of the polygon. The mean flux rate for the total vegetation period of the depression was  $53.2 \pm 8.7 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ , whereas the mean flux rate of the dryer rim part of the polygon was  $4.7 \pm 2.5 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ . The emission of methane from the depression covered dominantly by *Carex aquatilis* showed large seasonal fluctuations. Right from the start of soil thawing a relatively high methane flux rate of more than  $10 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$  was determined at the beginning of June (Table 7.1). This rate increased with advanced thawing of the active layer and reached the highest values with about  $120 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$  in the middle of July. In contrast to the polygon depression, methane fluxes of the rim, which is dominated by moss vegetations, showed minor seasonal fluctuations. During the whole season the rate was lower than  $12 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ .

The strong seasonal and spatial fluctuations are caused by the fundamental microbial processes of methane production and oxidation (chapter 7.3), which are

steered by numerous abiotic (e.g. soil temperature, soil moisture, active layer thaw depth) and biotic (organic matter quality, vegetation) variables:

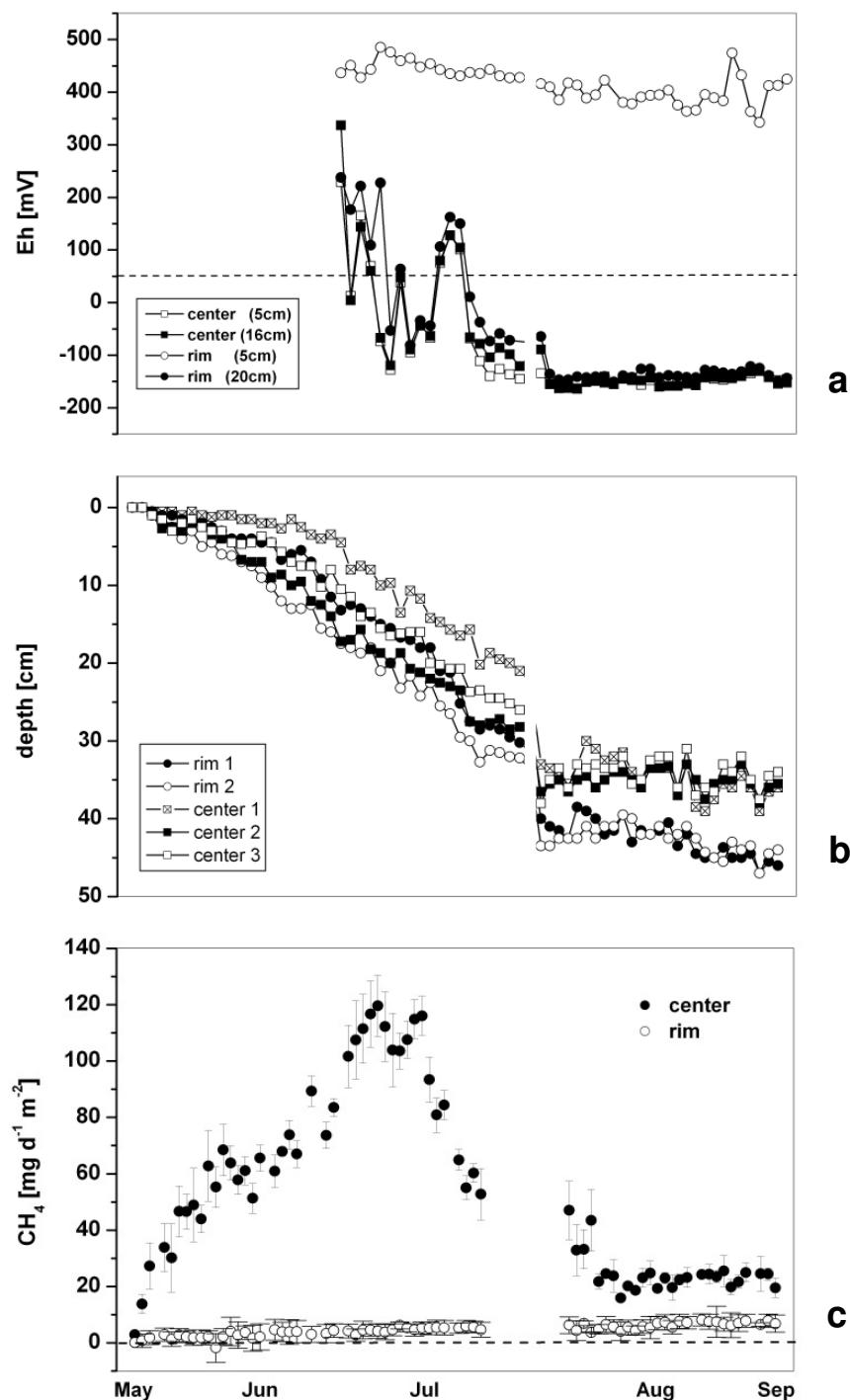
1. One important parameter controlling methane emissions is soil temperature, because the microbiological activity depends on temperature, as confirmed by numerous studies of methane emission using closed chamber or eddy covariance techniques (e.g. Nakano et al., 2000; Christensen et al., 2001; Hargreaves et al., 2001). However, this relationship has changes based on depth within the soil profile. The upper soil layers, dominated by mesophilic microorganisms, are characterized by high temperature fluctuations, while the bottom part, populated by psychrophilic microbes, showed lower *in situ* temperatures with slight seasonal variations (chapter 4.2; 4.3; 5.2). Therefore, the methane production and oxidation in the top soil is rather more influenced by temperature fluctuations than as by the processes in the bottom of the active layer.
2. Many studies identified the thaw depth of soils as an important predictor of methane emission (e.g. Friborg et al., 2000; Tsuyuzaki et al., 2001; van Huissteden et al., 2005). In the present study, the thaw depth was found to correlate weakly with the methane emissions and does not improve the applied flux model based on soil temperature (chapter 3.1). This observation can be explained in two ways. Firstly, during short flux studies, which use the closed chamber technique, the correlation between methane flux and thaw depth is usually spatial. A spatial correlation at one point in time does not imply the existence of a temporal correlation during the course of the season. Secondly, during distinct periods of the season, soil thaw depth and soil temperature are highly correlated. In this case, the methane fluxes were also significantly correlated with the thawing of the active layer shown for the early vegetation period in 1999 (Figure 7.3; chapter 4.2). However, when the time series included data from the periods of spring thaw and autumnal freeze-back, the different behaviour of the two variables became apparent and the ability of soil thaw depth to explain variations of methane flux diminished.
3. In many other studies, the water table position was identified as the main factor that controls methane emission (e.g. Friborg et al., 2000; Suyker et al., 1996). This was explained with the regulation of the methane production/oxidation balance through changes of the aerobic/anaerobic soil column ratio. However, no significant influence of the water table on methane flux was detected by high-resolution eddy covariance measurements, despite the great variations of the water table position through the season. This can be explained with respect to the microrelief elements prevalent in our study area: Firstly, in the polygon rims, the water table was always well below the soil surface, so the ratio of aerobic/anaerobic soil column was always high.

Furthermore, process studies have shown that oxidation activity in these soils is greatest near the aerobic-anaerobic interface, where psychrophytic methanotrophic bacteria exist and the substrate provision is at its optimum (chapter 4.3). Hence the methane flux from polygon rim areas does not respond strongly to variations of the water table position. Secondly, despite the variations in water table position, in most of the polygon centers, the water table was distinctly above the soil surface in most measurement periods, so that the change in the water table position could not influence the methane production/oxidation balance significantly. However, extreme draught could lower the water level below the soil surface in many polygon centers and lead to increased oxidation and overall decreased methane flux. This “on-off switch” effect was observed for single polygon center sites on Samoylov Island during the summer of 1999 (chapter 4.2).

4. The vegetation occupies a central position for the gas transport. Plants can have both enhancing and attenuating effects on methane emission. Through the aerenchyma of vascular plants, oxygen is transported from the atmosphere to the rhizosphere, thus stimulating methane oxidation in otherwise anoxic soil horizons (Van der Nat & Middelburg 1998; Popp et al. 2000). In opposite direction, the aerenchyma are a major pathway for methane transport from the anoxic horizons to the atmosphere, bypassing the oxic/anoxic interface in the soil, where methane oxidation is prominent (chapters 4.2 and 4.3). Furthermore, the vegetation provides the substrates for methanogenesis as decaying plant material and fresh root exudates (Whiting & Chanton 1992; Joabsson et al. 1999). Using self-constructed chambers, methane transport via *Carex aquatilis* was shown to account for between 27 and 68 % of overall methane emissions at the polygonal tundra on Samoylov Island (chapter 3.2).

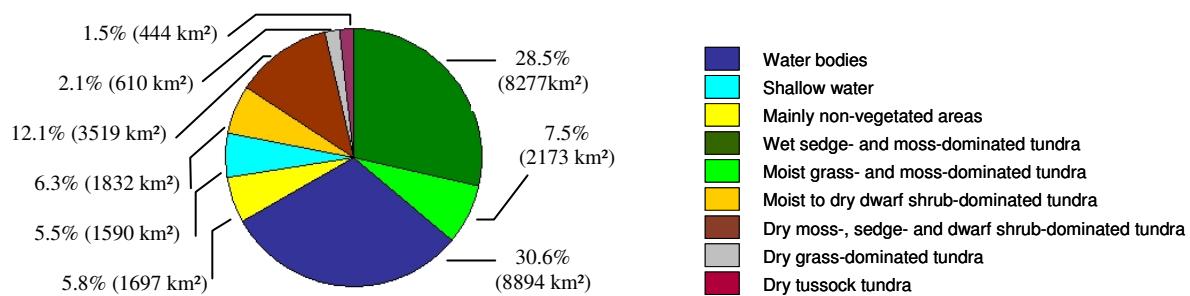
The eddy covariance measurements of methane fluxes from wet polygonal tundra (ecosystem scale) on Samoylov Island revealed daily summer fluxes of about 30 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> (chapter 3.1). These values are lower compared to methane emissions reported by other eddy covariance flux studies from arctic wetlands. Friborg et al. (2000) reported average methane fluxes of about 50 mg m<sup>-2</sup> d<sup>-1</sup> during July and August from a north-east Greenland fen (74°N) and Hargreaves et al. (2001) measured a mean methane emission of 38 mg m<sup>-2</sup> d<sup>-1</sup> from a Finnish mire (69°N) in August. These differences can be mainly attributed to: (i) the longer field campaign on Samoylov Island, including the period of spring thaw and autumnal freeze back (June-October) and (ii) the genesis of ice-wedge polygons, which lead to a strong spatial heterogeneity in soil and vegetation properties and to a high surface coverage of dry sites (> 50%). However, as the main driving parameters of methane emission on the ecosystem scale soil temperature and near surface atmospheric turbulence

was identified. A model based on these variables explained variations of methane flux corresponding to continuous processes of microbial methane generation and oxidation (chapter 7.3), and diffusion through soil and plants (chapter 3.2) reasonably well.



**Figure 7.3:** Redox potential (a), thaw depth (b) and methane emission (c) measured in a polygonal tundra for the main vegetation period in 1999 on Samoylov Island.

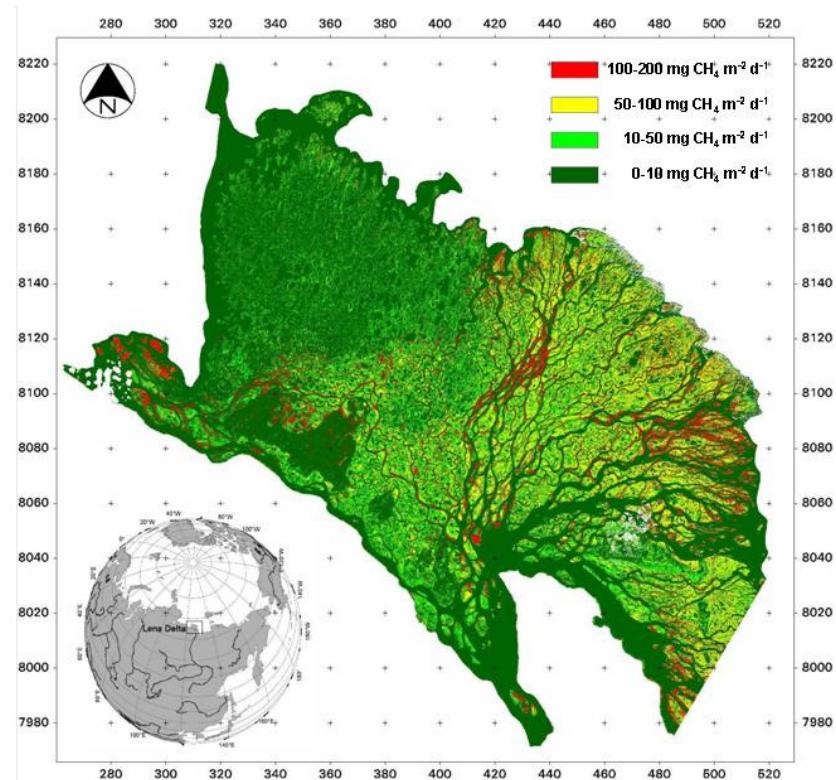
For the overall balance of methane emissions from the entire delta the first land cover classification was performed on the basis of Landsat 7 ETM+ images and applied for an upscaling of methane fluxes as inferred from closed chamber measurements (chapter 3.3). Nine land cover classes characterized by their vegetation, surface moisture, and topography could be defined by this approach for the Lena Delta region (Figure 7.4).



**Figure 7.4:** Results of land cover classification of the Lena Delta based on Landsat 7 ETM+ images (modified according to Schneider et al., submitted).

The total area of the Lena Delta was quantified with  $29036 \text{ km}^2$  with large spatial fluctuations in methane emission rates ranging from  $0\text{-}200 \text{ mg m}^{-2} \text{ d}^{-1}$  (Figure 7.5). Although the measured methane emissions on the plot scale (Figure 7.2) are similar to those reported from analogous areas in high latitudes (e.g. reviewed by Harriss et al., 1993, Corradi et al., 2005; van Huissteden et al., 2005), the regionally weighted mean daily methane fluxes of the Lena Delta ( $10 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ ) are less than those calculated for other arctic wetlands. Although long-term studies of methane emission rates are necessary for the refinement of global methane flux estimates (Matthews and Fung, 1987), most of the studies on methane fluxes in the high arctic consider only a measuring period of less than four weeks (Bartlett et al., 1992; Whalen and Reeburgh, 1992; Vourlitis et al., 1993; Christensen et al., 2000; Nakano et al., 2000; Tsuyuzaki et al., 2001). Most calculations are only based on measurements made through a couple of days for the whole season (Torn and Chapin, 1993; Christensen et al., 1995; Oberbauer et al., 1998; Verville et al., 1998). The low methane emission estimated in this study can be explained by the used integrative approach of long-term flux measurements and supervised land cover classifications, which has not been undertaken so far. The annual methane emission of the Lena Delta amounts to about 0.03 Tg. A comparison of the Lena Delta source strength with other arctic areas is difficult, since so far only few other studies were published presenting a balance of methane emissions for tundra environments (Whalen & Reeburgh, 1988;

Morrissey & Livingston, 1992; Christensen, 1993; MacDonald et al., 1998; Christensen et al., 2000).



**Figure 7.5:** Methane emission in the Lena Delta. Calculation based on supervised classification of satellite data and on methane flux studies (projection: UTM (WGS84), zone 52; classification: Landsat 7 ETM+, acquisition dates: 27.07.00 and 26.07.01; map according to Schneider 2005).

This study is the first attempt to assess the methane fluxes of the Lena Delta based on field measurements and on satellite data. The used approach provides a more realistic estimation of the real methane emissions on the regional scale as the hitherto published results, because of the high-resolution data base and the applied remote sensing methods. Despite some potential uncertainties, the results show that the Lena Delta contributes significantly to the global methane emission because of its extensive wetland areas. Furthermore, the study delivered the longest high-resolution time series of methane emissions on the ecosystem scale in the high Arctic using eddy covariance technique.

### 7.3 Microbial Processes and Communities Involved in the Arctic Methane Cycle

As the preceding notes indicate, the microbial processes of methane production and oxidation as well as the involved microbial communities must be known in order to understand the spatial and seasonal fluctuations of methane fluxes (chapter 7.2) and

for the assessment of the recent and future carbon dynamics in permafrost environments. The remarks in this chapter will mainly focus on the results obtained from the polygonal study site on Samoylov Island, unless otherwise stated.

Permafrost environments in the Arctic are characterised by extreme environmental conditions which demand a specific resistance of microorganisms to survive and to be metabolic active under these conditions. The seasonal unfrozen part of permafrost (active layer, approx. 0.5 m thickness at the study site) is subjected to freezing and thawing cycles during the year with an extreme surface temperature from about +25 °C to –45 °C. During the Quaternary, prolonged cryogenic processes led to the formation of patterned grounds like the low-centered ice-wedge polygons on the main investigation area on Samoylov Island (Figure 1.5). During the summer period, soils within these polygons are showing large gradients in temperature and geochemistry along their depths profiles (chapters 4.1, 4.2, 4.3, 5.2), which are the main environmental factors influencing the microbial communities in permafrost soils. The present study reveals differences of the micro-relief elements of the investigated low-center polygon between the elevated rim and the depressed center in respect to microbial activities, community structure, and soil characteristics on the ecosystem scale (centimetres to metres).

In spite of the extreme habitat conditions permafrost soils are colonized by high numbers of microorganisms including representatives of *Bacteria* and *Archaea* (chapters 4.3 and 5.1). The highest total cell counts were in the range of  $10^9$  cells g<sup>-1</sup> soil and decrease from the top to the bottom of the active layer. Detailed analyses of methanotrophic cell counts showed highest numbers in the polygon rim where they ranged between  $1.0 \times 10^8$  and  $3.0 \times 10^6$  cells g<sup>-1</sup> soil, while their numbers in the polygon center were two orders of magnitude lower compared to the rim part. In contrast, most methanogenic archaea were found in the upper soil horizon of the polygon center with  $3.0 \times 10^8$  cells g<sup>-1</sup> soil, which assigned to 22 % of total cell counts.

These high cell numbers are reflected also in the high microbial biomass (expressed in total phospholipid biomarker concentrations) with maximal values for the polygon rim and center, respectively, of 105.5 and 851.6 nmol g<sup>-1</sup> soil (chapter 5.2). They are significantly higher than in arable soils (35.2 – 59.4 nmol g<sup>-1</sup>, Göttinger *et al.*, 2002b, Zelles, 1999), rice paddies (44.7 – 90.9 nmol g<sup>-1</sup> dw, Bai *et al.*, 2000), and boreal Swedish peatlands (0.2 – 7.0 nmol g<sup>-1</sup> wet peat, Sundh *et al.*, 1997).

Independently of the harsh living conditions in permafrost ecosystems both methane production and methane oxidation could be proven in all examined soils. However, activities of methanogens and methanotrophs differed significantly in their rates and distribution patterns among the two investigated profiles of the main study site (chapters 4.2, 5.2 and 5.3).

The methane production and oxidation in the polygon rim showed the typical activity patterns as known from other hydromorphic soils (e.g. Krumholz *et al.*, 1995), which means in the case of methane production no or less activity in the dry and oxic upper horizons and increasing rates in the anoxic bottom layers. In contrast, this pattern was not observed in the polygon center. Here the highest methane production occurred in the upper soil horizons, which posses not the best conditions for methanogenesis shown by the redox status of this site (chapter 4.1).

These small-scale differences in the activity in the microrelief could be attributed primarily to the quality of organic matter. Although organic carbon is highly accumulated in permafrost soils, a substrate limitation was found by studying potential methane production rates (chapters 5.2, 5.3). Subsequent analyses revealed a decrease of bioavailable organic matter (BWEOC) along with an increasing humification index with increasing soil depth. This trend shows that there is actually a high quantity of organic matter, particularly in the wet center, but that this carbon is not available for the microorganisms.

Another important factor for microbial metabolism is the habitat temperature. Even if only few psychrophilic strains of methanogens and methanotrophs have been described so far (Cavicchioli, 2006; Trotsenko and Khmelenina, 2002), the present results indicate a shift within the microbial communities from mesophilic to psychrotolerant or psychrophilic organisms with increasing soil depth.

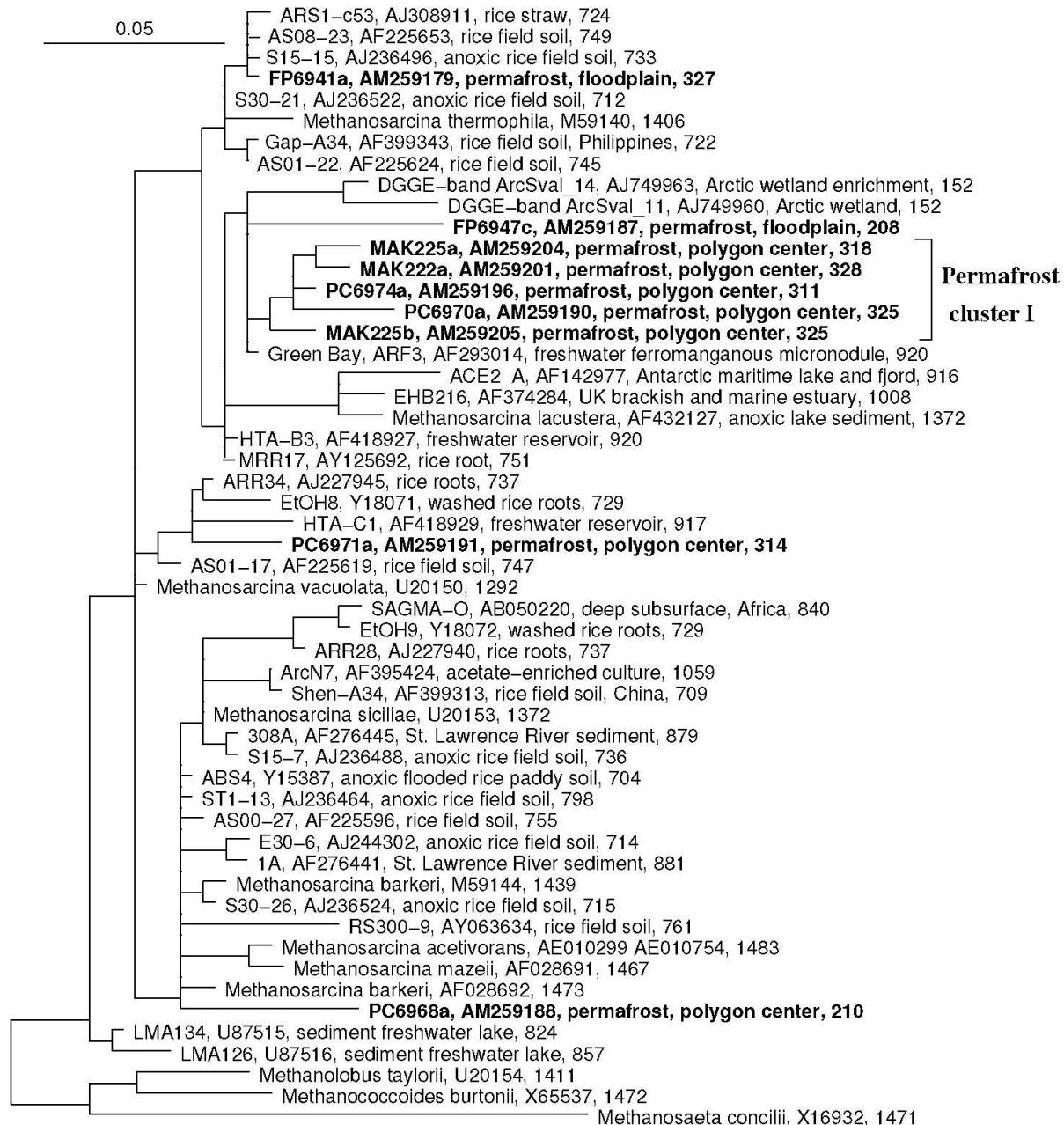
This adaptation was shown by phospholipid analyses, which reveal a shift of the overall composition of the microbiota with depth in both soils of the polygon. An increasing portion of iso- and anteiso-branched fatty acids related to the amount of straight chain fatty acids (both groups of biomarker are synthesised by different groups of bacteria; chapter 5.2) was determined. Branched chain fatty acids are typical found in cold-adapted microorganisms. This general trend was confirmed by the investigations on methanogenesis and methanotrophy in dependence of the temperature at the same site (chapters 4.3, 5.2 and 5.3).

The soils on Samoylov Island showed at least two optima of methane production activity within the vertical profile. Especially in the polygon rim, the highest activity occurred in the bottom of the active layer with an *in situ* temperature of around 1 °C.

The methane oxidation activity showed vertical shifts within the optimal temperature and within the distribution of type I and type II methanotrophs. In the upper active layer, maximum methane oxidation potentials were detected at 21 °C. Deep active layer zones that are constantly exposed to temperatures below 2 °C showed a maximum potential for methane oxidation at 4 °C. This indicates a dominance of psychrophilic methanotrophs close to the permafrost table.

These findings indicate an adaptation of the microbial communities involved in the methane cycle of permafrost soils to the low *in situ* temperatures. This is also in

accordance with the determination of high cell numbers of  $1.2 \times 10^8$  cells g<sup>-1</sup> soil in the boundary layer to the permafrost (chapter 5.1).



**Figure 7.6:** Phylogenetic tree illustrating the affiliation of methanogenic 16S rRNA gene sequences re-amplified from DGGE bands for *Methanosarcinaceae*. The “backbone” trees are based on maximum likelihood analysis of the dataset made with RAxML-IV and partial sequences of the permafrost DGGE bands (shown in bold) were added to the tree using parsimony addition tool of the ARB program package. The scale bar represents 0.05 changes per nucleotide. Identification of the bands is shown in chapter 5.3. Clone name, accession number, environment and length of each sequence are indicated.

Investigations on the microbial diversity in permafrost soils started only recently and are yet not fully completed. However, preliminary results of bacterial 16S rDNA clone library analyses revealed a distinct variability of the main phyla (e.g. *Bacteroidetes*, *Chloroflexi*, *Firmicutes* and *Proteobacteria*) within the soil of the polygon rim, while the community composition in the center soil is more homogenous (Liebner et al., in prep.). On the other hand, community structure analysis based on phospholipid profiling showed similarities between both soils for esterlinked PLFAs (biomarkers for bacteria) and differences in the fraction of unsaponifiable PLFAs and PLEs (biomarkers for anaerobes and archaea; chapter 5.2). The differences in the community structure both in the vertical profile and in the microrelief seem to be primarily referred to the differences in the soil moisture regime. The polygon rim is characterized by oxic conditions in the upper part, while anoxic conditions dominate above the permafrost table. In contrast, the polygon center is predominantly characterized by water saturation over the entire vertical profile.

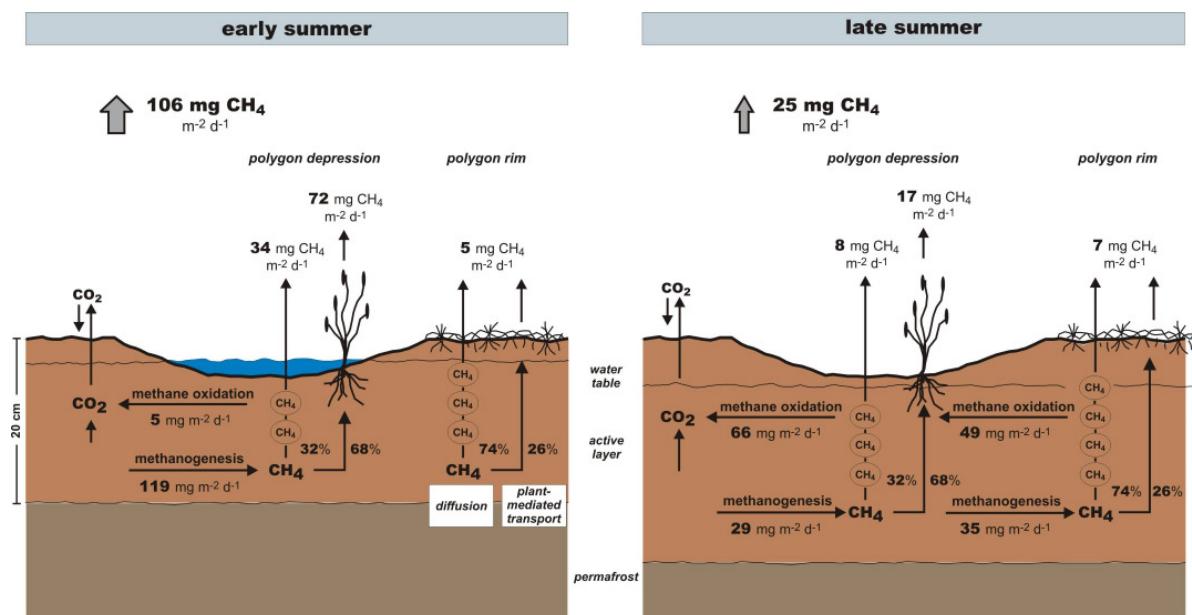
Detailed sequence analyses of methanogenic communities in three different permafrost soils based on 16S rRNA gene fingerprints revealed a distinct diversity of methanogenic archaea affiliated to *Methanomicrobiaceae*, *Methanosarcinaceae* and *Methanosaetaceae* (chapter 5.3). There were no restrictions of the detected families to specific depths or sites. Only sequences of *Methanosaetaceae* could not be detected in the polygon center soil of Mamontovy Klyk. According to statistic analyses out of the 28 sequences, 16 sequences can be assigned to four specific permafrost clusters. Figure 7.6 illustrates exemplarily the affiliation of methanogenic 16S rRNA gene sequences re-amplified from DGGE bands for *Methanosarcinaceae*. It is hypothesized from the obtained results, albeit somewhat speculative, that these clusters are formed by methanogenic archaea characterised by specific adaptation processes to the harsh permafrost conditions. However, a relationship between the activity and the diversity of methanogens in permafrost soils could not be proven.

Apart from the understanding of the ecological system (integration of soil variables with microbial processes and community structure), the seasonal change of the microbial processes is of importance for the knowledge of the large seasonal fluctuations in methane emissions (chapter 7.2). Therefore, the methane production and oxidation was quantified under *in situ* conditions in early and late summer in 1999 (chapter 4.2).

The microbiological processes of the polygon depression clearly correlate with the observed seasonal methane fluctuations. By the beginning of July, the methane production in the upper soil layer was unusually high with a rate of about 39 nmol CH<sub>4</sub> h<sup>-1</sup> g<sup>-1</sup>. At the same time, methane oxidation in the upper soil layer amounted to about 2 nmol CH<sub>4</sub> h<sup>-1</sup> g<sup>-1</sup>, whereas no activity was found in the deeper soil layers. Considering the analysed methane production and oxidation rates and the calculated

potential fluxes within the soil profile, only 4 % of the produced methane is oxidized by the methanotrophic bacteria in the upper soil layer. Compared with other ecosystems, where up to 90 % of the formed methane can be oxidized by methanotrophic bacteria (Frenzel *et al.*, 1990; Khalil *et al.*, 1998; Le Mer and Roger, 2001), the oxidation activity in the permafrost soil is very low at the beginning of the vegetation period. The high methane production rate together with the low methane oxidation rate can explain the highest methane flux rates determined in July 1999 (Figure 7.3c).

At the beginning of August, the methane production and oxidation activity of the microorganisms differed completely from those of July. The methane production in the upper soil layer drastically decreased to about  $4.5 \text{ nmol CH}_4 \text{ h}^{-1} \text{ g}^{-1}$ , while the methane oxidation, which could be detected now in nearly the whole profile, strongly increased to rates between 4 and  $7 \text{ nmol CH}_4 \text{ h}^{-1} \text{ g}^{-1}$ . The calculated balance of the methane production and oxidation for the whole profile showed, that the microbial methane oxidation capacity ( $66 \text{ mg m}^{-2} \text{ d}^{-1}$ ) in this period was about two times higher than the methane production ( $29 \text{ mg m}^{-2} \text{ d}^{-1}$ ). Nevertheless, a significant methane release from the polygon depression was also determined in August 1999, which can be attributed to the influence of the vegetation (chapter 3.2). Figure 7.7 summarizes the results of integrated investigations on methane fluxes and microbial processes for this case study.



**Figure 7.7:** Illustration of the microbial processes associated with the methane cycle as well as the methane flux rates of a polygonal tundra on the basis of the spring and late summer conditions (detailed explanations are given in chapter 4.2).

The degradation of permafrost and the associated release of climate-relevant trace gases, as a consequence of an enhanced turnover of organic carbon and from ancient methane reservoirs, represent a potential risk with respect to future global warming. At this point, it becomes important to know how the microorganisms and their potential activities behave in the perennially frozen ground.

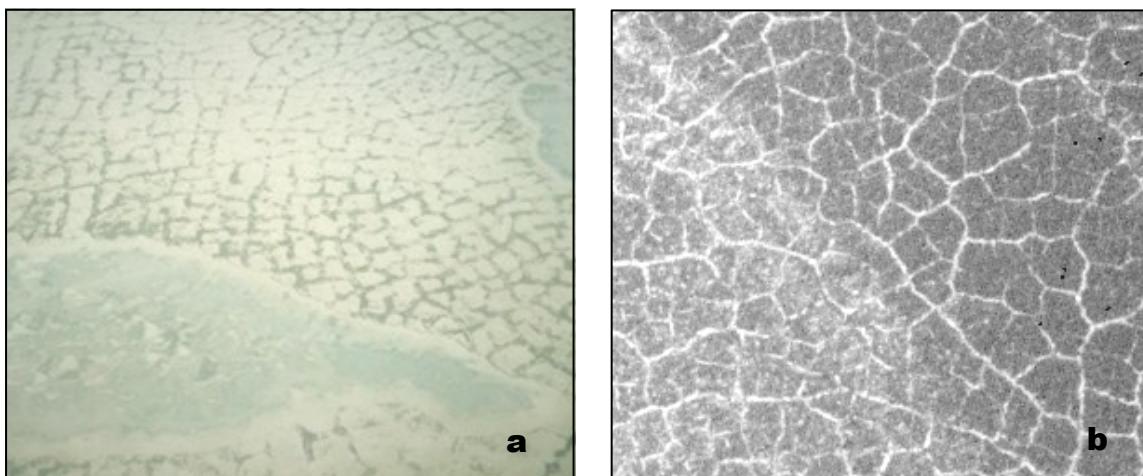
Therefore, a permafrost core of Holocene age was drilled in the Lena Delta in order to improve the prediction of the carbon dynamics in highly sensitive Arctic permafrost ecosystems (chapter 4.4). The organic carbon of the permafrost sediments varied between 0.6 and 4.9 % and was characterized by an increasing humification index with permafrost depth. A high methane concentration was found in the upper 4 m of the deposits, which correlates well with the methanogenic activity and archaeal biomass (expressed as PLEL concentration). Even the incubation of core material at  $-3^{\circ}\text{C}$  and  $-6^{\circ}\text{C}$  with and without substrates showed a significant methane production (range:  $0.04 - 0.78 \text{ nmol CH}_4 \text{ h}^{-1} \text{ g}^{-1}$ ).

This work shows for the first time that microorganisms (particularly methanogens and methanotrophs) do not only survive in permafrost habitats but also can be metabolic active in perennially frozen deposits. Despite the adaptation of the microorganisms to their cold environment, it was shown that a slight increase of the temperature can lead to a substantial increase of methanogenic activity. In dependence of the microrelief, the hydrological conditions and the vegetation, the methane oxidation can be an important sink for methane in permafrost ecosystems. However, the erosion and degradation of permafrost can lead to an extensive expansion of the methane reservoir by *in situ* methanogenesis as shown for the Holocene deposits, with their subsequent impacts on total methane budget.

#### 7.4 Survival of Methanogenic Archaea under Extreme Environmental Conditions

Terrestrial permafrost, in which microorganisms have survived for several millions of years (Vorobyova et al., 1997, Rivkina et al., 1998), is considered as a model for extraterrestrial analogues, because of their comparable environmental conditions (chapter 6.1). The Martian surface and terrestrial permafrost areas show similar morphological structures (Figure 7.8), which points to profound cryogenic processes (Kuzmin, 2005). Despite the harsh living conditions, terrestrial permafrost is colonized by high numbers of microorganisms such as methanogenic archaea (cp. chapter 7.3). Survival of microorganisms is sustained by anabiosis (dormant stage of life) or by reduced metabolic activity in unfrozen water films (chapter 4.4). Methanogenic archaea represent the most suitable model organisms for studying life under extreme

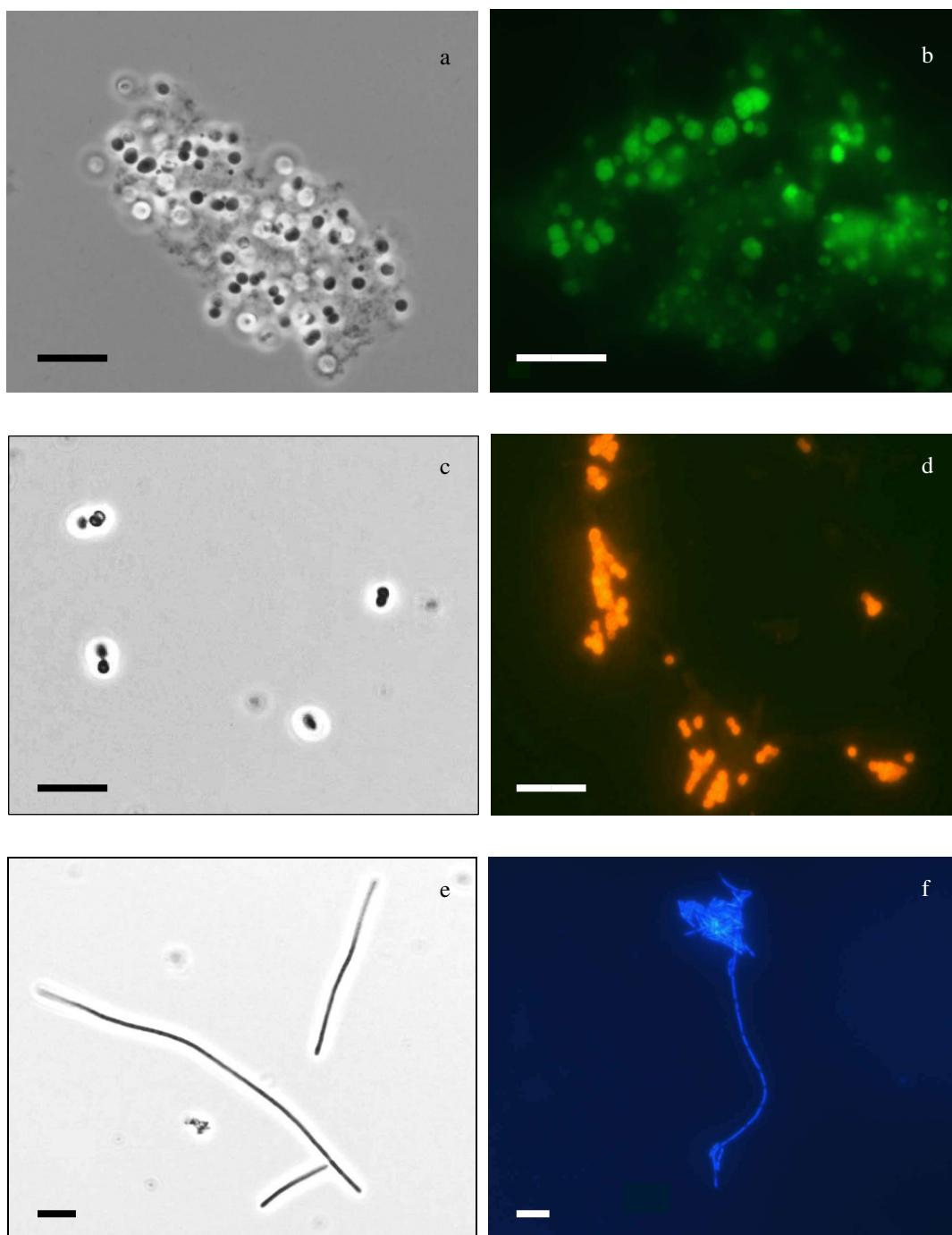
permafrost conditions, because of their potential to lithoautotrophic growth (chapter 6.1).



**Figure 7.8:** Polygonal patterned ground: a.) Lena Delta, Siberian Arctic (April 1999, photo W. Schneider, AWI) and b.) Mars, Northern Hemisphere, (May 1999, photo NASA).

For the investigation of the stress tolerance of methanogenic archaea, representatives of these organisms have been enriched and isolated from permafrost soils of Samoylov Island with hydrogen, methanol and acetate as substrates (Figure 7.9). Most of the experiments were carried out with *Methanosarcina* spec. SMA-21. The organism was grown on bicarbonate-buffered, oxygen-free OCM culture medium (Boone et al., 1989) under an atmosphere of H<sub>2</sub>/CO<sub>2</sub> (80:20 v/v, pressurised to 150 kPa). SMA-21 grew well at 28°C and slowly at low temperatures (4°C and 10°C). The cells grow as irregular cocci, with a diameter of 1-2 µm. Cell aggregates were regularly observed. All other methanogenic isolates were not phylogenetically described so far. However, cells of the strain SMA-16 were small irregular diplococci, 0.5–1 µm in diameter. Strain SMA-23 appeared as rod-shaped cells with about 1 µm in width and max. 8 µm in length. This strain often appears in the forms of long cell chains.

The survival potential of *Methanosarcina* SMA-21 was analyzed in comparison to methanogenic archaea from non-permafrost environments as reference organisms (*Methanosarcina barkeri* DSM 8687, *Methanobacterium* spec. MC-20; chapter 6.3). They were exposed to different environmental stress conditions comprising low temperature (down to -78.5 °C), high salinity (up to 6 M NaCl), starvation (up to 3 months), long-term freezing (up to 2 years), desiccation (up to 25 days) and oxygen exposure (up to 72 hours).



**Figure 7.9:** Phase contrast and fluorescence micrographs of methanogenic archaea isolated from Siberian permafrost. *Methanosarcina* SMA-21 (a, b), strain SMA-16 (c, d) and strain SMA-23 (e, f). The cells were stained with the general oligonucleotid probe for Archaea (ARC915 Cy3, d), DAPI (f) and self fluorescence by 420 nm (b). Bar = 10  $\mu$ m (photos according to Morozova et al, in press).

*Methanosarcina* SMA-21 showed remarkably high resistance to all of the tested stress conditions, while the reference organisms reacted very sensitively to the same stress parameters (chapter 6.3). Strain SMA-21 can be active even under salt saturated conditions. The activity was twice as high at 4 °C with 0.022 nmol CH<sub>4</sub> h<sup>-1</sup>

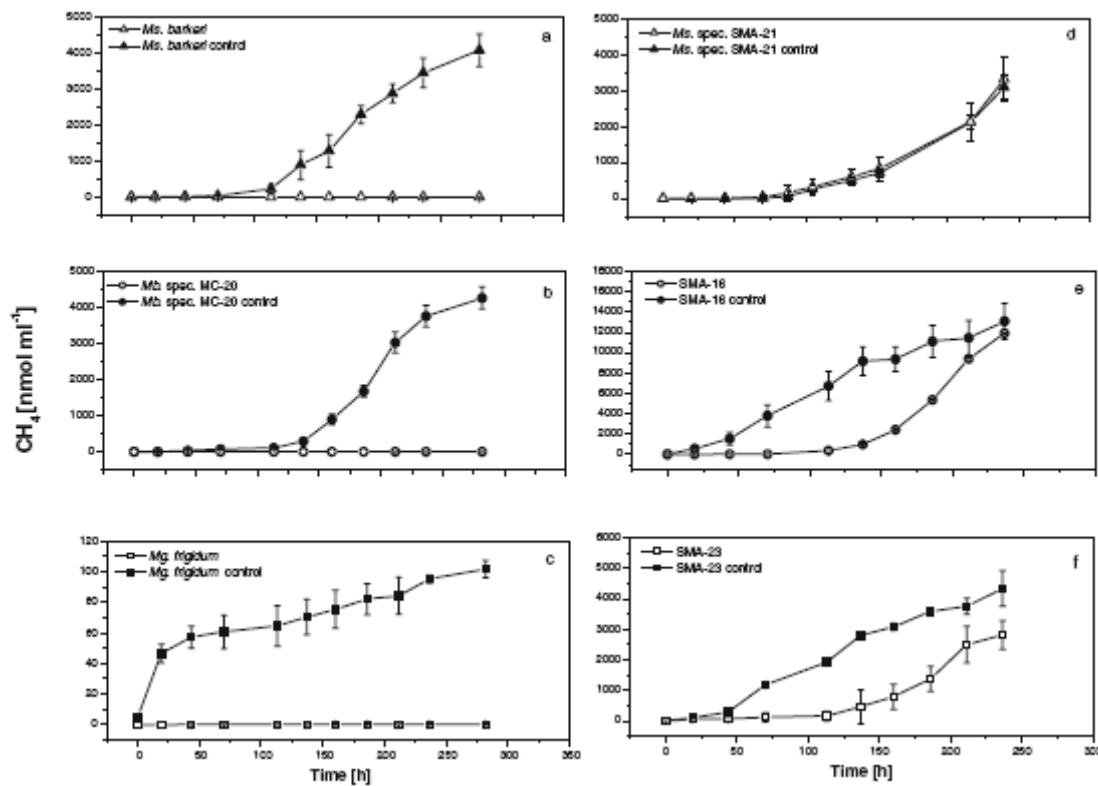
ml<sup>-1</sup> than it was at 28 °C (0.013 nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>) at the same salt concentration. This corresponds to the environmental conditions in permafrost in late autumn. During the freeze-back of the active layer the salt concentration in the remaining pore water increases. Furthermore, a high survival of more than 70% of the cells was observed after freezing at -78.5 °C. Pre-conditioning to cold temperatures (cold shock), known to increase the resistance to freezing of many microorganisms due to the expression of cold-responsive genes and cryoprotectant molecules (Kim & Dunn, 1997; Wouters *et al.*, 2001; Georlette, 2004; Weinberg *et al.*, 2005), does not increase the freezing tolerance of *Methanosarcina* SMA-21. This finding is consistent with the resistance to long-term freezing of this archaeon observed after two-year exposure at -20 °C without any pre-conditioning. Both results suggest that this strain is already adapted to the extreme temperature regime of the permafrost habitat.

Incubation of the reference organisms without any substrate was very efficient in reducing the survival potential of these methanogens, which do not survive after one month of starvation. In contrast, *Methanosarcina* SMA-21 maintained high survival rates even after being starved for three months. The same trend was observed for desiccation, which was lethal for the reference organisms, whereas *Methanosarcina* SMA-21 survived for at least 25 days.

Most striking was the ability of the permafrost strain to survive for several hours in the presence of oxygen without any decrease in cell numbers or methane production rates. Moreover, a significant portion (10 %) of *Methanosarcina* SMA-21 population survived up to 72 hours of oxygenation.

It has been shown by Kiener and Leisinger (1983) that the arrangement of cells in packets, which is typical for the genus *Methanosarcina*, protected these anaerobic colony-forming units during extended periods of environmental stress like varying oxygen concentrations. Thus, it was concluded that the outer cell aggregates of *Methanosarcina* SMA-21 also protect the inner cells from the damaging influence of the permafrost environments, such as caused by ice crystal formation at low temperatures that increases the salinity or leads to high oxygen concentrations. Apart from the physico-chemical conditions of permafrost, the physiological properties of methanogens are relevant for the adaptation to extreme conditions. The survival of methanogenic archaea in permafrost can be achieved, if they acquire the abilities of DNA repair, modification of cell membranes and other vital functions to maintain cell viability (Rivkina *et al.*, 2004). However, cell damages caused by different stress factors seem to be compensated by similar protecting mechanisms. For instance most of freeze protection mechanisms (synthesis of chaperones, accumulation of compatible solutes, modification of cytoplasm membrane, synthesis of antioxidants) overlap with tolerance mechanisms protecting cells against desiccation, starvation or high salt concentrations (Macario *et al.*, 1999; Georlette *et al.*, 2004).

Apart from the single stress experiments, the probability of methanogens to survive under present Martian conditions was studied by running a unique Mars simulation experiment within the HUMIDITY-Lab of the German Aerospace Center (chapter 6.4).



**Figure 7.10:** Methane production activities of the reference organisms *Methanosaerica barkeri* (a), *Methanobacterium MC-20* (b), *Methanogenium frigidum* (c) and methanogens isolated from Siberian permafrost *Methanosaerica SMA-21* (d), SMA-16 (e), SMA-23 (f) before and after exposure to simulated Martian conditions (the error bars represent the standard deviation, n=3).

Three strains of methanogens from Siberian permafrost (*Methanosaerica* SMA-21, SMA-23 and SMA-16) and three reference organisms from non-permafrost habitats, (*Methanosaerica barkeri* DSM 8687, *Methanogenium frigidum* DSM 16458 and *Methanobacterium* spec. MC-20), were exposed to conditions equivalent to those of the equatorial Martian surface for 22 days: the diurnal temperature during the simulation varied between -75 °C and +20 and the water activity between  $a_w$  values of 0.1 and 0.9 in an anoxic, Mars-like atmosphere dominated by carbon dioxide (95.3 %). The methanogens from permafrost and non-permafrost habitats showed distinct differences in their survival potential under Martian conditions.

The average cell number of *Methanosaerica* SMA-21 decreased from  $6.1 \times 10^7$  cells  $\text{ml}^{-1}$  at the beginning of the experiment to  $5.5 \times 10^7$  cells  $\text{ml}^{-1}$  at the end of the simulation, which equals to a survival rate of 90.4%. Strains SMA-16 and SMA-23 showed a survival rate of 67.3% and 60.6%, respectively. In comparison, only 1.1%

of *Methanobacterium* MC-20, 5.8% of *Methanogenium frigidum* and 0.3% of *Methanosarcina barkeri* survived under simulated Martian conditions. The decrease of cell numbers correlates well with the methane production rates of the cultures. Thus, activity of strains SMA-16, SMA-21 and SMA-23 measured prior to the exposure to simulated Martian conditions was similar to that after the simulation, whereas methane production of the reference organisms *Mb.* MC-20, *Mg. frigidum* and *Ms. barkeri* drastically decreased after the experiment (Figure 7.10). The methane production rates of *Ms.* SMA-21 slightly decreased after exposure to simulated Martian conditions from  $48.61 \pm 6.57$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> to  $44.11 \pm 5.08$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>. The activities of two other permafrost isolates, SMA-16 and SMA-23 were also only marginally affected by the Martian experiment. The methane production rates of SMA-16 decreased from  $52.77 \pm 6.18$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> at the beginning of the experiment to  $45.37 \pm 0.03$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> after the exposure. The methane production rates of SMA-23 decreased from  $22.13 \pm 1.94$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> to  $13.92 \pm 3.87$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup>. The activities of the reference organisms *Ms. barkeri* and *Mb.* MC-20 after the simulation experiment were almost extinct. Methane production rates of *Mg. frigidum* significantly decreased from  $2.76 \pm 0.07$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> measured before the experiment to  $0.003 \pm 0.005$  nmol CH<sub>4</sub> h<sup>-1</sup> ml<sup>-1</sup> after the exposure. Obviously methanogenic archaea from permafrost ecosystems more persistent under unfavourable, mars-like living conditions than those from non-permafrost environments.

## 8 Conclusions

The present thesis provides the first integrated research on methane dynamics in Arctic permafrost ecosystems that connects trace gas flux measurements with studies on microbial processes and communities. The findings demonstrate the close relationship between apparent methane fluxes and the modes and intensities of microbiological processes of methane production and oxidation in the polygonal tundra soils. The permafrost environment forces the adaptation of the microbial communities to low temperature conditions with species, which have been untraced in temperate ecosystems so far. An important finding is that in addition to soil characteristics and climate conditions, the activity and physiology of the microbial communities dictate trace gas fluxes in permafrost soils. In this manner, the conducted study underlines that the prediction and modeling of the future fate of the large carbon reservoir in permafrost ecosystems also has to involve a sophisticated view of the small-scale microbiological processes in permafrost soils. The following conclusions can be drawn from the specific results obtained in this study:

- The methane emission from the polygonal tundra is comparatively low regarding both daily summer fluxes (typically  $30 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ ) and the total annual flux ( $3 \text{ g CH}_4 \text{ m}^{-2}$ ). Reason for this may be (a) the very low permafrost temperature in the study region, (b) the sandy soil texture and low bio-availability of nutrients in the soils, and (c) the genesis of ice-wedge polygons which lead to a strong spatial heterogeneity in soil and vegetation properties and to a high surface coverage of dry micro-sites (> 50 %).
- The used upscaling approach for the calculation of methane flux balances, based on remote sensing and supervised image classification, provides a more realistic estimation of the methane emission on the regional scale than most of the published data for comparable environments.
- Although permafrost soils include a high amount of organic carbon, methanogenesis is substrate-limited because of a depth-dependence decrease of organic matter quality within the soils. This is an important finding for modeling and calculating trace gas fluxes from permafrost environments, because the known flux models only consider the total carbon amount.
- The microbial community analyses show that permafrost soils of the Siberian Arctic are composed by members of all three domains of life (*Archaea*, *Bacteria* and *Eukarya*), with a total biomass comparable to temperate soil ecosystems.
- Both groups of microorganisms (methanogens and methanotrophs) involved in the Arctic methane cycle are well adapted to the extreme environmental conditions of their habitat. This is reflected in a shift of the microbial

communities from mesophilic to psychrotolerant or psychrophilic species along the temperature gradient of permafrost soils.

- Phylogenetic analyses of methanogenic archaea reveal specific clusters of these organisms only found in the investigated permafrost ecosystems so far. The DNA sequences obtained from permafrost soils are belonging probable to methanogenic archaea characterized by specific adaptations to extreme permafrost conditions.
- Methanogenic archaea from terrestrial permafrost show an unexpected resistance against extreme living conditions and even survived under simulated Martian temperature and humidity conditions. In consideration of the results from the last Mars missions, which proved the presents of both the appropriate substrates ( $H_2 + CO_2$ ) for lithoautotrophic methanogenesis and the product ( $CH_4$ ) of methanogenic metabolism on Mars, organisms comparable to methanogens from permafrost can be seen as one of the most likely candidates for life on Mars.

Furthermore, the results show that methane of microbial origin in perennially frozen deposits probably represents an unconsidered source for the global methane budget. Methane release to the atmosphere from frozen ground is mediated by ongoing permafrost degradation through enhanced thermokarst formation and accelerated coastal erosion in the Arctic. Although the change in permafrost conditions by global warming is examined in the framework of several international projects (e.g. ACD: Arctic Coastal Dynamics, CALM: Circumpolar Active Layer Monitoring), these investigations should be linked more closely with microbiological process studies and biodiversity research. Microbial parameters important for the assessment of the carbon turnover (e.g. cell numbers, activities, biodiversity and stability of microbial communities) should be analysed at observation areas in the Arctic, where long-term ongoing monitoring programs are undertaken. The evaluation of microbiological data and their correlation with climatic and geochemical results represents the basis for the understanding of the role of permafrost in the global system, in particular feedback mechanisms related to material fluxes and greenhouse gas emissions in the scope of a warming Earth.

## 9 References

Since in two manuscripts (Chapter 5.1 and 6.1) the citations are specified after numbers, these are placed in front of the summarized list of references.

**References of Chapter 5.1: Characterisation of microbial community composition of a Siberian tundra soil by fluorescence in situ hybridisation**

- [1] Post, W.M., Emanuel, W.R., Zinke, P.J. and Stangenberger, A.G. (1982) Soil carbon pools and world life zones. *Nature* 298, 156-159.
- [2] Oechel, W.C. and Billings, W.D. (1992) Effects of global change on the carbon balance of arctic plants and ecosystems. In: Arctic ecosystems in a changing climate. An ecophysiological perspective. (Chapin, F.S., Ed.) Academic, San Diego, California.
- [3] Smith, L.C., MacDonald, G.M., Velichko, A.A., Beilman, D.W., Borisova, O.K., Frey, K.E., Kremenetski, K.V. and Sheng, Y. (2004) Siberian peatlands a net carbon sink and global methane source since the early holocene. *Science* 303, 353-356.
- [4] Gorham, E. (1991) Northern peatlands: role in the carbon cycle and probable responses to climatic warming. *Ecological Applications* 1, 182-195.
- [5] Nadelhoffer, K.J., Giblin, G.R., Shaver, G.R. and Linkins, A.E. (1992) Microbial processes and plant nutrient availability in arctic soils. In: Arctic ecosystems in a changing climate. An ecophysiological perspective. (Chapin, F.S., Ed.) Academic, San Diego, California.
- [6] Khalil, M.A.K. and Rasmussen, R.A. (1983) Sources, sinks, and seasonal cycles of atmospheric methane. *J. Geophys. Res.* 88, 5131-5144.
- [7] Christensen, T.R., Jonasson, S., Havström, M. and Livens, F. R. (1999) Carbon cycling and methane exchange in Eurasian tundra ecosystems. *Ambio* 28, 239-244.
- [8] Fung, I., John, J., Lerner, J., Matthews, E., Prather, M., Steele L. P. and Fraser, P. J. (1991) Three dimensional model synthesis of the global methane cycle. *J. Geophys. Res.* 96, 13033-13065.
- [9] IPCC (Intergovernmental Panel of Climate Change). (1990) Scientific Assessment. Cambridge Univ. Press, Cambridge.
- [10] Christensen, T. (1991) Arctic and sub-Arctic soil emissions: possible implications for global climate change. *Polar Record* 27, 205-210.
- [11] Chen, G., Zhu, H. and Zhang, Y. (2003) Soil microbial activities and carbon and nitrogen fixation. *Research in Microbiology* 154, 393-398.
- [12] Zyvagtsev, D. (1994) Vertical distribution of microbial communities in soils. In: Beyond the biomass (Ritz, K., Dighton, J., Giller, K., Eds.) pp. 29-37, Wiley, West Sussex, UK.
- [13] Blume, E., Bischoff, M., Reichert, J., Moorman, T., Konopka, A. and Turco, R. (2002) Surface and subsurface community structure and metabolic activity as a function of soil depth and season. *Appl. Soil Ecol.* 59, 1-11.
- [14] Skinner, F.A., Jones, P.C.T. and Mollison, J.E. (1952a) Comparison of a direct- and a plate-counting technique for the quantitative estimation of soil microorganisms. *J. Gen. Microbiol.* 6, 261-271.
- [15] Wagner, M., Amann, R., Lemmer, H. and Schleifer, K.-H. (1993) Probing activated sludge with oligonucleotides specific for proteobacteria: inadequacy of culture-dependent methods for describing microbial community structure. *Appl. Environ. Microbiol.* 59, 1250-1255.
- [16] Amann, R. I., Ludwig, W. and Schleifer, K.-H. (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *FEMS Microbiol. Rev.* 59, 143-169.
- [17] Poulsen, L. K., Ballard, G. and Stahl, D. A. Use of rRNA fluorescence in situ hybridization for measuring the activity of single cells in young and established biofilms. *Appl. Environ. Microbiol.* 59, 1354-1360.
- [18] Kemp, P.F., Lee, S. and LaRoche, J. (1993) Estimating the growth rate of slowly growing marine bacteria from RNA content. *Appl. Environ. Microbiol.* 59, 2594-2601.
- [19] DeLong, E. F., Wickham, G. S. and Pace, N. R. (1989) Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. *Science* 243, 1360-1362.
- [20] Hahn, D., Amann, R. I., Ludwig, W., Akkermann, A. D. L. and Schleifer, K.-H. (1992) Detection of micro-organisms in soil after in situ hybridization with rRNA-targeted, fluorescently labelled oligonucleotides. *J. Gen. Microbiol.* 138, 879-887.

- [21] Moter, A. and Göbel, U. B. (2000) Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. *J. Microbiol. Methods* 41, 85-112.
- [22] Amann, R., Glöckner, F.-O. and Neef, A. (1997) Modern methods in subsurface microbiology: in situ identification of microorganisms with nucleic acid probes. *FEMS Microbiol. Rev.* 20, 191-200.
- [23] Pernthaler, J., Glöckner, F.O., Schönhuber, W. and Amann, R. (2001) Fluorescence in situ hybridization with rRNA-targeted oligonucleotide probes. In: *Methods in Microbiology: Marine Microbiology*. Vol. 30. (Paul, J., Ed.) Academic Press Ltd., London.
- [24] Amann, R. I. (1995) In situ identification of micro-organisms by whole cell hybridization with rRNA-targeted nucleic acid probes. In: *Molecular Microbial Ecology Manual* (Akkemann, A.D.C., van Elsas, J.D. and de Brujin, F.J., Eds.) pp.1-15. Kluwer Academic Publishers, Dordrecht.
- [25] Christensen, H., Hansen, M. and Sørensen, J. (1999) Counting and size classification of active soil bacteria by fluorescence in situ hybridization with an rRNA oligonucleotide probe. *Appl. Environ. Microbiol.* 65, 1753-1761.
- [26] Bouvier, T. and del Giorgio, P.A. (2003) Factors influencing the detection of bacterial cells using fluorescence in situ hybridization (FISH): a quantitative review of published reports. *FEMS Microbiol. Ecol.* 44, 3-15.
- [27] Wagner, D., Kobabe, S., Pfeiffer, E.-M. and Hubberten, H.-W. (2003) Microbial controls on methane fluxes from a polygonal Tundra of the Lena Delta, Siberia. *Permafrost and Periglac. Process.* 14, 173-185.
- [28] Rachold, V. and Grigoriev M.N. (Eds) (2000). Russian-German Cooperation SYSTEM LAPTEV SEA 2000: The Expedition LENA 1999. *Reports on Polar Research* 354, 1-269.
- [29] Schwamborn, G., Rachold, V. and Grigoriev, M.N. (2002) Late Quaternary sedimentation history of the Lena Delta. *Quaternary International* 89, 119-134.
- [30] Bouvier, T. and del Giorgio, P.A. (2003) Factors influencing the detection of bacterial cells using fluorescence in situ hybridization (FISH): a quantitative review of published reports. *FEMS Microbiol. Ecol.* 44, 3-15.
- [31] Wagner, D., Kobabe, S., Pfeiffer, E.-M. and Hubberten, H.-W. (2003) Microbial controls on methane fluxes from a polygonal Tundra of the Lena Delta, Siberia. *Permafrost and Periglac. Process.* 14, 173-185.
- [32] Rachold, V. and Grigoriev M.N. (Eds) (2000). Russian-German Cooperation SYSTEM LAPTEV SEA 2000: The Expedition LENA 1999. *Reports on Polar Research* 354, 1-269.
- [33] Schwamborn, G., Rachold, V. and Grigoriev, M.N. (2002) Late Quaternary sedimentation history of the Lena Delta. *Quaternary International* 89, 119-134.
- [34] Schoeneberger, P.J., Wysocki, D.A.. Benham, E.C. and Broderson, W.D. (Eds.), (2002) Field book for describing and sampling soils, Version 2.0. Natural Resources Conservation service, National Soil Survey Center, Lincoln, NE.
- [35] Schlichting, E., Blume, H.-P. and Stahr, K. (1995) Bodenkundliches Praktikum – Pareys Studientexte 81. Blackwell Wissensverlag, Berlin.
- [36] Soil Survey Staff. (1998) Keys to Soil taxonomy, 8th edition. USDA-The national resources conservation service, Lincoln.
- [37] Kutzbach, L., Wagner, D. and Pfeiffer, E.-M. (2003) Effect of microrelief and vegetation on methane emission from wet polygonal tundra, Lena Delta, Northern Siberia. *Biogeochemistry*, in press.
- [38] Friedrich, K. (2001) Energie- und Wasserhaushalt eines Tundrenstandortes im Lena-Delta. Diploma-thesis, TU Dresden.
- [39] Sherr, B. F., Sherr, E. B. and Fallon, R. D. (1987) Use of monodispersed, fluorescently labelled bacteria to estimate in situ protozoan bacterivory. *Appl. Environ. Microbiol.* 53, 958-965.
- [40] Bloem, J., Veninga, M. and Shephard, J. (1995) Fully automatic determination of soil bacterium numbers, cell volumes, and frequencies of dividing cells by confocal laser scanning microscopy and image analysis. *Appl. Environ. Microbiol.* 61, 926-936.
- [41] Snaird, J., Amann, R., Huber, I., Ludwig, W. and Schleifer, K.-H. (1997) Phylogenetic analysis and in situ identification of bacteria in activated sludge. *Appl. Environ. Microbiol.* 63, 2884-2896.
- [42] Stahl, D.A. and Amann, R. (1991) Development and application of nucleic acid probes. In: *Nucleic acid techniques in bacterial systematics* (Stackebrandt, E. and Goodfellow, M., Eds.) pp. 205-248. Wiley and Sons, New York.
- [43] Daims, H., Ramsing, N.B., Schleifer, K.-H. and Wagner, M. (2001) Cultivation-independent, semiautomatic determination of absolute bacterial cell numbers in environmental samples by fluorescence in situ hybridization. *Appl. Environ. Microbiol.* 67, 5810-5818.

- [44] Friedrich, U., Naismith, M.M., Altendorf, K. and Lipski, A. (1999) Community analysis of biofilters using fluorescence in situ hybridization including a new probe for the *Xanthomonas* branch of the class Proteobacteria. *Appl. Environ. Microbiol.* 65, 3547-3554.
- [45] Felske, A., Akkermans, A. D. L. and de Vos, W. M. (1998) In situ detection of an uncultured predominant bacillus in Dutch grassland soils. *Appl. and Environ. Microbiol.* 64, 4588-4590.
- [46] Schmidt, N. (1999) Microbial properties and habitats of permafrost soils an Taymir peninsula, central Siberia. *Reports on Polar Research* 340, 1-164.
- [47] Dedysh, S.N., Dunfield, P.F., Derakshani, M., Stubner, S., Heyer, J. and Liesack, W. (2003) Differential detection of type II methanotrophic bacteria in acidic peatlands using newly developed 16S rRNA-targeted fluorescent oligonucleotide probes. *FEMS Microbiol. Ecol.* 43, 299-308.
- [48] Atlas, R.M. and Bartha, R. (1993) *Microbial Ecology Fundamentals and Applications*. The Benjamin/Cummings Publishing Company, Redwood City, California.
- [49] Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkina, E. and Vishnivetskaya, T. (1997) The deep cold biosphere: facts and hypothesis. *FEMS Microbiol. Rev.* 20, 277-290.
- [50] Vishnivetskaya, T., Kathariou, S., McGrath, J., Gilichinsky, D. and Tiedje, J.M. (2000) Low-temperature recovery strategies for the isolation of bacteria from ancient permafrost sediments. *Extremophiles* 4, 165-173.
- [51] Rivkina, E.M., Friedmann, E.I., McKay, C.P. and Gilichinsky, D.A. (2000) Metabolic activity of permafrost bacteria below the freezing point. *Appl. Environ. Microbiol.* 66, 3230-3233.
- [52] Zarda, B., Hahn, D., Chatzinotas, A., Schönhuber, W., Neef, A., Amann, R. I. and Zeyer, J. (1997) Analysis of bacterial community structure in bulk soil by in situ hybridization. *Arch. Microbiol.* 168, 185-192.
- [53] Lynch, J.M. (1998) Microorganisms in their natural environments: the terrestrial environment. In: *Micro-organisms in action: concepts and applications in microbial ecology* (Lynch, J.M. and Hobbie, J.E., Eds.), pp. 103-131 Blackwell Scientific Publications, London, England.
- [54] Smith, J.L. and Paul, E.A. (1990) The significance of soil microbial biomass estimations. In: *Soil biochemistry* (Bollag, J.-M. and Stotzky G., Eds.). Marcel Dekker, Inc., pp. 357-395. New York, N.Y..
- [55] Aldén, L., Demoling, F. and Bååth, E. (2001) Rapid method of determining factors limiting bacterial growth in soil. *Appl. Environ. Microbiol.* 67, 1830-1838.
- [56] Fierer, N., Schimel, J.P. and Holden, P.A. (2003) Variations in microbial community composition through two soil depth profiles. *Soil Biology & Biochemistry* 35, 167-176.
- [57] Holmes, B. The Genera *Flavobacterium*, *Sphingobacterium*, and *Weeksella* In: *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*, (Dworkin, M. et al., Eds.), 3rd edition, release 3.0, 1999, Springer-Verlag, New York, <http://link.springer-ny.com/link/service/books/10125/>.
- [58] Reichenbach, H. The order Cytophagales In: *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*, (Dworkin, M. et al., eds.), 3rd edition, release 3.0, 1999, Springer-Verlag, New York, <http://link.springer-ny.com/link/service/books/10125/>.
- [59] LaMontagne, M.G., Schimel, J.P. and Holden, P.A. (2003) Comparison of subsurface and surface soil bacterial communities in California grassland as assessed by terminal restriction fragment length polymorphisms of PCR-amplified 16SrRNA genes. *Microb. Ecol.* 46, 216-227.
- [60] Rovira A.D. (1956) Plant root excretions in relation to the rhizosphere effect II. A study of the properties of root exudates and its effect on the growth of micro-organisms isolated from the rhizosphere and control soil. *Plant Soil* 7, 195-208.
- [61] Bolton, H., Jr., Fredrickson, J.K. and Elliott, L.F. (1993) Microbial ecology of the rhizosphere. In: *Soil Microbial Ecology* (Metting, F.B., Jr., Ed.), pp. 27-63. Marcel Dekker, New York.
- [62] Armstrong, J., Armstrong, W., Beckett, P.M., Halder, J.E., Lythe, S., Holt, R. and Sinclair, A. (1996) Pathway of aeration and the mechanisms and beneficial effects of humidity- and Venturi-induced convections in *Phragmites australis* (Cav.) Trin.ex Steud. *Aquatic Botany* 54, 177-197.
- [63] Brix, H., Sorrell, B.K. and Schierup, H.H. (1996) Gas fluxes achieved by in situ convective flow in *Phragmites australis*. *Aquatic Botany* 54, 151-163.
- [64] Bendix, M., Tornbjerg, T. and Brix, H. (1994) Internal gas transport in *Typha latifolia* L. and *Typha angustifolia* L. 1. Humidity-induced pressurization and convective throughflow. *Aquatic Botany* 49, 75-89.
- [65] Hales, B.A., Edwards, C., Ritchie, D.A., Hall, G., Pickup, R.W. and Saunders, J.R. (1995) Isolation and identification of methaogen-specific DNA from blanket bog peat by PCR amplification. *Appl. Environ. Microbiol.* 62, 668-675.

- [66] Whitman, W.B., Bowen, T.C. and Boone, D.R. (1992) The methanogenic bacteria. In: The prokaryotes (Balows, A., Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H., Eds.) Vol. 1, pp. 719-767, Springer.
- [67] Wagner, D. and Pfeiffer, E.-M. (1997) Two temperature optima of methane production in a typical soil of the Elbe river marshland. FEMS Microbiol. Ecol. 22, 145-153.
- [68] Mayer, H.-P. and Conrad, R. (1990) Factors influencing the population of methanogenic bacteria and the initiation of methane production upon flooding of paddy soils. FEMS Microbiol. Ecol. 73, 103-112.
- [69] Peters, V. and Conrad, R. (1995) Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. Appl. Environ. Microbiol. 61, 1673-1676.
- [70] Wagner, D. and Pfeiffer, E.-M. (1997) Two temperature optima of methane production in a typical soil of the Elbe river marshland. FEMS Microbiol. Ecol. 22, 145-153.
- [71] Wachinger, G., Fiedler, S., Zepp, K., Gattinger, A., Sommer, M. and Roth, K. (2000) Variability of soil methane production on the micro-scale: spatial association with hot spots of organic material and archaeal populations. Soil Biol. Biochem. 32, 1121-1130.
- [72] Valentine, D.W., Holland, E.A. and Schimel, D.S. (1994) Ecosystem and physiological controls over methane production in northern wetlands. J. Geophys. Res. 99, 1563-1571.
- [73] Zepp-Falz, K., Holliger, C., Großkopf, R., Liesack, W., Nozhenikova, A.N., Müller, B., Wehrli, B. and Hahn, D. (1999) Vertical distribution of methanogens in the anoxic sediment of Rotsee (Switzerland). Appl. Environ. Microbiol. 65, 2402-2408.
- [74] Harris, J.M. and Tibbles, B.J. (1997) Factors affecting bacterial productivity in soils on isolated inland nunataks in continental Antarctica. Microb. Ecol. 33, 106-123.
- [75] Böltner, M., Blume, H.-P. and Kuhn, D. (1999) Soils and their microbiological properties from a transect from Cape Horn to the Antarctic Peninsula. Polar Bioscience 12, 54-67.
- [76] Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R. and Stahl, D.A. (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. 56, 1919-1925.
- [77] Daims, H., Brühl, A., Amann, R., Schleifer, K-H. and Wagner, M. (1999) The domain-specific probe EUB338 is insufficient for the detection of all Bacteria; development and evaluation of a more comprehensive probe set. System. and Appl. Microbiol. 22, 434-444.
- [78] Wallner, G., Amann, R. and Beisker, W. (1993) Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. Cytometry 14, 136-143.
- [79] Neef, A. (1997) Anwendung der in situ Einzelzell-Identifizierung von Bakterien zur Populationsanalyse in komplexen mikrobiellen Biozönosen. Ph.D. Thesis, Technische Universität München.
- [80] Manz, W., Amann, R., Ludwig, W., Wagner, M. and Schleifer, K-H. (1992) Phylogenetic Oligodeoxynucleotide probes for the major subclasses of proteobacteria: problems and solutions. System. Appl. Microbiol. 15, 593-600.
- [81] Roller C., Wagner M., Amann R., Ludwig W. and Schleifer, K.-H. (1994). In situ probing of Gram-positive bacteria with high DNA G+C content using 23S rRNA- targeted oligonucleotides. Microbiol. 140, 2849-2858.
- [82] Meier, H., Amann, R., Ludwig, W. and Schleifer, K-H. (1999) Specific oligonucleotide probes for in situ detection of a major group of gram-positive bacteria with low DNA G+C content. System. Appl. Microbiol. 22, 186-196.

**References of Chapter 6.1: Microbial life in terrestrial permafrost: methanogenesis and nitrification in gelisols as potentials for exobiological processes**

- 1 Wharton Jr., R.A., McKay, C.P., Mancinelli, R.L., Simmons Jr., G.M. (1989). Early martian environments: the antarctic and other terrestrial analogs. Adv. Space Res. 9: 147-153.
- 2 Horneck, G. (in press). The microbial world and the case for Mars. Planet. Space Sci.
- 3 Soil Survey Staff (1998). Keys to Soil Taxonomy. Lincoln, Nebraska. U.S. Department of Agriculture, Soil Conservation Service. Pocahontas Press, Blacksburg.
- 4 French, H.M. (1996). The periglacial environment. Longman, Westminster.
- 5 Herbert, R.A. (1996). The ecology and physiology of psychrophilic microorganisms, in (Herbert, R.A., Cod, G.A., eds.) Microbes in extreme environments. Academic Press, London: 1-23.
- 6 Yershov, E.D. (1998). General Geochryology. Cambridge University Press, Cambridge.
- 7 Gounot, A.M. (1991). Bacterial life at low temperature: physiological aspects and biotechnological implications. J. Appl. Bacteriol. 71: 386-397.

- 8 Russel, N.J., Hamamoto, T. (1998). Psychrophiles. In: (Horokoshi, K., Grant, W.D., eds.) *Extremophiles: microbial life in extreme environments*. Wiley, New York: 25-45.
- 9 Morita, R.Y. (1975). Psychrophilic bacteria. *Bacteriol. Rev.* 39: 144-167.
- 10 Rivkina, E., Friedmann, E.I., McKay, C.P., Gilichinsky, D. (2000). Metabolic activity of permafrost bacteria below the freezing point. *Appl. Environ. Microbiol.* 66: 3230-3233.
- 11 Morita, R.Y. (1992). Low-temperature environments. In: (Lederberg, J., ed.) *Encyclopedia of microbiology*. Academic Press, San Diego: 625-637.
- 12 Ananyan, A.A. (1970). Unfrozen water content in frozen clay at temperature from -0.6 °C to -40 °C - -60 °C. *Merzlotnye Issledovaniya* 10: 267-270 (in Russian).
- 13 Ostroumov, V.E., Siegert, C. (1996). Exobiological aspects of mass transfer in microzones of permafrost deposits. *Adv. Space Res.* 18: 79-86.
- 14 McGrath, J., Wagener, S., Gilichinsky, D.A. (1994). Cryobiological studies of ancient microorganisms isolated from the Siberian permafrost. In: (Gilichinsky, D.A., ed.) *Viable microorganisms in permafrost*. Pushchino Research Centre, Pushchino: 48-67.
- 15 Russel, N.J. (1990). Cold adaptation of microorganisms. *Phil. Trans. Roy. Soc. Lond.* 326: 595-611.
- 16 Shi, T., Reeves, R.H., Gilichinsky, D.A., Friedmann, E.I. (1997). Characterization of viable bacteria from siberian permafrost by 16S rDNA sequencing. *Microb. Ecol.* 33: 169-179.
- 17 E. Lebedeva, V. Soina: Nitrifying bacteria as a promising group for paleoecological investigations in the permafrost, in *Viable microorganisms in permafrost*, D.A. Gilichinsky (Ed.), 1th Int'l Conference on Cryopedology and Global Change, November 1992 (Pushchino Research Centre, Pushchino 1994) pp. 74-82
- 18 Gilichinsky, D.A., Vorobyova, E.A., Erokhina, L.G., Fyodorov-Dayvdov, D.G., Chaikovskya, N.R. (1992). Long-term preservation of microbial ecosystems in permafrost. *Adv. Space Res.* 12 (4): 255-263
- 19 Morita, R.Y. (2000). Is hydrogen the universal energy source for long-term survival? *Microbial Ecology* 38: 307-320.
- 20 Garcia, J.L. (1990). Taxonomy and ecology of methanogens. *FEMS Microbiol. Rev.* 87: 297-308.
- 21 Deppenmeier, U., Müller, V., Gottschalk, G. (1996). Pathways of energy conservation in methanogenic archaea. *Arch. Microbiol.* 165: 149-163.
- 22 Whitman, W.B., Bowen, T.C., Boone, D.R. (1992). The methanogenic bacteria. In: (Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H., eds.) *The Prokaryotes*. Springer Verlag, New York: 719-767.
- 23 Gounot, A.M. (1999). Microbial life in permanently cold soils. In: (Margesin, R., Schinner, F., eds.) *Cold-adapted organisms*. Springer, Berlin: 3-16.
- 24 Robertson, D.E., Noll, D., Roberts, M.F., Menaia, J.A., Boone, D.R. (1990). Detection of the osmoregulator betaine in methanogens. *Appl. Environ. Microbiol.* 56: 563-565.
- 25 Rivkina, E., Friedmann, E.I., McKay, C.P. Gilichinsky, D.A. (1998). Biogeochemical activity of anaerobic microorganisms from buried permafrost sediments. *Geomicrobiology* 15: 187-193.
- 26 Peters, V., Conrad, R. (1995). Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. *Appl. Environ. Microbiol.* 61: 1673-1676.
- 27 Stetter, K.O., Fiala, G., Huber, G., Huber, R., Segerer, A. (1990). Hyperthermophilic microorganisms. *FEMS Microbiol. Rev.* 75: 117-124.
- 28 Watson, S.W., Bock, E., Harms, H., Koops, H.P., Hooper, A.B. (1989). Nitrifying bacteria. In (Staley, J.T., Bryant, M.P., Pfennig, N., Holt, J.G., eds.) *Bergey's Manual of Systematic Bacteriology*, 1th Ed., Vol 3, (Williams & Wilkins Co., Baltimore pp. 1808-1834.
- 29 Bock, E. and Koops, H.-P. (1992). The genus *Nitrobacter* and related genera. In: (Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H., eds.) *The Prokaryotes*. Springer Verlag, New York: 2302-2309.
- 30 Koops H.-P., Möller U.C. (1992) The lithotrophic ammonia-oxidizing bacteria. In In: (Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H., eds.) *The Prokaryotes*. Springer Verlag, New York: 2625-2637.
- 31 Teske, A., Alm, E., Regan, J.M., Toze, S., Rittmann, B.E., Stahl, D.A. (1994). Evolutionary relationships among ammonia- and nitrite-oxidizing bacteria. *J. Bacteriol.* 176: 6623-6630.
- 32 Ehrlich, S., Behrens, D., Lebedeva, E., Ludwig, W., Bock, E. (1995). A new obligately chemolithoautotrophic, nitrite-oxidizing bacterium, *Nitrospira moscoviensis* sp. nov. and its phylogenetic relationship. *Arch. Microbiol.* 164: 16-23.
- 33 Speck, E., Bock, E. Genus *Nitrospira*. In (Garrity, G.M., Williams, S.T., Staley, J.T., Brenner, D.J., Holt, J.G., Boone, D.R., Castenholz, R.W., Krieg, N.R., Schleifer, K.H. eds.) *Bergey's Manual of Systematic Bacteriology*, , 2nd Ed., Vol 1 (Williams & Wilkins Co., Baltimore in press).
- 34 Stüven R., Bock E. (2000) Nitrification and denitrification as a source for NO and NO<sub>2</sub> production in high-strength wastewater. *Wat. Res.*, in press

- 35 Wilson, K., Sprent, J.I., Hopkins, D.W. (1997). Nitrification in antarctic soils. *Nature* 385: 404
- 36 Mansch, R. Bock, E. (1998) Biodegradation of natural stone with special reference to nitrifying bacteria. *Biodegradation* 9, 47-64
- 37 Fredrickson, J.K., Garland, T.R., Hicks, R.J., Thomas, J.M., Li, S.W., McFadden, K.M. (1989). Lithotrophic and heterotrophic bacteria in deep subsurface sediments and their relation to sediment properties. *Geomicrobiol. J.* 7: 53-66.
- 38 Lin, L. (1994). Kompatible Solute in nitrifizierenden Bakterien, Ph.D. Thesis, University of Hamburg, Hamburg.
- 39 Conrad R. (1996) Soil microorganisms as controllers of atmospheric trace gases ( $H_2$ , CO,  $CH_4$ , OCS,  $N_2O$ , and NO). *Microbiol. Rev.* 60: 609-640
- 40 Wagner, D., Pfeiffer, E.-M. (1997). Two temperature optima of methane production in a typical soil of the Elbe river marshland. *FEMS Microbiol. Ecol.* 22: 145-153.
- 41 Rivkina, E., Gilichinsky, D., Wagener, S., Tiedje, T., McGrath, J. (1998). Biogeochemical activity of anaerobic microorganisms from buried permafrost sediments. *Geomicrobiol.* 15: 187-193.
- 42 V.S. Soina, E.V. Lebedeva, O.V. Golyshina, D.G. Fedorov-Davydov, D.A. Gilichinsky. (1991) Nitrifying bacteria from permafrost deposits of the Kolyma lowland. *Microbiologia* 60, 187-190, in Russian
- 43 Friedmann, I. (1994). Permafrost as microbial habitat. in *Viable microorganisms in permafrost*, D.A. Gilichinsky (Ed.), 1th Int'l Conference on Cryopedology and Global Change, November 1992 (Pushchino Research Centre, Pushchino 1994) pp.21-26
- 44 Panikov, N.S. (1997). A kinetic approach to microbial ecology in arctic and boreal ecosystems in relation to global change. In: (Oechel, W.C., ed.) *Global change and arctic terrestrial ecosystems*. Springer, Berlin: 171-188.
- 45 Samarkin, V.A., Gundelwein, A., Pfeiffer, E.-M. (1999). Studies of methane production and emission in relation to the microrelief of a polygonal tundra in northern Siberia. In. (Kassens, H., Bauch, H.A., Dmitrenko, I.A., Eicken, H., Hubberten, H.-W., Melles, M., Thiede, J., Timokhov, L.A., eds.) *Land-ocean systems in the Siberian arctic*. Springer, Berlin: 329-342.
- 46 Pfeiffer, E.-M. Wagner, D., Becker, H., Vlasenko, A., Kutzbach, L., Boike, J., Quass, W., Kloss, W., Schulz, B., Kurchatova, A., Pozdnyakov, V.I., Akhmadeeva, I. (2000). Modern processes in permafrost affected soils. In: (Rachold, V., Gregoriev, M.N.,eds.) *Russian-German cooperation System Laptev Sea 2000: The Expedition Lean 1999*. Reports Polar Res. 354: 22-54.
- 47 Kotsyurbenko, O.R., Simankova, M.V., Nozhevnikova, A.N., Zhilina, T.N., Bolotina, N.P., Lysenko A.M., Osipov, G.A. (1995). New species of psychrophilic acetogens: *Acetobacterium bakii* sp. nov., *A. paludosum* sp. nov., *A. fimetarium* sp. nov. *Arch. Microbiol.* 163: 29-34.
- 48 Omelchenko, M.V., Vasilyeva, L.V., Zavarzin, G.A. (1993). Psychrophilic methanotroph from tundra soil. *Curr. Microbiol.* 27: 255-259.
- 49 Finne, G. and Matches, J.R. (1974). Low-temperature-growing clostridia from marine sediments. *Can. J. Microbiol.* 20: 1639-1645.
- 50 Janssen, H. (1994) Anreicherung, Isolierung und Charakterisierung nitrifizierender und methylotropher Bakterien aus Permafrostboden, Diploma Thesis, University of Hamburg, Hamburg
- 51 Franzmann, P.D., Liu, Y., Balkwill, D.L., Aldrich, H.C., Conway de Macario, E., Boone, D.R. (1997). *Methanogenium frigidum* sp. nov., a psychrophilic,  $H_2$ -using methanogen from Ace Lake, Antarctica. *Int. J. Syst. Bacteriol.* 47: 1068-1072.
- 52 Putzer, K.P., Buchholz, L.A., Lidstrom, M.E., Remsen, C.C. (1991). Separation of methanotrophic bacteria by using percoll and its application to isolation of mixed and pure cultures. *Appl. Environ. Microbiol.* 57: 3656-3659.
- 53 Amann, R.I., Ludwig, W., Schleifer, K.-H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59: 143-169.
- 54 Raskin, L., Stromley, J.M., Rittmann, B.E., Stahl, D.A. (1994). Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. *Appl. Environ. Microbiol.* 60: 1232-1240.
- 55 Bartosch, S., Wolgast, I., Speck, E., Bock, E. (1999) Identification of nitrite-oxidizing bacteria with monoclonal antibodies recognizing the nitrite oxidoreductase. *Appl. Environ. Microbiol.* 65: 4126-4133
- 56 Pinck C., Coeur C., Potier P., Bock E. (2001) Polyclonal antibodies recognizing the AmoB of ammonia oxidizers of the  $\beta$ -subclass of *Proteobacteria*. *Appl. Environ. Microbiol.* 67: 118-124
- 57 Hartwig, C. (1999) Anreicherung von *Nitrospira* aus Naturproben, Diploma Thesis, University of Hamburg, Hamburg
- 58 Bartosch, S., Hartwig, C., Speck, E., Bock, E. Detection of *Nitrospira*-like bacteria in various soils. submitted.
- 59 Vestal, J.R., White, D.C. (1989) Lipid Analysis in Microbial Ecology. *Bioscience* 39:535-541

- 60 Ohtsubo, S., Kanno, M., Miyahara, G., Kohno, S., Koga, Y., Miura, I. (1993). A sensitive method for quantification of aceticlastic methanogens and estimation of total methanogenic cells in natural environments based on an analysis of ether-linked glycerolipids. *FEMS Microbiol. Ecol.* 12: 39-50.
- 61 Lipski, A., Spieck, E., Makolla, A., Altendorf, KH. Fatty acid profiles of nitrite-oxidizing bacteria reflect their phylogenetic heterogeneity. submitted.
- 62 Rothfuss, F., Conrad, R. (1993). Vertical profiles of CH<sub>4</sub> concentrations, dissolved substrates and processes involved in CH<sub>4</sub> production in a flooded Italian rice field. *Biogeochem.* 18: 137-152.
- 63 Christensen, T. (1993). Methane emission from arctic tundra. *Biogeochem.* 21: 117-139.
- 64 Kotsyurbenko, O.R., Nozhevnikova, A.N., Soloviova, T.I., Zavarzin, G.A. (1996). Methanogenesis at low temperatures by microflora of tundra wetland soil. *Antonie van Leeuwenhoek* 69: 75-86.
- 65 Schramm, A., Larsen, L.H., Revsbech, N.P., Ramsing, N.B., Amann, R., Schleifer, K.-H. (1996). Structure and function of a nitrifying biofilm as determined by *in situ* hybridization and the use of microelectrodes. *Appl. Environ. Microbiol.* 62: 4641-4647.
- 66 Wagner, D., Pfeiffer, E.-M., Bock, E. (1999). Methane production in aerated marshland and model soils: effects of microflora and soil texture. *Soil Biol. Biochem.* 31: 999-1006.
- 67 Scharpenseel, H.W., Pfeiffer, E.M. (1997). Carbon turnover in different climates and environments. *Adv. Soil Sci.* 577-590
- 68 Pfeiffer, E.-M., Janssen, H. (1994). Characterization of organic carbon, using the δ<sup>13</sup>C-value of a permafrost site in the Kolyma-Indigirka-Lowland, Northeast Siberia. In: (Kibble, J., Ahrens, R.J., eds.) *Proceedings of the meeting on the classification, correlation, and management of permafrost-affected soils*, Alaska, USA. USDA, Lincoln: 90-98.
- 69 Rothschild, L.J., DesMarais, D. (1989). Stable carbon isotope fractionation in the search for life on early Mars. *Adv. Space Sci.* 9: 159-165.
- 70 Horneck, G. (in press). The microbial world and the case for Mars. *Plaet. Space Sci.*
- 71 Horneck, G. (1995). Exobiology, the study of the origin, evolution and distribution of life within the context of cosmic evolution: a review. *Planet. Space Sci.* 43: 189-217.

#### **Summarized List of References:**

- Abuzov SS, Mitskevich NI, Poglazova MN (1998) Microflora of the deep glacier horizon of Central Antarctica. *Microbiol.* 67: 451-458.
- Abuzov SS, Mitskevich NI, Poglazova MN, Barkov NI, Lipenkov VYa, Bobin NE, Kudryashov BB, Pashkevich VM (1999) Antarctic ice sheet as an object for solving some methodological problems of exobiology, *Adv. Space Res.* 23/2: 371-376.
- ACIA (2004), Impacts of a Warming Arctic, Arctic Climate Impact Assessment, Cambridge University Press, New York, USA.
- Akhmadeeva I, Becker H, Friedrich K, Wagner D, Pfeiffer E-M, Quass W, Zhurbenko M, and Zöller E (1999) Investigation site 'Samoylov'. p. 19-21. In V. Rachold (ed.) *Expeditions in Siberia in 1998. Reports on Polar Research* 315. Bremerhaven, Alfred-Wegener-Institut.
- Alexander V, and Schell DM (1973) Seasonal and spatial variation of nitrogen fixation in Barrow, Alaska, Tundra. *Arc. Alp. Res.* 5: 77-88.
- Amann R, Binder BJ, Olson RJ, Chisholm SW, Devereux R, and Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and Environmental Microbiology*, 56: 1919-1925.
- Amann R, Ludwig W, and Schleifer KH (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *FEMS Microbiology Reviews*, 59: 143-169.
- Ananyan AA (1970) Unfrozen water content in frozen clay at a temperature from -0.6 °C to -40 °C -60 °C. *Merzlotnye Issledovaniya* 10: 267-270 (in Russian).
- Andreev A, Tarasov P, Schwamborn G et al. (2004) Holocene paleoenvironmental records from Nikolay Lake, Lena River Delta, Arctic Russia. *Palaeogeography, Palaeoclimatology, Palaeoecology* 209: 197-217.
- Andreev A, Klimanov A (2005) Late-Glacial and Holocene in Chapter 5 East Siberia (Based on data obtained mainly in Central Yakutia), *Geological Society of America Bulletin* 382: 98-102.
- Anisomov OA, Nelson FE, and Pavlov AV (1999) Predictive scenarios of permafrost development under conditions of global climate change in the XXI century. *Earth Cryology*, 3: 15-25.
- Are F, and Reimnitz E (2000) An overview of the Lena River Delta setting: Geology, tectonics, geomorphology, and hydrology. *J. Coastal Res.* 16: 1083-1093.
- Armstrong W (1979) Aeration in higher plants. In Woolhouse HW (Ed) *Advances in Botanical Research* (pp 226-333). Academic Press, New York.

- Armstrong J, Armstrong W, Beckett PM, Halder JE, Lythe S, Holt R & Sinclair A (1996) Pathways of aeration and the mechanisms and beneficial effects of humidity- and Venturi-induced convections in *Phragmites australis* (Cav.) Trin. ex Steud. Aquat. Bot. 54: 177-197.
- Arndt JL, and Richardson JL (1989) Geochemical development of hydric soil salinity in a North Dakota prairie-pothole wetland system. Soil Sci. Soc. Am. J. 53: 848-855.
- Aselmann I, and Crutzen J (1989) Global distribution of natural freshwater wetlands and rice paddies, their net primary productivity, seasonality and possible methane emissions. J. Atmospheric Chem. 8: 307-358.
- Augustin J, Merbach W, Schmidt W and Reining E (1996) Effect of changing temperature and water table on trace gas emission from minerotrophic mires. J Appl Bot 70: 45-51.
- Bai Q, Gattinger A, and Zelles L (2000) Characterisation of microbial consortia in paddy rice soil by phospholipid analysis. Microbiol Ecol 39: 273-281.
- Bakermans C, Tsapin AI, Souza-Egipsy V, Gilichinsky DA and Nealson K (2003) Reproduction and metabolism at -10°C of bacteria isolated from Siberian permafrost, Environ. Microbiol. 5: 321-326.
- Banning N, Brock F, Fry JC, Parkes RJ, Hornbrook ER & Weightman AJ (2005) Investigation of the methanogen population structure and activity in a brackish lake sediment. Environ Microbiol 7: 947-960.
- Bartlett KB, Crill PM, Sass RL, Harriss RC, Dise NB (1992) Methane emissions from tundra environments in the Yukon-Kuskokwim Delta, Alaska. Journal of Geophysical Research 97 (D15): 16645-16660.
- Bartlett KB, Harriss RC (1993). Review and assessment of methane emissions from wetlands. Chemosphere, 26 (1-4): 261-320.
- Bartlett RJ, James BR (1995) System for categorising soil redox status by chemical field testing. Geoderma 68: 211-218.
- Basiliko N, Yavitt JB, Dees PM & Merkel SM (2003) Methane biogeochemistry and methanogen communities in two northern peatland ecosystems, New York State. Geomicrobiol J 20: 563-577.
- Bergman I, Klarqvist M, Nilsson M. 2000. Seasonal variation in rates of methane production from peat of various botanical origins: effects of temperature and substrate quality. FEMS Microbiology Ecology 33: 181-189.
- Berry ED & Foegeding PM (1997) Cold temperature adaptation and growth of microorganisms. J Food Protection 60 (12): 1583-1594.
- Beyer L, Sorge C, Blume H-P, and Schulten HR (1995) Soil organic matter composition and transformation in Gelic Histosols of coastal continental Antarctica. Soil Biol. Biochem. 27: 1279-1288.
- Beyer L, Pingpank K, Wriedt G, and Böltner M (2000) Soil formation in coastal continental Antarctica (Wilkes Land). Geoderma 95: 283-304.
- Bianchi TS, Freer ME, Wetzel RG (1996) Temporal and spatial variability, and the role of dissolved organic carbon (DOC) in methane fluxes from the Sabine River Floodplain (Southeast Texas, U.S.A.). Archiv für Hydrobiologie 136: 261-287.
- Bleich K and Stahr K (1978) Ein Eisen-Bändchen-Stagnogley in der Bodengesellschaft der mittleren Arktis Kanadas. Eiszeitalter und Gegenwart 28: 179-188.
- Bliss LC (1997) Arctic ecosystems of North America. p. 541-686. In Wielgolaski FE (ed.) Polar and Alpine Tundra. Ecosystems of the world 3. Elsevier, Amsterdam.
- Bockheim JG and Tarnocai C (1998) Recognition of cryoturbation for classifying permafrost-affected soils. Geoderma 81: 281-293.
- Bockheim JG, Everett LR, Hinkel KM, Nelson FE and Brown J (1999) Soil organic storage and distribution in Arctic Tundra, Barrow, Alaska. Soil Sci. Soc. Am. J. 63: 934-940.
- Bockheim JG, Hinkel KM and Nelson FE (2003) Predicting carbon storage in Tundra soils of Arctic Alaska. Soil Sci. Soc. Am. J. 67: 948-950.
- Boike J (1997) Thermal, hydrological and geochemical dynamics of the active layer at a continuous permafrost site, Taymyr Peninsula, Siberia. Reports on Polar Research 242: 1-67.
- Boike J, Roth K and Overduin PP (1998) Thermal and hydraulic dynamics of the active layer a continuous permafrost site (Taymyr Peninsula, Siberia). Water Res. Res. 34: 355-365.
- Boike J, Hinzman L, Overduin PP, Romanovsky V, Ippisch O, Roth K (2003) A comparison of snow melt at three circumpolar sites: Spitzbergen, Siberia, Alaska. Proceedings of the 8<sup>th</sup> International Conference on Permafrost, Zurich, Switzerland, 21-25 July 2003.
- Boike J and Wille C (2006) The meteorology, and energy and water balance of polygonal tundra in the Lena Delta, Siberia during wet and dry years, Journal of Geophysical Research, (in review).
- Boone D, Johnson R and Liu Y (1989) Diffusion of the interspecies electron carriers H<sub>2</sub> and formate in methanogenic ecosystems and its implications in the measurement of Km for H<sub>2</sub> or formate uptake. Appl Environ Microbiol 55: 1735-1741.

- Bowman JP, Sly LI, Nichols PD and Hayward AC (1993) Revised taxonomy of the methanotrophs: Description of *Methylobacter* gen. nov., emendation of *Methylococcus*, validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family Methylococcaceae includes only the group I methanotrophs. *Int J Syst Bacteriol* 43: 735-753.
- Bowman JP, McCammon SA and Skerratt JH (1997) *Methylosphaera hansonii* gen. nov., sp. Nov., a psychrophilic, group I methanotroph from Antarctic marine-salinity, meromictic lakes. *Microbiology* 143: 1451-1459.
- Bowman JP, McCammon SA, Lewis T, Skerratt JH, Brown JL, Nichols DS and McMeekin TA (1998) *Psychroflexus torquis* gen. nov., sp. Nov., a psychrophilic species from Antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson et al. 1993) as *Psychroflexus gondwanense* gen. nov., comb.nov. *Microbiology* 144: 1601-1609. Braun-Blanquet J (1964) Pflanzensoziologie. Springer, Wien.
- Börjesson G, Sundh I and Svensson B (2004) Microbial oxidation of CH<sub>4</sub> at different temperatures in landfill cover soils. *FEMS Microbiol Ecol* 48: 305-312.
- Böttcher J and Strelbel O (1988) Ermittlung des Redoxpotentials (Eh-Wert) von Böden und Grundwässern aus dem zeitlichen Verlauf der Elektrodendepolarisation. *Z. Pflanzenernaehr. Bodenkdl.* 151: 363-368.
- Brioukhanov AL, Netrusov AI and Eggen RIL (2006) The catalase and superoxide dismutase genes are transcriptionally up-regulated upon oxidative stress in the strictly anaerobic archaeon *Methanosarcina barkeiri*. *Microbiology* 152: 1671-1677.
- Brook RK & Kenkel NC (2002). A multivariate approach to vegetation mapping of Manitoba's Hudson Bay Lowlands. *International Journal of Remote Sensing*, 23 (21), 4761-4776.
- Brown J (1967) Tundra soils formed over ice wedges, Northern Alaska. *Soil Sci. Soc. Am. Proc.* 31: 686-691.
- Bubier JA, Moore TR, Bellisario L, Comer NT (1995) Ecological controls on methane emissions from a northern peatland complex in the zone of discontinuous permafrost, Manitoba, Canada. *Global Biogeochemical Cycles* 9: 455-470.
- Cao M, Marshall S, Gregson K (1996) Global carbon exchange and methane emissions from natural wetlands: Application of a process-based model, *Journal of Geophysical Research*, 101(D9): 14399-14414.
- Cao M, Gregson K, Marshall S (1998) Global methane emission from wetlands and its sensitivity to climate change. *Atmospheric Environment*, 32 (19): 3293-3299.
- Carr MH (1989) Recharge of the early atmosphere of Mars by impact-induced release of CO<sub>2</sub>. *Icarus* 79: 311-327.
- Carr MH (1996) Water on Mars. Oxford University Press, Oxford.
- Cavicchioli R (2006) Cold-adapted archaea. *Nature* 4: 331-343.
- Cavigelli MA and Robertson GP (2001) Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. *Soil Biology and Biochemistry*, 33, 297-310.
- Chan OC, Wolf M, Hepperle D & Casper P (2002) Methanogenic archaeal community in the sediment of an artificially partitioned acidic bog lake, *FEMS Microbiol Ecol* 42: 119-129.
- Chan OC, Claus P, Casper P, Ulrich A, Lueders T and Conrad R (2005) Vertical distribution of the methanogenic archaeal community in Lake Dagow sediment. *Environ Microbiol* 7: 1139-1149.
- Chanton JP & Dacey JWH (1991) Effects of vegetation on methane flux, reservoirs, and carbon isotopic composition. In: Sharkey TD, Holland EA and Mooney HA (Eds) *Trace Gas Emissions by Plants* (pp 65-89). Academic Press, San Diego.
- Chanton JP, Bauer JE, Glaser PA, Siegel DI, Kelly CA, Tyler SC, Romanowicz EH & Lazarus A (1995) Radiocarbon evidence for the substrates supporting methane formation within northern Minnesota peatlands. *Geochem Cosmochim Acta* 59: 3663-3668.
- Chapin III FS, McGuire AD, Randerson J, Pielke R, Baldocchi D, Hobbie SE, Roulet N, Eugster W, Kasischke E, Rastetter EB, Zimov SA, Running SW (2000) Arctic and boreal ecosystems of western North America as components of the climate system, *Global Change Biology*, 6(S1), 211-223.
- Chapman WL, Walsh JE (1993) Recent Variations of Sea Ice and Air Temperature in High Latitudes, *Bull. Am. Meteorol. Soc.*, 74(1), 33-47.
- Chavez PS (1996). Image-based atmospheric corrections – revised and improved. *Photogrammetric Engineering & Remote Sensing*, 62 (9), 1025-1036.
- Chernov YI and Matveyeva NV (1997) Arctic ecosystems in Russia. In: Wielgolaski FE (Ed) *Polar and Alpine Tundra - Ecosystems of the World* 3 (pp 361-507). Elsevier, Amsterdam.
- Chin KJ and Conrad R (1995) Intermediary metabolism in methanogenic paddy soil and the influence of temperature. *FEMS Microbiol Ecol* 18: 85-102.
- Choudhry GLG (1984) Photophysical and photochemical properties of soil and aquatic humic materials. *Residue Rev* 92: 59-112.

- Christensen TR (1993) Methane emission from arctic tundra. *Biogeochemistry* 21: 117-139.
- Christensen TR, Jonasson S, Callaghan TV, Havström M (1995) Spatial variation in high-latitude methane flux along a transect across Siberian and Eurasian tundra environments, *J. Geophys. Res.* 100, 21035-21045.
- Christensen TR, Prentice IC, Kaplan J, Haxeltine A, Sitch S (1996) Methane flux from northern wetlands and tundra: an ecosystem source modelling approach. *Tellus* 48B: 651-660.
- Christensen TR, Jonasson S, Havstroem M and Livens FR (1999) Carbon cycling and methane exchange in Estuarine tundra ecosystems. *AMBIO*, 28, 239-244.
- Christensen TR, Friberg T, Sommerkorn M, Kaplan J, Illeris L, Soegaard H, Nordstroem C, Jonasson S (2000) Trace gas exchange in a high-arctic valley: 1. Variations in CO<sub>2</sub> and CH<sub>4</sub> flux between tundra vegetation types. *Global Biogeochemical Cycle*, 14: 701-713.
- Christensen TR, Lloyd D, Svensson B, Martikainen P, Harding R, Oskarsson H, Friberg T, Søgaard H and Panikov N (2001) Biogenic controls on trace gas fluxes in northern wetlands. *IGBP-Global Change NewsLetter* 51: 9-15.
- Christensen PR, Wyatt MB, Glotch TD, Rogers AD, Anwar S, Arvidson RE, Bandfield JL, Blaney DL, Budney C, Calvin WM, Fallacaro A, Fergason RL, Gorelick N, Graft TG, Hamilton VE, Hayes AG, Johnson JR, Knudson AT, McSween HY, Mehall GL, Mehall LK, Moerisch JE, Morris RV, Smith MD, Squyres SW, Ruff SW and Wolff MJ (2004) Mineralogy at Meridiani Planum from the Mini-Tes experiment on the Opportunity Rover. *Science* 306: 1733-1739.
- Chuvilin EM, Ershov ED and Naletova NS (1998a) Ionic migration in frozen soils and ice. *Proc. 7<sup>th</sup> Int. Conf. of Permafrost*. Yellowknife, Canada, pp. 167-171.
- Chuvilin EM, Ershov ED and Smirnova OG (1998b) Mass transfer and structure formation in freezing. *Proc. 7<sup>th</sup> Int. Conf. of Permafrost*. Yellowknife, Canada, pp. 173-179.
- Cihlar J (2000). Land cover mapping of large areas from satellites: status and research priorities. *International Journal of Remote Sensing*, 21 (6 & 7), 1093-1114.
- Clark MH and Ping C-L (1997) Hydrology, morphology, and redox potentials in four soils of South Central Alaska. p. 113-131. In Vepraskas MJ and Sprecher SW (ed.) Aquic conditions and hydric soils: The problem soils. SSSA Spec. Publ. No. 50. Madison, WI.
- Clein JS and Schimel JP (1995) Microbial activity of tundra and taiga soils at sub-zero temperatures. *Soil Biol & Biochem* 27: 1231-1234.
- Cleland D, Krader P, McCree C, Tang J and Emerson D (2004) Glycine betaine as a cryoprotectant for prokaryotes. *J Microbiol Methods* 58: 31-38.
- Colwell FS, Nunoura T, Delwiche ME et al. (2005) Evidence of minimal methanogenic numbers and activity in sediments collected from the JAPEX/JNOC/GSC et al. Mallik 5L-38 Gas Hydrate Production Research Well. In: *Scientific Results from Mallik 2002 Gas Hydrate Production Research Well Program, Mackenzie Delta, Northwest Territories, Canada* (eds Dallimore SR, Collett TS), pp. 1-11. Bulletin 585. Geological Survey of Canada, Microbiology section.
- Congalton RG (1991) A review of assessing the accuracy of classifications of remotely sensed data. *Remote sensing of Environment*, 37, 35-46.
- Conrad R (1989) Control of methane production in terrestrial ecosystems, in *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere*, edited by M. O. Andreae and D. S. Schimel, pp. 39-58, John Wiley and Sons, Chichester, UK.
- Conrad R (2005) Quantification of methanogenic pathways using stable carbonisotopic signatures: a review and a proposal. *Organ Geochem* 36: 739-752.
- Corradi C, Kolle O, Walter K, Zimov SA, Schulze ED (2005) Carbon dioxide and methane exchange of a north-east Siberian tussock tundra, *Global Change Biology*, 11, 1910-1925.
- Daims H, Bruehl A, Amann R, Schleifer KH and Wagner M (1999) The domain specific probe EU338 is insufficient for the detection of all Bacteria, development and evaluation of a more comprehensive probe set. *Systematic and Applied Microbiology*, 22, 434-444.
- Davidson EA, Janssens IA and Luo Y (2006) On the variability of respiration in terrestrial ecosystems: moving beyond Q10. *Global Change Biology*, 12, 154-164.
- Deppenmeier U, Müller V, Gottschalk G (1996) Pathways of energy conservation in methanogenic archaea. *Archive of Microbiology* 165: 149-163.
- Dise NB (1992) Winter fluxes of methane from Minnesota peatlands. *Biogeochemistry* 17 (2): 71-83.
- Douglas LA and Tedrow JCF (1960) Tundra soils of arctic Alaska. *Trans. 7<sup>th</sup> Int. Cong. Soil Sci.* Madison, Vol. 4, Com. V, pp. 291-304.
- Dunfield P, Knowles R, Dumont R and Moore TR (1993) Methane production and consumption in temperate and subarctic peat soils: Response to temperature an pH. *Soil Biol. Biochem.* 25(3): 321-326.
- Durham R, Schmunk RB and Chamberlain JW (1989) Comparative analysis of the atmospheres of early Earth and Mars. *Adv Space Res* 9: 139-142.

- DSMZ – Deutsche Sammlung für Mikroorganismen und Zellkulturen: Medium No. 120: Methanoscincina, <http://www.dsmz.de/media/med120.htm>.
- DSMZ – Deutsche Sammlung für Mikroorganismen und Zellkulturen: Medium No. 141: Methanogenium, <http://www.dsmz.de/media/med141.htm>.
- Ehhalt DH, Schmidt U (1978) Sources and sinks of atmospheric methane. Pure and Applied Geophysics 116: 452-464.
- Eicher J (2001) Biotechnological uses of archaeal extremozymes. Biotechnology Advances 19: 261-278.
- Eller G, Stubner S and Frenzel P (2001) Group-specific 16 rRNA targeted probes for detection of type I and type II methanotrophs by fluorescence in situ hybridisation. FEMS Microbiology Letters, 198, 91-97.
- Eller G, Krüger M and Frenzel P (2004) Comparing field and microcosm experiments: a case study on methano- and methylotrophic bacteria in paddy soil. FEMS Microbiology Ecology, 51, 279-291
- Elovskaya LG (1987) Classification and diagnostics of Yakutian permafrost soils. Yakutian Section of the Siberian Branch of the Academy of Science USSR, Yakutsk.
- European Space Agency (2004) Water and Methane maps overlap on Mars: a new clue? [http://www.esa.int/SPECIALS/Mars\\_Express/SEML131XDYD\\_0.html](http://www.esa.int/SPECIALS/Mars_Express/SEML131XDYD_0.html).
- Everett KR and Parkinson RJ (1977) Soil and landform associations, Prudhoe Bay area, Alaska. Arct. Alp. Res. 9:1-19.
- Everett KR and Brown J (1982) Some recent trends in the physical and chemical characterization and mapping of tundra soils, arctic slope of Alaska. Soil Sci. 133: 264-280.
- Falge E, Baldocchi D, Olson R, Anthoni P, Aubinet M, Bernhofer C, Burba G, Ceulemans R, Clement R, Dolman H, Granier A, Gross P, Grünwald TH, Hollinger D, Jensen NO, Katul G, Keronen P, Kowalski A, Lai CT, Law BE, Meyers T, Moncrieff J, Moors E, Munger JW, Pilegaard K, Rannik Ü, Rebmann C, Suyker A, Tenhunen J, Tu K, Verma S, Vesala T, Wilson K, Wofsy S (2001), Gap filling strategies for defensible annual sums of net ecosystem exchange, Agric. Forest Meteorol., 107, 43-69.
- Farrell RE, Swerhone GDW and Van Kessel C (1991) Construction and evaluation of a reference electrode assembly for use in monitoring in situ soil redox potentials. Commun. Soil. Sci. Plant Anal. 22: 1059-1068.
- Felix NA & Binney DL (1989). Accuracy assessment of a LANDSAT-assisted vegetationmap of the coastal plain of the Arctic National Wildlife Refuge. Photogrammetric Engineering & Remote Sensing, 55 (4), 475-478.
- Ferguson RS (1991). Detection and classification of muskox habitat on Banks Island, Northern Territories, Canada, using LANDSAT Thematic Mapper data. Arctic, 44 (1), 66-74.
- Feustel IC, Dutilly A and Anderson MS (1939) Properties of soils from North American arctic region. Soil Sci. 48: 183-199.
- Fiedler S (1997) In-situ-Langzeitmessungen des Redoxpotentials in hydromorphen Böden einer Endmoränenlandschaft im württembergischen Alpenvorland. Hohenheimer Bodenkdl. Hefte 42: 1-135.
- Fiedler S, Wagner D, Kutzbach L, Pfeiffer EM (2004) Element redistribution along hydraulic and redox gradients of low-centered-polygons, Lena Delta, Northern Siberia, Soil Sci. Soc. Am. J., 68, 1004-1011.
- Foken T and Wichura B (1996) Tools for quality assessment of surface-based flux measurements, Agricultural and Forest Meteorology, 78(1), 83-105.
- Formisano V (2004) Detection of methane in the atmosphere of Mars. Science 306: 1758-1761.
- Franzmann PD, Springer N, Ludwig W, De Macario EC and Rohde M (1992) A methanogenic archaeon from Ace Lake, Antarctica – *Methanococcoides burtonii* sp. nov. Syst Appl Microbiol15: 573-58.
- Franzmann PD, Liu Y, Balkwill DL, Aldrich HC, Conway de Macario E, Boone DR (1997) *Methanogenium frigidum* sp. nov., a psychrophilic, H<sub>2</sub>-using methanogen from Ace Lake, Antarctica. International Journal of Systematic Bacteriology 47: 1068-1072.
- French HM (1996) The Periglacial Environment, Longman, London, UK.
- Frenzel P, Thebraath B, Conrad R (1990) Oxidation of methane in the oxic surface layer of a deep lake sediment (Lake Constance). FEMS Microbiology Ecology 73: 149-158.
- Frenzel P, Karofeld E (2000) CH<sub>4</sub> emission from a hollow-ridge complex in a raised bog: the role of CH<sub>4</sub> production and oxidation. Biogeochemistry 51: 91-112.
- Friborg T, Christensen TR, Søgaard H (1997) Rapid response of greenhouse gas emission to early spring thaw in a subarctic mire as shown by micrometeorological techniques. Geophysical Research Letters 24: 3061-3064.

- Friborg T, Christensen TR, Hansen BU, Nordstroem C, Soegaard H (2000) Trace gas exchange in a high-arctic valley 2. Landscape CH<sub>4</sub> fluxes measured and modeled using eddy correlation data, *Global Biogeochem. Cycles*, 14(3), 715-724.
- Friborg T, Soegaard H, Christensen TR, Lloyd CR and Panikov N (2003) Siberian wetlands: where a sink is a source. *Geophysical Research Letters*, 30, 2129-2131.
- Friedmann E (1994) Permafrost as microbial habitat, Viable microorganisms in permafrost, Institute of Soil Science and photosynthesis, Russian Academy of Science, Pushkino, pp. 21-26.
- Frolking S and Crill P (1994) Climate controls on temporal variability of methane flux from a poor fen in southeastern New Hampshire: Measurement and modeling, *Global Biogeochemical Cycles*, 8(4), 385-397.
- Frostegard A and Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol Fert Soils* 22: 59-65.
- Fung I, John J, Lerner J, Matthews E, Prather M, Steele LP, Fraser PJ (1991) Three-dimensional model synthesis of the global methane cycle. *J Geophysical Res* 96: 13033-13065.
- Galand PE, Saarnio S, Fritze H & Yrjälä K (2002) Depth related diversity of methanogen Archaea in Finnish oligotrophic fen. *FEMS Microbiol Ecol* 42: 441-449.
- Galand PE, Fritze H & Yrjälä K (2003) Microsite-dependent changes in methanogenic populations in a boreal oligotrophic fen. *Environ Microbiol* 5: 1133-1143.
- Galand PE, Juottonen H, Fritze H & Yrjälä K (2005) Methanogen communities in a drained bog: effect of ash fertilization. *Microb Ecol* 49: 209-217.
- Ganzert L, Zimmermann U & Wagner D (2004). Microbial process studies on methane fluxes from permafrost environments. In: Schirrmeyer L, Grigoriev MN, Kutzbach L, Wagner D, Bolshianov DYU (Eds.). Russian-German Cooperation System Laptev Sea: The expedition Lena-Anabar 2003, Reports on Polar and Marine Research, 489, 20-29.
- Ganzert L, Jurgens G, Münster U and Wagner D (2006) Methanogenic communities in permafrost-affected soils on the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. *FEMS Microbiol Ecology* DOI 10.1111/j.1574-6941.2006.00205.x.
- Ganzert L, Jurgens G, Münster U et al. (2006) Biodiversity of methanogenic archaea in permafrost environments of the Laptev Sea coast. *FEMS Microbiology Ecology*, awaiting acceptance.
- Gattinger A, Schloter M and Munch JC (2002a) Phospholipid etherlipid and phospholipid fatty acid fingerprints in selected euryarchaeotal monocultures for taxonomic profiling. *FEMS Microbiol Lett* 213: 133 –139.
- Gattinger A, Ruser R, Schloter M and Munch JC (2002b) Microbial community structure varies in different soil zones of a potato field. *J Plant Nutr Soil Sci* 165: 421-428.
- Gattinger A, Günthner A, Schloter M et al. (2003) Characterisation of Archaea in soil ecosystems by polar lipid analysis. *Acta Biotechnology*, 23, 21-28.
- Garcia JL, Patel BKC and Olliver B (2000) Taxonomic, phylogenetic and ecological diversity of methanogenic archaea. *Anaerobe* 6: 205-226.
- Georlette D, Blaise V, Collins T, D'Amico S, Gratia E, Hoyoux A, Marx J-C, Sonan G, Feller G and Gerday C (2004) Some like it cold: biocatalysis at low temperatures. *FEMS Microbiol Rev* 28: 25-42.
- Gersper PL, Alexander V and Barkley SA (1980) The soils and their nutrients. p. 219-254. In J. Brown (ed.) An arctic ecosystem. The coastal tundra at Barrow, Alaska. Hutchinson and Ross. Stoudsburg Dowden.
- Gilichinsky D, Soina VS and Petrova MA (1993) Cryoprotective properties of water in the Earth cryolithosphere and its role in exobiology, *Origins Life Evol. Biosphere* 23, 65-75.
- Gilichinsky D and Wagener S (1994) Microbial life in permafrost, in Gilichinsky D (ed.) Viable microorganisms in Permafrost, Pushchino Research Center, pp. 7-20.
- Gilichinsky D, Rivkina E, Shcherbakova V, Laurinavichuis K and Tiedje J (2003) Supercooled water brines within permafrost - an unknown ecological niche for microorganisms: a model for astrobiology. *Astrobiology* 3/2: 331-341.
- Glissman K, Chin KJ, Casper P and Conrad R (2004) Methanogenic pathway and archaeal community structure in the sediment of eutrophic Lake Dagow: effect of temperature. *Microb Ecol* 48: 389-399.
- Gounot AM (1999) Microbial life in permanently cold soils. In: Cold-adapted organisms (eds Margesin R, Schinner F) pp. 3-16, Springer, Berlin.
- Gorham E (1991) Northern peatlands role in the carbon cycle and probable responses to climatic warming. *Ecological Applications* 1: 182-195.
- Grigoriev MN (1993) Cryomorphogenesis of the Lena River mouth area, pp. 176, SB Russian Academy of Science, Yakutsk, Russia (in Russian).

- Grigoriev MN, Rachold V, Bolshiyanov DYu, Pfeiffer EM, Schirrmeyer L, Wagner D and Hubberten HW (2003) Russian-German Cooperation SYSTEM LAPTEV SEA: The expedition LENA 2002, Reports on Polar and Marine Research 466, pp.341.
- Gross MF, Hardinsky MA, Doolittle JA & Klemas V (1990) Relationships among depth to frozen soil, soil wetness, and vegetation type and biomass in tundra near Bethel, Alaska, USA. Arctic and Alpine Research, 22 (3), 275-282.
- Grosse G, Schirrmeyer L, Malthus TJ (2006) Application of Landsat-7 satellite data and a DEM for the quantification of the thermokarst-affected terrain types in the periglacial Lena-Anabar coastal lowland. Polar Research, 25 (1), 51-67.
- Grosskopf R, Janssen PH and Liesack W (1998a) Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. Appl Environ Microbiol 64: 960-969.
- Grosskopf R, Stubner S and Liesack W (1998b) Novel euryarchaeotal lineages detected on rice roots and in the anoxic bulk soil of flooded rice microcosms. Appl Environ Microbiol 64: 4983-4989.
- Große W, Büchel HB & Tiebel H (1991) Pressurized ventilation in wetland plants. Aquat. Bot. 39: 89-98.
- Grotenhuis J, Plugge C, Stams A and Zehnder A (1992) Hydrophobicities and electrophoretic mobilities of anaerobic bacterial isolates from methanogenic granular sludge, Appl. Environ. Microbiol. 58: 1054-1056.
- Grulke NE, Riechers GH, Oechel WC, Hjelm U and Jaeger C (1990) Carbon balance in tussock tundra under ambient and elevated atmospheric CO<sub>2</sub>. Oecologia 83: 485-494.
- Grünfeld S and Brix H (1999) Methanogenesis and methane emissions: effects of water table, substrate type and presence of *Phragmites australis*. Aquat. Bot. 64: 63-75.
- Gunny D, Hallsworth EG and DV Crawford (1965) The experimental study of the mobility of ions in soils, with particular reference to phosphorus. p. 149-156. In Hallsworth EG, and Crawford DV (ed.) Experimental pedology. Butterworths. London.
- Haag RW (1974) Nutrient limitations to plant production in two tundra communities. Can. J. Bot. 52: 103-116.
- Hales BA, Edwards C, Ritchie DA, Hall G, Pickup RW and Saunders JR (1996) Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. Appl Environ Microbiol 62: 668-675.
- Hallsworth EG, Beattie JA and Darley WE (1982) Formation of soils in an aridic environmental western New South Wales, Australia, Catena Suppl. 1: 83-102.
- Hansen J, Nazarenko L, Ruedy R, Sato M, Willis J, Del Genio A, Koch D, Lacis A, Lo K, Menon S, Novakov T, Perlitz J, Russell G, Schmidt GA and Tausnev N (2005) Earth's energy imbalance: conformation and implications. Science 308: 1431-1435.
- Hansen J, Fung I, Lacis A, Rind D, Lebedeff S, Ruedy R, Russell G, Stone P (1988) Global climate changes as forecast by Goddard Institute for Space Studies three-dimensional model. Journal of Geophysical Research 93: 9341-9364.
- Hanson RS and Hanson TE (1996) Methanotrophic Bacteria. Microbiological Reviews, 60 (2), 439-471
- Hargreaves KJ, Fowler D (1998) Quantifying the effects of water table and soil temperature on the emission of methane from peat wetland at the field scale. Atmospheric Environment 32: 3275-3282.
- Hargreaves KJ, Fowler D, Pitcairn CER, Aurela M (2001) Annual methane emission from Finnish mires estimated from eddy covariance campaign measurements, Theor. Appl. Climatol. 70, 203-213.
- Harriss R, Bartlett K, Frolking S and Crill P (1993) Methane emission from northern high-latitude wetlands. p. 449-485. In Oremland RS (ed.) Biogeochemistry of global change. New York: Chapman and Hall.
- Harvey HR, Fallon RD, Patton JS (1986) The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. Geochimica et Cosmochimica Acta, 50, 795-804.
- Hedderich R and Whitman WB (2005) Physiology and Biochemistry of the methane-producing Archaea. The Prokaryotes, An Evolving Electronic Resource for the Microbiological Community, 3rd edition, release 3.2, December 31, 2005 (Dworkin M et al., eds): Springer-Verlag, New York, <http://link.springer-ny.com/link/service/books/10125/>.
- Heijnen CE, Hok-A-Hin CH and Van Veen JA (1992) Improvements to the use of bentonite clay as a protective agent increasing survival levels of bacteria introduced into soil. Soil Biology & Biochemistry, 24, 533-538.
- Heikkinen JEP, Virtanen T, Huttunen JT, Elsakov V & Martikainen PJ (2004) Carbon balance in Easteuropean tundra. Global Biogeochemical Cycles, 18, GB1023, doi: 10.1029/2003GB002054.

- Henckel T, Roslev P and Conrad R (2000) Effects of O<sub>2</sub> and CH<sub>4</sub> on presence and activity of the indigenous methanotrophic community in rice field soil. *Environmental Microbiology*, 2 (6), 666-679.
- Heyer J and Suckow R (1985) Ökologische Untersuchungen der Methanoxydation in einem sauren Moorsee. *Limnologica* 16(2): 247-266.
- Hinkel KM and Outcalt SI (1994) Identification of heat transfer processes during soil cooling, freezing and thaw in central Alaska. *Permafrost and Periglacial Proc.* 5: 217-235.
- HMCR, Hydrometeorological Centre of Russia (2004) Russia's Weather, <http://meteo.infospace.ru>.
- Hogg EH (1993) Decay potential of hummock and hollow Sphagnum peats at different depths in a Swedish raised bog. *Oikos*, 66, 269-278.
- Høj L, Olsen RA and Torsvik VL (2005) Archaeal communities in High Arctic wetlands at Spitsbergen, Norway (78°N) as characterised by 16S rRNA gene fingerprinting. *FEMS Microbiol Ecol* 53: 89-101.
- Holzapfel-Pschorn A, Conrad R and Seiler W (1986) Production, oxidation and emission of methane in rice paddies. *FEMS Microbiol. Ecol.* 31: 343-351.
- Horz HP, Raghubanshi AS, Heyer J, Kammann C, Conrad and Dunfield R, PF (2002) Activity and community structure of methane-oxidising bacteria in a wet meadow soil. *FEMS Microbiology Ecology*, 41, 247-257.
- Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Xiaosu D (Eds.) (2001) Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change, 944 pp., Cambridge University Press, UK.
- Huang C, Townshend JRG, Zhan X, Hansen M, DeFries R & Solberg R (1998) Developing the spectral trajectories of major land cover change processes. *Proceedings of SPIE* (Beijing:SPIE), 155-162.
- Hubberten HW, Wagner D, Pfeiffer EM, Boike J and Gukov A Yu (2006) The Russian-German research station Samoylov, Lena Delta – a key site for polar research in the Siberian arctic. *Polarforschung* 73: 111-116.
- Intergovernmental Panel of Climate Change (2001) Climate Change 2001: Impacts, Adaption, and Vulnerability. Cambridge University Press, Cambridge.
- IPCC (2001) Climate change 2001: The scientific basis. Contribution of the working group I to the third assessment report of the Intergovernmental Panel on Climate Change. Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K and Johnson CA (Eds.) Cambridge University Press, Cambridge, New York, 881.
- ISSS-ISRIC-FAO (1998) World Reference Base for Soil Resources. FAO, World Soil Resources Report No. 84, Rome.
- IUPAC-IUB Commission on biochemical nomenclature (1977) The nomenclature of lipids. *Eur J Biochem* 79: 11-21.
- Iversen N and Blackburn H (1981) Seasonal Rates of Methane Oxidation in Anoxic Marine Sediments. *Applied and Environmental Microbiology*, 41(6), 1295-1300.
- Jeanthon C, L`Haridon S, Pradel N and Prieur D (1999) Rapid identification of hyperthermophilic methanococci isolated from deep-sea hydrothermal vents. *Int J Syst Bacteriol* 49: 591-594.
- Joabsson A, Christensen TR and Wallén B (1999) Influence of vascular plant photosynthetic rate on CH<sub>4</sub> emission from peat monoliths from southern boreal Sweden. *Polar Res.* 18: 215-220.
- Joabsson A and Christensen TR (2001) Methane emissions from wetlands and their relationship with vascular plants: an Arctic example. *Global Change Biology* 7, 919-932.
- John MK (1970) Colorimetric determination of phosphorus in soil and plant material with ascorbic acid. *Soil Sci.* 109: 214-220.
- Joria PE & Jorgenson JC (1996) Comparison of three methods for mapping tundra with Landsat digital data. *Photogrammetric Engineering & Remote Sensing*, 62 (2), 163-169.
- Juutinen H, Galand PE, Tuittila ES, Laine J, Fritze H and Yrjälä K (2005) Methanogen communities and Bacteria along an ecohydrological gradient in a northern raised bog complex. *Environ Microbiol* 7(10): 1547-1557.
- Jurgens G, Glöckner FO, Amann R, Saano A, Montonen L, Likolammi M and Münster U (2000) Identification of novel Archaea in bacterioplankton of a boreal forest lake by phylogenetic analysis and fluorescence in situ hybridisation. *FEMS Microbiol Ecol* 34: 45-56.
- Kaneda T (1991) Iso- and anteiso-fatty acids in bacteria: Biosynthesis, function, and taxonomic significance. *Microbiol Rev* 55: 288-302.
- Kattenberg A, Giorgi F, Grassel H, Meehl GA, Michell JFB, Stouffer RJ, Tokioka T, Weaver AJ, Wigley TML (1996) Climate models – projections of future climate. In *Climate Change 1995*, Houghton JT (ed). University Press: Cambridge; 285-357.

- Kelker D and Chanton J (1997) The effect of clipping on methane emissions from *Carex*. *Biogeochemistry* 39: 37-44.
- Kellogg CE (1934) Morphology and genesis of the Solonetz soils of western North Dakota. *Soil Sci.* 38: 483-501.
- Keough BP, Schmidt TM and Hicks RE (2003) Archaeal nucleic acids in picoplankton from great lakes on three continents. *Microbial Ecol* 46: 238-248.
- Kessler, MA and Werner BT (2003) Self-organization of sorted patterned ground. *Science* 299: 380-383.
- Khalil MAK, Rasmussen RA, Shearer MJ (1998) Effects of production and oxidation processes on methane emissions from rice fields. *Journal of Geophysical Research* 103: 25,233-25,239.
- Kiener A and Leisinger T (1983) Oxygen sensitivity of methanogenic bacteria. *Syst Appl Microbiol* 4: 305-312.
- Kim WS and Dunn NW (1997) Identification of a cold shock gene in lactic acid bacteria and the effect of cold shock on cryotolerance. *Curr Microbiol* 35: 59-63.
- Kimura H, Sugihara M, Yamamoto H, Patel BKC, Kato K and Hanada S (2005) Microbial community in a geothermal aquifer associated with the subsurface of the Great Artesian Basin, Australia. *Extremophiles* 9(5): 407-414.
- King GM and Adamsen APS (1992) Effects of temperature on methane consumption in a forest soil and in pure cultures of the methanotroph *Methylomonas rubra*. *Appl. Env. Microbiol.* 58(9): 2758-2763.
- King JY, Reeburgh WS and Regli SK (1998) Methane emission and transport by arctic sedges in Alaska: Results of a vegetation removal experiment. *J. Geophys. Res.* 103(D22): 29083-29092
- Kling GW, Klipphut GW & Miller MC (1992) The flux of CO<sub>2</sub> and CH<sub>4</sub> from lakes and rivers in arctic Alaska. *Hydrobiologia*, 240, 23-36.
- Klingelhofer G, Morris RV, Bernhard B, Schroder C, Rodnov DS, de Souza PA, Yen A, Gellert R, Evlanov EN, Zubkov B, Foh J, Bonnes U, Kankeleit E, Gutlich P, Ming DW, Renz F, Wdowiak T, Squyres SW and Arvidson RE (2004) Jarosite and hematite at Meridiani Planum from Opportunity's Mossbauer spectrometer. *Science* 306: 1740-1745.
- Knief C, Lipski A and Dunfield PF (2003) Diversity and activity of methanotrophic bacteria in different upland soils. *Appl Environ Microbiol* 69: 6703-6714.
- Knief C, Dunfield PF (2005) Response and adaptation of different methanotrophic bacteria to low methane mixing ratios. *Environmental Microbiology*, 7(9), 1307-1317.
- Knief C, Kolb S, Bodelier PLE, Lipski A, Dunfield PF (2006) The active methanotrophic community in hydromorphic soils changes in response to changing methane concentration. *Environmental Microbiology*, 8(2), 321-333.
- Kobabe S, Wagner D and Pfeiffer EM (2004) Characterization of microbial community composition of a Siberian tundra soil by fluorescence in situ hybridization. *FEMS Microbiol Ecol* 50: 13-23.
- Končalová H (1990) Anatomical adaptions to waterlogging in roots of wetland graminoids: limitations and drawbacks. *Aquat. Bot.* 38: 127-134.
- Kormann R, Mueller H, Werle P (2001) Eddy flux measurements of methane over the fen "Murnauer Moos", 11°11'E, 47°39'N, using a fast tunable diode laser spectrometer, *Atmos. Environ.* 35 (14) 2533-2544.
- Korotaev VN (1986) Geomorphology of river deltas on the Arctic coast of Siberia. *Polar Geography and Geology* 10: 139-147.
- Kotsyurbenko OR, Nozhevnikova AN and Zavarzin GA (1993) Methanogenic degradation of organic matter by anaerobic bacteria at low temperature. *Chemosphere* 27: 1745-1761.
- Kotsyurbenko OR, Chin KJ, Glagolev MV, Stubner S, Simankova MV, Nozhevnikova AN and Conrad R (2004) Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an West-Siberian peat bog. *Environ Microbiol* 6: 1159-1173.
- Kotlyakov V, Khromova T (2002) Permafrost, Snow and Ice, In, Stolbovoi V and McCallum I (eds) CD-ROM Land Resources of Russia, International Institute of Applied Systems Analysis and the Russian Academy of Science, Laxenburg, Austria.
- Kral TA, Bekkum CR and McKay CP (2004) Growth of methanogens on a Mars soil stimulant, Origins of Life and Evolution of the Biosphere 34: 615-626.
- Krueger M, Eller G, Conrad R and Frenzel P (2002) Seasonal variation in pathways of CH<sub>4</sub> production and in CH<sub>4</sub> oxidation in rice fields determined by stable carbon isotopes and specific inhibitors. *Global Change Biology*, 8, 265-280.
- Krumholz LR, Hollenback JL, Roskes SJ and Ringelberg DB (1995) Methanogenesis and methanotrophy within a *Sphagnum* peatland. *FEMS Microbiol. Ecol.* 18: 215-224.
- Kutzbach L (2000) Die Bedeutung der Vegetation und bodeneigener Parameter für die Methanflüsse in Permafrostböden. Master Thesis, University of Hamburg, Institute of Soil Science. 105p.

- Kutzbach L & Kurchatova A (2002) Methane-related investigations of soils and waters in the Sangadzhie region. In: Pfeiffer E-M & Grigoriev MN (Eds.). Russian-German Cooperation System Laptev Sea 2000: The expedition Lena 2001, Reports on Polar and Marine Research, 426, 76-87.
- Kutzbach L, Kurchatova AN, Stoof G (2003) The flora of Samoylov Island - A documentation, in Russian-German Cooperation SYSTEM LAPTEV SEA, The Expedition LENA 2002, Reports on Polar and Marine Research 466, edited by M. N. Grigoriev, 58-63, Alfred-Wegener-Institute, Bremerhaven, Germany.
- Kutzbach L, Wagner D and Pfeiffer EM (2004) Effect of microrelief and vegetation on methane emission from wet polygonal tundra, Lena Delta, Northern Siberia. *Biogeochemistry*, 69 (3), 341-362.
- Kutzbach L, Stoof G, Schneider W, Wille C, Abramova EN (2004a) Seasonal progression of active-layer thickness dependent on microrelief, in Expeditions in Siberia in 2003, Reports on Polar and Marine Research 489, edited by Schirrmeister L, 34-38, Alfred-Wegener-Institute, Bremerhaven, Germany.
- Kutzbach L (2006) The Exchange of Energy, Water and Carbon Dioxide between Wet Arctic Tundra and the Atmosphere at the Lena River Delta, Northern Siberia, Thesis, University of Hamburg, Reports on Polar and Marine Resrach (in press).
- Kuzmin RO (2005) Ground Ice in the Martian Regolith, in T. Tokano (ed.) *Water on Mars and Life*, Springer Verlag Berlin, pp. 155-183.
- Lange M and Ahring B (2001) A comprehensive study into the molecular methodology and molecular biology of methanogenic archaea. *FEMS Microbiol Rev* 25: 553-571.
- Lehmann-Richter S, Grosskopf R, Liesack W, Frenzel P and Conrad R (1999) Methanogenic archaea and CO<sub>2</sub>-dependent methanogenesis on washed rice roots *Appl Environ Microbiol* 65: 2402-2408.
- Le Mer J and Roger P (2001) Production, oxidation, emission and consumption of methane by soils: A review. *European Journal of Soil Biology* 37, 25-50.
- Liblik LK, Moore TR, Bubier JL, Robinson SD (1997) Methane emissions from wetlands in the zone of discontinuous permafrost: Fort Simpson, Northwest Territories, Canada. *Global Biogeochemical Cycles* 11: 485-494.
- Liebner S and Wagner D (2006) Abundance, distribution and potential activity of methane oxidising bacteria in permafrost soils from the Lena Delta, Siberia, *Environmental Microbiology* 9, 107-117
- Liebner S, Harder J and Wagner D. (in prep.) Microbial and methanotrophic diversity in Siberian permafrost soils from the Lena Delta, Siberia, based on 16S rDNA clone libraries and CARD FISH analysis.
- Lin C, Raskin L and Stahl DA (1997) Microbial community structure in gastrointestinal tracts of domestic animals: comparative analyses using rRNA-targeted oligonucleotide probes. *FEMS Microbiol Ecol* 22: 281-294.
- Lipski A, Altendorf K (1997) Identification of heterotrophic bacteria isolated from ammonia-supplied experimental biofilters. *System Applied Microbiology*, 20, 448-457.
- Lloyd J and Taylor JA (1994) On the temperature dependence of soil respiration. *Functional Ecology*, 8, 315-323.
- Lu Y & Conrad R (2005) In situ stable isotope probing of methanogenic archaea in the rice rhizosphere. *Science* 309: 1088-1090.
- Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadukumar, Buchner A, Lai T, Steppi S, Jobb G, Forster W, Brettske I, Gerbe S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, Konig A, Liss T, Lussmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A and Schleifer KH (2004) ARB: a software environment for sequence data. *Nucleic Acids Res* 25: 1363-1371.
- Lueders T and Friedrich M (2000) Archaeal population dynamics during sequential reduction processes in rice field soil. *Appl Environ Microbiol* 66: 2732-2742.
- Lundin LC and Johnsson H (1994) Ion dynamics of a freezing soil monitored in situ by time domain reflectometry. *Water Resour. Res.* 30: 3471-3478.
- Macalady JL, McMillan AM, Dickens AF, Tyler SC and Scow KM (2002) Population dynamics of type I and II methanotrophic bacteria in rice soils. *Environmental Microbiology*, 4(3), 148-157.
- Macario AJL, Lange M, Ahring BK and De Macario EC (1999) Stress genes and proteins in the archaea. *Microbiol Mol Biol Rev* 63/4: 923-967.
- MacDonald JA, Fowler D, Hargreaves KJ, Skiba U, Leith ID & Murray MB (1998) Methane emission rates from a northern wetland; response to temperature, water table and transport. *Atmospheric Environment*, 32 (19), 3219-3227.
- MacLean AH and Pawluck S (1975) Soil genesis in relation to groundwater and soil moisture regimes near Vegreville., Alberta. *Can. J. Soil Sci.* 26: 278-293.
- Mangelsdorf K, Haberer RM, Zink KG et al. (2005) Molecular indicators for the occurrence of deep microbial communities at the JAPEX/JNOC/GSC et al. Mallik 5L-38 Gas Hydrate Production

- Research Well. In: Scientific Results from Mallik 2002 Gas Hydrate Production Research Well Program, Mackenzie Delta, Northwest Territories, Canada (eds Dallimore SR, Collett TS), pp. 18-29. Bulletin 585. Geological Survey of Canada, Microbiology section.
- Martens CS, Kelley CA, Chanton JP & Showers WJ (1992) Carbon and hydrogen isotopic characterization of methane from wetlands and lakes of the Yukon-Kuskokwim Delta, Western Alaska. *Journal of Geophysical Research*, 97 (D15), 16689-16701.
- Martikainen PJ, Nykänen H, Alm J and Silvola J (1995) Changes in fluxes of carbon dioxide, methane and nitrous oxide due to forest drainage of mire sites of different trophy. *Plant Soil* 168-169: 571-577.
- Mathrani IM and Boone DR (1985) Isolation and characterization of a moderately halophilic methanogen from a solar saltern. *Appl Environ Microbiol* 50: 140-143.
- Matthews E (1983) Global vegetation and landuse: New high resolution data bases for climate studies. *Journal of Climate and Applied Meteorology*, 22, 474-487.
- Matthews E, Fung I (1987) Methane emission from natural wetlands: global distribution, area, and environmental characteristics of sources. *Global Biogeochemical Cycles* 1 (1): 61-86.
- Matthews E, (2000) Wetlands. In: Khalil MAK (Ed.). *Atmospheric methane, its role in the global environment*, Springer, 202-233.
- Matveyeva NV (1994) Floristic classification and ecology of tundra vegetation of the Taymyr Peninsula, northern Siberia. *J. Vegetation Science* 5: 813-828.
- Maxwell B (1997) Recent climate patterns in the Arctic. In: Oechel WC, Callaghan T, Gilmanov T, Holten JI, Maxwell B, Molau U and Sveinbjörnsson B (Eds) *Global Change and Arctic Terrestrial Ecosystems* (pp. 21-46) Springer, New York.
- McCown BH (1978) The interactions of organic nutrients, soil nitrogen, and soil temperature and plant growth and survival in the arctic environment. p. 435-456. In L.L. Tieszen (ed.) *Vegetation and Production Ecology of an Alaskan Arctic Tundra*. Ecological Studies 29 Springer, New York.
- McKay CP and Davis W (1991) Duration of liquid water habitats on early Mars. *Icarus* 90: 214-221.
- McKay CP, Friedman EI, Wharton RA and Davies WL (1992) History of water on Mars: a biological perspective. *Adv Space Res* 12: 231-238.
- McMillen R (1988) An eddy correlation technique with extended applicability to nonsimple terrain, *Boundary-Layer Meteorology*, 43, 231-245.
- Mehra OP and ML Jackson (1960) Iron oxide removal from soils and clays. *Clays Clay Miner.* 7: 317-327.
- Melillo JM, Steudler PA, Aber JD, Newkirk K, Lux H, Bowles FP, Catricala C, Magill A, Ahrens T and Morrisseau S (2002) Soil warming and carbon-cycle feedbacks to the climate system. *Science* 298: 2173-2176.
- Mellor RA, Crill PM (1996) Winter methane dynamics in a temperate peatland. *Global Biogeochemical Cycles* 10: 247-254.
- Meyer H (2003) Studies on recent cryogenesis, in Russian-German Cooperation SYSTEM LAPTEV SEA, The Expedition LENA 2002, Reports on Polar and Marine Research 466, edited by Grigoriev MN, 8-16, Alfred-Wegener-Institute, Bremerhaven, Germany.
- Michaelson GJ, Ping CL and Kimble JM (1996) Carbon storage and distribution in tundra soils of Arctic Alaska, USA. *Arct. Alp. Res.* 28: 414-424.
- Mindock CA, Petrova MA and Hollingsworth RI (2000) Re-evaluation of osmotic effects as a general adaptive strategy for bacteria in sub-freezing conditions. *Biophysical Chemistry* 89(1): 13-24.
- Mitsch WJ, Mitsch RH & Turner RE (1994) Wetlands of the Old and New Worlds: ecology and management. In: Mitsch WJ (Ed.). *Global wetlands: Old World and New*. Elsevier Science, Amsterdam, 3-56.
- Möhlmann D, Wernecke R and Schwanke V (2004) Measurement principle and equipment for measuring humidity contents in the upper Martian surface and subsurface, *ESA SP-543*, 163 - 168.
- Möhlmann D (2005) Adsorption Water-Related Potential Chemical and Biological Processes in the Upper Martian Surface, *Astrobiology* 5, 770 -777.
- Moncrieff JB, Massheder JM, de Bruin H, Elbers J, Friberg T, Heusinkveld B, Kabat P, Scott S, Soegaard H, Verhoef A (1997) A system to measure surface fluxes of momentum, sensible heat, water vapour and carbon dioxide, *J. Hydrol.*, 188-189, 589-611.
- Moore CJ (1986) Frequency response corrections for eddy correlation systems, *Boundary-Layer Meteorology*, 37, 17-35.
- Moore T, Roulet N, Knowles R (1990) Spatial and temporal variations of methane flux from 745 subarctic/northern boreal fens. *Global Biogeochemical Cycles*, Volume 4, Issue 1, p. 29-46.
- Moore TR and Roulet NT (1993) Methane flux: Water table relations in northern wetlands. *Geophys. Res. Lett.* 20: 587-590.

- Moosavi SC and Crill PM (1998) CH<sub>4</sub> oxidation by tundra wetlands as measured by a selective inhibitor technique. *J. Geophys. Res.* 103(D22): 29093-29106.
- Moran M, Miller JD, Kral T and Scott D (2005) Desert methane: implications for life detection on Mars, *Icarus* 178, 277-280.
- Morgenstern A (2005) GIS based analysis of the morphometry and spatial distribution of lakes in the Lena Delta, NE-Siberia. Diploma thesis, University of Potsdam, 70pp, (in German).
- Morita RY (1975) Psychrophilic bacteria. *Bacteriological Review* 39: 144-167.
- Morita RY(1992) Low-temperature environments. In *Encyclopedia of Microbiology*, Vol. 2, Lederbetrg J (ed) Academic Press: San Diego; 625-637.
- Morita RY (1995) Low-Temperature Environments. *Encyclopedia of Microbiology*, (Vol.2), Academic Press, San Diego.
- Morozova D, Möhlmann D and Wagner D (2006) Survival of methanogenic archaea from Siberian permafrost under simulated Martian thermal conditions. *Orig. Life Evol Biosph.* DOI 10.1007/s11084-006-9024-7.
- Morozova D, Möhlmann D and Wagner D (in press) Response of methanogenic archaea from Siberian permafrost to Martian thermo-physical conditions. *ESA Special Publication – Microorganisms and the Martian Environment*.
- Morrissey LA and Livingston GP (1992) Methane emissions from Alaska arctic tundra: An assessment of local spatial variability. *J. Geophys. Res.* 97 (D15): 16661-16670.
- Morrissey LA, Zobel DB, Livingston GB (1993) Significance of stomatal control on methane release from *Carex* dominated wetlands. *Chemosphere* 26(1-4): 339-355.
- Muller SV, Racoviteanu AE & Walker DA (1999). LANDSAT MSS-derived land-cover map of Northern Alaska: extrapolation methods and a comparison with photo-interpreted and AVHRR-derived maps. *International Journal of Remote Sensing*, 20 (15 & 16), 2921-2946.
- Müller K (1997) Patterned ground and properties of permafrost soils of the Northsiberian Lena Delta. *J. Plant. Nutr. Soil Sci.* 160: 497-503.
- Müller-Lupp W (2002) Freeze and Thaw in Siberian Permafrost Soils - Methods and Ecological Relevance. *Reports on Polar and Marine Research* 415: p. 145.
- Nadeau MJ, Grootes PM, Schleicher M et al. (1998) Sample throughput and data quality at the Leibniz-Labor AMS facility. *Radiocarbon*, 40, 239-245.
- Nadeau MJ, Schleicher M, Grootes PM et al. (1997) The Leibniz-Labor facility at the Christian Albrecht University, Kiel, Germany. *Nuclear Instruments and Methods in Physics Research*, 123, 22-30.
- Nakano T, Kuniyoshi S and Fukuda M (2000) Temporal variation in methane emission from tundra wetlands in a permafrost area, northeastern Siberia. *Atmos. Environ.* 34: 1205-1213.
- Nichols PD, Guckert JB, White DC (1986) Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulphide adducts. *Journal of Microbiological Methods*, 5, 49-55.
- NSIDC (2003) Circumpolar Active-Layer Permafrost System, CD-ROM, National Snow and Ice Data Center, University of Colorado, Boulder, Colorado, USA.
- Nykänen H, Alm J, Silvola J, Tolonen K and Martikainen PJ (1998) Methane fluxes on boreal peatlands of different fertility and the effect of long-term experimental lowering of the water table on flux rates. *Global Biogeochem. Cycles* 12: 53-69.
- Oberbauer SF, Starr G, Pop EW (1998) Effects of extended growing and soil warming on carbon dioxide and methane exchange of tussock tundra in Alaska. *Journal of Geophysical Research* 103: 29,075-29,082.
- Oechel WC, Hastings SJ, Jenkins M, Riechers G, Grulke NE and Vourlitis GL (1993) Recent change of arctic tundra ecosystems from a net carbon sink to a source. *Nature*. 361: 520-526.
- Oechel WC, Callaghan T, Gilmanov T et al. (1997) Global change and Arctic terrestrial ecosystems. *Ecological Studies* 124, Springer, Berlin, 493 pp.
- Omelchenko MV, Vasilyeva LV, Zavarzin GA (1993) Psychrophilic methanotrophs from tundra soils. *Current Microbiology* 27: 255-259.
- Osterkamp TE (2001) Subsea Permafrost. Chapter in: Steele, J.H., Thorpe, S.A. and Turekian, K.K. (Editors). *Encyclopedia of Ocean Sciences*, p. 2902-2912, Academic Press.
- Ostroumov V (1995) A physical and chemical characterization of Martian permafrost as possible habitat for viable organisms, *Adv. Space Res.* 15/3, 229-236.
- Ostroumov V, Siegert C (1996) Exobiological aspects of mass transfer in microzones of permafrost deposits. *Advances in Space Research*, 18, 79-86.
- Overduin PP and Young KL (1997) The effect of freezing on soil moisture and nutrient distribution at Levinson-Lessing Lake, Taymyr Peninsula, Siberia. p. 237-333. In: Iskandar IK, Wright EA, Radke JK, Sharratt BS, Groenevelt PH and Hinzman LD (ed) *International Symposium on Physics, Chemistry, and Ecology of Seasonally Frozen Soils*. Special Report 97-10. Fairbanks, Alaska.

- Panikov NS, Belyaev AS, Semenov AM & Zelenov VV (1993) Methane production and uptake in some terrestrial ecosystems of the former USSR. In: Oremland, R.S. (Ed.). Biogeochemistry of Global Change, Chapman & Hall Inc., 221-244.
- Panikov NS (1997) A kinetic approach to microbial ecology in arctic and boreal ecosystems in relation to global change. In Global Change and Arctic Terrestrial Ecosystems, Oechel WC (ed). Springer: Berlin; 171-188.
- Panikov NS, Dedysh SN (2000) Cold season CH<sub>4</sub> and CO<sub>2</sub> emission from boreal peat bogs (West Siberia) winter fluxes and thaw activation dynamics. Global Biogeochemical Cycles 14: 1071-1080.
- Panikov NS, Dedysh SN, Kolesnikov OM, Mardini AI, Sizova MV (2001), Metabolic and environmental control on methane emission from soils: mechanistic studies of mesotrophic fen in West Siberia, Water, Air, & Soil Pollution: Focus, 1(5-6), 415-428, doi:10.1023/A:1013153927194.
- Pernthaler J, Gloeckner FO, Schoenhuber W and Amann R (2001) Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. Methods in Microbiology: Marine Microbiology, 30, 207-226.
- Pfeiffer EM, Wagner D, Becker H, Vlasenko A, Kutzbach L, Boike J, Quass W, Kloss W, Schulz B, Kurchatova A, Pozdnyakov VI, Akhmadeeva I (2000) Modern processes in permafrost affected soils. In Russian-German Cooperation System Laptev Sea 2000: The Expedition Lena 1999, Rachold V, Grigoriev MN (eds). Reports on Polar Research 354; 22-54.
- Pfeiffer EM and MN Grigoriev (2002) Russian-German cooperation system Laptev Sea 2000: The expedition LENA 2001. Reports on Polar Research 426: 21-41.
- Pfeiffer EM, D Wagner, S Kobabe, Kutzbach L, Kurchatova AN, Stoof G, Wille C 760 (2002) Modern processes in permafrost affected soils, in Russian-German Cooperation SYSTEM LAPTEV SEA 2000, The Expedition LENA 2001, Reports on Polar Research 426, edited by Pfeiffer EM and Grigoriev MN, 21-41, Alfred-Wegener-Institute, Bremerhaven, Germany.
- Popp TJ, Chanton JP, Whiting GJ and Grant N (2000) Evaluation of methane oxidation in the rhizosphere of a *Carex* dominated fen in north central Alberta, Canada. Biogeochemistry 51: 259-281.
- Post WM, Emanuel WR, Zinke PJ and Stangenberger AG (1982) Soil carbon pools and world life zones. Nature, 298, 156-159, doi:10.1038/298156a0.
- Rachold V and Grigoriev MN (1999) Russian-German cooperation system Laptev Sea 2000: The Lena delta 1998 expedition. Reports on Polar Research 315: 1-259.
- Rachold V and Grigoriev MN (2000) Russian-German cooperation system Laptev Sea 2000: The expedition LENA 1999. Reports on Polar Research 354: pp. 303.
- Rachold V and Grigoriev MN (2001) Russian-German cooperation system Laptev Sea 2000: The expedition Lena 2000. Reports on Polar Research 388: pp. 135.
- Rachold V, Grigoriev MN, Are FE et al. (2000) Coastal erosion vs riverine sediment discharge in the Arctic Shelf seas. International Journal of Earth Sciences, 89, 450-460.
- Ramakrishnan B, Lueders T, Dunfield PF, Conrad R and Friedrich MW (2001) Archaeal community structures in rice soils from different geographical regions before and after initiation of methane production, FEMS Microbiol Ecol 37: 175-186.
- Ratledge C and Wilkinson SG eds. (1988) Microbial Lipids, Vol 1. London: Academic Press.
- Reeburgh WS, King JY, Regli SK, Kling GW, Auerbach NA, Walker DA (1998) A CH<sub>4</sub> emission estimate for the Kuparuk River basin, Alaska. Journal of Geophysical Research 103: 29,005-29,013.
- Rees WG, Williams M & Vitebsky P (2003) Mapping land cover change in a reindeer herding area of the Russian Arctic using LANDSAT TM and ETM+ imagery and indigenous knowledge. Remote Sensing of Environment, 85, 441-452.
- Richards JA & Jia X (1999) Remote sensing digital image analysis. An Introduction, 3.Edt., Springer, Berlin, Heidelberg, New York, 363.
- Richardson JL, Arndt JL and Montgomery JA (2001) Hydrology of wetland and related soils. p. 35-84. In Richardson JL and Vepraskas MJ (ed.) Wetland soils. Genesis, hydrology, landscape and classification. Lewis Publishers. Boca Raton, London, New York, Washington D.C.
- Richter-Menge J, Overland J, Proshutinsky A et al. (2006) State of the Arctic Report. NOAA OAR Special Report, NOAA/OAR/PME, Seattle, WA, 36 pp.
- Rieger S (1983) The genesis and classification of cold soils. New York: Academic Press. 230p.
- Ringelberg DB, Sutton S, White DC (1997) Biomass, bioactivity and biodiversity: microbial ecology of the deep subsurface: analysis of ester-linked phospholipid fatty acids. FEMS Microbiology Reviews, 20, 371-377.
- Rivkina EM, Gilichinsky DA (1996) Methane as a paleoindicator of the dynamics of permafrost deposits. Limnology and Mineral Resources, 31, 396-399.

- Rivkina EM, Gilichinsky D, Wagener S et al. (1998) Biochemical activity of anaerobic microorganisms from buried permafrost sediments. *Geomicrobiology*, 15, 187-193.
- Rivkina EM, Friedmann EI, McKay CP et al. (2000) Metabolic activity of permafrost bacteria below the freezing point. *Applied Environmental Microbiology*, 66, 3230-3233.
- Rivkina E, Laurinavicius K, McGrath J, Tiedje J, Shcherbakova V, Gilichinsky D (2004) Microbial life in permafrost, *Advances in Space Research*, 33(8), 770, 1215-1221, doi:10.1016/j.asr.2003.06.024.
- Romanovskii NN, Hubberten HW (2001) Results of permafrost modelling of the lowlands and shelf of the Laptev Sea region, Russia. *Permafrost and Periglacial Processes*. 12: 191-202.
- ROSHYDROMET, Russian Federal Service for Hydrometeorology and Environmental Monitoring (2004), Weather Information for Tiksi, <http://www.worldweather.org/107/c01040.htm> World Meteorological Organization.
- Roslev P and King GM (1996) Regulation of methane oxidation in a freshwater wetland by water table changes and anoxia. *FEMS Microbiology Ecology*, 19, 105-115.
- Rothschild LJ and Mancinelli RL (2001) Life in extreme environments. *Nature* 409, 1092-1101
- Roulet NT, Jano A, Kelly CA, Klinger LF, Moore TR, Protz R, Ritter JA and Rouse WR (1994) Role of the Hudson bay lowland as a source of atmospheric methane. *J. Geophys. Res.* 99 (D1): 1435-1454.
- Roura-Carol M and Freeman C (1999) Methane release from peat soils: effects of *Sphagnum* and *Juncus*. *Soil Biol. Biochem.* 31: 323-325.
- Russel NJ, Hamamoto T (1998) Psychrophiles. In: *Extremophiles: microbial life in extreme environments* (eds Horikoshi K, Grant WD), pp. 25-45, J. Wiley, New York.
- Samarkin VA, Fedorov-Davydov DG, Vecherskaya MS, Rivkina EM (1994) CO<sub>2</sub> and CH<sub>4</sub> emissions on cryosols and subsoil permafrost and possible global climate changes. In *Soil processes and greenhouse effect*, Lal R, Kimble JM, Levine E (eds) USDA Soil Conservation Service, National Soil Survey Center, Lincoln, NE; 55-71.
- Samarkin VA, Gundelwein A and Pfeiffer EM (1999) Studies of methane production and emission in relation to the microrelief of a polygonal tundra in northern Siberia. In Kassens H, Bauch HA, Dmitrenko IA, et al. (Eds) *Land-Ocean Systems in the Siberian Arctic, Dynamics and History* (pp 329-342). Springer, Berlin.
- Schachtschabel P, Blume HP, Brümmer, Hartge KH and Schwertmann U (1998) Scheffer/Schachtschabel - Lehrbuch der Bodenkunde. Enke, Stuttgart
- Schimel JP (1995) Plant transport and methane production as controls on methane flux from arctic wet meadow tundra. *Biogeochemistry* 28: 183-200.
- Schimel JP, Guldge J (1998) Microbial community structure and global trace gases. *Global Change Biology* 4: 745-758.
- Schink B and Zeikus JG (1982) Microbial ecology of pectin decomposition in anoxic lake sediments. *J. General Microb.* 128: 393-404.
- Schirrmeyer L, Grosse G, Schwamborn G, Andreev AA, Meyer H, Kunitsky VV, Kuznetsova TV, Dorozhkina MV, Pavlova EY, Bobrov AA, Oezen D (2003) Late Quaternary History of the Accumulation Plain North of the Chekanovsky Ridge (Lena Delta, Russia): A Multidisciplinary Approach. *Polar Geography*, 27 (4), 277-319.
- Schirrmeyer L and Wagner D (2007) Russian-German Cooperation System Laptev Sea 2000: The Expedition LENA 2005. *Reports on Polar and Marine Research* (in press).
- Schirrmeyer L, Grigoriev MN, Kutzbach L, Wagner D and Bolshiyanov DYU (2004) Russian-German Cooperation System Laptev Sea 2000: The Expedition Lena-Anabar 2003. *Reports on Polar and Marine Research* 489, pp.331.
- Schlüchtung E (1973) *Pseudogleye und Gleye - Genese und Nutzung hydromorpher Böden*. p. 1-6. In Schlüchtung E and Schwertmann U (ed.) *Pseudogley & Gley. Trans. Comm. V and VI Int. Soc. Soil Sci., Verlag Chemie, Weinheim*.
- Schlüchtung E, Blume HP and Stahr K (1995) *Bodenkundliches Praktikum - Pareys Studentexte* 81. Blackwell Wissenschaftsverlag, Berlin.
- Schneider J (2005) Bilanzierung von Methanemissionen in Tundrengebieten am Beispiel des Lena-Deltas, Nordostsibirien, auf der Basis von Fernerkundungsdaten und Geländeuntersuchungen. Diploma thesis, TU Dresden.
- Schneider J, Grosse G, Kutzbach L and Wagner D (2006) Land cover classification of tundra environments in the arctic Lena Delta based on Landsat 7 ETM+ data and its application for upscaling of methane emissions, Proceedings the Globwetland Symposium - Looking at wetlands from Space, 19-20 October 2006, Frascati, Italy.
- Schoeneberger PJ, Wysocki DA, Benham EC and Broderson WD (1998) Field book for describing and sampling soils. Natural Resources Conservation Service, USDA, National Soil Survey Center, Lincoln.

- Schuepp PH, Leclerc MY, MacPherson JI, Desjardins RL (1990) Footprint prediction of scalar fluxes from analytical solutions of the diffusion equation, *Boundary-Layer Meteorology*, 50 (1-4), 355-373, doi:10.1007/BF00120530.
- Schüller H (1969) Die CAL-Methode, eine neue Methode zur Bestimmung des pflanzenverfügbaren Phosphates in Böden. *Z. Pflanzenernaehr. Bodenkd.* 123: 48-63.
- Schütz H, Schröder P and Rennenberg H (1991) Role of plants in regulating the methane flux to the atmosphere. In: Sharkey TD, Holland EA & Mooney HA (Eds) *Trace Gas Emissions by Plants* (pp 29-63). Academic Press, San Diego.
- Schwamborn G, Rachold V, Grigoriev MN (2002) Late quaternary sedimentation history of the Lena Delta. *Quaternary International* 89: 119-134.
- Schwertmann U (1964) Differenzierung der Eisenoxide durch Extraktion mit Ammoniumoxalat-Lösung. *Z. Pflanzenernaehr. Bodenkd.* 101: 194-202.
- Sears DWG, Benoit PH, McKeever SWS, Banerjee D, Kral T, Stites W, Roe L, Jansma P, Mattioli G (2002) Investigation of biological, chemical and physical processes on and in planetary surfaces by laboratory simulation, *Planetary and Space Science* 50, 821-828.
- Sebacher DJ, Harriss RC and Bartlett KB (1985) Methane emissions to the atmosphere through aquatic plants. *J. Environ. Qual.* 14: 39-46.
- Segers R (1998) Methane production and methane consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry* 41: 23-51.
- Seibt A, Hoth P and Naumann D (2000) Gas solubility in formation waters of the North German Basin - implications for geothermal energy recovery. In: *Proceedings World Geothermal Congress 2000 Kyushu-Tohoku Japan May 28 - June 10 2000* (pp 1713-1718). International Geothermal Association IGA, Pisa, Italy.
- Semiletov IP, Pipko II, Pivovarov NYa, Popov VV, Zimov SA, Voropaev YuV & Daviodov SP (1996) Atmospheric carbon emission from north Asian lakes: a factor of global significance. *Atmospheric Environment*, 30 (10/11), 1657-1671.
- Senesi N, Miano TM, Provenzano MR et al. (1989) Spectroscopic and compositional comparative characterization of I.H.S.S reference and standard fulvic and humic acids of various origin. *Sci Total Environ*, 81/82, 143-156.
- Serreze MC, Walsh JE, Chapin III, Osterkamp FS, Dyurgerov T, Romanovsky M, Oechel V, Morison WC, Zhang J, T and Barry RG (2000) Observational evidence of recent change in the northern high-latitude environment. *Climatic Change*, 46, 159-206.
- Sherer P, Lippert H and Wolff G (1983) Composition of the major elements and trace elements of 10 methanogenic bacteria determined by inductively coupled plasma emission spectrometry. *J Biol Trace Elem Res* 5: 149-163.
- Shi T, Reeves R, Gilichinsky D et al. (1997) Characterization of viable bacteria from Siberian permafrost by 16S rDNA sequencing. *Microbial Ecology*, 33, 167-179.
- Shuisong N and Boone D (1998) Extremophilic methanogenic archaea and their adaptation mechanisms. *Extremophiles: Microbial Life in Extreme Environments*: 211-232.
- Slobodkin AI, Panikov NS, Zavarzin GA (1992) Formation and uptake of methane in tundra and boreal wetlands. *Microbiology* 61: 683-691.
- Simankova MV, Kotsyurbenko OR, Lueders T, Nozhevnikova AN, Wagner B, Conrad R and Friedrich MW (2003) Isolation and characterization of new strains of methanogens from cold terrestrial habitats. *Syst Appl Microbiol* 26: 312-318.
- Smith J, Stone R and Fahrenkamp-Uppenbrink J (2002) Trouble in polar paradise: Polar science (Introduction). *Science* 297: 1489.
- Smith LC, MacDonald GM, Velichko AA, Beilman WD, Borisova OK, Frey KE, Kremenetski KV, Sheng Y (2004) Siberian peatlands: a net carbon sink and global methane source since the early Holocene. *Science* 303: 353-356.
- Soil Survey Staff, Soil Conservation Service, USDA (1998) *Keys to Soil Taxonomy* 8th Edition. Pocahontas, Blacksburg, Virginia.
- Soil Survey Staff (1999) *Soil Taxonomy - a basic system of soil classification for making and interpreting soil surveys*, 2nd ed., 869 pp., US Government printing Office, Washington D.C.
- Sommer M and Schlichting E (1997) Archetypes of catenas in respect to matter - a concept for structuring and grouping catenas. *Geoderma* 76: 1-33.
- Sommer M, Thies H, Kolb E; Bächle H and Stahr K (1997) Biogeochemistry of a cirque-lake landscape: An interdisciplinary study in a catchment of the northern Black Forest, Germany. *Water Resour. Res.* 33: 2129-2142.
- Sorrel BK and Boon PI (1994) Convective gas flow in *Eleocharis sphacelata* R.Br.: methane transport and release from wetlands. *Aquat. Bot.* 47: 197-212.
- Spirina EV and Fedorov-Davydov DG (1998) Microbiological characterization of cryogenic soils in the Kolymskaya Lowland. *Eurasian Soil Sci* 31: 1331-1344.

- Spott O (2003) Frostmusterbedingte Seen der Polygonalen Tundra und ihre Funktion als Quellen atmosphärischen Methans, Diploma Thesis, 125 pp., University of Leipzig, Leipzig, Germany.
- Stamatakis A, Ludwig T and Meier H (2005) RAxML-III: A fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatic 21: 456-463.
- Stein LY, La Duc MT, Grundl TJ and Nealson KH (2001) Bacterial and archaeal populations associated with freshwater ferromanganese micronodules and sediments. Environ Microbiol 3: 10-18.
- Stein LY, Jones G, Alexander B, Elmund K, Wright-Jones C and Nealson KH (2002) Intriguing microbial diversity associated with metal-rich particles from a freshwater reservoir. FEMS Microbiol Ecol 42: 431-440.
- Steinwald AL and Richardson JL (1989) Gypsum occurrence in soils on the margin of semi permanent prairie pothole wetlands. Soil Sci. Soc. Am. J. 53: 836-842.
- Stetter KO, Fiala G, Huber G, Huber R and Segerer A (1990) Hyperthermophilic microorganisms. FEMS Microbiol Rev 75: 117-124.
- Steudler PA, Jones RD, Castro MS, Melillo JM and Lewis DL (1996) Microbial controls on methane oxidation in temperate forest and agricultural soils. In Microbiology of atmospheric trace gases. Murrell JC and Kelly DP (eds) Berlin: Springer, pp. 69-81.
- Story M & Congalton RG (1986) Accuracy assessment: A user's perspective. Photogrammetric Engineering and Remote Sensing, 52 (6), 835-841.
- Stotzky G (1966) Influence of clay minerals on microorganisms. III. Effect of particle size, cation exchange capacity and surface area on bacteria. Canadian Journal of Microbiology, 12, 1235-1246
- Stow D, Hope A, Boynton W, Phinn S, Walker D & Auerbach N (1998) Satellite-derived vegetation index and cover type maps for estimating carbon dioxide flux for arctic tundra regions. Geomorphology, 21, 313-327.
- Ström L, Ekberg A, Mastepanov M and Christensen TB (2003) The effect of vascular plants on carbon turnover and methane emissions from a tundra wetland. Global Change Biol 9: 1185-1192.
- Stuiver M, Reimer PJ, Braziunas TF (1998) High-precision radiocarbon age calibration for terrestrial and marine samples. Radiocarbon, 40, 1127-1151.
- Sundh I, Nilsson M and Borgå P (1997) Variation in microbial community structure in two boreal peatlands as determined by analysis of phospholipid fatty acid profiles. Appl Environ Microbiol 63: 1476-1482.
- Suyker AE, Verma SB, Clement RJ, Billesbach DP (1996) Methane flux in a boreal fen: Season-long measurement by eddy correlation, J. Geophys. Res., 101(D22), 28637-28648, doi:10.1029/96JD02751.
- Svensson BH and Rosswall T (1984) In situ methane production from acid peat in plant communities with different moisture regimes in a subarctic mire. Oikos 43: 341-350.
- Takeuchi W, Tamura M & Yasuoka Y (2003) Estimation of methane emission from West Siberian wetland by scaling technique between NOAA AVHRR and SPOT HRV. Remote Sensing of Environment, 85, 21-29.
- Tarnocai C and SC Zoltai (1978) Earth hummocks of the Canadian arctic and subarctic. Arct. Alp. Res. 10: 581-594.
- Tedrow JCF, Drew JV and Douglas LA (1958) Major genetic soils of the Arctic Slope of Alaska. J. Soil Sci. 9: 33-45.
- Tedrow JCF (1977) Soils of the polar landscapes. Rutgers University Press, New Brunswick, New Jersey.
- Thomas KL, Benstead J, Davies KL and Lloyd D (1996) Role of wetland plants in the diurnal control of CH<sub>4</sub> and CO<sub>2</sub> fluxes in peat. Soil Biol. Biochem. 28: 17-23.
- Thomsson E, Gustafsson L and Larsson C (2005) Starvation response of *Saccharomyces cerevisiae* grown in anaerobic nitrogen- or carbon-limited chemostat cultures. Appl Environ Microbiol 71/6: 3007-3013.
- Torn MS and Chapin FS (1993) Environmental and biotic controls over methane flux from arctic tundra. Chemosphere 26: 357-368.
- Tornbjerg T, Bendix M and Brix H (1994) Internal gas transport in *Typha latifolia* L. and *Typha angustifolia* L. 2. Convective throughflow pathways and ecological significance. Aquat. Bot. 49: 91-105.
- Traina SJ, Novak J and Smeck NE (1990) An ultraviolet absorbance method for estimating the percent aromatic carbon content of humic acids. J. Environ. Qual. 19: 151-153.
- Trotsenko YA and Khmelenina VN (2005) Aerobic methanotrophic bacteria of cold ecosystems. FEMS Microbiology Ecology, 53, 15-26.
- Tsuyuzaki S, Nakano T, Kuniyoshi S and Fukuda M (2001) Methane flux in grassy marshlands near Kolyma River, north-eastern Siberia. Soil Biol. Biochem. 33: 1419-1423.

- Tung HC, Bramall NE and Price PB (2005) Microbial origin of excess methane in glacial ice and implications for life on Mars, PNAS 102 (in press).
- Valentine DW, Holland EA and Schimel DS (1993) Ecological controls over methane and carbon dioxide fluxes along a successional gradient. J Geophys Res 99: 1563-1571.
- Van Bodegom P, Stams F, Mollema L, Boeke S, Leffelaar P (2001) Methane oxidation and the competition for oxygen in the rice rhizosphere. Applied and Environmental Microbiology 67: 3586-3597.
- Van der Nat FJWA and Middelburg JJ (1998) Seasonal variation in methane oxidation by the rhizosphere of *Phragmites australis* and *Scirpus lacustris*. Aquat. Bot. 61(2): 95-110.
- Van Huissteden J, Maximov TC, Dolman AJ (2005) High methane flux from an arctic floodplain (Indigirka Lowlands, eastern Siberia), J. Geophys. Res., 110, G02002, doi: 10.1029/2005JG000010.
- Van Vliet-Lanoë B (1991) Differential frost heave, load casting and convection: converging mechanisms; a discussion of the origin of cryoturbations. Permafrost Periglac Process. 2:123-139.
- Vecherskaya MS, Galchenko VF, Sokolova EN, Samarkin VA (1993) Activity and species composition of aerobic methanotrophic communities in tundra soils. Current Microbiology 27: 181-184.
- Vepraskas MJ and SP Faulkner (2001) Redox chemistry of hydric soils. p. 85-105. In Richardson JL and Vepraskas MJ (ed.) Wetland soils. Genesis, hydrology, landscape and classification. Lewis Publishers. Boca Raton, London, New York, Washington D.C.
- Verville JH, Hobbie SE, Chapin III FS, Hooper DU (1998) Response of tundra CH<sub>4</sub> and CO<sub>2</sub> flux to manipulation of temperature and vegetation. Biogeochemistry 41: 215-235.
- Virtanen T, Mikkola K & Nikula A (2004) Satellite image based vegetation classification of a large area using limited ground reference data: a case study in the Usa Basin, north-east European Russia. Polar Research, 23 (1), 51-66.
- Vishnivetskaya T, Kathariou S, McGrath J, Gilichinsky D and Tiedje J (2000) Low-temperature recovery strategies for the isolation of bacteria from ancient permafrost sediments. Extremophiles 4: 165-173.
- Von Zezschwitz E, Schwertmann U and Ulrich B (1973) Die Podsolierungsstadien von Braunerden aus Schieferschutt. Z. Pflanzenernähr. Bodenkd. 136: 40-52.
- Vorobyova EA, Gilichinsky DA, Sonina VS et al. (1998), Antarctic permafrost as microbial habitat, in Lake Vostok Study: Scientific Objectives and Technological Requirements, International Workshop , March 24-26, St. Petersburg, Russia, p.97.
- Vourlitis GL, Oechel WC, Hastings SJ, Jenkins MA (1993) The effect of soil moisture and thaw depth on CH<sub>4</sub> flux from wet coastal tundra ecosystems on the north slope of Alaska. Chemosphere 26: 329-337.
- Wachinger G, Fiedler S, Zepp K, Gattinger A, Sommer M and Roth K (2000) Variability of soil methane production on the micro-scale: spatial association with hot spots of organic material and archaeal populations. Soil Biol & Biochem 32: 1121-1130.
- Waddington JM, Roulet NT and Swanson RV (1996) Water table control of CH<sub>4</sub> emission enhancement by vascular plants in boreal peatlands. J. Geophys. Res. 101: 22775-22785.
- Wagner D and Pfeiffer EM (1997) Two temperature optima of methane production in a typical soil of the Elbe river marshland. FEMS Microbiol Ecol 22: 145-153.
- Wagner D, Pfeiffer EM and Bock E (1999) Methane production in aerated marshland and model soils: effects of microflora and soil texture. Soil Biology and Biochemistry 31: 999-1006.
- Wagner D, Kurchatova A and Stoff G (2001a) Modern processes in permafrost affected soils. p. 16-23. In Rachold V and Grigoriev MN (ed.) Russian-German Cooperation System Laptev Sea 2000. The Expedition LENA 2000. Reports on Polar Research 388.
- Wagner D, Speck E, Bock E and Pfeiffer EM (2001b) Microbial life in terrestrial permafrost: methanogenesis and nitrification in Gelisols as potentials for exobiological processes. p.143-159. In Horneck G and Baumstark-Khan C (ed.) Astrobiology: The quest for the conditions of life. Springer. Berlin, Heidelberg, New York.
- Wagner D, Kobabe S, Pfeiffer EM et al. (2003a) Microbial controls on methane fluxes from a polygonal tundra of the Lena Delta, Siberia. Permafrost Periglac Process 14, 173-185.
- Wagner D, Wille C, Kobabe S et al. (2003b) Simulation of freezing-thawing cycles in a permafrost microcosm for assessing microbial methane production under extreme conditions. Permafrost and Periglacial Processes, 14, 367-374.
- Wagner D, Lipski A, Embacher A, Gattinger A (2005) Methane fluxes in permafrost habitats of the Lena Delta: effects of microbial community structure and organic matter quality. Environ Microbiol 7: 1582-1592.
- Wagner D, Gattinger A, Embacher A, Pfeiffer EM, Schloter M, Lipski A (2006) Methanogenic activity and biomass in Holocene permafrost deposits of the Lena Delta, Siberian Arctic and its implication for the global methane budget, Global Change Biology (submitted).

- Wagner D. and Bolshiyanov D. (2006) Russian-German Cooperation System Laptev Sea 2000: The Expedition LENA 2004. *Reports on Polar and Marine Research* 539, pp.123.
- Wagner D., Kobabe S., Kurchatova A., Ganzert L., Zimmermann U., Morozova D., Liebner S., Joseph J. and Pfeiffer E.-M. (in prep.) Spatial and temporal variations of methane fluxes from polygonal tundra of the Lena Delta, Siberia: a eight-year study.
- Wallner G, Amann R and Beisker W (1993) Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry*, 14, 136-143.
- Walter BP, Heimann M, Shannon RD and White JR (1996) A processed-based model to derive methane emissions from natural wetlands. *Geophysical Research Letters*, 23(25), 3731-3734.
- Walter BP and Heimann M (2000) A processed-based, climate-sensitive model to drive methane emissions from natural wetlands: Applications to five wetland sites, sensitivity to model parameters, and climate. *Global Biochemical Cycles*, 14(3), 745-765.
- Wardlaw AC (1985) Practical statistics for experimental biologists. John Wiley and Sons, Chichester.
- Washburn A L (1979) Geocryology, A Survey of Periglacial Processes and Environments, 406 pp., Arnold, London, UK.
- Watanabe T, Asakawa S, Nakamura A, Nagaoka K and Kimura M (2004) DGGE method for analyzing 16S rDNA of methanogenic archaeal community in paddy field soil. *FEMS Microbiol Lett* 232: 153-163.
- Wein N (1999) Sibirien. Perthes Regionalprofile. Gotha: Klett-Perthes. 248p.
- Weinberg MV, Schut GJ, Brehm S, Datta S and Adams MW (2005) Cold shock of a hyperthermophilic archaeon: Pyrococcus furiosus exhibit multiple responses to a suboptimal growth temperature with a key role of membrane-bound glycoproteins. *J Bacteriol* 187(1): 336-348.
- Whalen SC, Reeburgh WS (1988) A methane flux time series for tundra environments. *Global Biogeochemical Cycles* 2 (4): 399-409.
- Whalen SC & Reeburgh WS (1990) A methane flux transect along the trans-Alaska pipeline haul road. *Tellus*, 42 (B), 237-249.
- Whalen SC and Reeburgh WS (1992) Interannual variations in tundra methane emission: A 4-year time series at fixed sites, *Global Biogeochemical Cycles*, 6(2) 139-159.
- Whalen SC, Reeburgh WS and Reimers CE (1996) Control of tundra methane emission by microbial oxidation. In: Reynolds JF and Tenhunen JD (Eds) Landscape function and disturbance in Arctic tundra, *Ecological Studies* 120 (pp 257-274) Springer, Berlin, Germany.
- Wharton Jr RA, McKay CP, Mancinelli RL and Simmons Jr GM (1989) Early Martian environments: the Antarctic and other terrestrial analogs. *Adv Space Res* 9/6: 147-153.
- White DC, Davis WM, Nickels JS et al. (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia*, 40, 51-62].
- Whiting GJ and Chanton JP (1992) Plant-dependent CH<sub>4</sub>-emission in a subarctic Canadian fen. *Global Biogeochem. Cycles* 9: 225-231.
- Whittig LD and Janitzky P (1963) Mechanisms of formation of sodium carbonate in soils I. manifestations of biologic conversions. *J. Soil Sci.* 14: 322-333.
- Wiegand CL, Lyles L and Cater DL (1966) Interspersed salt-effected and unaffected dryland soils of the lower Rio Grande Valley: II. Occurrence of salinity in relation to infiltration rates and profile characteristics. *Soil Sci. Soc. Am. Proc.*
- Wille C (2004) Air photography and surface classification of Samoylov Island, in Expeditions in Siberia in 2003, *Reports on Polar and Marine Research* 489, edited by L. Schirrmeister, 39-40, Alfred-Wegener-Institute, Bremerhaven, Germany.
- Wise MG, McArthur JV and Shimkets LJ (2001) *Methylosarcina fibrata* gen. nov., sp. Nov. and *Methylosarcina quisquiliarum* sp. Nov., novel type I methanotrophs. *Int J Syst Bacteriol* 51: 611-621.
- Worthy DEJ, Levin I, Hopper F, Ernst MK & Trivett NBA (2000) Evidence for a link between climate and northern wetland methane emissions. *Journal of Geophysical Research*, 105 (D3), 4031-4038.
- Wouters JA, Frenkel H, de Vos WM, Kuipers OP and Abbe T (2001) Cold shock proteins of *Lactococcus lactis* MG1363 are involved in cryoprotection and in the production of cold-induced proteins. *Appl Environ Microbiol* 67(11): 5171-5178.
- Wuebbles J and Hayhoe K (2002) Atmospheric methane and global change. *Earth-Science Reviews*, 57, 177-210.
- WWIS (2004). World Weather Information Service, <http://worldweather.org/107/c01040.html>.
- Yamamoto S, Alcauskas JB and Crozier TE (1976) Solubility of methane in distilled water and seawater. *J. Chem. Eng. Data* 21(1) 78-80.

- Yavitt JB, Williams CJ, Wieder RK (2000) Controls on microbial production of methane and carbon dioxide in three *Sphagnum*-dominated peatland ecosystems as revealed by a reciprocal field peat transplant experiment. *Geomicrobiological Journal* 17: 61-88.
- Yershov ED (1998) General geochryology. Cambridge University Press, Cambridge, pp. 580.
- Zarda B, Hahn D, Chatzinotas A, Schoenhuber W, Neef A, Amann R and Zeyer J (1997) Analysis of bacterial community structure in bulk soil by in situ hybridization. *Archives of Microbiology*, 168, 185-192.
- Zelles L, Bai QY (1993) Fractionation of fatty acids derived from soil lipids by solid phase extraction and their quantitative analysis by GC-MS. *Soil Biology and Biochemistry*, 25, 495-507.
- Zelles L and Bai QY (1994) Fatty acid patterns of phospholipids and lipopolysaccharids in environmental samples. *Chemosphere* 28: 391-411.
- Zelles L (1997) Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere* 35: 275-294.
- Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils*, 29, 111-129.
- Zhang T, Barry RG, Knowles K, Heginbotton JA and Brown J (1999) Statistics and characteristics of permafrost and ground-ice distribution in the northern hemisphere. *Polar Geography*, 23(2), 132-154.
- Zhang W, Culley DE, Nie L and Brockman FJ (2006) DNA microarray analysis of anaerobic *Methanosarcina barkeri* reveals responses to heat shock and air exposure. *J Ind Microbiol Biotechnol* DOI 10.1007/s10295-006-0114-3.
- Zimov SA, Voropaev YV, Semiletov IP, Davidov SP, Prosiannikov SF, Chapin III FS, Chapin MC, Trumbore S & Tyler S (1997) North Siberian lakes: a methane source fueled by Pleistocene carbon. *Science*, 277, 800-802.
- Zimov SA, Schuur EAG and Chapin III FS (2006) Permafrost and the global carbon budget. *Science* 312: 1612-1613.
- Zsolnay A, Baigar E, Jiminez M et al. (1999) Differentiation with fluorescence spectroscopy the sources of dissolved organic carbon. *Chemosphere*, 38, 45-50.
- Zsolnay A (2003) Dissolved organic matter: artefacts, definitions, and functions. *Geoderma*, 113, 187-209.

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## Appendix

### Correspondence for the Publications under Revision:

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## List of Oral and Poster Presentations

### *Invited Talks*

- Wagner, D.** (2006) Long-term studies on methane fluxes from permafrost environments of the Lena Delta, Siberia: microbial processes and communities, TUMSS Research Cluster Seminar Series, ETH Zurich, Switzerland, 23.11.2006.
- Wagner, D.**, Morozova D., Möhlmann D., Rettberg P. (2006) Response of methanogenic archaea from Siberian permafrost to Martian thermo-physical conditions, European Planetary Science Congress 2006, Berlin, Germany, 18.-22. September 2006.
- Wagner, D.** (2006) Methane fluxes from Siberian permafrost: microbial communities and their astrobiological relevance, Colloquium, German Research Centre for Biotechnology, Braunschweig, 12.09.2006.
- Wagner, D.** (2006) Response of methanogenic archaea from Siberian permafrost to Martian conditions, Adlershofer Planetenseminar, Institut für Planetenforschung, Deutsches Luft- und Raumfahrtzentrum, 26.04.2006.
- Morozova, D. and **Wagner, D.** (2005) Tolerance limits of methanogenic life in terrestrial permafrost, DFG Colloquium: Mars and the Terrestrial Planets, Berlin, 29.-30. August 2005.
- Wagner, D.** (2005) Cold adapted microorganisms: natural processes and possibilities of their technical utilization, Workshop on Challenges of Permafrost Degradation of Siberian Soils to Science and Technology, Institute of Forest (RAS), Krasnoyarsk, Russia, 16.-19. März 2005.
- Wagner, D.** (2004) Langzeitstudien zur Methanfreisetzung aus Tundrenböden des Lena Deltas, Sibirien, Geowissenschaftliches Institutskolloquium, Institut für Geophysik und Geologie, Universität Leipzig, 10.06.2004.
- Wagner, D.** (2004) Methane fluxes from tundra environments of the Lena Delta: long-term studies and astrobiological relevance, Colloquium, Max Planck Institute for Biogeochemistry, Jena, 03.06.2004.
- Wagner, D.** and Hubberten, H.-W. (2004) Long-term studies on methane fluxes from tundra environments of the Lena Delta: microbial processes, Biogeochemistry and Permafrost Modeling, International Workshop on Permafrost-Carbon-Climate Interactions, Paris, France, 17.-18. Februar 2004.
- Wagner, D.** (2003) Methanogene Mikrobengemeinschaften im terrestrischen Permafrost und ihre Relevanz für mögliches Leben auf dem Mars, Astrobiology Colloquium, Deutsches Zentrum für Luft- und Raumfahrt, Institut für Luft- und Raumfahrtmedizin, Köln, 27.11.2003.
- Wagner, D.**, Speck, E. and Pfeiffer, E.-M. (2003) Tolerance limits of microbial life in terrestrial permafrost, DFG Colloquium: Mars and the Terrestrial Planets, Münster, 20.-21. August 2003.
- Wagner, D.** (2002) Mikrobiologische Prozessstudien zum Methankreislauf in Permafrostböden, Kolloquiumsvortrag, Aktuelle Themen der Erdsystemforschung, Universität Hamburg, Institut für Bodenkunde, 13.11.2002.
- Wagner, D.** (2002). Microbial controls on methane emission from Siberian tundra environments, Kolloquiumsvortrag, Bodenökologisches Seminar, GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Bodenökologie, Neuherberg, 06.11.2002.
- Wagner, D.** (2002) Microbial archives in permafrost: methanogenic archaea as key-organisms, HELMERT-Summerschool, Potsdam, 12.-18. September 2002.
- Wagner D.** (2001) Methanflüsse aus Permafrostböden Sibiriens: Ergebnisse und offene Fragen, Kolloquiumsvortrag, Universität Kiel, Institut für Polarökologie, 05.02.2001.
- Wagner D.** (2000) Saisonale Variabilität von rezenten Stoffumsetzungen: Cryosole und Methanogenese, Kolloquiumsvortrag, Universität Hohenheim, Institut für Bodenkunde und Standortlehre, Stuttgart, 17.05.2000.
- Wagner D.** (1998) Methane production and release of trace gases from sub-arctic tundra, Siberia, Second TUNDRA Meeting, Utrecht, The Netherlands, 15.-19. Dezember 1998.

*Oral Presentations (for the last 5 years)*

- Liebner, S., Harder, J. and **Wagner, D.** (2006) Stability of methane oxidising communities in Siberian permafrost soils in the context of global climate change, International Conference on Alpine and Polar Microbiology, Innsbruck, Austria, 27.-30. March 2006.
- Morozova, D. and **Wagner, D.** (2006) Methanogene Archaeen aus sibirischem Permafrost als Modellsysteme für das Leben auf dem Mars, DPG-AEF Tagung, Heidelberg, Deutschland, 13.-16. März 2006.
- Morozova, D. and **Wagner, D.** (2006) Methanogenic archaea in terrestrial permafrost as a model for probable microbial life on Mars, International Conference on Alpine and Polar Microbiology, Innsbruck, Austria, 27.-30. March 2006.
- Morozova, D. and **Wagner, D.** (2006) Methanogenic archaea from Siberian permafrost as prime candidates for life on Mars, DFG Workshop, Cologne, Germany, 4.-5. May 2006.
- Rettberg, P., **Wagner, D.**, Ott, S., Ondruschka, J. and Möhlmann, D. (2006) Survival potential of terrestrial organisms under Martian conditions, ESA-CNES Workshop on Special Regions, European Space Research and Technology Centre, Noordwijk, The Netherlands, 25. January 2006.
- Cannone, N., Guglielmin, M., **Wagner, D.** and Hubberten, H.-W. (2005) Active layer characteristics and bacterial occurrence across a latitudinal gradient in Victoria Land (continental Antarctica) as indicator of functional processes in permafrost environments and ecosystems, Second European Conference on Permafrost, EUCOP 2005, Potsdam, Germany, 12.-16. June 2005.
- Gattinger, A., Embacher, A., Labrenz, M., **Wagner, D.** and Schloter, M. (2005) Die Bedeutung der organischen Bodensubstanz für die Anpassung und Entwicklung von Archaeengemeinschaften in Böden, Jahrestagung der Deutschen Bodenkundlichen Gesellschaft, Marburg, Deutschland, 3.-11. September 2005.
- Liebner, S. and **Wagner, D.** (2005) Spatial variance of methane oxidation rates in Siberian permafrost soils in dependence of the temperature: an indicator for microbial changes of structure and diversity? Workshop on Development and Control of Functional Biodiversity at Micro- and Macro-scales, Neuherberg, Germany, 5.-7. October 2005.
- Liebner, S. and **Wagner, D.** (2005) Temperature-Dependence of Methane Oxidation Rates in Permafrost Soils of the Lena Delta, Siberia, 22. International Polar Meeting, Jena, Germany, 18.-24. September 2005.
- Meyer, H., Yoshikawa, K., Schirrmeister, L., Andreev, A. A., **Wagner, D.** and Hubberten, H.-W. (2005) The Vault Creek permafrost tunnel - Late Quaternary climate and environmental history of the Fairbanks region, Alaska, Second European Conference on Permafrost, EUCOP 2005, Potsdam, Germany, 12.-16. June 2005.
- Morozova, D. and **Wagner, D.** (2005) Tolerance limits of methanogenic archaea from Siberian permafrost: effects of high salinity and low temperatures, Second European Conference on Permafrost EUCOP 2005, Potsdam, Germany, 12.-16. June 2005.
- Morozova, D. and **Wagner, D.** (2005) Microbial life under extreme environments of permafrost: tolerance limits of methanogenic archaea as keystone organisms for the investigation of extraterrestrial life, 22. International Polar Meeting, Jena, Germany, 18.-24. September 2005.
- Morozova, D. and **Wagner, D.** (2005) Survival potential of methanogenic archaea from Siberian permafrost: investigation of possible extraterrestrial life, VAAM Annual Meeting, Gottingen, Germany, 25.-28. September 2005.
- Morozova, D. and **Wagner, D.** (2005) Methanogenic archaea under permafrost extreme conditions: a model for putative life on Mars, International workshop on Astrobiology: Life in extreme conditions, Oslo, Norway, 21.-22. November 2005.
- Morozova, D. and **Wagner, D.** (2005) Methanogenic archaea from Siberian permafrost: fit for Mars? 5th European Workshop on Astrobiology, Budapest, Hungary, 10.-12. October 2005.
- Schneider, J., Kleber, A. and **Wagner, D.** (2005) Balance of methane emissions from tundra environments of the Lena Delta based on remote sensing, 22. International Polar Meeting, Jena, Germany, 18.-24. September 2005.
- Wagner, D.**, Gattinger, A., Lipski, A. and Schloter, M. (2005) Effects of microbial communities and organic matter quality on methane fluxes in different areas of a Siberian polygon tundra, Second European Conference on Permafrost, EUCOP 2005, Potsdam, Germany, 12.-16. June 2005.

- Wagner, D.** and Morozova, D. (2005) Methanogenic archaea in terrestrial permafrost: a model for probable microbial life in Martian permafrost, ARCHAEA - The First Generation, Hohenheimer Castle Academy, Munich, Germany, 2.-4. June 2005.
- Wagner, D.**, Gattinger, A., Lipski, A. and Schloter, M. (2005) Methane fluxes, microbial activities and community structures in a wet tundra of the Lena Delta, 22. International Polar Meeting, Jena, Germany, 18.-24. September 2005.
- Cannone, N., Guglielmin, M., **Wagner, D.** and Hubberten, H.-W. (2004) Active layer characteristics across a latitudinal gradient in Victoria Land (Continental Antarctica) as indicator of functional processes in permafrost environments and ecosystems, XXVIII SCAR Meeting, Bremen, Germany, 25.-31. July 2004.
- Kobabe, S., Liebner, S., Pfeiffer, E.-M. and **Wagner, D.** (2004) Characterisation of microbial community controlling methane emission from Siberian tundra soils by FISH, International Conference on Arctic Microbiology, Rovaniemi, Finland, 22.-25. March 2004.
- Morozova, D., **Wagner, D.** and Hubberten, H.-W. (2004) Subsurface microbial life as a model for extraterrestrial permafrost habitats, El`gygytgyn Lake Workshop, Leipzig, Germany, 24.-26. March 2004.
- Pfeiffer, E.-M., Spott, O., Kutzbach, L. and **Wagner, D.** (2004) Patterned ground related soils and lakes and their role as sources for methane, International Conference "Cryosphere of Oil-And-Gas Bearing Provinces", Tyumen, Russia, 22.-29. May 2004.
- Wagner, D.**, Kurchatova, A. and Gattinger, A. (2004) Methanogenesis in late Pleistocene permafrost sediments of the Lena Delta, Siberia, International Workshop on Geomicrobiology, Aarhus, Denmark, 28.-31. January 2004.
- Wagner, D.** and Morozova, D. (2004) Tolerance limits of methanogenic archaea in terrestrial permafrost, International Conference "Cryosphere of Oil-And-Gas Bearing Provinces", Tyumen, Russia, 22.-29. May 2004.
- Wagner, D.**, Embacher, A., Lipski, A. and Gattinger, A. (2004) Methane fluxes in different areas of a Siberian polygon tundra: effects of organic matter quality and microbial communities, VAAM Annual Meeting 2004, Braunschweig, Germany, 28.-31. March 2004.
- Wagner, D.** and Gattinger, A. (2004) Archaeal activity and biomass in Holocene permafrost deposits of the Lena Delta, Siberia, International Conference on Arctic Microbiology, Rovaniemi, Finland, 22.-25. March 2004.
- Pfeiffer, E.-M. and **Wagner, D.** (2003) Permafrost related methane fluxes and their importance for climate change effects, Agriculture in Northern Ecosystems: Effects of Global Change on Soil Ecological Processes, Vechta, Germany, 2.-4. April 2003.
- Wagner, D.**, Pfeiffer, E.-M. and Hubberten, H.-W. (2003) Methane cycle in permafrost soils: first evidence of cold adaptation of methanogenic archaea and methylotrophic bacteria, Workshop on Cold Adaptation of Aquatic Microorganisms, Bremen, 11.-14. Mai 2003.
- Wagner, D.** and Pfeiffer, E.-M. (2003) Mikrobielle Methanbildung unter extremen Umweltbedingungen in Permafrostböden: Ein Modell für exobiologische Prozesse? 21. Internationale Polartagung, Kiel, Deutschland, 16.-21. März 2003.
- Wagner, D.**, Pfeiffer, E.-M. and Hubberten, H.-W. (2003) Long-term studies on methane fluxes from tundra environments in the Lena Delta, Siberia, NECC Conference: The Carbon Balance of Aquatic and Terrestrial Ecosystems and their Interaction, Lammi, Finland, 26.-28. October 2003.
- Kobabe, S., **Wagner, D.**, Schröder, H., Damm, E., Kassens, H. and Pfeiffer, E.-M. (2002) Methane contents in different compartment of the Laptev Sea - Preliminary results, Climate Drivers of the North, Kiel, Germany, 8.-11. May 2002.
- Kutzbach, L., **Wagner, D.**, Wille, C., Kobabe, S. and Pfeiffer, E.-M. (2002) Permafrostlandschaften als Quellen atmosphärischen Methans: Feldstudien zur zeitlichen und flächenhaften Variabilität der Methanemission aus Tundren des Lena-Deltas, Geo 2002 - Jahrestagung der Gesellschaften der Festen Erde in der Bundesrepublik Deutschland, Würzburg, Deutschland, 1.-5. Oktober 2002.
- Pfeiffer, E.-M. and **Wagner, D.** (2002) Methane studies in the active layer: methanogenesis as a model process for the extreme habitat development in permafrost, Extreme Phenomena in the Cryosphere, Pushchino, Russia, 5.-8. May 2002.

- Wagner, D.** and Pfeiffer, E.-M. (2002) Microbial life in permafrost soils: methanogenesis as analogues for exobiological processes, Water in the Upper Martian Surface, Potsdam, Germany, 17.-19. April 2002.
- Wagner, D.**, Pfeiffer, E.-M. and Samarkin, V. (2002) Microbial controls on methane emission from Siberian tundra environments: open questions and future perspectives, Climate Drivers of the North, Kiel, Germany, 8.-11. May 2002.
- Wagner, D.**, Kobabe, S. and Pfeiffer, E.-M. (2002) Microbial controls on methane emission from Siberian tundra environments, VAAM Annual Meeting 2002, Göttingen, Germany, 24.-27. March 2002.
- Wagner, D.**, Kobabe, S. and Pfeiffer, E.-M. (2002) Bedeutung der mikrobiellen Methanbildung und -oxidation für die Methanfreisetzung aus arktischen Tundren Sibiriens, GEO 2002- Jahrestagung der Gesellschaften der Festen Erde in der Bundesrepublik Deutschland, Würzburg, Deutschland, 1.-5. Oktober 2002.
- Wagner, D.**, Pfeiffer, E. -M. and Hubberten, H.-W. (2002) The consequences of microbial processes for the methane emission from Siberian permafrost environments, AGU 2002 Fall Meeting, San Francisco, USA, 6.-10. December 2002.
- Wagner, D.** and Pfeiffer, E.-M. (2002) Methanogenesis as a model process for extreme habitat development: process studies in the active layer of different permafrost sites, Second European Workshop on Exo/Astrobiology, Graz, Austria, 16.-19. September 2002.

*Poster Presentations (for the last 5 years)*

- Jurgens, G., Münster, U., Grigoriev, M. and **Wagner, D.** (2006) Archaeal activity and diversity in Late Pleistocene permafrost sediments of the river Lena Delta, Siberia, Russia, 11th International Symposium on Microbial Ecology, Vienna, Austria, 20.-25. August 2006.,
- Liebner, S., Harder, J. and **Wagner, D.** (2006) Bacterial diversity in Siberian permafrost soils based on 16S rDNA clone libraries and *in situ* cell counting, 11th International Symposium on Microbial Ecology, Vienna, Austria, 20.-25. August 2006.
- Morozova, D. and **Wagner, D.** (2006) Response of methanogens from Siberian permafrost to extreme conditions of terrestrial and extraterrestrial permafrost, VAAM Annual Meeting 2006, Jena, Germany, 19.-22. March 2006.
- Morozova, D. and **Wagner, D.** (2006) Response of methanogens from Siberian permafrost to extreme conditions of terrestrial and extraterrestrial permafrost, VAAM Annual Meeting 2006, Jena, Germany, 19.-22. March 2006.
- Morozova, D. and **Wagner, D.** (2006) Highly resistant methanogenic archaea from Siberian permafrost as candidates for the possible life on Mars, 6th European Workshop on Astrobiology, Lyon, France, 16.-18. October 2006.
- Sachs, T., Wille, C. and **Wagner, D.** (2006) Trace gas flux measurements on Samoylov Island, Lena Delta, 8th Workshop SYSTEM LAPTEV SEA, St. Petersburg, Russia, 7.-9. February 2006.
- Schneider, J., Grosse, G., Kutzbach, L. and **Wagner, D.** (2006) Land cover classification of tundra environments in the Arctic Lena Delta based on Landsat 7 ETM+ data and its application for up-scaling of methane emissions, GlobWetland Symposium - Looking at Wetlands from Space, ESA-ESRIN, Frascati, Italy, 19.-20. October 2006.
- Wagner, D.**, Kobabe, S., Lipski, A. and Gattinger, A. (2006) Effects of microbial community structure and soil organic matter on methanogenesis in Siberian permafrost soils, International Conference on Alpine and Polar Microbiology, Innsbruck, Austria, 27.-30. March 2006.
- Wagner, D.** and Morozova, D. (2006) Methanogenic archaea as potential candidates for life on Mars, 6th European Workshop on Astrobiology, Lyon, France, 16.-18. October 2006.
- Ganzert, L. and **Wagner, D.** (2005) Biodiversity of methanogenic archaea in permafrost affected soils of the Lena Delta, Siberia, 22. International Polar Meeting, Jena, Germany, 18.-24. September 2005.
- Ganzert, L. and **Wagner, D.** (2005) Composition of methanogenic archaeal communities in permafrost soils of Northern Siberia, VAAM Annual Meeting 2005, Göttingen, Germany, 23.-26. September 2005.

- Gattinger, A., Embacher, A., Labrenz, M., **Wagner, D.** and Schloter, M. (2005) The Importance of soil organic matter for adaptation and evolution of archaeal communities in terrestrial ecosystems, ARCHAEA - The First Generation, Munich, Germany, 2.-4. June 2005.
- Liebner, S. and **Wagner, D.** (2005) Cold loving methanotrophic communities in permafrost soils of the Lena Delta, Siberia, VAAM Annual Meeting 2005, Göttingen, Germany, 23.-26. September 2005.
- Liebner, S. and **Wagner, D.** (2005) Adaptation and composition of methanotrophic communities in permafrost soils of the Lena Delta, Siberia, 2nd European Conference On Permafrost, Potsdam, Germany, 12.-16. June 2005.
- Meyer, H., Schirrmeister, L., Andreev, A., **Wagner, D.**, Hubberten, H.-W., Yoshikawa, K. and Brown, J. (2005) Multi-proxy approach to a buried ice-wedge system, Barrow, Alaska, 1st CLIC International Science Conference, Beijing, China, 11.-15. April 2005.
- Meyer, H., Yoshikawa, K., Schirrmeister, L., Andreev, A., **Wagner, D.** and Hubberten, H.-W. (2005) Palaeoenvironmental record of a Late Quaternary permafrost tunnel in the Vault Creek, Fairbanks region, Alaska, 1st CLIC International Science Conference, Beijing, China, 11.-15. April 2005.
- Meyer, H., Schirrmeister, L., Andreev, A. A., **Wagner, D.**, Hubberten, H.-W., Yoshikawa, K. and Brown, J. (2005) A buried ice-wedge system as archive for the Late Quaternary environmental history near Barrow, Alaska, EUCOP II, 2nd European Conference on Permafrost, Potsdam, Germany, 12.-16. June 2005.
- Wagner, D.** and Morozova, D. (2005) Methane production in terrestrial permafrost: a model for possible microbial life in Martian permafrost, VAAM Annual Meeting 2005, Göttingen, Germany, 23.-26. September 2005.
- Kurchatova, A. N., Pfeiffer, E.-M., Slagoda, E. A. and **Wagner, D.** (2004) Ranks of cryostructures of ice complex sequences of the Lena Delta, Russia, International Conference "Cryosphere of Oil-And-Gas Bearing Provinces", Tyumen, Russia, 22.-29. May 2004.
- Wagner, D.**, Kobabe, S., Pfeiffer, E. -M. and Hubberten, H.-W. (2003) Methanogenesis under extreme environmental conditions in permafrost soils: a model for exobiological processes? 8th International Conference on Permafrost, Zurich, Switzerland, 21.-25. July 2003.
- Wagner, D.**, Liebner, S. and Kobabe, S. (2003) Activity and diversity of methanogens and methanotrophs under extreme environmental conditions in permafrost soils, International Symposium on Structure and Function of Soil Microbiota, Marburg, Germany, 18.-20. September 2003.
- Kobabe, S., **Wagner, D.** and Pfeiffer, E.-M. (2002) Methane production in Siberian tundra soils: Influence of temperature and substrates, VAAM Annual Meeting 2002, Göttingen, Germany, 24.-27. March 2002.
- Kutzbach, L., Wille, C., **Wagner, D.** and Pfeiffer, E.-M. (2002) The eddy covariance technique as a tool to determine trace gas fluxes on the ecosystem scale, Climate Drivers of the North Conference, Kiel, Germany, 8.-11. May 2002.
- Pfeiffer, E.-M., Kobabe, S. and **Wagner, D.** (2002) Methane fluxes in Siberia and their relevance for the permafrost-related gas hydrate research, Climate Drivers of the North,