



Seasonal patterns of bacterial communities in the coastal brackish sediments of the Gulf of Finland, Baltic Sea

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ABSTRACT

Coastal areas are critical in mitigating the impact of nutrient runoffs and downstream eutrophication of aquatic ecosystems. In the Gulf of Finland, the easternmost sub-basin of the Baltic Sea, seasonal and long-term oxygen depletion at the surface of the sediment feeds back the eutrophication loop by promoting the release of nutrients locked in the sediment matrix. In order to understand how the bacterial community responds to the seasonal variations, we sequenced ribosomal gene fragments from the top sediment layer at two coastal sites in southern Finland in spring, summer and late autumn during two consecutive years. Analysis of the samples collected at a shallow (11 m) and deep site (33 m) revealed that the overall community composition was rather constant over time with an extensive collection of shared operational taxonomic units (OTU) between sites. The dominant taxa were related to organo-heterotrophs and sulfate reducers and the variation in community structure was linked to the availability of organic matter in the surface sediment. *Proteobacteria* formed the most abundant and diverse group. The taxa characteristic of spring samples belonged primarily to *Actinobacteria*, possibly of fresh water origin and linked to humic carbon. Summer communities were characterized by an increase in the number of reads associated with heterotrophic bacteria such as *Bacteroidetes* which feed on labile organic matter from spring bloom. Taxa typical of autumn samples were linked to *Cyanobacteria* and other bloom-forming bacteria from the overlying water and to bacteria feeding on organic matter drifting from the phytal zone.

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1. Introduction

The wide distribution, large biomass and importance in biogeochemical transformations make microbes globally important in all ecosystems (Whitman et al., 1998; Falkowski et al., 2008). Hence, one of the central themes in molecular microbial ecology is the understanding of links between microorganisms and ecosystem processes. To this end, the availability of molecular techniques such as ribosomal gene sequencing has enabled tremendous upgrades to the knowledge of environmental microbes

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by allowing comprehensive description of natural communities (Lane et al., 1985; Weisburg et al., 1991; Hugenholtz and Pace, 1996). Further advances in molecular methods have shed light on the immense diversity of complex bacterial communities (Sogin et al., 2006) and have allowed the detailed description of geographic and temporal patterns created by microbial populations (Lauber et al., 2009; Gilbert et al., 2009, 2011). High-throughput molecular methods are thus ideal to study intricate microbial communities such as those found in marine sediments (Nealson, 1997; Lozupone and Knight, 2007; Baker et al., 2015).

The Baltic Sea is an interesting environment to investigate microbial processes because it comprises several overlapping and biologically-relevant gradients, such as salinity and oxygen gradients, across its various sub-basins (Conley et al., 2009). Added to this, there are intense seasonal variations in temperature due to its high latitudinal location. Within the Baltic, the Gulf of Finland is

characterized by severe eutrophication that culminates in sediment phosphorus release into the water column (Lehtoranta, 2003). It has been suggested that mitigating the impact of sediment nutrient release to the overlying water body will involve a reduction in the flow of labile organic matter to the sediment (Lehtoranta et al., 2008), which causes oxygen depletion upon mineralization. Because the seasonality in the Gulf of Finland is so polarized, with intense algal blooms in surface waters in spring and summer (Kanoshina et al., 2003) and ice cover during winter, there is a need to understand how the flow of energy and organic matter affect sediment microbial communities throughout the year. Most importantly, there is a need to examine the microbial communities in the coastal sediments because of their importance as an interface between the land and the sea (Hänninen et al., 2000; Lundberg et al., 2005) and act as filters that limit nutrients and organic matter transport towards the deeper and oxygen-poorer parts of the Gulf.

In the present study, the bacterial community composition at the surface of the sediment was investigated during different seasons at two archipelago sites influenced by the Karjaanjoki river estuary in southern Finland. Using a whole-community approach, the aim was to identify plausible spatial and temporal patterns in the bacterial community structure and link them to seasonal changes in environmental conditions. Based on data available in the literature, the bacterial community structure is linked with bacterial metabolism in order to gain a more holistic view of seasonal events. The 454 pyrosequencing technique (Margulies et al., 2005) targeting the V1 to V3 hypervariable regions of bacterial 16S rDNA genes was used to generate libraries of approx. 400 bp reads and compare samples collected during two consecutive years. Our data highlights the presence of reoccurring microbial assemblages that drive the main biogeochemical processes and organic matter mineralization in the surface sediment of this area.

2. Materials and methods

2.1. Study site, sampling and measurements

The sampling sites at Storfjärden (depth 33 m, 59°51.31' N, 23°18.81' E) and Muncken (depth 11 m, 59°51.14' N, 23°14.70' E) are located near the Tvärminne Research Station in southwestern Finland northern Gulf of Finland in the Baltic Sea. The two sites represent typical habitats in the outer archipelago zone, with Muncken as a shallow transportation channel between two islands and Storfjärden a local accumulation basin. Intact sediment samples were taken using a Gemax twin corer (9 cm Ø acrylic plastic cylinders). Three cores were taken at each sampling site and time.

The sampling sites were visited in April, August, and November 2008 and April, August and December 2009 (Table 1). Several subsamples (1 ml) were transferred from the sediment surface (0.0–0.5 cm) to micro centrifuge tubes using cut-off pipet tips and placed on dry ice until they could be stored in a –70 °C deep freezer (within two hours of sampling).

2.2. Physical and chemical analyses

Temperature and salinity were recorded using a conductivity, temperature, and depth device SiS CTD plus 100, SiS, Schwentimental, Germany Oxygen, NO_x (combined nitrite and nitrate), and NH₄⁺ concentrations in the bottom water were measured from water samples collected 5 cm above the sediment surface. Oxygen concentration was determined by titration. NH₄⁺ and NO_x were measured by ion chromatography (Lachat QuickChem, Hach Company, Loveland, Colorado, United States of America (U.S.A.)). Sediment organic content was determined as loss on ignition (LOI) by combusting desiccated subsamples (105 °C, 12 h) of the top 1 cm slice at 550 °C for 3 h. Diffusive oxygen uptake (DOU) was inferred from triplicate oxygen concentration profiles in subsamples (2.5 cm Ø acrylic cylinders) of the larger cores. The height of the sediment in the subsamples was at least 5 cm. Triplicate oxygen concentrations profiles were measured at *in situ* temperatures with a Clark-type O₂ microelectrode (Unisense OX-100, Unisense, Aarhus, Denmark) and DOU calculated with the following equation (adapted from Glud et al., 2003): $DOU (\text{mmol m}^{-2} \text{d}^{-1}) = D_o (dC/dZ)$, where D_o is the molecular diffusion coefficient of oxygen ($\text{cm}^2 \text{s}^{-1}$) at the given conditions (Table 1) and dC/dZ is the slope of the linear oxygen concentration gradient in the diffusive boundary layer at the top of the sediment.

2.3. DNA extraction

Once thawed, each 1 ml sediment sample was briefly centrifuged to remove water before processing. DNA was extracted using the method of Griffiths et al. (2000). Concentration and purity of the DNA were determined by spectrometry (Nanodrop, Thermo Scientific, Wilmington, DE, USA). For each sample, triplicate DNA extractions were combined to account for extraction bias.

2.4. Sample preparation and PCR

Fragments spanning the V1, V2 and V3 regions of bacterial 16S rRNA genes were amplified using the primer 27F (5'–AGAGTTT-GATCMTGGCTCAG – 3') (Wilson et al., 1990) and 536R (5' –GTATTACCGCGGCTGCTG – 3') (Lane et al., 1985). The initial PCR

Table 1
Description and environmental variables for each sample.

Site	Season	Date	z (m)	T (°C)	S	O ₂ (µM)	LOI (%DM)	DOU (mmol m ⁻² d ⁻¹)	NH ₄ ⁺ (µmol l ⁻¹)	NO _x (µmol l ⁻¹)
Storfjärden	Spring	Apr 08	33	2.4	5.4	410	17.7	4.5 ± 0.4	2.5	2.6
		Apr 09		0.8	5.3	393	13.2	6.7 ± 0.2	0.1	6.9
	Summer	Aug 08		16.2	5.9	205	21.0	15.4 ± 1.6	6.2	1.0
		Aug 09		5.8	6.6	178	31.6	16.8 ± 2.2	1.8	3.5
	Autumn	Nov 08		6.4	6.5	424	10.8	6.8 ± 0.4	0.3	5.3
		Dec 09		7.0	6.3	290	8.6	5.2 ± 1.0	1.4	4.0
Muncken	Spring	Apr 08	11	2.7	5.3	410	7.5	4.3 ± 0.4	0.0	1.1
		Apr 09		1.7	5.1	424	5.2	5.8 ± 0.1	0.2	4.0
	Summer	Aug 08		16.6	5.9	223	9.3	11.5 ± 0.8	4.9	0.7
		Aug 09		14.3	5.8	267	7.8	24.6 ± 0.4	1.4	2.2
	Autumn	Nov 08		6.3	6.5	360	7.6	8.1 ± 0.5	0.2	5.4
		Dec 09		5.5	4.5	360	8.6	5.3 ± 0.3	0.1	4.3

S: salinity.

LOI: loss on ignition.

DOU: diffusive oxygen utilization.

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