Towards the outwelling hypothesis in a Patagonian estuary: First support from lipid markers and bacterial communities

Germán A. Kopprio [a,b,c,*], Ana Martínez [d], Anna Fricke [e], Michael Hupfer [b], Rubén J. Lara [c], Martin Graeve [f], Astrid Gärdes [f,g]

[a] Robert Koch Institute, Berlin, Germany
[b] Leibniz Institute for Freshwater Ecology and Inland Fisheries, Berlin, Germany
[c] Instituto Argentino de Oceanografía - Consejo Nacional de Investigaciones Científicas y Técnicas, Bahía Blanca, Argentina
[d] Universidad Nacional del Sur, Bahía Blanca, Argentina
[e] Leibniz Institute of Vegetable and Ornamental Crops, Großbeeren, Germany
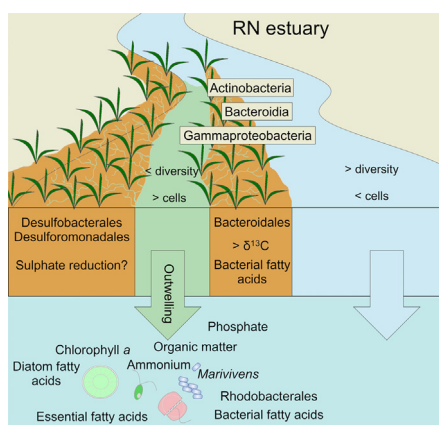[f] Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
[g] University of Applied Sciences, Bremerhaven, Germany

HIGHLIGHTS

- Essential fatty acids were exported from the wetland in the RN estuary.
- Actinobacteria and Bacteroidetes indicated copiotrophic conditions.
- Several Rhodobacterales were outwelling indicators and potential early colonizers.
- The exportation of organisms as proposed by Odum was inferred.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Biogeochemical markers in combination with bacterial community composition were studied at two contrasting stations at the Río Negro (RN) estuary to assess the outwelling hypothesis in the Argentinian Patagonia. Inorganic nutrients and dissolved organic matter were exported clearly during the last hours of the ebb at the station Wetland. Moreover, a considerable outwelling of polyunsaturated fatty acids (PUFA), particulates and microalgae was inferred by this combined approach. The exported 22:6(n-3) and 20:5(n-3) contributed very likely to sustain higher trophic levels in the coasts of the Southwest Atlantic. The stable isotopes did not evidence clearly the outwelling; nevertheless, the combination of $\delta^{13}C$ with fatty acid bacterial markers indicated organic matter degradation in the sediments. The dominance of Desulfobacterales and Desulfuromonadales suggested sulphate reduction in the sediments, a key mechanism for nutrient outwelling in salt marshes. *Marivivens* and other Rhodobacterales (Alphaproteobacteria) in the suspended particulate matter were clear indicators of the nutrient outwelling. The colonization of particles according to the island biogeography theory was a good hypothesis to explain the lower bacterial biodiversity at the wetland. The copiotrophic conditions of the RN estuary and particularly at the wetland were deduced also by the dynamic of some

* Corresponding author at: Centre for Biological Threats and Special Pathogens (ZBS 2), Robert Koch Institute, Seestraße 10, 13353 Berlin, Germany.
E-mail address: KopprioG@rki.de (G.A. Kopprio).

Actinobacteria, Bacteroidia and Gammaproteobacteria. This high-resolution snapshot combining isotopic, lipid and bacterial markers offers key pioneer insights into biogeochemical and ecological processes of the RN estuary.

1. Introduction

Temperate estuaries are characterized by large fluxes of nutrients and organic matter from diverse sources and by extremely variable environmental conditions. Estuaries are hot spot for the cycling of organic compounds and elements (Canuel and Hardison, 2016) and provide numerous ecosystem services sustaining biodiversity, protecting fisheries and reducing pollution and eutrophication (Barbier et al., 2011; Duarte et al., 2015). Estuaries are usually the gates to cities and ports, represents the interface between continental and marine environments, and are currently worldwide endangered by numerous anthropogenic impacts (e.g., Kopprio et al., 2018; Cunha et al., 2022; Lu et al., 2022). Due to the high complexity of hydrological, biogeochemical and ecological processes in estuaries, several gaps in estuarine research are currently recognized (reviewed by Khojasteh et al., 2021). This original study uses several complementary indicators to provide a better understanding of these processes and to fill some of the gaps in knowledge on estuarine areas of the Southwest Atlantic.

Stable isotopes of carbon and nitrogen and fatty acids have been widely used to elucidate biogeochemical process, organic matter fluxes and anthropogenic impacts. The $\delta^{13}C$ is an isotopic marker of the origin of the organic matter and elevated $\delta^{15}N$ may indicate a higher trophic level or presence of microzooplankton in the suspended particulate matter (e.g., Kopprio et al., 2015b). The 16:1(n-7), 16:2(n-4), 16:3(n-4) and 20:5 (n-3) fatty acids are diatom markers, the 15:0 and 18:1(n-7) are bacterial markers, the 18:4(n-3) and 22:6(n-3) are indicators of flagellates or dinoflagellates, and higher proportions of n-3 and n-6 polyunsaturated fatty acids (PUFA) are typical of zooplankton or higher trophic levels (reviewed by Dalsgaard et al., 2003; Both et al., 2020). Moreover, some n-3 and n-6 PUFA such as the 22:6(n-3) and the 20:5(n-3) are essential for the growth and development of marine fish larvae and other higher predators (e.g., Sargent et al., 1995). Stable isotopes in combination with fatty acid markers offer a multidimensional approach for the in-depth study of trophic webs and the biogeochemistry of the organic matter (Nyssen et al., 2005; Le Croizier et al., 2016; Both et al., 2020). For example, elevated values of $\delta^{13}C$ in combination with higher proportions of the fatty acids 20:5(n-3) and 16:1(n-7) indicated primary production driven by diatoms (Kopprio et al., 2015b), while the combination of the bacterial marker 18:1(n-7) and the depletion of ^{15}N were markers of strong sewage pollution (Kopprio et al., 2018).

Bacteria are also valuable indicators of anthropogenic impacts and drive important biogeochemical cycles in coastal and marine systems. Bacteria contribute to a considerable proportion of the global primary production, are able to mineralize a wide variety of substrates, and are key for nutrient and element cycling in aquatic ecosystems (Falkowski et al., 2008; Fuhrman et al., 2015; Bunse and Pinhassi, 2017). Bacteria have an active role in the microbial loop of the organic matter (Jiao et al., 2010) and some of them are important bioremediators (e.g., Liu et al., 2017) or indicators of polluted environments (Kopprio et al., 2021; Alonso et al., 2022; Zhao et al., 2022). Estuarine bacteria are subject to ever-changing environmental gradients, which originate diverse successional patterns in their communities (Ghosh and Bhadury, 2019; Kopprio et al., 2020; Huang et al., 2022). In the case of the Southwest Atlantic, Alphaproteobacteria seem to be pivotal players in the cycle of the organic matter (Garzón-Cardona et al., 2021).

In coastal waters of the Patagonia in the Southwest Atlantic, the outwelling of organic matter was practically unstudied. The outwelling hypothesis states that the organic matter and nutrients from wetlands are crucial to sustain the biological productivity of adjacent marine waters (Odum, 1968). Although considerable research has been performed, outwelling

science is still in its infancy (Santos et al., 2021; Ray et al., 2021). The Southwest Atlantic has been little investigated using lipid markers and more information of this region is urgently needed to perform latitudinal comparisons (Kattner and Hagen, 2009; Dutto et al., 2014). Furthermore, most of the Southern Hemisphere lacks consistent observations on microbial dynamics (Buttigieg et al., 2018). Outwelling research has been conducted worldwide mainly focusing on nutrient and organic matter flows; nevertheless, and to our knowledge, no study combines nutrient and organic matter flows with stable isotopes, lipid markers and bacterial communities. The complementary integration of these multiple variables will offer an excellent resolution for the study of biogeochemical processes and cycles in estuarine systems. Aims of this study were 1) to provide first insights into the role of a Patagonian salt marsh on the exportation of organic matter and nutrients to the South Western Atlantic, 2) to assess the combination of stable isotopes, fatty acids and bacterial communities for the study of nutrient fluxes and biogeochemical processes at two contrasting stations in the Río Negro (RN) estuary. We hypothesize that an important outwelling from the wetland occurs during the ebb at the RN estuary and that isotopic and lipid markers of detritus and terrigenous organic matter together with "early-colonizer" bacteria are exported from the salt marsh to the marine system.

2. Methods

2.1. Study site

The RN estuary is a mesotidal system with numerous banks and islets located in the north of the Argentinian Patagonia (Fig. 1). The RN river with a drainage area of 95,000 km^2 has a nival regime modified by the presence of dams (Depetris et al., 2005), and is the principal source of freshwater of the semi-arid region of the northern Patagonia. The RN wetlands at the estuary offer several ecosystem services and a Ramsar site is expected to be created in the region. Eutrophication, raw sewage, exotic species, potential pathogenic bacteria, heavy metals and organic pollutants are some of the impacts described for the system (Kopprio et al., 2015a; Kopprio et al., 2018; Arias et al., 2021; Perillo et al., 2022). The salt marsh vegetation at the RN estuary is dominated by the cordgrass *Spartina* spp. and the saltwort *Sarcocornia* sp.

2.2. Sampling strategy and in-situ measurements

Sampling in the RN estuary was performed hourly during a tidal cycle of ~3 m range on December 18th of 2017 at two stations simultaneously, named "Wetland" and "River" (Fig. 1). The station Wetland is situated at the mouth of a channel of ~60 m width and ~ 2.5 m depth, in which the outflow of an extensive salt marsh of ~730 ha discharges. The station River is located in the main channel of the estuary, with ~2400 m width and a maximum depth of ~6 m, and consequently less influenced by the wetland outflow. A flow of 798 m^3 s^{-1} during ebb and 397 m^3 s^{-1} during flow was estimated across the main channel with an Acoustic Doppler Current Profiler (ADCP, Workhouse, Teledyne RD Instrument). Salinity, conductivity, dissolved oxygen and pH were measured at 30 cm below the surface with electronic probes (PCE-PHD 1, PCE Instruments) at every time and station. Water samples were collected at 30 cm of the surface with 1 L sterile glass flasks for microbiological studies and with 5 L chemically clean HDPE bottles for biogeochemical analyses. Additionally, water samples were fixed with buffered paraformaldehyde (1 % final concentration) and stored in PE 50 mL bottles. At station Wetland, sterile PP tubes were inserted hourly into the sediments at 10 cm below the water level
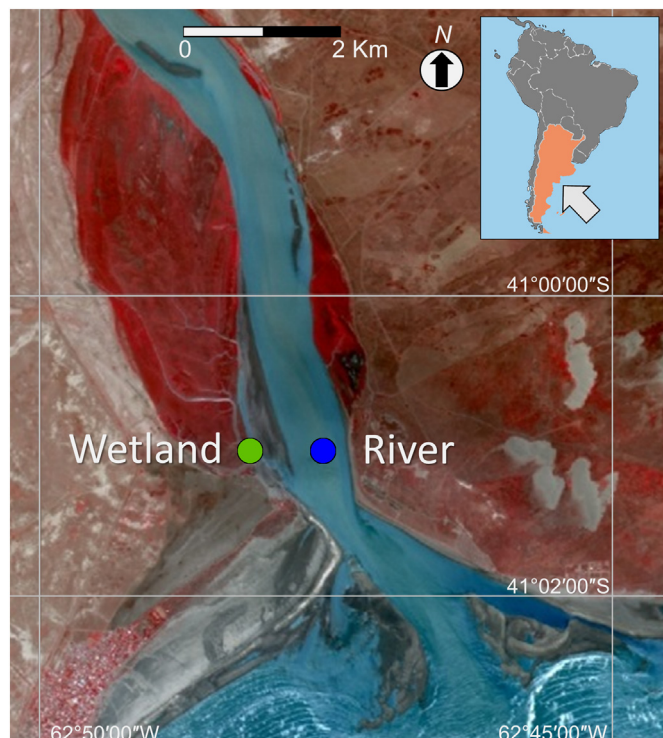
Fig. 1. Location of the sampling stations at the Río Negro (RN) estuary in the north of the Argentinian Patagonia.

and 10 samples were gathered to assess the possible effect of sediment resuspension in the water column. The sediment sample during ebb at time 4 (E4) was lost and only 10 h of the tidal cycle were covered because of operational reasons. All samples were transported in insulated boxes and processed under laboratory conditions within 6 h.

2.3. Chlorophyll, nutrient, isotope and lipid determinations

Estuarine water (from 250 to 500 mL) was filtered by triplicate through precombusted (500 °C, 5 h) GF/F glass-fibre filters of 0.7 μm pore size. Filters for chlorophyll were preserved frozen at −20 °C and pigments were extracted after an overnight incubation in acetone 90 % (Sintorgan). Chlorophyll a concentration was measured photometrically according to Lorenzen (1967). Filtrates were collected in chemically clean 50 mL bottles for dissolved inorganic nutrient and in 10 mL precombusted glass ampoules for dissolved organic matter measurements. Inorganic nutrients were determined according to standard methods (Hansen and Koroleff, 1999) with an automated analyser (Evolution III, Alliance Instruments). Dissolved organic carbon (DOC) and total dissolved nitrogen concentrations were measured with a Shimadzu TOC-VCPN coupled with a Shimadzu TNM-1 analyser. Dissolved organic nitrogen (DON) was calculated by the difference between total dissolved nitrogen and dissolved inorganic nitrogen.

Filters and sediments for particulate organic matter and stable isotopes were dried for 12 h at 50 °C and stored in a vacuum desiccator. Afterwards, the samples were acidified with hydrochloric acid (1 N) and placed in tin capsules for particulate organic nitrogen (PON) and in silver capsules for particulate organic carbon (POC) measurements. Carbon and nitrogen content in the capsules were estimated after a combustion at 1000 °C under pure oxygen in an elemental analyser (EURO EA, HEKAtech). The stable isotopes ^{13}C and ^{15}N were measured with a mass spectrometer (Thermo Finnigan Delta Plus, Thermo Fisher Scientific) coupled with an elemental analyser (Flash EA 1112, Thermo Fisher Scientific). Stable isotopes were reported as delta (δ) in parts per thousand (‰): nitrogen relative to the air and carbon relative to Pee Dee Belemnite. Peptone and acetanilide (HEKAtech) were used as internal standards and the isotopes ratios were

determined in accordance with reference standards of the International Atomic Energy Agency (IAEA-N-1 and IAEA-N-2) and the United States Geological Survey (USGS-24).

Filters and sediments for lipid analytic were stored in dichloromethane-methanol (2:1 by volume) under nitrogen atmosphere in 4 mL precombusted amber glass vials with PTFE caps (Agilent) at −20 °C. The 23:0 fatty acid standard was added, the samples were sonicated for 15 min and lipids were extracted according to Folch et al. (1957) using dichloromethane instead of chloroform. The extracted fatty acids were transesterificated with 3 % concentrated sulphuric acid in methanol for 4 h at 80 °C under nitrogen atmosphere. The resulting fatty acid methyl esters (FAMEs) were extracted with hexane and analysed by gas-liquid chromatography (Hewlett Packard 6890 GC) on a 60 m wall-coated capillary column (inner diameter 0.25 mm, film thickness 0.25 μm; liquid phase DB-FFAP) after Kattner and Fricke (1986). FAMEs were quantified with the internal standard and identified with standard mixtures and in some cases by mass spectrometry (GC–MS). Data were acquired and analysed with the software Clarity 8.0 (DataApex).

2.4. Microbiological studies and DNA extraction

For the determination of heterotrophic bacteria, water was directly spread on Plate Counting Agar (PCA, Britania) and Colony Forming Units (CFU) were counted after an overnight incubation at 30 °C. For total bacterial abundance, 30 mL of paraformaldehyde-fixed samples were filtered through GTTP Isopore membranes (Merck Millipore) of 0.2 μm pore size and placed in sterile Petri dishes at −20 °C. Filters were stained with DAPI (1 μg mL^{-1}) and bacteria were counted across random grids (120 × 120 μm). For DNA extraction, 200 mL of water were filtered through two types of membranes: 1) the mentioned GTTP of 0.2 μm pore size for suspended particulate matter (SPM), which includes free-living and particle-attached bacteria, and 2) GTTP Isopore membranes (Merck Millipore) of 5 μm pore size for particle-attached (PA) bacteria. The procedure of Griffiths et al. (2000) was used for DNA extraction. Basically, the DNA of filters or sediments was extracted firstly with hexadecyltrimethylammonium bromide (CTAB, Carl Roth) and subsequently with phenol–chloroform-isoamyl alcohol (25:24:1, Sigma-Aldrich). The samples were centrifuged at 16,000g for 10 min and the DNA was precipitated for 2 h with 2 volumes of 30 % polyethylene glycol 6000 (Sigma-Aldrich) – 1.6 M NaCl at 4 °C. Afterwards, a centrifugation step at 17,000g at 4 °C for 90 min was performed and the pellets were washed with ice-cold 70 % ethanol (Roth). Finally, the pellets were suspended in 20 μL sterile nuclease-free DEPC-treated water (Roth) and stored at −20 °C until further analyses.

2.5. DNA amplification and bioinformatics

The hypervariable region V3-V4 of the 16S rRNA gene was amplified according to Klindworth et al. (2012) with the primer set: Bact-341F (5′-CCT ACG GGN GGC WGC AG -3′) and Bact-785R (GAC TAC HVG GGT ATC TAA KCC). The selected amplicon was sequenced with an Illumina MiSeq sequencer on a 2 × 300 bp paired end run. Sequencing, demultiplexing from raw paired-end reads and removal of primer sequences were performed by the company LGC genomics. Trimming, quality filtering, dereplication, merging of paired reads, removal of chimeras and operational taxonomic unit (OTU) table assembly was performed with R 3.5.1 (R Core Team, 2021) and the package DADA2 1.9.3 (Callahan et al., 2016). For taxonomic assignment, OTU representative were submitted to SilvaNGS (v132, https://ngs.arb-silva.de/silvangs/) using a similarity threshold for creating OTUs of 1 or 100 %. Archaeal, mitochondrial, dobleton and singleton sequences were removed from the analysis. The occurrence of eukaryotic phytoplankton was inferred through the16S gene of the chloroplasts (e.g., Needham et al., 2017). The 16S sequences of the chloroplasts were aligned against a NCBI refseq database (date accessed: February 11th, 2019) containing chloroplast sequences from cultivated organisms. All sequences were deposited in the European Nucleotide Archive

(ENA) under accession number PRJEB43016. The data brokerage service of the German Federation for Biological Data (GFBio, Diepenbroek et al., 2014) was used for sequence submission, in compliance with the Minimal Information about any (x) Sequence (MIxS) standard (Yilmaz et al., 2011).

2.6. Statistics

The relations between the different variables were evaluated using Pearson correlations of log-transformed data. A total of 46 fatty acids were detected and those >1 % (mass % of total fatty acids) were selected for further analysis. Saturated fatty acids with a pair number of carbon atoms are ubiquitous among organisms and detritus and were therefore clustered together as SFA even. Furthermore, the bacterial markers 15:0 and 17:0 (including branched-chain iso and anteiso) were grouped as odd-chain saturated fatty acids (SFA odd) and the diatom markers 16:2(n-4), 16:3(n-4) and 16:4(n-1) as polyunsaturated fatty acids (PUFA) of 16 carbon atoms (PUFA 16C). A principal component analysis (PCA) was conducted to ordinate the SPM and sediment samples based on their fatty acid (mass % of total fatty acids) and stable isotope (‰) proportions. For the bacterial communities, data curation, taxa diversity and abundance calculations were conducted with R 3.5.1 and additional packages such as vegan (Oksanen et al., 2019) and iNEXT (Hsieh et al., 2019). The OTUs from the sediments, SPM and particles >5 μm were ordinated by Nonmetric Multi-Dimensional Scaling (NMDS). The OTU numbers were transformed $(\log[(x + \bar{x})/\bar{x}])$ and a Bray Curtis similarity matrix was calculated. Difference between the source of the organic matter (river, wetland or sediments) and the type of water sample (SPM and particles >5 μm) were evaluated using analysis of similarities (ANOSIM). In case of significance differences, similarity percentage analysis (SIMPER) was conducted to detect the main taxa contributing to dissimilarities. Graphics were performed with Xact, while correlations with R, PCA with XLSTAT and MDS, ANOSIM and SIMPER with Primer.

3. Results

Salinity values were generally higher at station River and a maximum of ~32 was detected at the slack high tide (HT) (Fig. 2). The δ^{13}C of POC followed a similar tidal dynamic and was positively correlated with salinity ($r = 0.73$, $p < 0.001$); nevertheless, the δ^{13}C dynamic remained stable at the station Wetland (~ -21.5 ‰) even during the salinity drop at the ebb. Lower δ^{15}N values (< 7.5 ‰) of PON were characteristic at the end of the flow at both stations. Wetland outwelling was evidenced by the peaks of DOC, ammonium and phosphate during the ebb at time 3 (E3), E4 and E5. Moreover, a similar increasing trend at E4 and E5 was observed on the concentrations of PON, chlorophyll a, the essential fatty acids 22:6 (n-3) and 20:5(n-3), and total fatty acids. Elevated total fatty acid contents (>80 μg mgC^{-1}) were detected generally at the wetland, particularly during the end of the ebb and the flow. The CFU abundance of terrestrial heterotrophic bacteria increased during the ebb at station Wetland and followed a similar trend than the nutrient outwelling. Total bacteria abundance was generally more elevated at the wetland (>12 × 10^5 cells mL^{-1}); nevertheless, the influence of the nutrient discharge was not evident. In general, the diversity indexes were richer in the river in comparison with the wetland, and poorer during the ebb at both stations.

Table 1 summarizes the correlations of ammonium and DOC as main outwelling indicators with other biogeochemical variables. A similar correlation pattern was observed between these indicators and DON, nitrite and chlorophyll a. No significant correlations were detected between both indicators with δ^{13}C and δ^{15}N. Most of the concentrations (μg L^{-1}) of fatty acid markers of diatoms, microzooplankton, flagellates, and bacteria in SPM showed strong significant correlations with ammonium and DOC. At lower r, the content (μg mgC^{-1}) of some fatty acids in SPM was significantly correlated with ammonium and DOC. The PUFA 16C content as a diatom marker was correlated at $r > 0.6$ with both outwelling indicators.

The PCA (Fig. 3) explains the ~56 % of the variation: 37.4 % the first component (PC1) and 18.3 % the second (PC2). Most of the correlations among components and markers are summarized in the PCA. PC1 could be associated with the origin of the organic matter: at the negative side were ordinated the sediment samples richer in detrital and bacterial fatty acids and δ^{13}C, while at the positive axis the SPM samples with higher proportions of n-3 polyunsaturated fatty acids, 20:4(n-6) and δ^{15}N. At the positive side, the PC2 could be related to the influence of the wetland and the ebb stations at time 4 and 5 (E4 and E5) with higher percentages of the diatom markers 16:1(n-7) and PUFA 16C were grouped at this extreme.

The bacterial community composition of the SPM at both stations (Fig. 4) was dominated mainly by the orders Frankiales (class Actinobacteria), Flavobacteriales (Bacteroidia), Betaproteobacteriales (Gammaproteobacteria) and Rhodobacterales (Alphaproteobacteria). The genus hgcl clade of the Frankiales incremented its relative sequence abundance during the ebb period at the river, while followed a bell shape with a peak at E1 and E2 in the wetland channel. The Rhodobacterales and particularly its genus *Marivivens* were clear indicators of the wetland outwelling. At order level, the relative sequence abundance of Rhodobacterales, *Candidatus Falkowbacteria* and *Candidatus Komeilibacteria* (phylum Patescibacteria), Clostridiales (class Clostridia) and Campylobacterales were strongly correlated with ammonium and DOC (Table 2). Moreover, several OTUs of *Marivivens Salinihabitans*, *Roseivivax*, *Roseobacter* and *Marivita* (Rhodobacterales) together with *Candidatus Aquiluna* (Micrococcales), BD1-7 clade (Cellvibrionales), NS3a marine group (Flavobacteriales) and with *Marinobacterium* (Oceanospirillales) were significantly correlated with both outwelling indicators.

Regarding to the 16S sequences of the chloroplasts, which were aligned against a NCBI database, the dominant genera of eukaryotic phytoplankton were the dinoflagellates *Durinskia* and *Kryptoperidinium*; the diatoms *Cylindrotheca*, *Phaeodactylum*, *Coscinodiscus*, *Thalassiosira*, *Asterionellopsis* and *Trieres;* the flagellates *Guillardia*, *Triparma* and *Pyramimonas;* and other microalgae of the genera *Nannochloropsis, Pycnococcus, Chlorella* and *Marvania.*

The NMDS (Fig. 5) showed a clear ordination between the origin of the samples but not between the fractions of the water column (SPM from 0.2 μm pore size membrane or PA from 5 μm). ANOSIM revealed significant differences between the sediments at the wetland and particulates (SPM and PA) from the water column at both stations (Global $R = 0.54$, $p < 0.001$ at order level and Global $R = 0.61$, p < 0.001 at OTU level). According to pairwise tests, the sediments were significantly different from the water particulates at the wetland ($R = 0.86$, p < 0.001 for orders and $R = 0.85$, p < 0.001 for OTUs) and the river ($R = 0.77$, p < 0.001 and $R = 0.91$ p < 0.001). At lower R ($R = 0.30$, $p = 0.002$ and $R = 0.39$, $p = 0.001$), significant differences were detected between the water particulates from the wetland and the river. No significant differences were found between the SPM and PA samples at OTU level (Global $R = 0.029$, $p = 0.162$).

The Frankiales, Desulfobacterales and Micrococcales (Table 3) were the main orders contributing to the dissimilarities between the different sources of organic matter: water particulates from the river, water particulates from the wetland or wetland sediments. The Desulfobacterales, Desulfuromonadales, Clostridiales and Bacteroidales dominated in the sediments and were important contributors to the dissimilarities between this fraction and the water particulates (River or Wetland). The percentages of total dissimilarity were higher at OTU level, but the contribution of each OTU to the total dissimilarities was lower than at the order level. *Marivivens* sq. 9, *Hydrogenophaga* sq. 42, *Marinobacterium* sq. 162, *Marinobacterium* sq. 238 and *Sulfitobacter* sp. 192 dominated in the wetland and contributed to the highest dissimilarities values between the stations River and Wetland. Several OTUs of hgcl clade, *Rhodobacter* sq. 10 and NS11-12 marine group unclassified were the main contributors to the dissimilarities between the water (River or Wetland) and the sediments. *Malonomonas* sq. 275 of the order Desulfuromonadales was an OTU typical of the sediments and contributed to elevated percentages of dissimilarities.
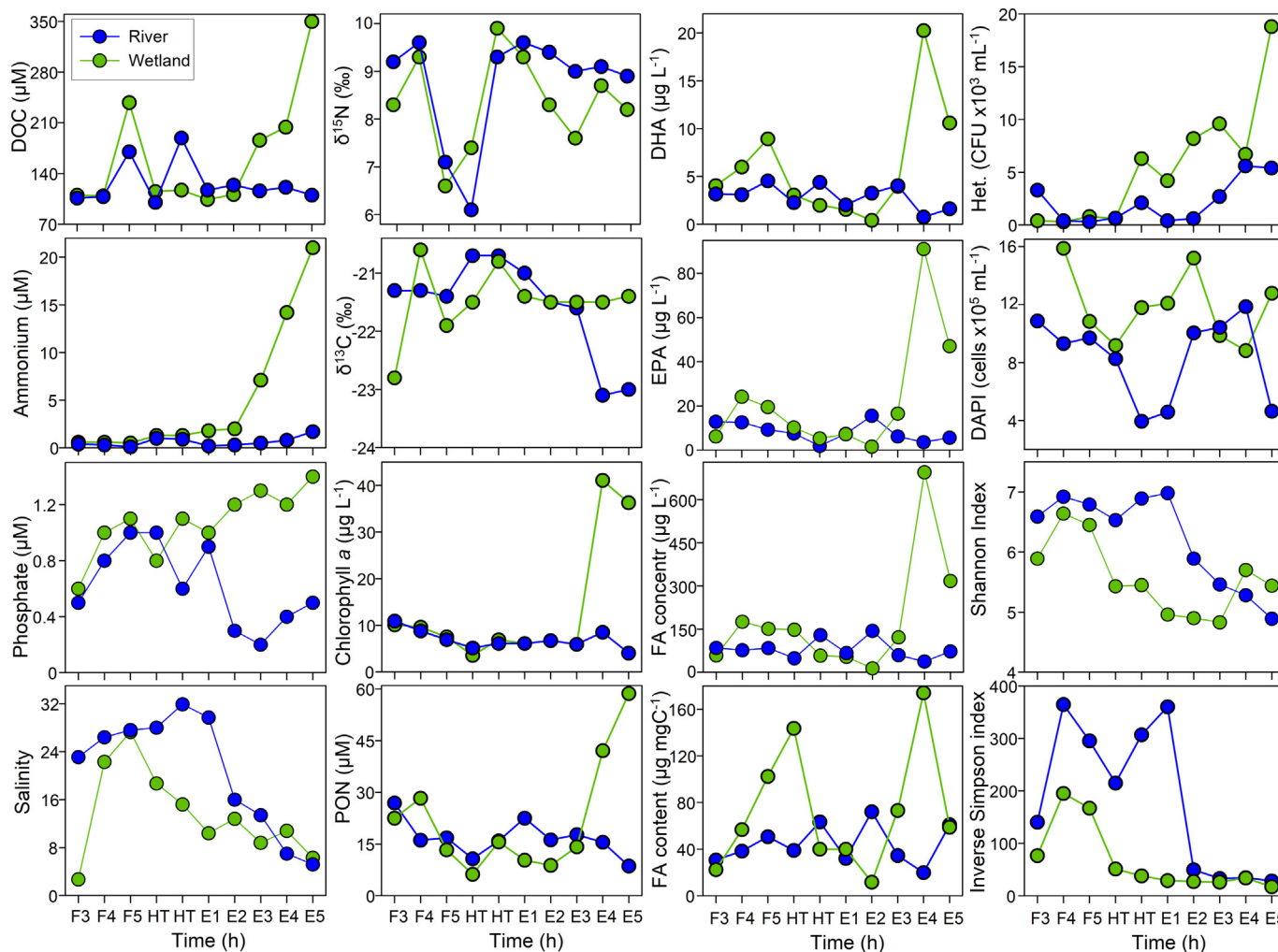
Fig. 2. Tidal dynamic of main biogeochemical and bacterial parameters. F: flow, E: ebb, HT: high tide, PON: particulate organic nitrogen, DOC: dissolved organic carbon, DHA: docosahexaenoic acid or 22:6(n-3), EPA: eicosapentaenoic acid or 20:5(n-3), FA: fatty acid, concentr: concentration, DAPI: 4′,6-diamidino-2-phenylindole counts or total bacterial abundance, Het.: heterotrophic bacteria, CFU: colony forming unit.

4. Discussion

4.1. Outwelling of dissolved nutrients and organic matter

A clear exportation of inorganic nutrients and dissolved organic matter from the salt marsh to the Southwest Atlantic was evidenced at the end of the ebb at the Station Wetland but not at the Station River. Although some initial controversy, it has been long recognized that salt marshes and mangroves release considerable amounts of organic matter and inorganic nutrients to nearby marine systems (Dame et al., 1986; Dittmar and Lara, 2001; Santos et al., 2019). The wetland outwelling is a well-known phenomenon observed in several coastal system of the world and now deduced in the Argentinian Patagonia. This phenomenon stimulates the primary production, drives the microbial pump of adjacent marine waters and sustains secondary consumers and coastal fisheries.

4.2. Sulphate reduction as a potential outwelling driver

Key mechanisms triggering nutrient outwelling in mangroves and salt marshes are the sulphate reduction in the sediments followed by tidal flushing of burrows, macropores and vegetal detritus (Bouillon et al., 2007; Santos et al., 2021; Ray et al., 2021). As reviewed by Santos et al. (2021), sulphate in sea water is about three orders of magnitude higher than oxygen and sulphate is often the dominant oxidant used by microorganisms when consuming organic matter in coastal sediments. Sulphate reduction in the

sediments of the RN estuary was suspected by the orders Desulfobacterales and Desulfuromonadales as well as its OTU *Malonomonas* sq. 275 (class Deltaproteobacteria). Secondarily, nitrate reduction may be important in some coastal systems (Kristensen et al., 2011) and nitrite in the RN estuary was linked to the outwelling, this may indicate that nitrate is used also as oxidant by the microbes in the sediments.

The Desulfobacterales, Desulforomonadales and many other Deltaproteobacteria are sulphate-reducing microorganisms and sulphate is generally the dominant oxidant used by bacteria when consuming organic matter in marine sediments (Jørgensen et al., 2019). Particularly, the order Desulfobacterales play an active role in the sulfur cycle of estuarine salt marshes (Wang et al., 2020). Moreover, *Sulfurovum* (Order Campylobacterales) is a chemolithotroph named because of their ability to oxidize sulfur (Inagaki et al., 2004) and may contribute to important redox processes in the sediments of the RN estuary. *Sulfurovum* sq. 282 in the water particulates at the Station Wetland may suggest the contribution of suspended sediments to this fraction.

4.3. Indicators of degradation processes in sediments

The order Bacteroidales may indicate higher concentrations of organic matter in the sediments and may contribute with the degradation of complex polymers and cycling of organic matter. Bacteroidetes have several enzymes to catabolize high molecular weight compounds and many adaptations to growth attached to particles or surfaces (Fernández-Gómez et al., 2013;

Table 1
Main Pearson correlations (r) of ammonium and dissolved organic carbon (DOC) as indicators of wetland outwelling with other nutrients, chlorophyll *a*, stable isotopes and main fatty acids.

	Ammonium			DOC		
	Variable	r	p	Variable	r	p
Nutrients, chlorophyll and stable isotopes	DON	0.78	<0.001	DON	0.87	<0.001
	Nitrite	0.76	<0.001	Nitrite	0.70	<0.001
	Chlorophyll *a*	0.66	0.001	Ammonium	0.64	0.002
	DOC	0.64	0.002	Chlorophyll *a*	0.59	0.006
	Phosphate	0.57	0.009	PON	0.51	0.022
Fatty acid content (μg mgC^{-1})	PUFA 16C	0.67	0.001	PUFA 16C	0.63	0.003
	SFA odd	0.51	0.022	16:1(n-7)	0.58	0.007
				22:6(n-3)	0.56	0.010
				18:4(n-3)	0.56	0.011
Fatty acid concentration (μg L^{-1})	PUFA 16C	0.71	<0.001	22:6(n-3)	0.70	<0.001
	16:1(n-7)	0.69	<0.001	PUFA 16C	0.69	<0.001
	20:4(n-6)	0.69	<0.001	18:4(n-3)	0.69	<0.001
	SFA odd	0.67	0.001	16:1(n-7)	0.66	0.001
	20:5(n-3)	0.64	0.002	SFA odd	0.63	0.003
	18:4(n-3)	0.62	0.004	18:1(n-7)	0.62	0.003
	18:1(n-7)	0.61	0.004	SFA even	0.61	0.004
				18:2(n-6)	0.56	0.010
				20:4(n-6)	0.54	0.013

DON: dissolved organic nitrogen, PON: particulate organic nitrogen, PUFA 16C: polyunsaturated fatty acids of 16 carbon atoms, SFA odd: saturated fatty acids of odd carbon atoms, SFA even: saturated fatty acids of pair carbon atoms, n.s.: not significant.

Yilmaz et al., 2016). A considerable amount of DOC with a sedimentary or porewater origin may be deduced by the correlations of DOC with Desulfobacterales, Campylobacterales and Clostridiales. The Clostridiales and Ruminococcaceae may indicate the usual hypoxic conditions in the sediments of salt marshes. Ammonium is also a hypoxia indicator and may be originated in the same process of remineralisation of the sedimentary organic matter. The higher concentration of phosphate during the outwelling may have the same origin than ammonium. The exportation of ammonium and phosphate from salt marshes suggests that major decomposition processes are taking place in the system (Dame et al., 1986). Furthermore, the close ordination of δ^{13}C, detrital and bacterial fatty acid markers with sediment samples indicated an elevated microbial activity within this fraction.

4.4. Isotopic and lipid markers in the suspended particulate matter

The δ^{13}C of the SPM was a good indicator of the origin of the organic matter (marine or freshwater) as showed by the correlative evidence with
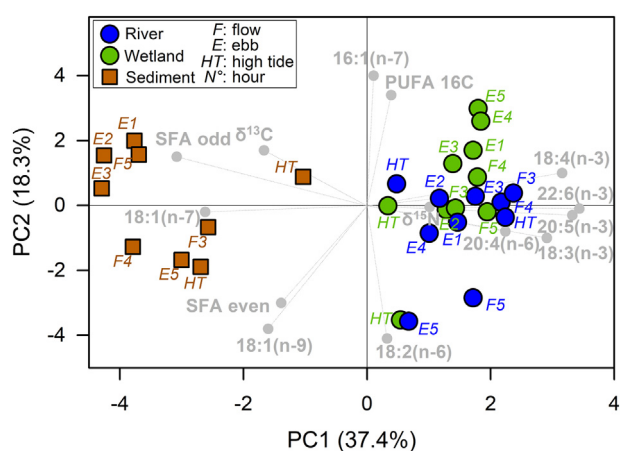

Fig. 3. Principal Component analysis (PCA) of the proportions of stable isotopes (‰) and fatty acids (%) in the suspended particulate matter and sediments. SFA odd: saturated fatty acids of odd carbon number, SFA even: saturated fatty acids of even carbon number, PUFA 16C: polyunsaturated fatty acids of 16 atoms of carbon and two or more double bonds.

salinity. Anyway, it was not a good indicator of the outwelling and there was not a clear distinction of δ^{13}C between the two sampling stations. Estuaries have typically increasing δ^{13}C values from freshwater to marine stations (Ke et al., 2017; Kopprio et al., 2018). The δ^{13}C values remained stable during the last hours of the ebb at the wetland, even though a lower salinity, this phenomenon may be linked to the influence of the sediments or phytoplankton in the SPM during the outwelling. Anyway, δ^{13}C values around ~22 ‰ indicated the dominance of phytoplankton (Ray et al., 2021). The terrestrial markers 18:3(n-3) and 18:2(n-6) did not characterize the outwelling; nevertheless, the nutrient outwelling at the wetland was dominated by fatty acid markers of microalgae, particularly those from diatoms.

The δ^{15}N in SPM was not a good indicator of the origin of the organic matter and its composition may be explained by the planktonic composition. The lower proportions of δ^{15}N seems not to be influenced by the dynamic of cyanobacteria (mostly from the order Synechococcales in the RN estuary) nor the concentrations of ammonium. Values of δ^{15}N near 0 in the SPM in combination with elevated values of ammonium, 18:1(n-7) and coliforms were evidenced at the sewage discharge in the RN estuary (Kopprio et al., 2018). This exceptional signature was not detected at the wetland. The close ordination of δ^{15}N with n-3 PUFA suggests the presence of secondary consumers in the SPM such as microzooplankton or heterotrophic dinoflagellates.

4.5. Exportation of organisms and their essential fatty acids

Coastal wetlands are not only a relevant source of dissolved carbon and nitrogen to the coastal ocean, but also in this study case, of PUFA, chlorophyll *a* and particulate organic matter in the form of planktonic organisms or at least fresh particles derived from them. Odum (2002) developed the idea that salt marsh production may be outwelled also as living organisms to the surrounding coastal waters. Essential fatty acids like the 22:6(n-3) and 20:5(n-3) were directly exported from the salt marsh and likely contributed to sustain higher trophic levels and fisheries in coastal waters of the Argentinian Patagonia, as mentioned in the introduction, the growth and development of marine fishes is limited by essential fatty acids. Higher proportions of PUFA are typical of living particles (Dutto et al., 2014), organisms or highly nutritional organic matter are exported from the wetland to coastal waters, which is in general agreement with Odum (2002). Our study revealed higher concentrations of essential fatty acids together with fatty acids markers of diatoms, dinoflagellates, flagellates and bacteria during the outwelling. Moreover, the identity of some of these organisms was confirmed by the information from the sequences of the chloroplasts.

The elevated concentrations of organic matter at the wetland may explain the higher abundance of total bacterioplankton at this station. During the outwelling occurred an export of culturable heterotrophic bacteria, probably oligohaline organisms with an active metabolism deduced from their ability to growth in culture media. The exportation of bacterioplankton from the wetland was also reflected by the dynamic of bacterial fatty acid markers such as SFA odd and 18:1(n-7). The bacterial community composition in the RN estuary was likely dominated by copiotrophic bacteria and early colonizers.

4.6. Hypothetical colonizers of the organic matter

The colonization process according to the theory of island biogeography is a good hypothesis to explain the diversity patterns in the RN estuary. Relatively few bacteria have the ability to colonize the outwelled organic matter or the "new island", this explains also the lower biodiversity observed in the SPM at station Wetland. *Marivivens* and other Rhodobacterales were clear indicators of the outwelling and may play key roles in primary colonization of the organic matter. *Marivivens* was inferred as an early colonizer with the ability to produce extracellular polymeric substances and to facilitate the settlement of other bacteria in a tropical estuary (Kopprio et al., 2020); therefore, a similar role of
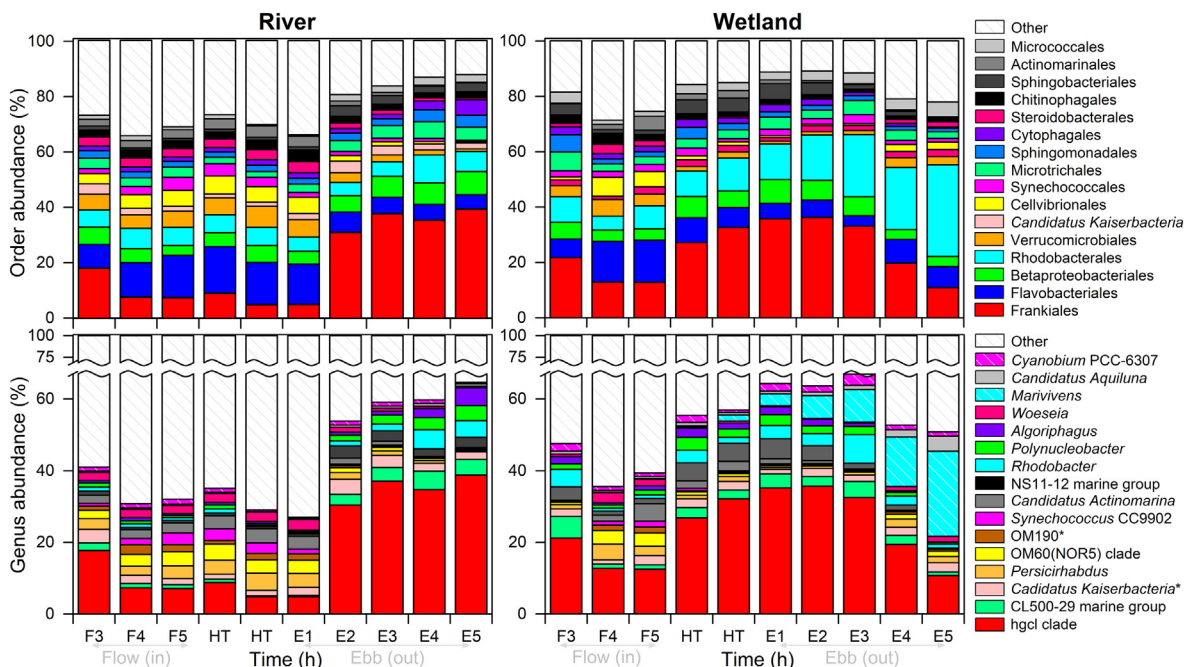
Fig. 4. Bacterial community composition at order and genus level in the suspended particulate matter (SPM) of the Río Negro (RN) estuary across a tidal cycle. A similar color indicates correspondence between order and genus.

Marivivens spp. is suggested for the RN estuary. The Rhodobacterales are primary surface colonizer in temperate coastal Atlantic waters (Dang et al., 2008). Furthermore, some strains of *Marivivens* are potential aromatic compounds degraders (Chen et al., 2020) and their abundance may be related to the mentioned hydrocarbon pollution at the RN estuary.

Table 2
Main Pearson positive correlations (r) of ammonium and dissolved organic carbon (DOC) as indicators of wetland outwelling with relative sequence abundance of orders and operational taxonomic units (OTUs) in the suspended particulate matter.

		Ammonium			DOC		
	Taxa	r	p	Taxa	r	p
Order	Rhodobacterales[Rh]	0.91	<0.001	*Cand Komeilibacteria*	0.76	<0.001
	Cand Falkowbacteria	0.90	<0.001	*Cand Falkowbacteria*	0.73	<0.001
	Campylobacterales	0.83	<0.001	Clostridiales	0.71	<0.001
	Clostridiales	0.78	<0.001	Campylobacterales	0.66	0.002
	Cand Komeilibacteria	0.71	<0.001	Desulfobacterales	0.63	0.003
	Micrococcales[Mi]	0.67	0.001	Gracilibacteria uncl	0.60	0.005
	Oceanospirillales[Oc]	0.67	0.001	Rhodobacterales[Rh]	0.57	0.008
OTU	*Salinihabitans* sq645[Rh]	0.95	<0.001	*Cand Aquiluna* sq324[Mi]	0.75	<0.001
	Roseivivax sq243[Rh]	0.95	<0.001	*Roseivivax* sq243[Rh]	0.75	<0.001
	Marivivens sq320[Rh]	0.94	<0.001	*Cand Aquiluna* sq470[Mi]	0.74	<0.001
	Marinobacterium sq711[Oc]	0.93	<0.001	*Roseobacter** sq264[Rh]	0.73	<0.001
	Marivivens sq9[Rh]	0.92	<0.001	*Salinihabitans* sq645[Rh]	0.72	<0.001
	BD1-7 clade sq 448	0.92	<0.001	*Marivita* sq725[Rh]	0.72	<0.001
	NS3a mar gr sq268	0.92	<0.001	*Marivivens* sq320[Rh]	0.68	<0.001
	Cand Aquiluna sq324[Mi]	0.89	<0.001	NS3a mar gr sq268	0.63	0.003
	*Roseobacter** sq264[Rh]	0.89	<0.001	BD1-7 clade sq448	0.62	0.004
	Marivita sq725[Rh]	0.86	<0.001	*Marivita* sq182[Rh]	0.59	0.006
	Flavobacterium sq246	0.86	<0.001	Rhodobact uncl sq542[Rh]	0.58	0.007
	Marinobacterium sq162[Oc]	0.84	<0.001	*Roseobacter*** sq512[Rh]	0.57	0.008
	Cand Aquiluna sq470[Mi]	0.84	<0.001	*Marinobacterium* sq711[Oc]	0.57	0.008
	Cand Aquiluna sq51[Mi]	0.79	<0.001	*Marivivens* sq9[Rh]	0.57	0.009
	Marinobacterium sq616[Oc]	0.70	<0.001	*Flavobacterium* sq246	0.56	0.010
	Marinobacterium sq541[Oc]	0.70	<0.001	*Cand Kaiserbact* uncl sq613	0.56	0.010
	Sulfitobacter sq192[Rh]	0.65	0.002			
	Marinobacterium sq238[Oc]	0.64	0.002			
	Hydrogenophaga sq42	0.64	0.002			
	Marinobacterium sq386[Oc]	0.64	0.002			
	Rhodobacter sq461[Rh]	0.62	0.004			

Cand: Candidatus, mar gr: marine group, uncl: unclassified, *Kaiserbact: Kaiserbacteria*, Rhodobact: Rhodobacteraceae. Caption superscrit and color indicate correspondence between order and OTU (e.g., *Salinihabitans* sq645[Rh]: belongs to the order Rhodobacterales[Rh]).
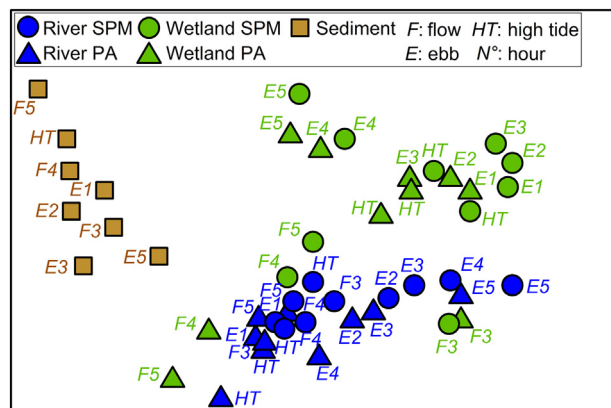(*) *Roseobacter* clade NAC11-7 lineage, (**) *Roseobacter* clade CHAB-I-5 lineage

Fig. 5. Non-metric Multi-Dimensional Scaling (NMDS) ordination of the suspended particulate matter (SPM), particle attached (PA > 5 μm) and sediment samples across a tidal cycle at the stations Wetland and River.

The "new island" was likely composed by plankton or their fresh particulates as evidenced by the fatty acid and chlorophyll dynamics. Furthermore, the lack of differences between the community composition of the SPM and PA fractions suggest the dominance of particle-attached bacteria as occurs typically in eutrophic systems (e.g., Tang et al., 2017). Rhodobacterales bacteria interact strongly with marine phytoplankton and possess unique enzymatic pools to access phytoplankton exudates (Isaac et al., 2021). Other Rhodobacterales of the genera *Roseobacter*, *Salinihabitans*, *Roseivivax*, *Marivita* and *Sulfitobacter* were deduced as important early colonizers of the particles or phytoplankton as well as outwelling markers. For example, *Roseobacter* clades occur particle-attached in nutrient hot-spots and play a major role in cycling organic matter from phytoplankton (Zhang et al., 2016; Bakenhus et al., 2017; Wang et al., 2020).

Salinihabitans spp. have been associated with eukaryotes in estuarine areas of Southern California (Diner et al., 2021) and *Marivita* spp. have been described as an early colonizer of microbeads (Wang et al., 2021).

4.7. Main bacterial taxa and their links with biogeochemical processes

The dominance of Actinobacteria, Bacteroidia, Gammaproteobacteria evidenced the eutrophic and changing conditions of the RN estuary and several OTUs from them were relevant outwelling markers. Actinobacteria are copiotrophic microorganisms, play a key role in carbon cycling of estuarine systems, and degrade complex polymers such as cellulose, lignin and chitin (Ghosh and Bhadury, 2019; Dhal et al., 2020). Moreover, some Actinobacteria have the ability to remove heavy metals and organic pollutants (Alvarez et al., 2017) and may have an important role in the self-detoxification of the RN estuary. The hgcI clade is an indicator of eutrophication and has an important role in global cycle of nitrogen (Ruprecht et al., 2021). Within the Actinobacteria, the order Micrococcales and its genus *Candidatus Aquiluna* were associated with the nutrient-rich conditions of the wetland and the outwelling in the RN estuary.

A similar association was observed in OTUs of NS3a marine group and *Flavobacterium*. As mentioned before the Bacteroidetes are copiotrophic bacteria with the ability to degrade large molecules. Some taxa within the order Flavobacteriales are indicators of eutrophic conditions (Kopprio et al., 2021) and for this study case, a similar role was inferred for NS3a marine group and *Flavobacterium*, indicating higher concentrations of dissolved inorganic nutrients and organic matter. Within Gammaproteobacteria, some OTUs of *Marinobacterium* (order Oceanospirillales), *Hydrogenophaga* (Betaproteobacteriales) and BD1-7 clade (Cellvibrionales) were markers of the outwelling as well as the copiotrophic conditions of the wetland. Gammaproteobacteria are copiotrophic organisms (Fuhrman et al., 2015) and particularly, the Oceanospirillales are fast-surface-colonizers in temperate coastal Atlantic waters (Dang et al., 2008), while the Betaproteobacteriales are primary

Table 3

Total dissimilarities and contributions of the orders and operational taxonomic units (OTUs) with higher percentages of dissimilarties in the comparison between the different sources of organic matter.

	River vs. Wetland	%	Dom	River vs. Sediment	%	Dom	Wetland vs. Sediment	%	Dom
Order	Total	25.5		Total	35.0		Total	41.9	
	Frankiales[Fr]	2.5	Wet	Frankiales[Fr]	4.2	Riv	Frankiales[Fr]	4.4	Wet
	Micrococcales[Mi]	2.1	Wet	Desulfobacterales	2.9	Sed	Desulfobacterales	2.6	Sed
	Steroidobacterales[St]	2.0	Riv	*Candidatus Falkowbacteria*	2.3	Sed	Micrococcales[Mi]	2.6	Wet
	Cellvibrionales[Ce]	2.0	Riv	Betaproteobacteriales[Be]	2.1	Riv	Bacteroidales	2.0	Sed
	OM190 uncl	1.9	Riv	Micrococcales[Mi]	1.9	Riv	Desulfuromonadales[De]	1.9	Sed
	Pedosphaerales	1.9	Riv	Desulfuromonadales[De]	1.9	Sed	Rhodobacterales[Rh]	1.7	Wet
	Actinomarinales[Ac]	1.9	Riv	Clostridiales[Cl]	1.8	Sed	Betaproteobacteriales[Be]	1.7	Wet
OTU	Total	49.8		Total	61.1		Total	65.6	
	Marivivens sq9[Rh]	0.13	Wet	hgcI clade sq2[Fr]	0.11	Riv	hgcI clade sq2[Fr]	0.12	Wet
	Hydrogenophaga sq42[Be]	0.11	Wet	NS11-12 mar gr uncl sq14	0.10	Riv	*Rhodobacter* sq10[Rh]	0.12	Wet
	Marinobacterium sq162	0.09	Wet	*Rhodobacter* sq10[Rh]	0.09	Riv	NS11-12 mar gr uncl sq14	0.11	Wet
	Marinobacterium sq238	0.08	Wet	hgcI clade sq7[Fr]	0.09	Riv	hgcI clade sq7[Fr]	0.11	Wet
	Sulfitobacter sq192[Rh]	0.08	Wet	hgcI clade sq12[Fr]	0.09	Riv	hgcI clade sq12[Fr]	0.11	Wet
	Marinobacterium sq267	0.07	Wet	*Algoriphagus* sq22	0.09	Riv	*Marivivens* sq9[Rh]	0.10	Wet
	*Roseobacter** sq264[Rh]	0.07	Wet	*Malonomonas* sq275[De]	0.09	Sed	*Algoriphagus* sq22	0.10	Wet
	Marivivens sq320[Rh]	0.07	Wet	*Cand Actinomarina* sq38[Ac]	0.09	Riv	hgcI clade sq18[Fr]	0.10	Wet
	NS3a mar gr sq268	0.07	Wet	*Gaetbulibacter* sq218	0.09	Sed	hgcI clade sq28[Fr]	0.10	Wet
	Roseivivax sq243[Rh]	0.07	Wet	hgcI clade sq18[Fr]	0.09	Riv	hgcI clade sq20[Fr]	0.10	Wet
	Marinobacterium sq386	0.07	Wet	hgcI clade sq34[Fr]	0.09	Riv	hgcI clade sq45[Fr]	0.09	Wet
	Marinobacterium sq388	0.07	Wet	hgcI clade sq20[Fr]	0.09	Riv	Roseiflexaceae uncl sq53	0.09	Wet
	Flavobacterium sq246	0.06	Wet	Sphingomonadaceae uncl sq33	0.08	Riv	Sphingomonadaceae uncl sq33	0.09	Wet
	hgcI clade sq167[Fr]	0.06	Wet	hgcI clade sq28[Fr]	0.08	Riv	*Cand Aquiluna* sq51[Mi]	0.09	Wet
	Sulfurovum sq282	0.06	Wet	*Cand Actinomarina* sq63[Ac]	0.08	Riv	hgcI clade sq34[Fr]	0.09	Wet
	BD1-7 clade sq448[Ce]	0.06	Wet	hgcI clade sq45[Fr]	0.07	Riv	*Rhodoluna* sq55	0.09	Wet
	NS5 mar gr sq104	0.06	Riv	*Planktomarina* sq56[Rh]	0.07	Riv	*Cand Planktoluna* sq40[Mi]	0.09	Wet
	Woeseia sq80[St]	0.06	Riv	Roseiflexaceae* sq53	0.07	Riv	*Malonomonas* sq275[De]	0.08	Sed
	Leptothrix sq544[Be]	0.06	Riv	*Sulfurovum* sq282	0.07	Sed	*Polynucleobacter* sq62[Be]	0.08	Wet
	Cand Aquiluna sq324[Mi]	0.06	Wet	Ruminococcaceae** sq294[Cl]	0.07	Sed	CL500-29 mar gr sq41	0.08	Wet
	Burkholderiaceae* q128[Be]	0.05	Riv	*Polynucleobacter* sq62[Be]	0.07	Riv	*Sediminibacterium* sq83	0.08	Wet

Dom: dominance, Riv: river, Wet: wetland, Sed: sediment, *Cand*: *Candidatus*, mar gr: marine group, uncl: unclassified, caption superscrit indicates correspondence between order and OTU (e.g., *Marivivens* sq9[Rh]: belongs to the order Rhodobacterales [Rh]) (*) *Roseobacter* clade NAC11-7 lineage (**) Ruminococcaceae UCG-012

colonizers (van der Kooij et al., 2018). Gammaproteobacteria and specially the BD1-7 clade dominated degrading particles in the California Current Ecosystem (Valencia et al., 2021).

5. Conclusions

Organisms, particulates and dissolved nutrients were exported from the RN wetland to the Atlantic Ocean, supporting the pioneer findings of Odum. Coastal wetlands provide numerous ecosystem services, which included in our study case, the delivery of n-3 polyunsaturated fatty acid to marine coastal waters, with their essential role for higher trophic levels and consequently for fisheries. Several Alphaproteobacteria were deduced as early colonizer of the exported organic matter, Bacteroidetes indicated copiotrophic conditions in the wetland, and certain Deltaproteobacteria suggested sulphate reduction in the sediment. The copiotrophic and changing estuarine conditions in the water column were evidenced by the dominance of some Actinobacteria, Bacteroidia and Gammaproteobacteria. This study cases offer a high-resolution snapshot of biomarkers, which are essential for the understanding of biogeochemical and ecological process in temperate mesotidal estuaries of the Southern Hemisphere.

CRediT authorship contribution statement

Germán A. Kopprio: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Visualization. **Ana Martínez:** Formal analysis, Investigation, Methodology. **Anna Fricke:** Formal analysis, Investigation, Methodology. **Michael Hupfer:** Writing – review & editing. **Rubén J. Lara:** Conceptualization, Visualization, Writing – review & editing. **Martin Graeve:** Conceptualization, Data curation, Methodology, Writing – review & editing. **Astrid Gärdes:** Conceptualization, Visualization, Writing – review & editing.

Data availability

Sequences are available at ENA (https://www.ebi.ac.uk/ena/data/view/PRJEB43016).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Alonso, C., Pereira, E., Bertoglio, F., De Cáceres, M., Amann, R., 2022. Bacterioplankton composition as an indicator of environmental status: proof of principle using indicator value analysis of estuarine communities. Aquat. Microb. Ecol. 88, 1–18. https://doi.org/10.3354/ame01979.

Alvarez, A., Saez, J.M., Davila Costa, J.S., Colin, V.L., Fuentes, M.S., Cuozzo, S.A., Benimeli, C.S., Polti, M.A., Amoroso, M.J., 2017. Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. Chemosphere 166, 41–62. https://doi.org/10.1016/j.chemosphere.2016.09.070.

Arias, A.H., Macchi, P.A., Abrameto, M., Solimano, P., Migueles, N., Rivas, F.G., Funes, A.I., Calabrese, G., Soricetti, M., Bernardis, A., Baggio, R.B., Labaut, Y., Marcovecchio, J.E., 2021. Negro River environmental assessment. In: Torres, A.I., Campodonico, V.A. (Eds.), Environmental Assessment of Patagonia's Water Resources. Springer International Publishing, Cham, pp. 95–126.

Bakenhus, I., Dlugosch, L., Billerbeck, S., Giebel, H.-A., Milke, F., Simon, M., 2017. Composition of total and cell-proliferating bacterioplankton community in early summer in the North Sea – Roseobacters are the most active component. Front. Microbiol. 8, 1771. https://doi.org/10.3389/fmicb.2017.01771.

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., Silliman, B.R., 2011. The value of estuarine and coastal ecosystem services. Ecol. Monogr. 81, 169–193. https://doi.org/10.1890/10-1510.1.

Both, A., Byron, C.J., Costa-Pierce, B., Parrish, C.C., Brady, D.C., 2020. Detrital subsidies in the diet of *Mytilus edulis*; macroalgal detritus likely supplements essential fatty acids. Front. Mar. Sci. 7, 561073. https://doi.org/10.3389/fmars.2020.561073.

Bouillon, S., Middelburg, J.J., Dehairs, F., Borges, A.V., Abril, G., Flindt, M.R., Ulomi, S., Kristensen, E., 2007. Importance of intertidal sediment processes and porewater exchange on the water column biogeochemistry in a pristine mangrove creek (Ras Dege, Tanzania). Biogeosciences 4, 311–322. https://doi.org/10.5194/bg-4-311-2007.

Bunse, C., Pinhassi, J., 2017. Marine bacterioplankton seasonal succession dynamics. Trends Microbiol. 25, 494–505. https://doi.org/10.1016/j.tim.2016.12.013.

Buttigieg, P.L., Fadeev, E., Bienhold, C., Hehemann, L., Offre, P., Boetius, A., 2018. Marine microbes in 4D—using time series observation to assess the dynamics of the ocean microbiome and its links to ocean health. Curr. Opin. Microbiol. 43, 169–185. https://doi.org/10.1016/j.mib.2018.01.015.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Canuel, E.A., Hardison, A.K., 2016. Sources, ages, and alteration of organic matter in estuaries. Annu. Rev. Mar. Sci. 8, 409–434. https://doi.org/10.1146/annurev-marine-122414-034058.

Chen, X., Ye, Q., Sanders, C.J., Du, J., Zhang, J., 2020. Bacterial-derived nutrient and carbon source-sink behaviors in a sandy beach subterranean estuary. Mar. Pollut. Bull. 160, 111570. https://doi.org/10.1016/j.marpolbul.2020.111570.

Cunha, S.C., Ferreira, R., Marmelo, I., Vieira, L.R., Anacleto, P., Maulvault, A., Marques, A., Guilhermino, L., Fernandes, J.O., 2022. Occurrence and seasonal variation of several endocrine disruptor compounds (pesticides, bisphenols, musks and UV-filters) in water and sediments from the estuaries of Tagus and Douro Rivers (NE Atlantic Ocean coast). Sci. Total Environ. 838, 155814. https://doi.org/10.1016/j.scitotenv.2022.155814.

Dalsgaard, J., St, M., John, G., Kattner, D.Müller-Navarra, Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. Adv. Mar. Biol. 46, 225–340. https://doi.org/10.1016/S0065-2881(03)46005-7.

Dame, R., Chrzanowski, T., Bildstein, K., Kjerfve, B., McKellar, H., Nelson, D., Spurrier, J., Stancyk, S., Stevenson, H., Vernberg, J., 1986. The outwelling hypothesis and North inlet, South Carolina. Mar. Ecol. Prog. Ser. 33, 217–229.

Dang, H., Li, T., Chen, M., Huang, G., 2008. Cross-ocean distribution of Rhodobacterales bacteria as primary surface colonizers in temperate coastal marine waters. Appl. Environ. Microbiol. 74, 52–60. https://doi.org/10.1128/AEM.01400-07.

Depetris, P.J., Gaiero, D.M., Probst, J.L., Hartmann, J., Kempe, S., 2005. Biogeochemical output and typology of rivers draining Patagonia's Atlantic seaboard. J. Coast. Res. 21, 835–844. https://doi.org/10.2112/015-NIS.1.

Dhal, P.K., Kopprio, G.A., Gärdes, A., 2020. Insights on aquatic microbiome of the Indian Sundarbans mangrove areas. PLOS ONE 15, 0221543. https://doi.org/10.1371/journal.pone.0221543.

Diepenbroek, M., Glöckner, F.O., Grobe, P., Güntsch, A., Huber, R., König-Ries, B., Kostadinov, I., Nieschulze, J., Seeger, B., Tolksdorf, R., 2014. Towards an integrated biodiversity and ecological research data management and archiving platform: the German Federation for the Curation of Biological Data (GFBio). Informatik 2014 – Big Data Komplexität meistern. GI-Edition: Lecture Notes in Informatics (LNI) – Proceedings, Köllen Verlag, pp. 1711–1724.

Diner, R.E., Kaul, D., Rabines, A., Zheng, H., Steele, J.A., Griffith, J.F., Allen, A.E., Bernstein, H.C., Seyler, L.M., 2021. Pathogenic *Vibrio* species are associated with distinct environmental niches and planktonic taxa in Southern California (USA) aquatic microbiomes. mSystems 6. https://doi.org/10.1128/mSystems.00571-21 571-521.

Dittmar, T., Lara, R.J., 2001. Driving forces behind nutrient and organic matter dynamics in a mangrove tidal creek in North Brazil. Estuar. Coast. Shelf Sci. 52, 249–259. https://doi.org/10.1006/ecss.2000.0743.

Duarte, C.M., Borja, A., Carstensen, J., Elliott, M., Krause-Jensen, D., Marbà, N., 2015. Paradigms in the recovery of estuarine and coastal ecosystems. Estuar. Coasts 38, 1202–1212. https://doi.org/10.1007/s12237-013-9750-9.

Dutto, M.S., Kopprio, G.A., Hoffmeyer, M.S., Alonso, T.S., Graeve, M., Kattner, G., 2014. Planktonic trophic interactions in a human-impacted estuary of Argentina: a fatty acid marker approach. J. Plankton Res. 36, 776–787. https://doi.org/10.1093/plankt/fbu012.

Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The microbial engines that drive Earth's biogeochemical cycles. Science 320, 1034–1039. https://doi.org/10.1126/science.1153213.

Fernández-Gómez, B., Richter, M., Schüler, M., Pinhassi, J., Acinas, S.G., González, J.M., Pedrós-Alió, C., 2013. Ecology of marine Bacteroidetes: a comparative genomics approach. ISME J. 7, 1026–1037. https://doi.org/10.1038/ismej.2012.169.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5.

Fuhrman, J.A., Cram, J.A., Needham, D.M., 2015. Marine microbial community dynamics and their ecological interpretation. Nat. Rev. Microbiol. 13, 133–146. https://doi.org/10.1038/nrmicro3417.

Garzón-Cardona, J.E., Guinder, V.A., Alonso, C., Martínez, A.M., Pantoja-Gutiérrez, S., Kopprio, G.A., Krock, B., Lara, R.J., 2021. Chemically unidentified dissolved organic carbon: a pivotal piece for microbial activity in a productive area of the Northern Patagonian shelf. Mar. Environ. Res. 167, 105286. https://doi.org/10.1016/j.marenvres.2021.105286.

Ghosh, A., Bhadury, P., 2019. Exploring biogeographic patterns of bacterioplankton communities across global estuaries. MicrobiologyOpen 8, 00741. https://doi.org/10.1002/mbo3.741.

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl. Environ. Microbiol. 66, 5488–5491. https://doi.org/10.1128/AEM.66.12.5488-5491.2000.

Hansen, H.P., Koroleff, F., 1999. Determination of nutrients. In: Grasshoff, K., Kremling, K., Ehrhardt, M. (Eds.), Methods of Seawater Analysis, pp. 159–228 https://doi.org/10.1002/9783527613984.ch10.

Hsieh, T.C., Ma, K.H., Chao, A., 2019. iNEXT: iNterpolation and EXTrapolation for species diversity. R package version 2.0.19. http://chao.stat.nthu.edu.tw/blog/software-download/.

Huang, J., Zhu, J., Liu, S., Luo, Y., Zhao, R., Guo, F., Li, B., 2022. Estuarine salinity gradient governs sedimentary bacterial community but not antibiotic resistance gene profile. Sci. Total Environ. 806, 151390. https://doi.org/10.1016/j.scitotenv.2021.151390.

Inagaki, F., Takai, K., Nealson, K.H., Horikoshi, K., 2004. *Sulfurovum lithotrophicum* gen. nov., sp. nov., a novel sulfur-oxidizing chemolithoautotroph within the ε-Proteobacteria isolated from Okinawa Trough hydrothermal sediments. Int. J. Syst. Evol. Microbiol. 54, 1477–1482. https://doi.org/10.1099/ijs.0.03042-0.

Isaac, A., Francis, B., Amann, R.I., Amin, S.A., 2021. Tight adherence (Tad) pilus genes indicate putative niche differentiation in phytoplankton bloom associated Rhodobacterales. Front. Microbiol. 12, 718297. https://doi.org/10.3389/fmicb.2021.718297.

Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W., Kirchman, D.L., Weinbauer, M.G., Luo, T., Chen, F., Azam, F., 2010. Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat. Rev. Microbiol. 8, 593–599. https://doi.org/10.1038/nrmicro2386.

Jørgensen, B.B., Findlay, A.J., Pellerin, A., 2019. The biogeochemical sulfur cycle of marine sediments. Front. Microbiol. 10, 849. https://doi.org/10.3389/fmicb.2019.00849.

Kattner, G., Fricke, H.S.G., 1986. Simple gas-liquid chromatographic method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J. Chromatogr. A 361, 263–268. https://doi.org/10.1016/S0021-9673(01)86914-4.

Kattner, G., Hagen, W., 2009. Lipids in marine copepods: latitudinal characteristics and perspective to global warming. Lipids in Aquatic Ecosystems. Springer, pp. 257–280 https://doi.org/10.1007/978-0-387-89366-2_11.

Ke, Z., Tan, Y., Huang, L., Zhao, C., Jiang, X., 2017. Spatial distributions of $\delta^{13}C$, $\delta^{15}N$ and C/N ratios in suspended particulate organic matter of a bay under serious anthropogenic influences: Daya Bay, China. Mar. Pollut. Bull. 114, 183–191. https://doi.org/10.1016/j.marpolbul.2016.08.078.

Khojasteh, D., Glamore, W., Heimhuber, V., Felder, S., 2021. Sea level rise impacts on estuarine dynamics: a review. Sci. Total Environ. 780, 146470. https://doi.org/10.1016/j.scitotenv.2021.146470.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2012. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, 1. https://doi.org/10.1093/nar/gks808.

Kooij, D.v.d., Veenendaal, H.R., Italiaander, R., Mark, E.J.v.d., Dignum, M., Björkroth, J., 2018. Primary colonizing Betaproteobacteriales play a key role in the growth of *Legionella pneumophila* in biofilms on surfaces exposed to drinking water treated by slow sand filtration. Appl.Environ.Microbiol. 84. https://doi.org/10.1128/AEM.01732-18 1732-1718.

Kopprio, G.A., Biancalana, F., Fricke, A., Garzón Cardona, J.E., Martínez, A., Lara, R.J., 2015a. Global change effects on biogeochemical processes of Argentinian estuaries: an overview of vulnerabilities and ecohydrological adaptive outlooks. Mar. Pollut. Bull. 91, 554–562. https://doi.org/10.1016/j.marpolbul.2014.08.021.

Kopprio, G.A., Lara, R.J., Martínez, A., Fricke, A., Graeve, M., Kattner, G., 2015b. Stable isotope and fatty acid markers in plankton assemblages of a saline lake: seasonal trends and future scenario. J. Plankton Res. 37, 584–595. https://doi.org/10.1093/plankt/fbv023.

Kopprio, G.A., Dutto, M.S., Garzón Cardona, J.E., Gärdes, A., Lara, R.J., Graeve, M., 2018. Biogeochemical markers across a pollution gradient in a Patagonian estuary: a multidimensional approach of fatty acids and stable isotopes. Mar. Pollut. Bull. 137, 617–626. https://doi.org/10.1016/j.marpolbul.2018.10.059.

Kopprio, G.A., Neogi, S.B., Rashid, H., Alonso, C., Yamasaki, S., Koch, B.P., Gärdes, A., Lara, R.J., 2020. Vibrio and bacterial communities across a pollution gradient in the Bay of Bengal: unraveling their biogeochemical drivers. Front. Microbiol. 11, 594. https://doi.org/10.3389/fmicb.2020.00594.

Kopprio, G.A., Cuong, L.H., Luyen, N.D., Duc, T.M., Ha, T.H., Huong, L.M., Gärdes, A., 2021. Carrageenophyte-attached and planktonic bacterial communities in two distinct bays of Vietnam: eutrophication indicators and insights on ice-ice disease. Ecol. Indic. 121, 107067. https://doi.org/10.1016/j.ecolind.2020.107067.

Kristensen, E., Mangion, P., Tang, M., Flindt, M.-R., Holmer, M., Ulomi, S., 2011. Microbial carbon oxidation rates and pathways in sediments of two Tanzanian mangrove forests. Biogeochemistry 103, 143–158. https://doi.org/10.1007/s10533-010-9453-2.

Le Croizier, G., Schaal, G., Gallon, R., Fall, M., Le Grand, F., Munaron, J.-M., Rouget, M.-L., Machu, E., Le Loc'h, F., Laë, R., De Morais, L.T., 2016. Trophic ecology influence on metal bioaccumulation in marine fish: inference from stable isotope and fatty acid analyses. Sci. Total Environ. 573, 83–95. https://doi.org/10.1016/j.scitotenv.2016.08.035.

Liu, S.-H., Zeng, G.-M., Niu, Q.-Y., Liu, Y., Zhou, L., Jiang, L.-H., Tan, X.-F., Xu, P., Zhang, C., Cheng, M., 2017. Bioremediation mechanisms of combined pollution of PAHs and heavy metals by bacteria and fungi: a mini review. Bioresour. Technol. 224, 25–33. https://doi.org/10.1016/j.biortech.2016.11.095.

Lorenzen, C.J., 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343–346. https://doi.org/10.4319/lo.1967.12.2.0343.

Lu, M., Jones, S., McKinney, M., Wagner, R., Ahmad, S.M., Kandow, A., Donahoe, R., Lu, Y., 2022. Sources and composition of natural and anthropogenic hydrocarbons in sediments from an impacted estuary. Sci. Total Environ. 838, 155779. https://doi.org/10.1016/j.scitotenv.2022.155779.

Needham, D.M., Sachdeva, R., Fuhrman, J.A., 2017. Ecological dynamics and co-occurrence among marine phytoplankton, bacteria and myoviruses shows microdiversity matters. ISME J. 11, 1614–1629. https://doi.org/10.1038/ismej.2017.29.

Nyssen, F., Brey, T., Dauby, P., Graeve, M., 2005. Trophic position of Antarctic amphipods—enhanced analysis by a 2-dimensional biomarker assay. Mar. Ecol. Prog. Ser. 300, 135–145. https://doi.org/10.3354/meps300135.

Odum, E.P., 1968. A research challenge: evaluating the productivity of coastal and estuarine water. Proceedings of the Second Sea Grant Conference. University of Rhode Island Newport, pp. 63–64.

Odum, E.P., 2002. Tidal marshes as outwelling/pulsing systems. Concepts And Controversies in Tidal Marsh Ecology. Springer, pp. 3–7 https://doi.org/10.1007/0-306-47534-0_1.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., et al., 2019. vegan: Community Ecology Package. R package version 2.5-4. https://CRAN.R-project.org/package=vegan.

Perillo, V.L., Bohn, V.Y., Menéndez, M.C., Ronda, A.C., Vitale, A.J., Perillo, G.M.E., Piccolo, M.C., Cuadrado, D.G., 2022. Spatial and seasonal dynamics of phosphorous and physicochemical variables in the Negro River Estuary (Argentina): a preliminary approach. Environ. Sci. Pollut. Res. 29, 15490–15500. https://doi.org/10.1007/s11356-021-16890-z.

R Core Team, 2021. R: A Language And Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/.

Ray, R., Miyajima, T., Watanabe, A., Yoshikai, M., Ferrera, C.M., Orizar, I., Nakamura, T., San Diego-McGlone, M.L., Herrera, E.C., Nadaoka, K., 2021. Dissolved and particulate carbon export from a tropical mangrove-dominated riverine system. Limnol. Oceanogr. 66, 3944–3962. https://doi.org/10.1002/lno.11934.

Ruprecht, J.E., Birrer, S.C., Dafforn, K.A., Mitrovic, S.M., Crane, S.L., Johnston, E.L., Wemheuer, F., Navarro, A., Harrison, A.J., Turner, I.L., et al., 2021. Wastewater effluents cause microbial community shifts and change trophic status. Water Res. 200, 117206. https://doi.org/10.1016/j.watres.2021.117206.

Santos, I.R., Maher, D.T., Larkin, R., Webb, J.R., Sanders, C.J., 2019. Carbon outwelling and outgassing vs. burial in an estuarine tidal creek surrounded by mangrove and saltmarsh wetlands. Limnol. Oceanogr. 64, 996–1013. https://doi.org/10.1002/lno.11090.

Santos, I.R., Burdige, D.J., Jennerjahn, T.C., Bouillon, S., Cabral, A., Serrano, O., Wernberg, T., Filbee-Dexter, K., Guimond, J.A., Tamborski, J.J., 2021. The renaissance of Odum's outwelling hypothesis in 'Blue Carbon' science. Estuar. Coast. Shelf Sci. 255, 107361. https://doi.org/10.1016/j.ecss.2021.107361.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1995. Requirement criteria for essential fatty acids. J. Appl. Ichthyol. 11, 183–198. https://doi.org/10.1111/j.1439-0426.1995.tb00418.x.

Tang, X., Chao, J., Gong, Y., Wang, Y., Wilhelm, S.W., Gao, G., 2017. Spatiotemporal dynamics of bacterial community composition in large shallow eutrophic Lake Taihu: high overlap between free-living and particle-attached assemblages. Limnol. Oceanogr. 62, 1366–1382. https://doi.org/10.1002/lno.10502.

Valencia, B., Stukel, M.R., Allen, A.E., McCrow, J.P., Rabines, A., Palenik, B., Landry, M.R., 2021. Relating sinking and suspended microbial communities in the California Current Ecosystem: digestion resistance and the contributions of phytoplankton taxa to export. Environ. Microbiol. 23, 6734–6748. https://doi.org/10.1111/1462-2920.15736.

Wang, W., Tao, J., Liu, H., Li, P., Chen, S., Wang, P., Zhang, C., 2020. Contrasting bacterial and archaeal distributions reflecting different geochemical processes in a sediment core from the Pearl River Estuary. AMB Express 10, 16. https://doi.org/10.1186/s13568-020-0950-y.

Wang, J., Lu, J., Zhang, Y., Wu, J., Luo, Y., 2021. Unique bacterial community of the biofilm on microplastics in coastal water. Bull. Environ. Contam. Toxicol. 107, 597–601. https://doi.org/10.1007/s00128-020-02875-0.

Yilmaz, P., Kottmann, R., Field, D., Knight, R., Cole, J.R., Amaral-Zettler, L., Gilbert, J.A., Karsch-Mizrachi, I., Johnston, A., Cochrane, G., et al., 2011. Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MIxS) specifications. Nat. Biotechnol. 29, 415–420. https://doi.org/10.1038/nbt.1823.

Yilmaz, P., Yarza, P., Rapp, J.Z., Glöckner, F.O., 2016. Expanding the world of marine bacterial and archaeal clades. Front. Microbiol. 6, 1524. https://doi.org/10.3389/fmicb.2015.01524.

Zhang, Y., Sun, Y., Jiao, N., Stepanauskas, R., Luo, H., Voordouw, G., 2016. Ecological genomics of the uncultivated marine *Roseobacter* lineage CHAB-I-5. Appl. Environ. Microbiol. 82, 2100–2111. https://doi.org/10.1128/AEM.03678-15.

Zhao, Y., Gao, J., Wang, Z., Cui, Y., Zhang, Y., Dai, H., Li, D., 2022. Distinct bacterial communities and resistance genes enriched by triclocarban-contaminated polyethylene microplastics in antibiotics and heavy metals polluted sewage environment. Sci. Total Environ. 839, 156330. https://doi.org/10.1016/j.scitotenv.2022.156330.