

Archaeal Communities of Frozen Quaternary Sediments of Marine Origin on the Coast of Western Spitsbergen

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Abstract—The archaeal composition of permafrost samples taken during the drilling of frozen marine sediments in the area of the Barentsburg coal mine on the east coast of Grønfjord Bay of Western Spitsbergen has been studied. This study is based on an analysis of the V4 region of the 16S rRNA gene, carried out using next-generation sequencing. The general phyla of the Archaea domain are *Euryarchaeota*, *Bathyarchaeota*, *Thaumarchaeota*, and *Asgardarchaea*. As a result of a phylogenetic analysis of the dominant operational taxonomic units, representatives of methanogenic and methane- and ammonium-oxidizing archaea, as well as heterotrophic archaea, are found. The methanogenic archaea of *Euryarchaeota* phylum, *Methanobacteria* class, are found in permafrost with controversial genesis, while the methane-oxidizing archaea of *Methanomicrobia* class *Methanosarcinales* order are found in the marine permafrost at cape Finneset: the ANME-2a, -2b group in layers of 8.6 and 11.7 m and the ANME-2d group (*Candidatus Methanoperedens*) in a layer of 6.5 m. Ammonium-oxidizing archaea of phylum *Thaumarchaeota* is present in all types of permafrost, while the order of *Nitrososphaerales* is found in permafrost with controversial genesis and the order *Nitrosopumilales* is in permafrost with marine and controversial genesis. Representatives of phylum *Bathyarchaeota* are found stratigraphically in the most ancient samples under study. *Asgardarchaeota* superphylum is excluded in the layers of permafrost with marine genesis and is represented by the phyla *Lokiarchaeota*, *Thorarchaeota*, and an unclassified group belonging to this superphylum. The presence of methane, ethylene, and ethane in the permafrost of the first sea terrace of Cape Finneset at a depth of 11.7 m, as well as the composition of the archaeal community, give us reason to assume that, before freezing, microbiological processes of anaerobic methane oxidation took place in it, probably received from Tertiary rocks. The results of both this and previous works present the Spitsbergen permafrost as a rich archive of genetic information of little-studied prokaryotic groups.

Keywords: Western Spitsbergen, permafrost, marine sediments, domain Archaea, V4 region of 16S rRNA, methane

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INTRODUCTION

The Spitsbergen is a unique region of the Arctic where fast processes associated with climate warming are recorded. According to meteorological data from Long-

yearbyen, the average annual air temperature increased during the 20th century from about -9 to -4°C (Humlum et al., 2003). The area of glaciers in the vicinity of Longyearbyen and Barentsburg decreased by about

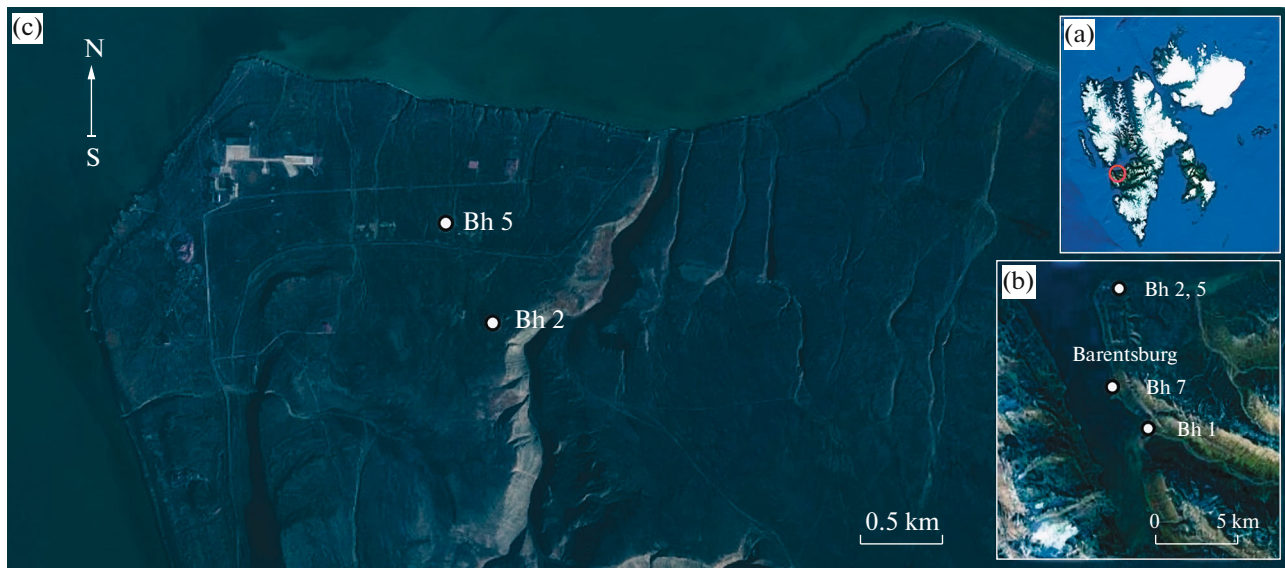


Fig. 1. Region of the study area in the Spitsbergen archipelago (a); satellite image of a permafrost test site in Barentsburg showing the locations of boreholes 1, 2, 5, and 7 (b); and the precise locations of boreholes 2 and 5 (c) (using maps from <https://google.ru/maps> and <https://toposvalbard.npolar.no>).

50% from 1936 to 2017 (Chernov et al., 2018). Spitsbergen permafrost is the warmest within the High Arctic due to the warming influence of currents and air masses carried from the Atlantic with the West Spitsbergen current. In 2016, as part of the creation of a permafrost test site of the Russian Science Center on the eastern bank of Grønfjord Bay, boreholes were drilled to study the structural features of permafrost and monitor ground temperatures (Demidov et al., 2016, 2019). Permafrost coastal–marine sediments frozen in the late Pleistocene and Holocene after they emerged from under the sea level, were drilled. This study is a continuation of the ongoing work on a comprehensive study of permafrost in the vicinity of the Barentsburg mine. Comparative microbiological studies of the Arctic systems of seawater–marine sediment, as well as terrestrial–subsea permafrost, show the differences between the dominant prokaryotic groups. For the Archaea domain, in the same seawater–marine sediments transition, an increase in the proportions of the orders of *Methanomicrobia* and *Methanococci* and a decrease in the order of *Methanobacteria* of the phylum *Euarchaeota*, as well as a significant increase in the proportion of *Thermoprotei* (*Crenarchaeota*) and the appearance of the order *Thermoplasmata* (*Euarychaeota*), were observed (Hamdan et al., 2013). The dominant groups of the Archaea domain in permafrost in the Cape Mamontov Klyk area were anaerobic methanogenic, ammonium-oxidizing, and methane-oxidizing Archaea. The permafrost in the Buor-Khaya Bay (southern Laptev Sea) was dominated by groups of methanogenic and methane-oxidizing Archaea (Winkel et al., 2018). In West Antarctica in the South Atlantic Ocean, the composition of the Archaea domain of King George Island—

rich in microbial methane and about 7500 years old—the dominance of methanogenic and ammonium-oxidizing Archaea was revealed using the V3–V5 and V1–V5 regions of 16S rRNA (Karaevskaya et al., 2014). In the study of 2-m strata of marine sediments from the offshore of Northwestern Spitsbergen with a nonvertical methane emission, representatives of phylum *Crenarchaeota*, Miscellaneous Crenarchaeotal group (at the top of the strata), as well as phylum *Euarchaeota*, ANME-1 group of anaerobically methane-oxidizing archaea (in the lower part of the strata), were discovered (Treude et al., 2020). In the biofilms from boreholes drilled in marine gas hydrate pingo approximately 50 km from the south-southwest of South Cape Island (Spitsbergen archipelago), representatives of *Methanomicrobia* class, ANME-1a and -b groups of phylum *Eurarchaeota*; representatives of the recently discovered *C. methanofastidiosales* order, carrying out metallotrophic methanogenesis (Vanwonterghem et al., 2016); representatives of phylum *Bathyarchaeota*, which is supposedly capable of methanogenesis (Evans et al., 2015); and representatives of phyla *Thermoplasmata* and *Woesearchaeota* (Gründer et al., 2019) were discovered. However, the studies on the ancient, deep permafrost on Spitsbergen using the new generation sequencing method have not yet been carried out. The aim of our work was to characterize permafrost coastal marine sediments covering the entire altitude and age range of the ladder of marine terraces in the Barentsburg region using the methods of analyzing the V4 region of the 16S rRNA gene in order to gain an idea of the structure of archaeal communities and corresponding microbiological processes that took place in marine sediments before their freezing for a long time. This work represents the second part of the study

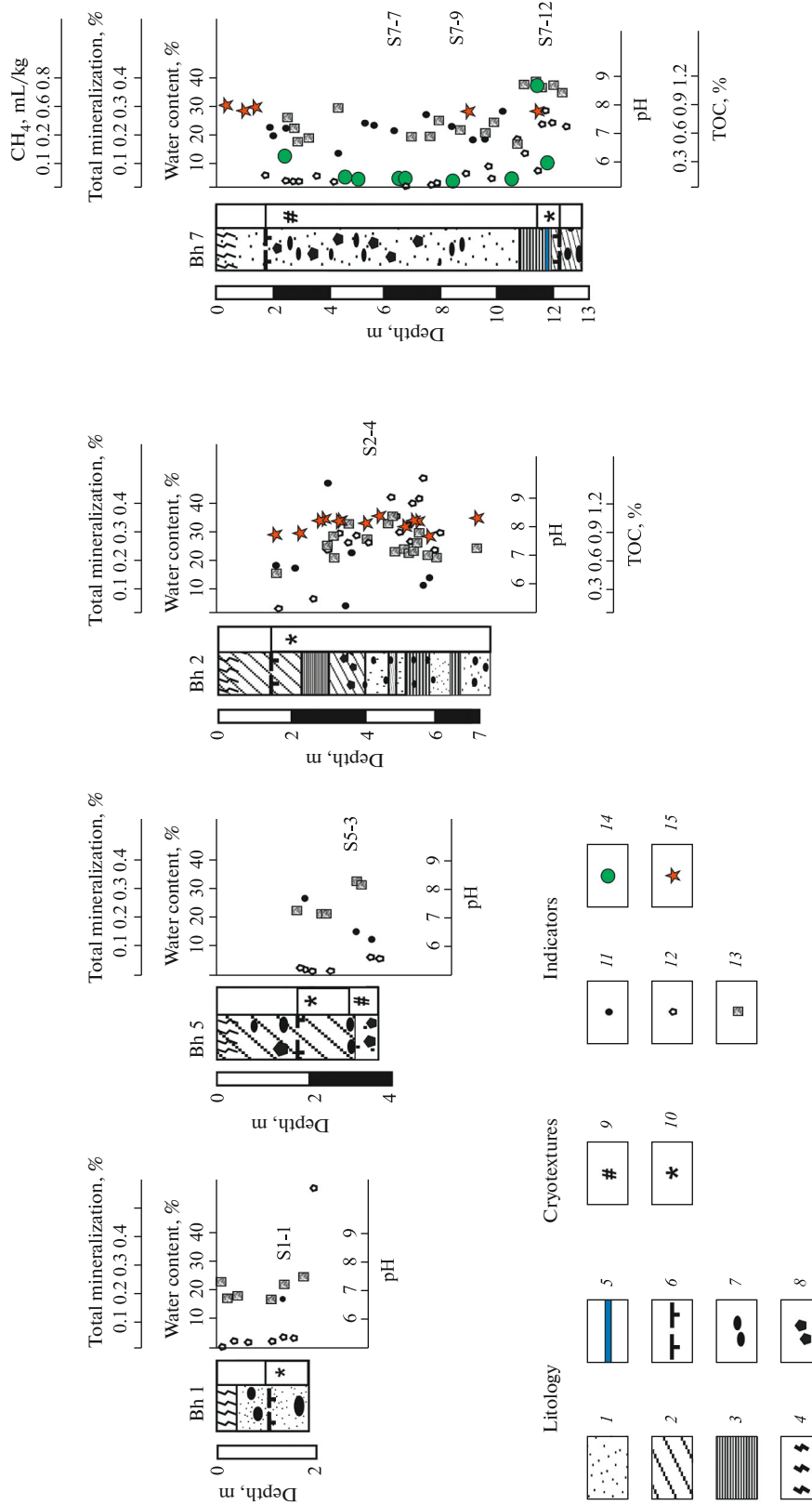


Fig. 2. Cryolithological columns of boreholes, some biogeochemical parameters of samples. The names of studying samples are shown opposite the corresponding depths.

on the Archaeal domain; the first part was dedicated to the Bacterial domain.

SAMPLING SITE

This study used permafrost cores from borehole (Bh) 1 (78.02289° N, 14.29845° E, 2.0 m above sea level (MSL)) drilled at the mouth of the Grøn River; Bh 2 (78.09504° N, 14.24096° E, 75.5 m MSL) and 5 (78.09856° N, 14.23299° E, 43.0 m MSL), drilled on the eastern bank of the Isfjord Bay; and Bh 7 (78.04703° N, 14.21962° E, 8.0 m MSL), drilled at Cape Finneset (Fig. 1). These sediments were represented by sands, sandy loams, loams, and clays of coastal–marine genesis (Fig. 2) which accumulated during the middle and late Holocene and then, during a sharp drop in relative sea level, came to the surface, were frozen, and then were covered with a thin cover of continental sediments of various genesis (Forman et al., 2004; Svendsen, Mangerud, 1997). The average annual temperature of Bh 2 (September 25, 2018–August 25, 2019) at a depth of 5.5 m was -2.17°C and a one-time measurement (September 12, 2016) of the Bh 7 temperature at a depth of 12.5 m was -0.87°C (Demidov et al., 2020). Despite the fact that in the modern era these sediments are frozen, samples from depths less than 3 m could have been thawed during the Holocene warming, when the seasonal thawing depth exceeded the modern one. In the case of Bh 1, a short-term rise in sea level might have caused the temporarily thawing of sediments which are now frozen (Solovieva et al., 2018; Salvigsen, Høgvard, 2005). Samples taken for next-generation sequencing were numbered according to Bh numbers and sampling depths (S1-1, S2-4, S5-3, S7-7, S7-9, and S7-12).

METHODS

Sampling. Drilling was carried out in August and September 2016 using the UKB 12/25 drilling machine (Vorovskiy Head, Yekaterinburg, Russia). The coring was undertaken without washing and without adding chemical reagents. Thin-walled core pipes with an external diameter of 76 to 112 mm were used. After cleaning the surface of the frozen core segments with a sterile scalpel, the samples were placed in sterile bags (Whirl-Pak®, Nasko, United States) and stored at temperatures -4 to -10°C . On 28 October 2016, they were transported to the laboratory, where they were stored in a freezer at -18°C until analysis in the period from October 2016 to February 2020.

Analysis of carbon monoxide, carbon dioxide, methane and ethylene concentrations. For an analysis of gases, we selected Bh7, since it is the deepest (12 m) and abundant in samples for gas extraction from permafrost. Gas was collected using headspace degassing in 150 mL syringes (Alperin et al., 1985).

Analyses of carbon monoxide, carbon dioxide, methane, ethylene, and ethane concentrations were

carried out on a Chromatek-Kristall 5000 chromatograph with packed and capillary columns and a temperature in the column oven by isotherm at 80°C (NRC Kurchatov Institute – IREA, Moscow, Russia). Three detectors were used: PID-1 (for carbon oxides and methane) and PID-2 (for C_2 - C_4 hydrocarbons); the temperature of both was 200°C ; RTA was used to determine nitrogen, oxygen, and hydrogen; the temperature was 160°C . Reference gas consumption (helium) was 15 mL/min. The carrier gas was helium (99.9999%); pressure was 25 kPa. Hydrogen consumption was 25 mL/min and air consumption was 500 mL/min. The gas mixtures used were $\text{O}_2/\text{CO}/\text{CO}_2/\text{CH}_4/\text{N}_2/\text{He}$ with mole fraction of components (ppm) 2.2/2.2/2.3/2.3/2.6/remainder and 7.8/7.7/7.6/7.7/7.6/remainder respectively, C_2H_4 , C_2H_2 , C_2H_6 , C_3H_6 , C_3H_8 , $i\text{-C}_4\text{H}_{10}/n\text{-C}_4\text{H}_{10}$, N_2 with mole fraction of components (%) 0.00105/0.00097/0.00108/0.00104/0.00105/0.00111/0.00107/remainder.

DNA isolation and preparation and sequencing of amplicon libraries. DNA from the samples was isolated using Fast DNA Spin Kit for Soil according to the manufacturer's method (MP Biomedicals, United States). The concentration was measured using a Qubit 2.0 fluorimeter with a dsDNA HS reagent KIT (Invitrogen™, United States). Amplicon libraries were created using PCR with universal primers for region V4 in accordance with the previously described methodology (Fadrosh et al., 2014) at the Vernadsky Institute of Microbiology. Primers were selected for the most objective ratio of Bacteria and Archaea domains: 515F (5'-GTGBCAGCMGCCGCGG-TAA-3') (Hugerth et al., 2014) and Pro-mod-805R (5'-GACTACNVGGTMTCTAATCC-3') (Merkel et al., 2019). Sequencing was performed on a MiSeq system (Illumina, United States) at Biospark (Moscow, Troitsk, Russia) using a reaction MiSeq Reagent Micro KIT v2 that reads 150 nucleotides from each end. Each sample was read in two technical duplicates, including the reagents and air control sample used to subtract the contaminant sequences from the study sample. A total of 164 843 sequences were obtained.

Bioinformatic and statistical analysis. Demultiplexing, as well as subsequent processing and sequence analysis, were performed using the appropriate scripts in QIIME 2 ver2019.1 software (Bolyen et al., 2019). Operational Taxonomic Units (OTUs) were identified using the SILVAngs 1.4 pipeline (<https://ngs.arb-silva.de/silvangs/>) and BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) programs. The coverage index of amplicon libraries was calculated by the formula

$$C = 1 - n/N$$

where n is the number of OTUs represented by one amplicon and N is the total number of amplicons (Good, 1953). The Chao1 index (Chao, 1984) was calculated by the formula

Table 1. Concentrations of carbon-containing gases in samples from Bh 7 by a Chromatek-Kristall 5000 chromatograph

Depth, m bs	Sample no.	Concentration, mL kg ⁻¹				
		CO	CO ₂	CH ₄	C ₂ H ₄	C ₂ H ₆
4.5		5.76202	13.46128	0.14902	b.d.l.*	b.d.l.
6.5	S7-7	1.94538	8.84039	0.09029	b.d.l.	b.d.l.
11.7	S7-12	4.85901	28.13825	0.48315	0.29511	0.74120

* b.d.l. indicates below the detected limit.

Table 2. Characteristics of amplicon libraries: archaeal DNA content, total number of obtained sequences and phylotypes (OTUs) in samples, coverage and diversity indices

Bh no.	Depth, m	Sample no.	Archaea, %	OTU, number	Amplicons, number	Good index, %	Shannon–Weaver index, %	Chao1 index, %
1	1.3	S1-1	0.04	3338 ± 427	30404 ± 5471	96.3 ± 4.3	5.0 ± 0.1	8540 ± 1156
2	4.2	S2-4	1.00	164 ± 18	6678 ± 604	98.3 ± 1.1	2.5 ± 0.1	857 ± 761
5	3.1	S5-3	0.02	531 ± 56	19829 ± 4061	98.7 ± 0.1	2.8 ± 0.1	1207 ± 387
7	6.5	S7-7	0.02	783 ± 60	14899 ± 2308	98.2 ± 0.1	4.7 ± 0.1	1239 ± 142
7	8.6	S7-9	1.00	96 ± 35	164 ± 32	53.6 ± 9.6	4.1 ± 0.6	452 ± 169
7	11.7	S7-12	0.14	799 ± 23	10274 ± 549	96.8 ± 0.4	4.3 ± 0.1	1242 ± 83

$$\text{Chao1} = S_{\text{obs}} + \left(\frac{a^2}{2b} \right),$$

where S_{obs} is the identified number of phylotypes (OTU), a is the number of phylotypes (OTU) represented by one amplicon, and b is the number of phylotypes represented by two amplicons. The Shannon–Weaver Index (Magurran, 1988) was calculated according to the formula

$$H' = \sum_i -p_i \ln p_i,$$

where p_i is the relative abundance of the i th phylotype (OTU). The relative abundance of technical replications was combined to indicate the relative content for analysis of the bacterial community using bubble graph diagrams. The analysis of main components and cluster analysis were performed using the Past3 statistical package (Hammer, 2017) using the paired group algorithm (UPGMA) and the Euclidean affinity index.

Deposit at GenBank. The sequence of regions V4 16S rRNA were loaded into the NCBI database as bio-project PRJNA625477. The gene sequences of 16S rRNA isolates were transferred to the NCBI database under the numbers MN599988–MN599993.

RESULTS

Methane and Other Carbon-Containing Gases in Samples of Bh 7 Near Cape Finneset

The methane content in Bh 7 samples ranged from 0.09029 to 0.48315 mL/kg (Table 1). The concentra-

tion of carbon dioxide was about 58–98 times higher than the methane concentration and 2.2–5.8 times higher than the carbon monoxide concentration. In the sample S7-12, ethylene and ethane were detected at comparable levels with methane content concentrations (Table 1).

Profiling of the V4 region of 16S rRNA. Genomic DNA was isolated from all samples: in concentrations from 0.1 to 0.8 ng/μL for Bh 1 and 7 samples and about 0.02 ng/μL for Bh 2 and 5 samples. The content of archaeal DNA in the total prokaryotic DNA did not exceed 1% (Table 2).

A total of 44 OTUs of the **Archaea domain** were revealed (all of them are dominant, ≥1%): 7, 5, 7, 8, 1, and 16 for samples S1-1, S2-4, S5-3, S7-7, S7-9, and S7-12, respectively.

It was represented by the phyla *Euryarchaeota* (5.6–100%), *Bathyarchaeota* (22.1–81.0%), *Thaumarchaeota* (5.6–54.0%), *Asgardarchaea* (6.3–79.2%), and *Woesearchaeota* (5.6–52.2%) (Figs. 3, 4). Methanogenic Archaea were found in samples S1-1, S2-4, S5-3, and S7-7 and were represented by four genera: *Methanocalculus*, *Methanobacterium*, *Methanobrevibacter*, and *Methanothermobacter*. In phylum *Euryarchaeota*, the *Methanobacteria* class was the most diverse in the studied samples. Its representatives were found in all boreholes. Therefore, in the S1-1 sample, 32.7% was the genus *Methanobacterium* and 15.4% was the genus *Methanothermobacter*. This is the only sample out of six in which the proportion of methanogenic Archaea exceeded 20.0%, amounting to 46%. This genus was also discovered as the only representative of methano-

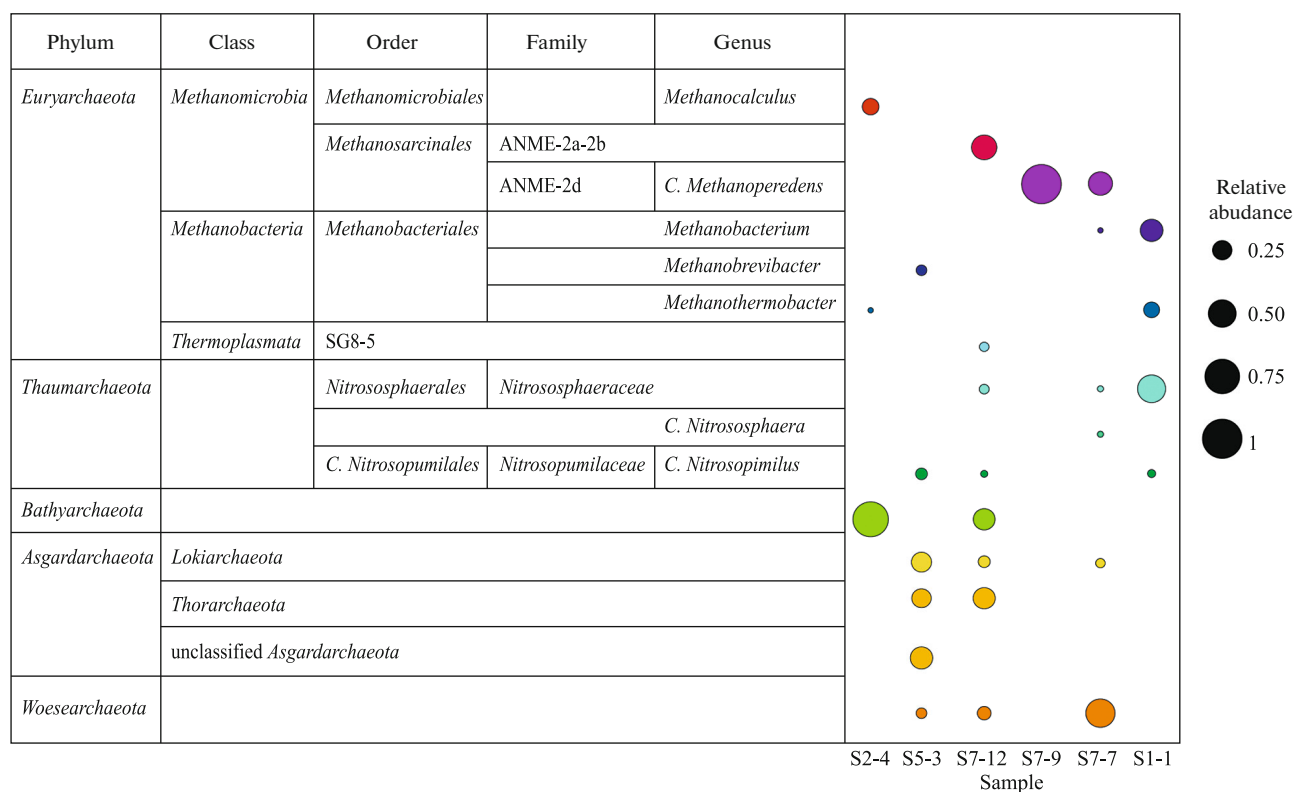


Fig. 3. Relative abundance of archaeal taxa in the studied samples. Sample names are explained in the text. Bubbles represent the mean value of relative abundances from two technical repetitions.

genic Archaea in sample S7-7. The genus *Methanothermobacter* was also found in sample S2-4. The genus *Methanobrevibacter* was the only identified representative of methanogens in sample S5-3. The *Methanomicrobia* class is represented in our samples by the genus *Methanocalculus* and was found only in sample S2-4, where it amounted to 18% of the archaeal community. The *Thermoplasmata* class was represented by the SG8-5 group and was found only in sample S7-12 (2.8%). Other members of the *Methanosarcinales* order belonging to Archaea involved in anaerobic methane oxidation were found in Bh 7: groups ANME-2a, -2b (S7-9 100% and S7-12 30.3%), and -2d (*Candidatus Methanoperedens*) (S7-7 36.1%). The phylum *Bathyarchaeota* was found in samples S2-4 (80.6%) and S7-12 (17.8%). Representatives of ammonium-oxidizing Archaea of the order of *Nitrososphaerales* were present in samples S1-1 (50%), S7-7 (3.6%), and S7-12 (5.6%) and those of the order *Nitrosopumilales* in samples S1-1 (3.9%), S5-3 (10.0%), and S7-12 (2.8%). Inside the *Asgardarchaeota* superfila, phyla *Lokiarchaeota* (S5-3 22.2%, S7-7 6.8%, and S7-12 12.2%), *Candidatus Thorarchaeota* (S5-3 27.7% and S7-12 29.3%), and unclassified *Asgardarchaeota* by the 16S rRNA gene (S5-3 33.3%) were discovered. Representatives of *Lokiarchaeota* in sample S5-3, as well as unclassified *Asgardarchaeota* in samples S5-3 and S7-12, were identified thanks to

the phylogenetic tree on which they appeared on common branches by representatives of *Lokiarchaeota* from samples S7-7 and S7-12. *Woesearchaeota* phylum was found inside the DPANN superfila (S5-3 3.9%, S7-7 52.2%, and S7-12 9.4%).

DISCUSSION

The content of carbon-containing gases in permafrost of Cape Finneset. The presence of ethylene and ethane in the sample S7-12 can be explained both by the natural composition of the gas phase rising through the permafrost to the surface from the bedrock, and by the anaerobic processes of ethylene splitting by archaea to ethane and methane described earlier (Koene-Cottaar and Schraa, 1997; Xie et al., 2017). Ethylene and acetylene are known to inhibit melanogenesis in marine sediments (Oremland, Taylor, 1975).

Characteristics of amplicon libraries (the characteristics are also given in the first part of the study about Bacteria domain (Karaevskaya et al., 2021)). Sample S1-1 was characterized by the greatest variety; its Shannon–Weaver index was 5.0 ± 0.1 and the Chao1 index was 8540 ± 1156 , while the library coverage was $96.3 \pm 4.3\%$ (Table 2). The samples of Bh 7 were less diverse, for which the Shannon–Weaver index was 4.1 ± 0.6 to 4.7 ± 0.1 and Chao1 was 452 ± 169 to

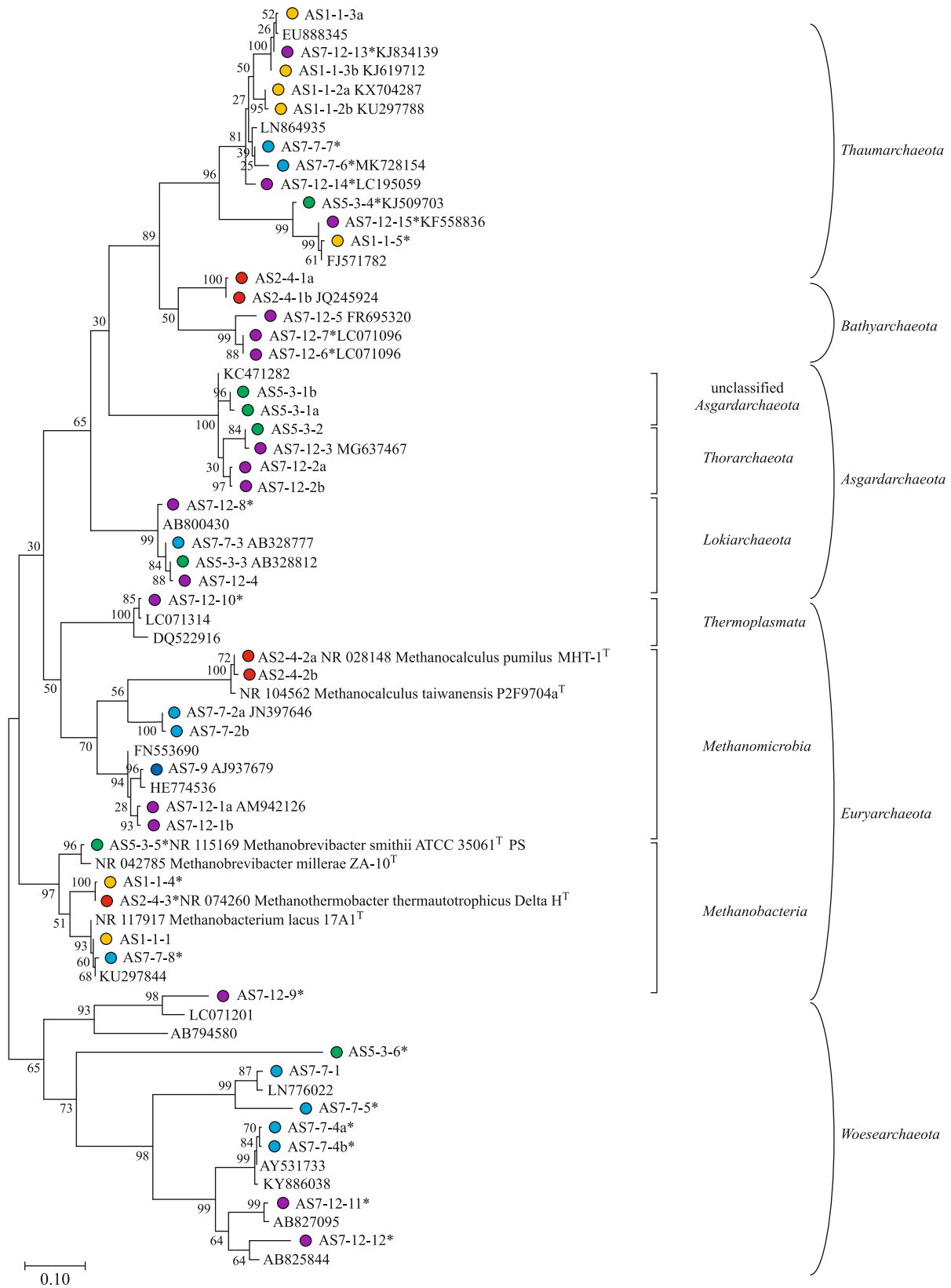


Fig. 4. Phylogenetic position of the archaeal OTU relative to the nearest homologues and type strains and from NCBI database (Maximum Likelihood, Tamura-Nei model, bootstrap method with 500 replicates).

1242 ± 83. Sample S7-9, with the lowest diversity indices and low library coverage, 53.6 ± 9.6%, was knocked out of the general picture when compared with the other two samples, S7-7 and S7-12 (96.8 ± 0.4 – 98.2 ± 0.1%). This phenomenon may exist due to unfavorable conditions for the conservation or isolation of DNA in this sample, which differs from others in its high content of plant residues. Samples S2-4 (2.5 ± 0.1) and S5-3 (2.8 ± 0.1) were characterized by the lowest diversity by the Shannon–Weaver index, while the library coverage in them was very good and amounted to 98.3 ± 1.1 and 98.7 ± 0.1, respectively. This may be due to the very low output concentrations of DNA, and this, in turn, to their older age when compared to the samples of the lower marine terraces exposed by Bh 1 and 7.

Taxonomy of OTUs of the Archaea domain. Here we considered the taxonomy of OTUs comprising the Archaea domain (Figs. 3, 4; Table 3), excluding those that make up less than 5% of all archaeal amplicons, which is below the error level of the method, marked with an asterisk in Table 3.

The OTUs of the phylum *Euryarchaeota* of the genus *Methanobacterium* revealed in samples S1-1 and S7-7 were found to be related to the type strain of hydrogenotrophic freshwater methanogenic archaea *M. lacus 17A1*^T (Borrel et al., 2012). The genus *Methanocalculus* unites halotolerant species and was represented in the S2-4 OTU sample, related to the type strain of *M. pumilus* MHT-1^T hydrogenotrophic archaea (Mori et al., 2000), which is resistant to high concentrations of several heavy metals, such as CdCl₂ and CuSO₄ (Fargeau et al., 2019).

OTUs of marine anaerobic methane-oxidizing archaea ANME-2a, -2b (Beuling et al., 2019) were found in samples S7-9 and S7-12, and freshwater ones—ANME-2d (Kurth et al., 2019)—were found in sample S7-7.

The phylum *Bathyarchaeota*, found in samples S2-4 and S7-12, is an archaea that occurs inter alia in Na²⁺-saline habitats in symbiosis with the class *Methanomicrobia*. In the *Bathyarchaeota* metagenome, genes encoding the methyl-coenzyme M-reductase (MKP) complex were found (Evans et al., 2015; Kallistova et al., 2017), participating in methanogenesis and anaerobic oxidation of methane (Thauer, 1998; Shima and Thauer, 2005).

The family *Nitrososphaeraceae* (found in samples S1-1, S7-7, and S7-12) and the family *Nitrosopumilaceae* (genus *Candidatus Nitrosopumilus*) (found in samples S1-1, S5-3, and S7-12) of the phylum *Thaumarchaeota* are anaerobic ammonium-oxidizing archaea; the former is found in soil and in freshwater and marine ecosystems and the latter is found in deep-sea marine ecosystems (Park et al., 2012).

Archaea of the superphylum *Asgardarchaeota* were found only in wells 5 and 7. It is assumed that the

archaea of this superphylum are obligate anaerobes; can have autotrophic, heterotrophic, and phototrophic types of nutrition; and can also participate in the reduction of iron and manganese in the presence of methanogenic archaea and sulfate-reducing bacteria (Jørgensen et al., 2013). The phylum *Lokiarchaeota*, found in samples S5-3, S7-7, and S7-12, was found in hydrothermal vents in Japan (Takai, Horikoshi, 1999), bottom marine sediments of the Atlantic Ocean (Vetriani et al., 1999), and in terrestrial anaerobic/microaerophilic aquatic ecosystems (Sorensen, Teske, 2006) and deep-sea hydrotherms of the North Atlantic on the Knipovich Ridge (Jørgensen et al., 2012; 2013). The phylum *Thorarchaeota* found in samples S5-3 and S7-12 was found in lacustrine, mangrove, and hydrothermal marine bottom sediments; presumably they are capable of transforming proteins and hydrocarbons, as well as acetogenesis and sulfate reduction (Seitz et al., 2016). All representatives of *Asgardarchaeota* closest to the OTUs obtained by us were found in marine ecosystems. It should be noted that the identified OTUs related to the phylum *Asgardarchaeota* practically did not intersect with each other in different samples, with the exception of AS5-3-2 and AS7-12-3, AS5-3-3 and AS7-12-4, which indicates their varied composition depending on the location and time of sedimentation (Fig. 4).

Genetic relationship of samples. Based on the analysis of the principal components, according to the taxonomic diversity of the Archaea, the samples most similar each other were S7-7 and S7-12, as well as S1-1. The isolation of sample S7-9 in Bh 7 can be explained by the increased content of fragments of plant material in it, which is most likely due to sedimentation conditions that are different from the other two samples. By the presence of superphylum, *Asgardarchaeota* made it possible to combine samples from Bh 5 (S5-3) and 7 (S7-7 and S7-12) and phylum *Thorarchaeota* (S5-3 and S7-12) (Fig. 5b). Another cluster is formed by samples S5-3 and S2-4 (Fig. 5b). The archaeal diversity of sample S2-4 is the most different of all samples. This may be due to the different conditions of sediment formation and the different age of the sediments of Bh 1, 3–7, and Bh 2.

Most likely, the archaeal communities of the layers corresponding to samples S5-3 and S7-9 were formed in strictly anaerobic conditions favorable for the processes of the anaerobic oxidation of methane and ammonium. Whereas the communities of the layers corresponding to samples S1-1, S2-4, S7-7, and S7-12 formed in both aerobic and anaerobic conditions, methanogenesis was added to other processes. The reason for the appearance of methanogenesis may be connected not so much with aerobic conditions arising in the process of the evolution of the sediments, but with the influence of saturated carbon dioxide in groundwaters on land ecosystems (Demidov et al., 2020).

Table 3. Identification of Archaeal OTUs according to the NCBI database

OTU name	% of all sample reads	Phylum, Order, Family	Nearest OTU's and/or type strains (% similarity, Genbank number)	Isolation site	References
AS1-1-1	32.70 ± 24.47	<i>Euryarchaeota, Methanobacteriales</i>	100% LN796335	Spring of 2011 from natural gas source in Berkhout, Noord-Holland, The Netherlands;	Timmers et al., 2016
AS1-1-2	26.79 ± 2.52	<i>Thaumarchaeota, Nitrososphaeraceae</i>	<i>Methanobacterium lacus 17A1</i> ^T (99.6% NR_117917)	Deep sediments, freshwater lake, France	Borrel et al., 2012
AS1-1-3	23.22 ± 2.52		99.6–100% KP905836	Kasatochi Island, Alaska soil or post-volcanic pyroclastic surface	Zeglin et al., 2016
AS1-1-4*	15.39 ± 21.76	<i>Euryarchaeota, Methanobacteriales</i>	<i>Nitrososphaera viennensis</i> EN76 ^T (95.6–96.0% NR_134097)	Vienna soil	Stieglmeier et al., 2014
AS1-1-5*	3.85 ± 5.44		99.6–100% JX099326	Mineral Atacama soil (6000 m MSL), Chile	Lynch et al., 2012
AS2-4-1	80.59 ± 5.54	<i>Candidatus Bathyarchaeota</i>	<i>Methanothermobacter thermoautotrophicus</i> Delta H ^T (99.6% NR_074260)	Wastewater, Germany	Smith et al., 1997
AS2-4-2	18.01 ± 7.53	<i>Euryarchaeota, Methanomicrobiales</i>	99.6% JQ224980	Ocean water marine sample, Saanich Inlet, Station S3, 100 m depth	Wright et al., 2013
AS2-4-3*	5.07 ± 7.17		<i>Nitrosopumilus</i>	<i>Nitrosopumilus oxycylinae</i> HCE1 ^T (98.0% NR_159207)	Seawater 17 m depth, Hood Canal, Washington, United States
AS5-3-1	29.23 ± 13.05	<i>Unclassified Asgardarchaeota</i>	99.6–100% JN876885	Forest in Guanabara Bay, Brazil	Pires et al., 2012
AS5-3-2	27.7 ± 17.40	<i>Asgardarchaeota, Candidatus Thorarchaeota</i>	archaeon isolate 218F3-36 ^T (97.0–97.5% MH268269)	Bay sediment, China	Pan et al., 2019
			<i>Methanocalculus pumilus</i> MHT-1 ^T (99.6–100% NR_028148)	Waste-disposal site, Osaka, Japan	Mori et al., 2000
			<i>Methanothermobacter thermoautotrophicus</i> Delta H ^T (100% NR_074260)	Wastewater, Germany	Smith et al., 1997
			99.2–100% MG638772) <i>Unclassified Asgardarchaeota</i>	Aarhus Bay Site M5, 1–50 cm depth sediment, Denmark	Jørgensen et al., 2019
			99.6% MG637467	Aarhus Bay Site M5, 1–50 cm depth sediment, Denmark	Jørgensen et al., 2019
			93.8% MH268358	Bay sediment, China	Pan et al., 2019

Table 3. (Contd.)

OTU name	% of all sample reads	Phylum, Order, Family	Nearest OTU's and/or type strains (% similarity, Genbank number)	Isolation site	References
AS5-3-3	25.39 ± 7.62	<i>Asgardarchaeota, Lokiarchaeota</i>	100% MK120404 <i>Candidatus Prometheoarchaeum syntrophicum</i> MK-D1 ^T (90.8% CP042905)	Marine sediment of Ulleung Basin, East Sea	Cho et al., 2017
AS5-3-4*	10.0 ± 14.14	<i>Thaumarchaeota, Candidatus Nitrosopumilus</i>	100% KJ509703 <i>Nitrosopumilus cobalaminigenes</i> HCA1 ^T (96.8% NR_159206)	Seawater, Yangshan port, China	Xie et al., 2017
AS5-3-5*	3.85 ± 5.44	<i>Euryarchaeota, Methanobacteriales</i>	<i>Methanobrevibacter smithii</i> ATCC 35061 ^T (100% NR_115169)	Human and ruminant gut	Miller et al., 1982; Joblin et al., 1990; Jarvis et al., 2000
AS5-3-6*	3.85 ± 5.44	<i>Woesearchaeota</i>	95.7% FJ351287	Lake Pontchartrain water, United States	Amaral-Zettler et al., 2008
AS7-7-1	46.23 ± 10.38		97.6% LN776022	Freshwater lake water, Pyrenees, Spain	Ortiz-Alvarez et al., 2020
AS7-7-2	36.11 ± 15.71	<i>Euryarchaeota, AMNE-2d, Candidatus Methanoperedens</i>	99.6–100% JN397646	Taipei spring pits, Jing-Mei River, China	Lai et al., 2010
AS7-7-3	6.75 ± 5.61	<i>Asgardarchaeota, Lokiarchaeota</i>	100% AB328777	Marine sediment of Nankai Trough, Japan	Okita et al., 2019
AS7-7-4*	3.18 ± 0.56		<i>Candidatus Prometheoarchaeum syntrophicum</i> MK-D1 ^T (90.4% CP042905)		Imachi et al., 2020
AS7-7-5*	2.78 ± 3.93	<i>Woesearchaeota</i>	99.2% AY531733	Lake Stechlin sediment, Germany	Conrad et al., 2007
AS7-7-6*	1.79 ± 2.52		99.6% KJ566494	Southern German cold freshwater spring	Probst et al., 2015
AS7-7-7*	1.79 ± 2.52	<i>Thaumarchaeota, Nitrososphaeraceae</i>	100% LC296534	Niigata soil, Japan	Oshiki et al., 2018
			<i>Nitrososphaera viennensis</i> EN76 ^T (96.0% NR_134097)	Vienna soil	Stieglmeier et al., 2014
			99.2% MK724236	Colorado soil, United States	Bueno de Mesquita et al., 2019
			<i>Nitrososphaera viennensis</i> EN76 ^T (98.4% NR_134097)	Vienna soil	Stieglmeier et al., 2014

Table 3. (Contd.)

OTU name	% of all sample reads	Phylum, Order, Family	Nearest OTU's and/or type strains (% similarity, Genbank number)	Isolation site	References
AS7-7-8*	1.39 ± 1.97	<i>Euryarchaeota</i> , <i>Methanobacteriales</i>	99.6% LC240735 <i>Methanobacterium lacus 17A1</i> ^T (98.8 %)	Niigata anaerobic UASB reactor fed with domestic sewage, Japan Lake Pavin sediment, France	Oshiki et al., 2018 Borrel et al., 2012
AS7-9	50.00 ± 70.81	<i>Euryarchaeota</i> , ANME-2a, 2b	100% AJ937679	Carpathian Mountains mud from a terrestrial volcano	Alain et al., 2006
AS7-12-1	30.34 ± 11.48		99.6–100% AM942126	Marenes-Oleron Bay subsurface marine sediment	Roussel et al., 2009
AS7-12-2	19.88 ± 4.53	<i>Asgardarchaeota</i> , <i>Candidatus Thorarchaeota</i>	<i>Methanobolus zinderi</i> SD1 ^T (90.7–91.1% NR_044558)	Deep subsurface coal seam, Louisiana, United States	Doerfert et al., 2009
AS7-12-3	9.40 ± 2.42		97.2–98.4% KC471282	Marine sediment at Yonaguni Knoll in the Okinawa Trough, Japan	Hoshino and Inagaki, 2013
AS7-12-4	8.34 ± 11.79	<i>Asgardarchaeota</i> , <i>Lokiarchaeota</i>	92.6–93.1% MH268358 (100% MG637467); (94.3% MH268358)	Bay sediment, China Aarhus Bay Site M5, 1–50 cm depth sediment, Denmark; bay sediment, China	Pan et al., 2019 Jorgensen et al., 2019 Pan et al., 2019
AS7-12-5	8.34 ± 11.79		100% MK120404	Marine sediment of Ulleung Basin, East Sea	Cho et al., 2017
AS7-12-6*	5.56 ± 7.86	<i>Candidatus Bathyarchaeota</i>	<i>Candidatus Prometheoarchaeum syntrophicum</i> MK-D1 ^T (90.4% CP042905)	Marine sediment of Nankai Trough, Japan	Imachi et al., 2020
AS7-12-7*	3.85 ± 5.44		100% HE978814	Southern North Sea intertidal sediment	Seidel et al., 2012
			100% JF931639	Marine sediments microcosms, Brest, France	Stauffert et al., 2014
			100% FR695320	Sulphate-methane transition zone of diffusion-controlled sediments of Aarhus Bay, Denmark	Webster et al., 2011

Table 3. (Contd.)

OTU name	% of all sample reads	Phylum, Order, Family	Nearest OTU's and/or type strains (% similarity, Genbank number)	Isolation site	References
AS7-12-8*	3.85 ± 5.44	<i>Asgardarchaeota, Lokiarchaeota</i>	99.6% MK120380 <i>Promethearchaeum syntrophicum</i> MK-D1 ^T (90.4% CP042905)	Marine sediment of Ulleung Basin, East Sea Nankai Trough marine sediment, Japan	Cho et al., 2017 Imachi et al., 2020
AS7-12-9*	3.85 ± 5.44	<i>Woesearchaeota</i>	91.7% LN776086 Archaeon GW2011_AR15 (90.0% CP010425)	Freshwater lake water, Pyrenees, Spain Colorado River groundwater, United States	Ortiz-Alvarez et al., 2020 Castelle et al., 2015
AS7-12-10*	2.78 ± 3.93	<i>Thermoplasmata, SG8-5</i>	99.6% AB858774 Archaeon LL37A29 (90.0% AJ745146)	Southern Mariana Trough seafloor massive sulfide sediments Netherlands soil	Kato et al., 2015 Kemnitz et al., 2005
AS7-12-11*	2.78 ± 3.93	<i>Woesearchaeota</i>	98.0% AB827095	Hydrothermal vent of the Taketomi submarine hot spring field, Yaeyama Archipelago, Japan	Nunoura et al., 2013
AS7-12-12*	2.78 ± 3.93		92.0% AB825844	Okinawa Trough, Iheya North sediment, Japan	Yanagawa et al., 2013
AS7-12-13*	2.78 ± 3.93		100% KU539639 <i>Nitrososphaera viennensis</i> EN76 ^T (95.6% NR_134097)	<i>Myrtillocactus geometrizans</i> and <i>O. robusta</i> , Mexico Vienna soil	Fonseca-Garcia et al., 2016 Stieglmeier et al., 2014
AS7-12-14*	2.78 ± 3.93	<i>Thaumarchaeota</i>	100% LN776037 <i>Nitrososphaera viennensis</i> EN76 ^T (97.2% NR_134097)	Freshwater lake water, Pyrenees, Spain Vienna soil	Ortiz-Alvarez et al., 2020 Stieglmeier et al., 2014
AS7-12-15*	2.78 ± 3.93		100% KF558836 <i>Nitrosopumilus oxycilinae</i> HCE1 ^T (98.8% NR_159207)	Surface seawater, Enderby Land, East Antarctica Puget Sound estuary, lower euphotic zone of coastal waters, United States	Hernández et al., 2015 Qin et al., 2017

The asterisk marks the OTUs that make up at least 5% (the level of the method error) of all archaeal amplicons.

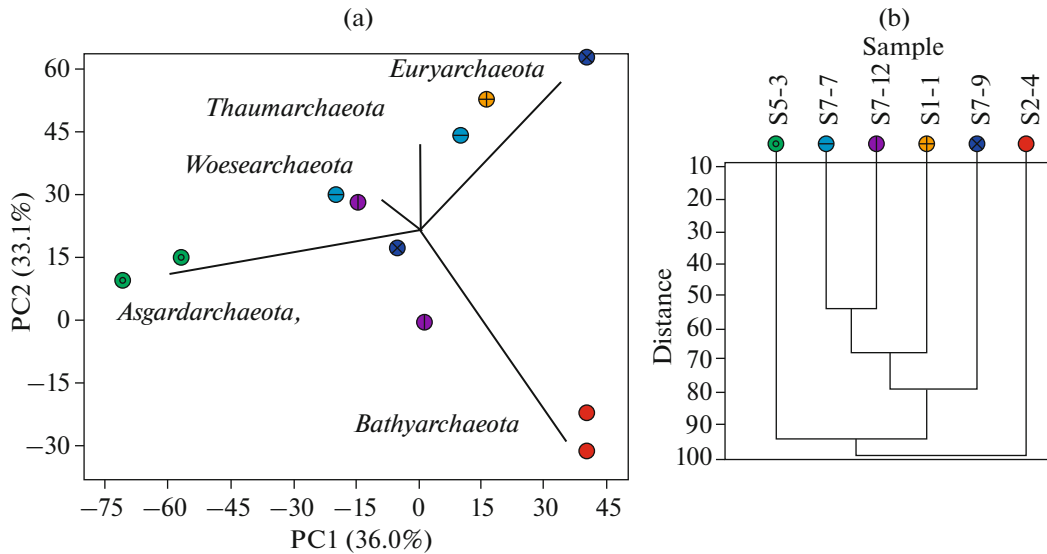


Fig. 5. PCA of phyla content for two technical repetitions of domain Archaea (a). Vectors show selected taxonomical factors that are mainly responsible for the variance between samples. Cluster analysis of average abundance values using the paired group algorithm (UPGMA) and the Euclidean affinity index (b).

CONCLUSIONS

A comparative study of Spitsbergen permafrost representing marine terraces located in different sites of the east bank of Grønfjord was carried out for the first time, using mutually complementary methods: profiling the V4 region of the 16S rRNA gene for the Archaeal domain.

Characterized by the predominance of archaeal phyla *Euryarchaeota*, *Bathyarchaeota*, *Thaumarchaeota*, and *Asgardarchaeota*, the communities were similar to the communities of modern coastal and marine coastal sediments. Presumably, they were formed mainly under anaerobic, but also under mixed aerobic–anaerobic conditions.

It had been previously established that the isotopic composition of methane and carbon dioxide in Bh 7, as well as the predominance of methane oxidizing of the ANME-2a, -2b and -2d groups over methanogenic of the *Methanobacteria* order, suggests that these deposits could have been formed under conditions of gas intake from Tertiary rocks.

The presence of methane, ethylene, and ethane in the sample from Bh 7 at a depth of 11.7 m, as well as the structure of archaeal communities, suggests the presence of microbiological processes of anaerobic oxidation of methane in this layer before freezing, probably coming from Tertiary rocks.

Thus, combining the results from domains Bacteria and Archaea, the functional role of the studied prokaryotic communities seems to be reduced to heterotrophic psychrophilic activity in all samples; methanogenesis in samples S1-1 and S2-4; the anaerobic oxidation of methane by bacteria of the genus *Methylobacter* in sample S1-1; the anaerobic oxidation of

methane by archaeal groups ANME-2a, -2b, and -2d in samples S7-7, S7-9, and S7-12; the sulfate-reducing activity of bacteria of the phyla *Firmicutes* and *Nitrospirae* in samples S2-4, S7-7, and S7-9; and anaerobic ammonium oxidation by Archaea of the phylum *Thaumarchaeota* in samples S1-1, S5-3, S7-7, and S7-12. Also, all samples assume the presence of microbiological processes of conversion of hydrocarbons.

The results obtained here and in our previous article (Karaevskaya et al., 2021) give motivation for further research in the field of the phylogeny of prokaryotic communities of Spitsbergen's permafrost, in particular that of marine archaea of the recently discovered superphylum *Asgardarchaeota*. Their metabolism, as well as the reasons for their quite wide variety in this permafrost, is of great interest. Due to the very low fraction of the Archaea domain in the prokaryotic communities under study, approaches with primers for the gene of interest of certain archaeal groups must be used for molecular genetic studies in this area.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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