

Sediment Bacterial Communities in Nutrient Cycling and in the History of the Baltic Sea

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LIST OF ORIGINAL PUBLICATIONS

- I** **Hanna Sinkko**, Kaarina Lukkari*, Abdullahi S. Jama* , Leila M. Sihvonen, Kaarina Sivonen, Mirja Leivuori , Matias Rantanen , Lars Paulin , Christina Lyra (2011) Phosphorus Chemistry and Bacterial Community Composition Interact in Brackish Sediments Receiving Agricultural Discharges. PLoS ONE 6: e21555. 10.1371/journal.pone.0021555.

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THE AUTHOR'S CONTRIBUTION

- I** Hanna Sinkko participated in the design of the study, conducted some of the laboratory analyses, analysed the data, interpreted the results and wrote the manuscript.
- II** Hanna Sinkko participated in the design of the study, conducted most of the laboratory analyses, analysed the data, interpreted the results and wrote the manuscript.
- III** Hanna Sinkko conducted some of the laboratory analyses, analysed and interpreted part of the statistical analysis and contributed to the writing of the manuscript.

ABBREVIATIONS

Anammox	Anaerobic ammonium oxidation with nitrite
AOM	Anaerobic oxidation of methane
ATP	Adenosine triphosphate
bp	Base pairs
CAP	Constrained analysis of principal coordinates
DNA	Deoxyribonucleic acid
DNRA	Dissimilatory nitrate/nitrite reduction to ammonium
GOF	Gulf of Finland
HCl	Hydrochloric acid
NaBD	Sodium dithionite in sodium bicarbonate
NaOH	Sodium hydroxide
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
T-RFLP	Terminal restriction fragment length polymorphism
T-RF	Terminal restriction fragment
cal yr. BP	Calendar years before the present (1950)

ABSTRACT

The continental Baltic Sea has been influenced by land uplift and sea-level rises, resulting in many fresh- and brackish, as well as oxic and hypoxic water phases. These features, the complex flow of saline water through the Danish Straits and the stratified water column, make the Baltic Sea naturally hypoxic. Recently, agriculture-driven eutrophication has caused spreading of hypoxic areas in the Baltic Sea and internal feedback mechanisms, such as the release of phosphorus from sediment to water, which sustain hypoxia.

Bacteria may participate in release of nutrients, such as phosphorus and nitrogen, either directly e.g. by mineralizing organic matter or indirectly by altering the sediment's ability to retain nutrients. Although the chemical background of hypoxia-induced phosphorus release, especially from iron oxyhydroxides is widely studied, less work has been done to assess how sediment bacterial communities affect the release of phosphorus in hypoxic aquatic systems.

In deep anoxic sediments, most microbes are inactive, dormant, dead or only their DNA is preserved, thus representing mainly the remains of the preceding sedimentary communities. However, it is uncertain whether the relationships of bacterial communities in sediment simply reflect organic matter mineralization or the past historical phases of the sea basin.

This work investigated current horizontal basin-scale and vertical environmental changes and variation in bacterial communities in the northeastern Baltic Sea (the Archipelago Sea and the Gulf of Finland (GOF)) along the gradients of different chemical forms of phosphorus and elements related to its cycling, as well as organic matter in the sediment. The associations of bacteria with sediment chemistry were studied from the standpoint of nutrient recycling. The study also aimed to elucidate whether the bacterial communities reflect the agriculture-driven eutrophication and progressive hypoxia in the Baltic Sea.

Downcore changes in the bacterial communities were investigated in a sedimentary record covering the last 8000 years of the central GOF to determine whether mineralization shifts and historical sea phases, such as sea-level rise culminations, can be inferred from bacterial community data. Bacterial community data were also evaluated to determine whether they could be used as a palaeomicrobiological tool.

Current and historical environments were studied using terminal restriction fragment length polymorphism (T-RFLP) analysis. The data obtained were examined with sediment composition and dating, as well as with the sediment chemical, spatial and other properties, using multivariate statistics. In addition, sequenced 16S rRNA genes of the deep sediment core were analysed by phylogenetics.

This study showed that the bacterial community composition changed mainly along the gradients of chemical forms of phosphorus and organic matter. Sulphate-reducing bacteria predominated in the hypoxic open-sea surface sediments and correlated with organic

phosphorus and nitrogen as well as reducible manganese. In the estuary, they correlated with redox-sensitive iron and the phosphorus bound to it. The correlations indicated that sulphate reducers participated in the release of phosphorus, e.g. by producing sulphide, which captures iron, or by reducing iron oxyhydroxides. The predominance of sulphate reducers, even in the sediment surface of most areas, suggests that eutrophication-driven hypoxia has progressed in the late phase where bacteria process most of the benthic energy.

In the most organic-rich surface sediments overlain by the oxic bottom water, *Flavo*-, *Sphingo*-, *Alphaproteo*- and *Gammaproteobacteria* prevailed and correlated with organic carbon, nitrogen and phosphorus. The correlations suggest that these bacteria were important in the initial degradation of organic matter and promoted the release of nutrients from organic compounds.

Bacterial community composition also varied vertically. Bacteria belonging to the family *Anaerolineaceae* (phylum *Chloroflexi*) increased downwards in the uppermost 25 cm of sediment. Based on the 16S rRNA sequences, *Chloroflexi* was common throughout the sediment core spanning the 8000-year history of the Baltic Sea. The results suggest that these bacteria were important in terminal mineralization and that the Baltic Sea has been relatively organic-rich throughout its history.

The heterogeneity of the bacterial communities, based on Bray-Curtis dissimilarity of the terminal restriction fragments and their relative abundance in sediment samples, varied nonlinearly with depth. From the surface down to 306 cm, the heterogeneity decreased and reached a plateau in the 4500-year-old sediment, suggesting the downcore mineralization of organic matter. A sudden increase in the heterogeneity of Litorina Sea sediments from depths of 388–422 cm was explained by salinity changes and thus suggests that a salinity maximum occurred in the central GOF approximately 6200–6600 years ago.

The bacterial communities of the Early Litorina and Late Litorina Sea layers were separated from the communities of the Litorina Sea layers, which were associated with elevated concentrations of uranium and strontium trace elements, used as palaeooxygen and palaeosalinity proxies. The results suggest that salinity was the major parameter affecting the bacterial communities. Thorough analysis of sediment core spanning the latest 8000 years suggested that the sediment layers were historically comparable below the plateau of mineralization.

The study showed that entire bacterial communities reflected both ancient and contemporary events, such as salinity changes and ongoing mineralization processes. Knowledge of sediment bacterial communities contributing to nutrient cycling should be taken in consideration in managing the eutrophication of coastal marine ecosystems. Bacterial community data may be used as an additional tool in ocean-drilling projects that aim to detect mineralization plateaus by determining historically comparable portions of sediment cores and historical events, such as sea-level rise culminations.

1 THE REVIEW OF THE LITERATURE

The following introduction briefly reviews the basics of the Baltic Sea and the central issues concerning its eutrophic state, as well as the consequences of the eutrophication. In addition, the history of the Baltic Sea will be briefly introduced. The introduction also describes the functions of sediment bacterial communities in biogeochemical nutrient cycling, especially in the cycling of phosphorus, and touches on the role of bacteria in aquatic palaeoenvironments, emphasizing these topics from the standpoint of the Baltic Sea. To give an idea of how sediment bacterial communities participate in nutrient cycling, their role in organic matter mineralization and nutrient fluxes, as well as factors that affect bacterial variation in the sediment, are presented.

1.1 The Baltic Sea

The present Baltic Sea (415 000 km²) is a shallow (average depth 60 m), brackish, semi-enclosed continental sea with a watershed populated by over 85 million people. It is composed of a series of basins, of which the main basins are The Bothnian Bay, Bothnian Sea, the Gulfs of Finland (GOF) and Riga, and the Baltic Proper. The drainage area is approximately four times larger than the sea itself, which causes a surplus of riverine freshwater input to the basin (Ehlin, 1981; Winterhalter *et al.*, 1981; Helcom, 2007). Inflow of water from the North Sea, including episodic major inflows, through the shallow and narrow Danish Belts and the Swedish Sound from Skagerrak/Kattegat is the source of saline water (Ehlin, 1981; Helcom, 2007). The inflow of riverine freshwater from the drainage area and the saline water via The Danish Straits results in a horizontal salinity gradient of the surface water from more saline southern (app. 8–10) towards less saline central (app. 7–8 in the Baltic Proper) and northern Baltic Sea (app. 3–5 in the GOF, Bothnian Sea and Bothnian Bay) (Zillen *et al.*, 2008). The inflow of fresh surface water and denser saline water also results in a stratified water column with a halocline, where the salinity steeply increases, usually at depths of 40–80 m (Kullenberg, 1981).

1.2 Eutrophication of the Baltic Sea and its consequences

Nutrients such as phosphorus and nitrogen are constituents of many macromolecules in cells and are essential for microbes, including primary and secondary producers, in aquatic systems. However, when the normal uptake capacity of nutrients is exceeded in aquatic systems, eutrophication develops. Geological records indicate that the Baltic Sea was an oligotrophic clear-water body in the last century. However, the semienclosed Baltic Sea basin with long residence times of water and nutrients is sensitive to external nutrient loading and the present Baltic Sea is severely eutrophic. The eutrophication is believed to have developed due to nutrient inputs, largely of nitrogen and phosphorus from anthropogenic sources, of which agriculture accounts for the most part (Zillen *et al.*, 2008). Indeed, in the Baltic Sea and worldwide, the riverine nutrient loading of phosphorus and nitrogen has been increased by 18–180 and 6–50 times, respectively, from the turn of the last century to the present (Conley, 2000).

The present Baltic Sea is sensitive to both nitrogen and phosphorus (Conley *et al.*, 2009). Nitrogen boosts the spring blooms of phytoplankton, which settle and enhance the oxygen consumption in the bottom areas, resulting in increased phosphorus availability in the water column. This, in turn, accelerates growth of nitrogen-fixing cyanobacteria blooms (Conley *et al.*, 2002).

1.2.1 Hypoxia

Hypoxia is one of the detrimental consequences of eutrophication. The term hypoxia is used to describe aquatic environments in which the concentration of dissolved oxygen is decreased to the level that is harmful to organisms and causes detrimental responses in ecosystems. With a consensus, hypoxia has been defined as $< 2 \text{ mg l}^{-1}$ or part per million (ppm) ($= 1.4 \text{ ml l}^{-1}$) dissolved oxygen (Renaud, 1986; Rabalais *et al.*, 2002).

Hypoxia occurs in productive aquatic systems when high amounts of organic matter, e.g. phytodetritus, settles in the bottom water below the halocline and ultimately onto the seafloor, where microbes and deposit-feeding benthic organisms degrade the organic matter and consume oxygen. Although hypoxia occurs naturally in the deepest parts of the Baltic Sea due to the halocline, which hinders the vertical mixing of the water column, hypoxic areas in the Baltic as well as worldwide have noticeably increased since the 1960s, hand in hand with increasing agricultural activities (Karlson *et al.*, 2002; Diaz & Rosenberg, 2008). In the Baltic, the distribution of laminated sediments, which indicates bottom-water hypoxia, increased approximately fourfold from the 1960s to the 1980s (up to $70\,000 \text{ km}^2$) (Jonsson *et al.*, 1990) and during the millennium the average hypoxic area covered around 10% ($41\,000 \text{ km}^2$) of the total Baltic Sea area annually (Conley *et al.*, 2002). Recently, Conley *et al.* (2011) reported that hypoxia of the shallow Baltic coastal zone is increasing.

Hypoxia results in severe ecosystem disturbances (Diaz & Rosenberg, 1995) and alters the biogeochemical cycles of nutrients such as phosphorus and nitrogen (Kemp *et al.*, 2005; Vahtera *et al.*, 2007; Conley *et al.*, 2009). The lack of oxygen decrease the benthic macrofauna, which interrupts the food webs and influences fish habitats and fisheries (Rabalais *et al.*, 2002), as well as causes the loss of biomass due to lowered secondary production. In fact, during the persistent hypoxia, the upward flow of energy in the food chain is interrupted and, instead, is directed downwards to the sediment microbes (Diaz & Rosenberg, 2008). These hypoxic zones with severe disturbance of benthic macrofauna and fisheries are called dead zones (Rabalais *et al.*, 2002), of which the Baltic Sea was estimated to be the largest in 2008. Dead zones are currently widespread and the hypoxia-related ecosystem-level changes are showing global significance, with over 400 sites suffering from their effects (Diaz & Rosenberg, 2008).

1.2.2 Phosphorus in sediments

Due to decades of heavy nutrient loading and the consequent productivity of the water column, Baltic Sea sediments are rich in nutrients such as phosphorus, which, if recycled back to the water column, reinforces the eutrophication. However, not all forms of

phosphorus accelerate eutrophication (Table 1), since the bioavailability and degradability of different chemical forms of phosphorus vary with the environment. Phosphorus compounds in sediments can be coarsely divided into mobile (reactive, labile) and immobile (nonreactive, recalcitrant) fractions, based on their potential for release from the sediment to the water column. This division is also dependent on the extraction method used for distinguishing the chemical forms of phosphorus.

In the recipient body of water, the forms of phosphorus are dependent on the amount and quality of external loading and on the sedimentation environment. For example, in the estuary environment where riverine freshwater meets the saline seawater, humic and particulate material aggregates and settles, also depositing phosphorus bound to their iron (Fe) oxyhydroxide coatings and Fe-phosphorus complexes (Sholkovitz, 1976; Boyle *et al.*, 1977). For these reasons, estuarial and coastal sediments can be rich in Fe-bound phosphorus, which can be released to dissolved form in case of oxygen deficiency and be bioavailable in the sediment. In addition to the concentration of Fe and oxygen, the potential bioavailability of phosphorus in the sediment is also dependent on other elements affecting phosphorus binding (see the next paragraph), as well as on the ratio of terrestrial and marine organic matter reaching the seafloor.

1.2.2.1 Phosphorus-binding mechanisms and the eutrophication-driven regeneration of phosphorus and nitrogen

One of the most detrimental effects of eutrophication and consequent bottom-water hypoxia is the release of deposited nutrients from sediment to pore water, from which they diffuse or are mixed in the water column (internal nutrient loading) and reinforce the eutrophication (Conley, 2000; Rabalais *et al.*, 2002). The magnitude of the internal phosphorus loading is most importantly regulated by oxygen conditions (e.g. Mortimer, 1941, 1942), but also by pH (Hingston *et al.*, 1967, 1972), ionic strength and temperature (Jensen & Andersen, 1992; YliHalla & Hartikainen, 1996), resuspension, and biological as well as microbial processes such as sulphate reduction (Caraco *et al.*, 1989). It has been suggested that in the Baltic Sea, the internal phosphorus loading at any given moment is an order of magnitude higher than the external phosphorus loading (Conley *et al.*, 2009).

Under the poor oxygen (and nitrate) conditions in the bottom water and sediment, phosphorus is released as a phosphate or as organic phosphorus compounds from ferric oxyhydroxides, since iron is reduced to the ferrous form, and oxyhydroxides are disrupted and can no longer bind phosphorus (Mortimer, 1941, 1942; Froelich *et al.*, 1979). Since reduced sediment surfaces cannot bind the phosphate that is released from organic compounds by microbial degradation (Mortimer, 1971), hypoxia in the sediment also enhances the mineralization-induced phosphorus release from organic compounds (Gächter *et al.*, 1988; Ingall *et al.*, 1993; Ingall & Jahnke, 1994, 1997; Jilbert *et al.*, 2011). Although phosphorus is preferentially bound to amorphous Fe oxides, it can also be associated with manganese (Mn) oxides under oxic conditions and be released in anoxic/hypoxic conditions. Mn compounds, however, have lower capacities for binding

phosphorus than those of Fe (Christensen *et al.*, 1997). In addition, phosphorus can be bound to aluminium (Al) and calcium (Ca) compounds (Hingston *et al.*, 1972; McLaughlin *et al.*, 1981; Bohn *et al.*, 1985), but the relatively immobile forms of Ca-bound phosphorus, such as apatite, and Al oxide-bound phosphorus are not sensitive to reduction and can thus also retain phosphorus in sediment also under anoxic conditions. However, they are sensitive to pH (Hingston *et al.*, 1972; Bohn *et al.*, 1985) and thus can be affected by bacterial activity. Furthermore, silicate competes with phosphate for the sorption sites on metal oxyhydroxides (Hingston *et al.*, 1967; Ryden *et al.*, 1987) and can therefore increase the release of phosphorus at organic-rich sites (Hartikainen *et al.*, 1996).

The lack of oxygen also influences nitrogen cycling and can result in the accumulation of ammonium in the sediment. When oxygen is present, nitrifying bacteria oxidize a large part of the ammonium to nitrate, which is further removed from aquatic systems as N₂ gas by denitrification. However, under anoxic conditions, oxygen-dependent nitrification is disturbed and hydrogen sulphide (H₂S), produced by sulphate-reducing bacteria, inhibits nitrifying bacteria. Furthermore, ammonium is accumulated as a result of dissimilatory nitrate reduction to ammonium (DNRA) (Kemp *et al.*, 2005). However, the role of anaerobic oxidation of ammonium to N₂ (anammox), which removes nitrogen from aquatic systems, is not well quantified (Conley *et al.*, 2009).

1.3 History of the Baltic Sea basin and the Baltic Sea

The postglacial history of the Baltic Sea basin involves a series of transgressions (sea-level rises) and regressions, followed by interactions, e.g. of land subsidence and uplifts, glacial water fluxes and phases of sea-level rise in both the Baltic Sea basin and the global ocean (Björck, 1995; Zillen *et al.*, 2008). Sea-level rises in the Baltic regulate the connection between the Baltic basin and the global ocean (Zillen *et al.*, 2008). The resulting connections to or isolations from the global ocean caused water salinity changes that characterized the main postglacial phases of the Baltic Sea basin, comprised of, by tradition, the freshwater Baltic Ice Lake (ca. 16 000 calendar years before the present (cal. yr BP), the partly brackish Yoldia Sea (11 600–10 700 cal. yr BP), the freshwater Ancylus Lake (10 700–10 000 cal. yr BP) and the Litorina Sea (ca. 10 000 cal. yr BP to the present)(Björck, 1995; Andren *et al.*, 2000; Berglund *et al.*, 2005).

The Litorina Sea, with three subphases (Zillen *et al.*, 2008), covers the history of the Baltic Sea. The Early (or Initial) Litorina Sea (or the Mastogloia Sea, ca. 10 000–7 400 cal. yr BP), which was the transition from the Ancylus Lake to the brackish water phase, was due to land uplift in the northern parts of the basin and subsequent inflow of saltwater from the south. Marine influxes via the broadened Öresund Strait evolved into the more saline Litorina Sea phase (ca. 8 500–3 500 cal. yr BP) and the formation of a permanent halocline. At this time, the surface water of the Baltic Proper had a higher salinity (10–15) than the present (7–8). For the GOF, the estimate was four units higher than the present.

At the transition of the middle Litorina Sea phase, precipitation of organic matter could be detected in the sediment record (Zillen *et al.*, 2008). A previous study from the Baltic

Proper (Sohlenius *et al.*, 1996) showed that increased salinity in the Early Litorina Sea contributed to the formation of a halocline and consequently nutrient enrichment in the bottom water. Later, due to replacement of the bottom water by brackish water via the Danish Straits, nutrients rose to the photic zone and accelerated primary production, which caused anoxia in the sediment during the Litorina Sea, 6 500–4 500 years ago and preservation of organic matter. The Litorina Sea phase was followed by a brackish Late Litorina Sea phase (Zillen & Conley, 2010).

1.3.1 Palaeoenvironmental methods

The environmental history of the sea basin can be studied, based on the sedimentary record. The history of the Baltic Sea recorded in sediment cores has been determined, e.g. using plant macrofossils or diatoms (Sohlenius *et al.*, 1996; Berglund *et al.*, 2005), lithostratigraphical features such as organic-rich laminae (Zillen *et al.*, 2008) and trace elements (Sternbeck *et al.*, 2000). Organic-rich laminae are an indication of hypoxia, since they are formed in the absence of bioturbation by benthic animals, of which the majority cannot live under hypoxic conditions (Zillen *et al.*, 2008). Trace elements can also be used as a proxy of hypoxia since, many of them such as uranium (U) precipitate in sediments under reduced conditions (Nath *et al.*, 1997; Zheng *et al.*, 2003). Some trace elements, such as chromium (Cr) accumulate in sediments with organic matter (Sohlenius *et al.*, 1996; Sternbeck *et al.*, 2000). In addition, strontium (Sr) and U in sediment are used as indicators of palaeosalinity of seawater (Lopez-Buendia *et al.*, 1999; Vincent *et al.*, 2006). The best method to date for estimating salinity changes is the diatom record, which, however declines due to several environmental factors, such as bioturbation (Flower, 1993). Hence, there is a need for new tools to reconstruct historical sediments.

1.3.1.1 Sediment extracellular DNA as a reserve of prokaryotic sequence information

As part of the sedimentary organic matter, extracellular DNA comprises most of the total DNA pool in sediments and is well preserved both in the surface as well as subsurface sediments, particularly under anoxic conditions. It escapes from DNase activities and thus accumulates in deeper sediments (Corinaldesi *et al.*, 2011). For instance, fossil DNA sequences, even from up to 217 000-year-old sapropels, could be amplified (Coolen & Overmann, 2007). Therefore, the total DNA pool represents a large reserve of prokaryotic DNA sequences that can be used to study prokaryotes, either in recently deposited sediments or in palaeoenvironments (Inagaki *et al.*, 2006). However, whether historical environmental changes in a sea basin can be inferred from DNA-based bacterial community composition data is a currently open question.

1.4 Sediment bacteria

Before the 1950s, when high numbers of viable bacteria were found in the first samples of sediments deeper than 10 000 m, the marine seabed was considered to be biologically inert. The inertia was assumed, owing to extreme conditions such as high pressure, darkness (no photosynthetic production) and low temperatures that are present at the seafloor. During recent decades, researchers have shown that sediments, even in the deep seas, are highly diverse and rich in microbial communities (Jørgensen & Boetius, 2007) and that sediments

play a critical role in aquatic ecosystems, particularly in freshwaters and coastal marine seas, where the water column is relatively shallow (Nedwell & Brown, 1982).

Generally, bacteria can account for 90–99% of the biomass in sediments and are the dominant group of organisms (Nealson, 1997). For example, up to 1.63×10^9 bacterial cells ml^{-1} were detected in a surface sediment sample from the Baltic Sea (Ekebom, 1999). Bacteria also predominate in terms of metabolic potential (Nealson, 1997), since they effectively mineralize organic matter in sediment, both aerobically and anaerobically (Suzumura & Kamatani, 1995; Burdige, 2007) and participate in biogeochemical processes, such as phosphorus and nitrogen cycling (Gächter & Meyer, 1993; Thamdrup & Dalsgaard, 2008). Recent reports showed that in contrast to microbial communities in seawater, microbial communities in sediment at the same location have wider capacity to degrade high-molecular-weight substrates and hydrolyse a broader range of substrates (Arnosti, 2011).

1.4.1 Role of sediment bacteria in phosphorus cycling

Traditionally, bacteria have been considered to play only an indirect role in the release of phosphorus by altering the ability of sediment to retain nutrients, e.g. by consuming oxygen, nitrate, Mn and Fe oxides or producing sulphide, all of which increase the release of Fe-bound phosphorus. Sulphide forms ferrosulphides with Fe and therefore Fe is not available for binding phosphorus in oxidized sediment layers (Berner, 1970). However, microbial processes are important in mobilization and fixation of phosphorus in sediment (Gächter & Meyer, 1993)(Table1), although the role of bacteria in phosphorus cycling has been poorly quantified. Sterilization of sediments decreased their sorption capacity for soluble reactive phosphorus (Gächter *et al.*, 1988). In addition, there are indications that bacteria can utilize even recalcitrant phosphorus forms (Benitez-Nelson *et al.*, 2004), which can enable the mobilization of immobile phosphorus.

Bacteria can affect the phosphorus flux directly by releasing phosphorus from organic compounds to the interstitial water in decomposition processes (Berner & Rao, 1994; Hupfer & Lewandowski, 2008; Jilbert *et al.*, 2011) or releasing phosphate form of polyphosphates (Gächter *et al.*, 1988; Gächter & Meyer, 1993; Hupfer *et al.*, 2007). Bacteria accumulate phosphate under oxic conditions as polyphosphates, which are hydrolysed intracellularly in anoxia for the synthesis of ATP and simultaneously phosphate also diffuses outside the cell. Several studies indicate that polyphosphate can constitute substantial proportions of total phosphorus in the uppermost sediment and polyphosphate-accumulating bacteria may be of high ecological importance (Hupfer *et al.*, 2007).

Gächter *et al.* (1988) noted that fixation and release of phosphorus by bacteria was redox-dependent. Many studies later suggested that bacteria regenerate reactive (dissolved) phosphorus from organic matter more effectively under anoxic than oxic conditions (Ingall & Jahnke, 1997; Jilbert *et al.*, 2011). In decomposition processes, phosphorus is preferentially remineralized with respect to carbon and nitrogen, and its rate increases

under reduced conditions (Ingall *et al.*, 1993; Ingall & Jahnke, 1994; Jilbert *et al.*, 2011). Interestingly, a recent investigation of organic-rich and anoxic sediments showed that under carbon limitation, bacteria use phosphatases to make the carbon moiety more accessible and phosphate is released (Steenbergh *et al.*, 2011). Thus a limited availability of carbon can increase the flux of phosphorus. Under oxic conditions, in contrast, bacteria can apparently convert a large fraction of assimilated phosphorus to the refractory form (Ingall & Jahnke, 1997), as noted also in oligotrophic lake sediments (Gächter & Meyer, 1993).

Regeneration or burial of organic phosphorus is complex. Whether bacteria release phosphorus when they mineralize organic detritus is dependent on their need to fulfil a phosphorus requirement and several other factors such as the C:P ratio of organic matter. Jilbert *et al.* (2011) showed that although the regeneration of phosphorus from organic matter increased during hypoxia, burial of organic phosphorus also increased during prolonged (multidecadal) and expanded hypoxia, due to higher net burial rates of organic matter. Under hypoxic conditions, supersaturation of phosphate by polyphosphate-accumulating bacteria can lead to the precipitation of authigenic phosphorus minerals (Ingall & Jahnke, 1997; Hupfer *et al.*, 2007).

1.4.2 Organic matter mineralization - energy for sediment bacteria

Bacteria gain energy and carbon in marine sediments, either by oxidizing organic compounds or using chemical energy (hydrogen, methane, hydrogen sulphide and Fe), transported upwards from the subsurface by geological processes (Jørgensen & Boetius, 2007). Organic compounds settle into the sediment as particulate organic matter or are synthesized in sediments (authigenic). Basically, bacteria transform organic matter back to CO₂ and harbour a fraction of the carbon as cellular biomass. The residual fraction is buried in the sediments. However, the organic matter buried is also very slowly remineralized (Jørgensen, 2011).

In the shallow and productive continental seas that receive riverine loading and runoff from land, the sediments are rich in organic matter and are the place where the most of the marine benthic carbon is mineralized. In addition to the distance from land, the quality and quantity of organic matter reaching the seafloor is dependent on the sedimentation rate and depth, as well as the productivity of the water column, where organic matter from both terrestrial and marine sources is exposed to microbial degradation (Jørgensen, 1982; Hartnett *et al.*, 1998).

Oxygen penetrates only a few millimetres into the organic-rich sediments (Revsbech *et al.*, 1980), whereas in the deep-sea sediments the oxic seafloor typically extends to 1 m below the sediment-water interface (Jørgensen & Boetius, 2007), or even deeper (Jørgensen, 2012). Since most of the sediments in this study contained high amounts of organic matter and were hypoxic, anaerobic mineralization is of importance here. In contrast to aerobic organisms, which are able to completely oxidize organic carbon, anaerobic decay occurs stepwise by several types of bacteria (Kristensen *et al.*, 1994). Generally, complex high-

molecular-weight organic macromolecules are first hydrolysed to smaller moieties, which are then fermented to fatty acids, mainly to acetate (Kristensen *et al.*, 1994) and dissolved inorganic nutrients.

Anaerobic or facultative aerobic bacteria largely oxidize the organic compounds by reducing inorganic electron acceptors in a sequence of Mn oxides, nitrate, Fe oxides, sulphate, and ultimately CO₂ (Canfield *et al.*, 1993a). Reduced electron acceptors, yielded via respiration or fermentation, are reoxidized by chemolithotrophs. The sediments are thus redox-stratified environments. Below the oxic layer, an anoxic but oxidized zone with nitrate, Mn and Fe oxides exists and is followed by the sulphidic zone and ultimately the sulphate-methane-transition as well as the methane zone. The higher the organic input into the sediment, the closer to the surface is the sulphidic zone (Jørgensen, 1982; Nedwell & Brown, 1982).

The biogeochemical stratification and mineralization of organic matter can be influenced by sediment-reworking processes, e.g. by bioturbating benthic animals in the bottom areas where the oxygen concentration is high enough for the success of the eukaryotic benthos. The benthos burrows in the sediment, which mixes the vertical layers and brings the oxygen to deeper anoxic layers (Kristensen *et al.*, 1994).

Due to the vertical stratification of electron acceptors, the bacterial community composition also varies with depth. For instance, Edlund *et al.* (2008) found that representatives of the phylum *Planctomycetes* and class *Betaproteobacteria*, linked with anammox, were redox-specific. Shubenkova *et al.* (2010), who studied the microbial community of reduced pockmark sediments of the southern Baltic Sea, found that Eubacteria predominated in the uppermost 10 cm, but *Archaea* increased in the 10- to 30-cm layers. However, the vertical variation in bacterial communities is far less studied than the biogeochemical redox stratification.

In general, species richness and diversity are expected to decrease with sediment depth, due to poorer substrate quality and quantity (Bowman & McCuaig, 2003), although contrasting trends have been observed (Wu *et al.*, 2011). Active bacteria decrease downwards and in the deep sediments covering timescales from hundreds to hundreds of thousands of years, bacteria are largely inactive, dormant or dead, and are termed as necromass. Recent investigations showed that the necromass comprises 96% of the amino-acid carbon, whereas vegetative cells and endospores comprise together 4% of the sedimentary amino-acid carbon (Langerhuus *et al.*, 2012; Lomstein *et al.*, 2012) and of the total organic matter of bacterial origin (Lomstein *et al.*, 2006). Microbial biomass turnover times (or bacterial generation times) can be hundreds to thousands of years, and the necromass is recycled even more slowly, during hundreds of thousands of years (Langerhuus *et al.*, 2012; Lomstein *et al.*, 2012).

Horizontally, it has been estimated that most part of the marine carbon in organic-rich coastal areas is mineralized via different anaerobic processes (Canfield *et al.*, 1993a; Canfield *et al.*, 2005). Of all the anaerobic processes, sulphate reduction is substantial,

since sulphate-reducing bacteria can mineralize half of the organic matter in coastal marine areas (Jørgensen, 1982; Canfield, 2005). Since methanogenesis and sulphate reduction compete with the electron donors, methanogenesis predominates in low-sulphate environments. In the brackish Baltic Sea, both processes are significant. For instance, high rates of sulphate reduction and methanogenesis were observed recently (Pimenov *et al.*, 2008, 2010, 2012). Among the anaerobic processes, reduction of Mn and Fe can also be significant in the Fe- or Mn-rich sediments, such as in the Baltic-North Sea transition (Canfield *et al.*, 1993b; Jensen *et al.*, 2003) or in the Barents Sea (Vandieken *et al.*, 2006).

1.4.3 Variation in sediment bacterial community composition

Due to the high levels of environmental heterogeneity, bacterial community compositions vary greatly in different sediments. The factors that shape the bacterial communities include the quality and quantity of organic matter and the environmental factors affecting organic matter before it reaches the sediment. Such factors include the depth and productivity of the water column as well as the sediment accumulation rate. In addition to the quantity of organic matter, the bioavailability of organic matter affected by the adsorption of organic compounds in mineral phases (Burdige, 2007) and bioturbation (Kunihiro *et al.*, 2011) drives the bacterial community composition. For instance, changes in bacterial community composition were reported along an organic pollution gradient (Edlund *et al.*, 2006) and redox gradients (Edlund *et al.*, 2008), as well as before and after dredging of contaminated sediments (Edlund & Jansson, 2006). Bacterial community compositions can also be influenced by spatial distance, i.e. small local or large geographical scales (Green & Bohannon, 2006) and temporal factors, as observed by Böer (2009) in sandy sediments. Other factors influencing bacterial communities include species interactions, competition, predators and disturbance. In fact, sediment chemical, environmental, spatial, temporal and biological factors are interlinked and are, in addition, entwined with evolutionary adaptation as well as history at deeper vertical scales.

1.4.3.1 Sediment bacterial communities in the Baltic Sea

Although sediment bacteria are widely studied worldwide, extensive investigations of sediment bacterial community composition in the Baltic Sea are few, particularly in the deep biosphere. Edlund *et al.* (2006, 2008) and Edlund & Jansson (2006) determined bacterial community composition in the Baltic Sea sediments of coast of Sweden. Their study (Edlund *et al.*, 2008) showed that most abundant active bacteria belonged to the alpha-, beta-, delta-, and gammaproteobacterial classes, and the phyla of *Bacteroidetes*, *Chloroflexi*, *Actinobacteria* and *Planctomycetes*. Recently, Tamminen *et al.* (2011) screened the bacterial community composition of fish farm sediments and reference pristine sites in the Archipelago Sea. They found that *Actinobacteria*, *Chloroflexi* and *Firmicutes* were abundant at fish farms, whereas at pristine sites the prominent clusters consisted of *Alphaproteobacteria*, *Cyanobacteria*, *Deltaproteobacteria* and *Verrucomicrobia*.

Table 1. Phosphorus forms, their potential biodegradability in sediment and their potential environmental effects (The table originates from I).

Used definition of phosphorus forms ^a	Classification of phosphorus forms	Examples of phosphorus compounds ^e	Potential biodegradability or bioavailability ^j	Potential environmental effect
Pore water P, loosely adsorbed P ^b	Dissolved inorganic P	Phosphate (PO ₄ -P)	Already biodegraded or released from sorption sites	Increases eutrophication
Iron-bound P, redox-sensitive P ^b	P bound to hydrated oxides of reducible metals, mainly those of Fe	Phosphate (PO ₄ -P) bound to hydrated oxides of Fe ³⁺	Biodegradable or released if Fe-compounds are reduced	Increases eutrophication
Labile organic P ^b	Low molecular weight dissolved organic P ^c	Orthophosphate monoesters ^f and diesters ^g , poly-P compounds ^h	Partly biodegradable (includes also degradation products)	Increases eutrophication
Refractory organic P ^b	High molecular weight particulate organic P ^d	E.g. phosphonates	Slowly biodegradable ^j , mainly recalcitrant	Mainly buried with sediment in shallow seas, decreases eutrophication
Aluminium-bound P ^b	P bound to hydrated oxides of non-reducible metals, mainly those of Al	Phosphate (PO ₄ -P) bound to hydrated oxides of Al ³⁺	Mainly unavailable, bioavailable only if released from Al-compounds	Buried with sediment, decreases eutrophication
Apatite P ^b	P in apatite minerals	Detrital apatite minerals, may include authigenic apatite	Mainly unavailable, may be slowly biodegradable ^k	Buried with sediment, decreases eutrophication if includes authigenic apatite-P forms

^a See phosphorus fractionation method in Table S2 in I.

^b In this fractionation method, according to a coarse division, pore water and loosely adsorbed P, redox-sensitive (iron-bound) P, and labile organic P are considered mobile (or reactive) phosphorus forms while refractory P, aluminium-bound P, and apatite-P are considered immobile phosphorus forms (Lukkari *et al.*, 2007a; Lukkari *et al.*, 2008).

^c Particle size <0.4 µm

^d Particle size >0.4 µm

^e (Ahlgren, 2006; Lukkari *et al.*, 2007a)

^f e.g. sugar phosphates, mononucleotides, phospholipids, inositol P

^g e.g. sugar DNA-P, lipid P, teichoic-P

^h e.g. adenosine triphosphate

ⁱ Biodegraded to or chemically released phosphorus which is bioavailable.

^j (Ternan *et al.*, 1998; Kononova & Nesmeyanova, 2002; Nausch & Nausch, 2006)

^k (Welch *et al.*, 2002; Hutchens *et al.*, 2006)

2 AIMS OF THE STUDY

Here I focused on describing the variation in sediment bacterial community composition and environmental heterogeneity to determine whether various sediment environments affected the bacterial community composition and whether the bacterial communities reflected the severe agriculture-driven eutrophic and hypoxic state of the Baltic Sea (I, II). Thus, the bacterial community composition of the northeastern Baltic Sea sediments was studied along different horizontal and vertical gradients of 1) various chemical forms of phosphorus and elements involved in its cycling (I) and 2) organic carbon, nitrogen and phosphorus indicating the amount of organic matter (II). The results were discussed from the standpoint of nutrient cycling in the Baltic Sea, emphasizing phosphorus. The important objective was to determine whether bacterial communities reflected the historical environment, such as sea-level-derived changes and different historical sea phases of the Baltic Sea, and whether the DNA-based bacterial community data could be utilized as a novel palaeomicrobiological tool (III). To detect past environmental changes and the sea phases, the structure of the bacterial community composition was examined with depth, covering approximately the latest 8000 years (III).

The specific aims of this thesis were to examine:

1. The present and past sediment bacterial community composition in the northeastern Baltic Sea (I–III),
2. The role of bacteria in the release of nutrients from the sediment to the water column by determining the bacteria-chemistry associations in sediment (I, II),
3. The bacterial communities that mineralize organic matter (I–III) and downcore mineralization shifts (III) and
4. The historical salinity and oxygen changes in the GOF by determining the bacteria-geochemistry associations in deep organic-rich laminae (III).

3 MATERIALS AND METHODS

3.1 Research area and sediment properties

The study area covered the GOF, the Archipelago Sea and two estuaries, Paimionlahti Bay and Ahvenkoskenlahti Bay in the northeastern Baltic Sea (Fig. 1) The GOF is a shallow (mean depth 37 m), nontidal and brackish inner bay (29 571 km²), with low salinity water (5–10 practical salinity units (PSU)). In the Archipelago Sea, which is even shallower (mean depth 23 m), the salinity ranges from 4 to 7 PSU (Winterhalter *et al.*, 1981). The water column of the GOF is widely stratified with a halocline at depths of about 60–80 m (Kullenberg, 1981). At the shallowest coastal stations C63, BISA1 and BZ1, a halocline is not formed. These areas have patchy bottom topography that can also hinder the supply of oxygen to the bottom water (Winterhalter *et al.*, 1981).

Since the GOF is one of the most severely eutrophic and heavily loaded areas of the Baltic Sea (Helcom 2004), the bottom waters of the GOF are widely hypoxic. Therefore, at the time of sampling, most of the sites were hypoxic or barely oxic (Table 2), and the open-sea stations A7, E3, GF2F and XV1 as well as BISA1 on the coast had white bacterial mats growing at the sediment surface (or its remnants) and the strong smell indicated the presence of H₂S (Lukkari *et al.*, 2009a, 2009b). The open-sea sites and coastal site BISA1 as well as BZ1 commonly showed grey and black laminae below the surface layer (Lukkari *et al.*, 2009a, 2009b). The oxygen concentrations and other important sediment properties are summarized in Table 2.

Generally, the modern sediments in the study area were muddy clays rich in humic matter and Fe (Winterhalter *et al.*, 1981; Conley *et al.*, 1997; Carman, 1998). In the Paimionlahti Bay estuary and the Archipelago Sea, phosphorus was abundant (Figure 1C and Dataset 2 in I). The sediment was rich in Fe-bound, redox-sensitive phosphorus (and thus also Fe) of riverine origin from the agriculture-intensive area of southwestern Finland (Helcom, 1998). The concentration of Fe-bound phosphorus decreased and organic phosphorus increased along the transect from Paimionlahti Bay and the Archipelago Sea towards the open Baltic Proper and the western GOF (Figure 1 in I), which were also rich in apatite (I), (Lukkari *et al.*, 2008; Lukkari *et al.*, 2009a, 2009b).

In the GOF, organic loading into the sediments increased towards the eastern coast, where the sediments contained the highest concentrations of organic carbon, nitrogen and phosphorus (II), (Lukkari *et al.*, 2009a, 2009b) due to riverine nutrient loading e.g. from the Kymijoki and Neva rivers (Carman, 1998; Pitkänen *et al.*, 2001; Helcom, 2004). The Ahvenkoskenlahti Bay estuary, which is located on the northern coast of the GOF, was also relatively organic-rich (II). The estuary received loading from the Kymijoki River (Helcom, 1998).

The concentrations of total carbon and nitrogen throughout the study area were similar to those of organic carbon and nitrogen (Conley *et al.*, 1997; Carman, 1998), which is why the terms organic carbon and organic nitrogen were used instead of total carbon and nitrogen (II), even though, the total concentrations (TC and TN) were used previously (I).

Here, for simplicity, the terms organic carbon and nitrogen concerned the sediment samples from both I and II.

3.2 Sediment sampling

The sediments were collected from soft bottoms, with the exception of the sediment from site C63, which was from transportation area. The sediment cores (n = 18) down to 25 cm below the seafloor were collected during cruises on the r/v Aranda, assisted by the r/v Aurelia and r/v Muikku in September 2003, April 2004 and August 2004, and subsampled from different core depths, as shown in Table 3 (I, II). A summary of the sampling technique was presented (I,II) and described in detail by Lukkari *et al.* (2008, 2009a, 2009b). In addition, a deep sediment core down to 534 cm, spanning the latest 8000 years (site GF2, Fig. 2) was collected from the central GOF, subsampled to depth sections (n = 130) and described in III.

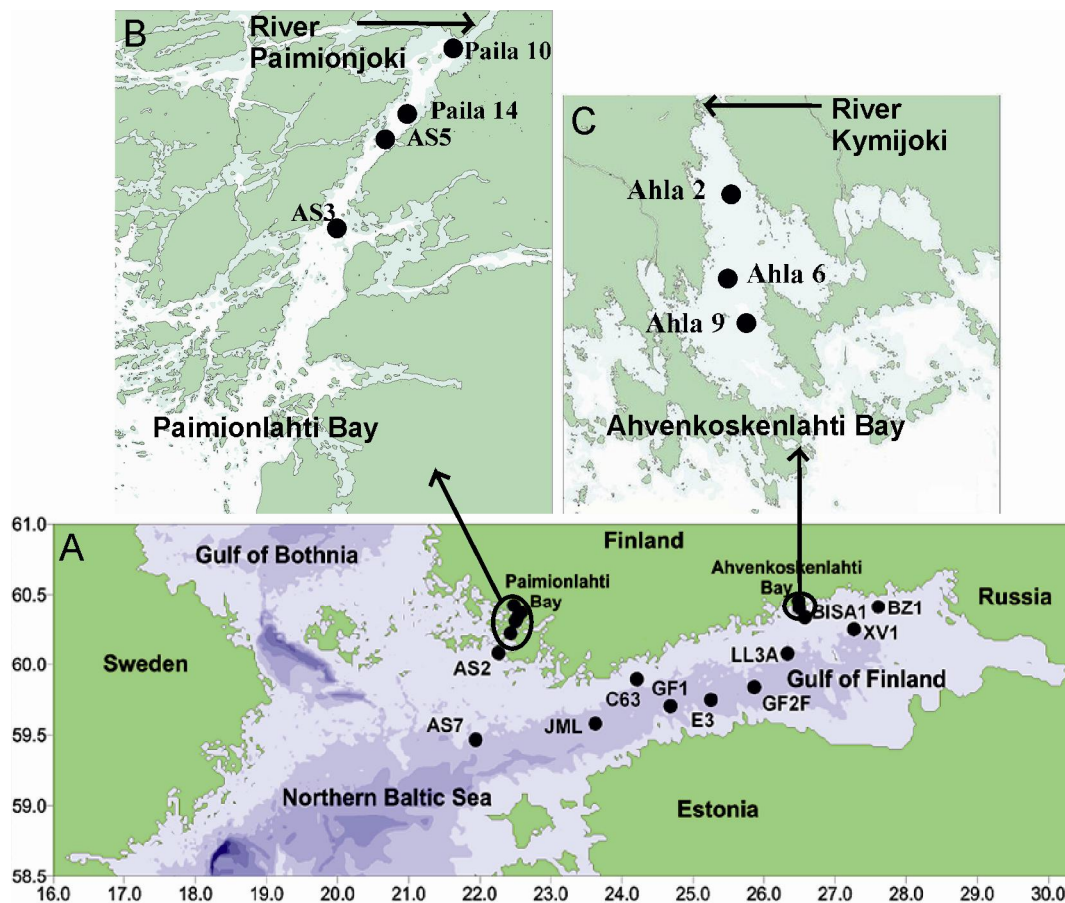


Fig. 1. (A) Sampling sites of the study from the north-eastern Baltic Sea covering the Baltic Proper (AS7), Archipelago Sea (AS2), Gulf of Finland divided into the western (JML, C63, GF1 and E3), central (GF2, GF2F and LL3a) and eastern parts (BISA1, XV1 and BZ1), as well as the estuaries of the Paimionlahti Bay (AS3 and AS5) and the Ahvenkoskenlahti Bay (Ahla2, Ahla 6 and Ahla 9). (B) Blowup of the Paimionlahti Bay and (C) the Ahvenkoskenlahti Bay, and (B, C) the locations of the sampling sites in the estuaries.

In this study, the sediment samples from 0 cm down to 25 cm below the seafloor (I,II) are termed the modern or recently deposited sediments and the sediment samples of the deep sediment core from the central GOF (III) are termed the deep or historical sediments.

3.3 Data types and the methods used

The methods that were used in this study are presented in Table 4 and described in detail in the original publications (I–III). Briefly, the entire bacterial community composition was determined from all subsamples of the sediment cores (Table 3), using terminal restriction fragment length polymorphism (T-RFLP) analysis (Liu *et al.*, 1997), which is based on polymerase chain reaction (PCR)-amplified 16S rRNA genes of the total bacterial DNA (I–III). To identify sediment bacteria, the 16S rRNA genes were cloned and sequenced from the various surface sediments (0–1 cm, I, II) of the estuaries (Paila10, Ahla2), the coastal (BISA1), as well as the open GOF (JML, GF1, E3) and from the several depth sections (91, 101, 330, 422 and 533 cm, III) of the the central GOF (GF2), and assigned to taxa by a naive Bayesian classifier and the seqmatch tool of the Ribosomal Database Project (RDP). Terminal restriction fragments (T-RFs) were identified by *in silico* (virtually) T-RFLP analysis of the assigned 16S rRNA gene sequences and by *in vitro* T-RFLP of the assigned 16S rRNA gene clones.

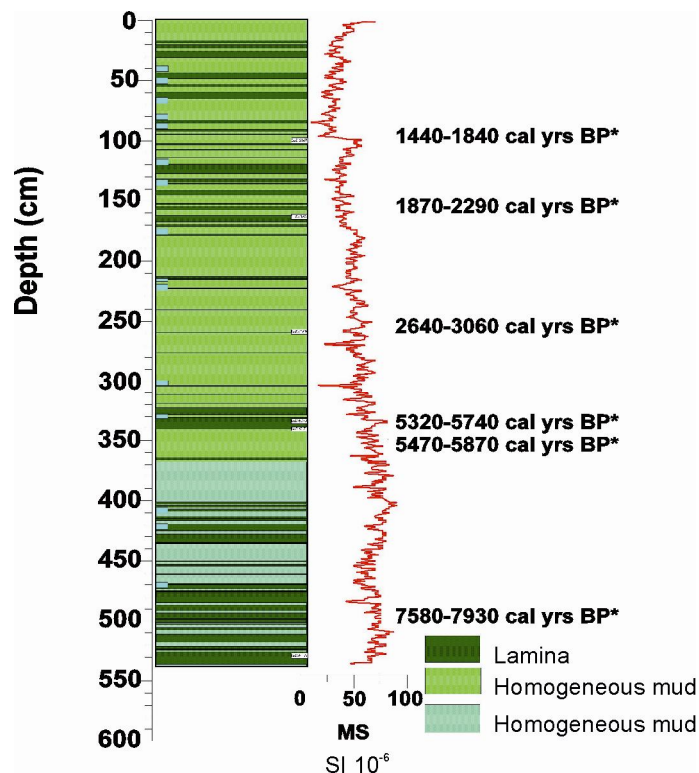


Fig. 2. Deep sediment core from the accumulation basin (GF2) of the central Gulf of Finland (III).

Statistical nonparametric and distance-based multivariate analyses (Table 4) were used to determine the relationships between the T-RFs, which represented bacterial communities, and sediment chemical, geochemical, environmental and spatial parameters, as well as sediment composition and dating data, which are presented in Table 5. Constrained analysis of principal coordinates (CAP) (Anderson & Willis, 2003; Oksanen *et al.*, 2011) was used to define the relationships between the T-RFs and chemical or geochemical parameters, and discriminant analysis (Anderson & Robinson, 2003) was used to discriminate the bacterial communities according to a *priori* assumption. Multivariate multiple regression (McArdle & Anderson, 2001) and variance partition (Borcard *et al.*, 1992; Anderson & Gribble, 1998) were used to determine the proportion of the explanatory variable (i.e. chemical parameter) or the set of the explanatory variables in the variation in the bacterial communities, respectively. Finally, the piecewise Mantel correlogram (Goslee & Urban, 2007) was used to determine the vertical structure (autocorrelation) of the bacterial community composition along the sediment depth. In all statistical analyses, Bray-Curtis dissimilarity was used between pairwise comparisons and 9999 permutations were used to calculate the significance.

Table 2. Characteristics of the sampled sediments, overlying water column and near-bottom water of the Baltic Sea. Geographical coordinates of sampling sites, redox potentials of the surface and near-surface sediment layers, water depth, sediment accumulation rate (SAR), concentrations of oxygen (O₂), incubation-derived phosphate (PO₄-P) flux, manganese (Mn²⁺), ammonium (NH₄⁺), nitrate (NO₃⁻) and phosphate (PO₄-P) as well as salinity in the near-bottom water (5 cm above sediment). Data from Lukkari *et al.* (2008, 2009a, 2009b).

	Site ^a	Coordinates ^b		Redox potential (mV) ^c			pH	Water depth (m)	SAR ^e (g m ⁻² y ⁻¹)	PO ₄ -P flux (μmol m ⁻² d ⁻²)	O ₂ (ml l ⁻¹)	Mn ²⁺ (μmol l ⁻¹)	NH ₄ ⁺ (μmol l ⁻¹)	NO ₃ ⁻ (μmol l ⁻¹)	PO ₄ -P (μmol l ⁻¹)	Salinity ^h (PSU)	C:N ⁱ	C:orgP ⁱ
		latitude	longitude	1 cm	2 cm	5/7 cm												
Open sea	AS7	59.2800	21.5650	10	na	na	7.2	71	840	198	1.6	2.2	5.8	5.5	3.4	8.6	9.7	280
	JML	59.3491	23.3760	237	na	-98 ^d	6.8	79	354 ^f	-209	1.8	4.3	2.5	3.6	3.9	8.2	10.9	254
	GF1	59.4231	24.4092	55	na	-193 ^d	7	83	800 ^f	1370	nd	na	6.6	0.4	5.2	8.5	9.3	273
	E3	59.4495	25.1506	381	309	-153	6.6	89	690 ^g	207	0.4	16.6	8.3	1.6	3.8	9.7	10.9	220
	GF2F	59.5038	25.5185	182	-75	-126	7.6	84	958	357	0.8	2.4	2.5	6.9	3.3	9.5	10.4	253
	LL3A	60.0466	26.1972	na	201	-42	na	63	329	-245	2.4	1.6	0.8	10.4	3.1	8.2	9.7	203
Coast	AS2	60.0488	22.1588	432	na	58 ^d	6.7	47	900	46	4.9	na	3.5	3.6	1.4	6.6	8.7	180
	C63	59.4372	24.1270	391	287	115	na	45	690 ^g	na	8.6 ^h	0.4	0.6	3.3	1.0	6.0	8.9	229
	Bisa1	60.2012	26.3455	-89	-160	-196	7.4	29	990	1.065	3.8	5.2	8.0	5.4	2.8	5.7	9.0	122
	XV1	60.1494	27.159	na	-88	-117	7.1	58	330	1.774	2.5	13.9	5.1	11.6	2.8	7.0	10.4	160
	BZ1	60.2464	27.3632	288	-39	-198	na	40	430	193	3.4	15.0	10.8	11.9	4.3	6.1	8.5	141
Estuary	Paila10	60.2215	22.3448	341	na	-3 ^d	7.4	12	4,580	na	6.2	na	5.1	0.4	0.4	na	10.8	148
	Paila14	60.1950	22.3130	291	na	25 ^d	6.7	29	5,540	278	2.9	na	7.7	7.6	0.7	6.2	11.1	156
	AS5	60.1847	22.3003	292	na	-14 ^d	6.7	19	4,650	-4.2	1.7	na	7.2	14.1	0.9	6.3	7.8	174
	AS3	60.1327	22.2598	341	na	90 ^d	7.3	33	840	24.4	6.1	na	2.3	1.5	0.3	6.3	12.2	173
	Ahla 2	60.2731	26.2898	278	264	23	6.9	4	730	na	6.4 ^h	na	8.5	3.6	0.9	na	13.9	303
	Ahla 6	60.253	26.286	119	92	36	7.1	7	660	na	4.3 ^h	na	8.6	3.1	1.6	na	13.2	208
	Ahla 9	60.2412	26.2973	137	92	235	7.2	13	1,373	671	4.5	na	10.8	3.6	2.9	na	10.2	210

na = Not available, nd = Not detectable

^a The following sampling site numbers refer to numbers in II as follows: AS7= 1, JML= 2, C63 = 3, E3 = 4, GF2F = 5, LL3A = 6, BISA1 = 7, XV1 = 8, BZ1 = 9, Ahla2 = 10, Ahla 6 = 11 and Ahla9 = 12.

^b WGS84 coordinate system.

^c Considered only suggestive, due to common problems involved in measuring redox potential with electrodes (Drever, 1997).

^d 7 cm below the seafloor.

^e Sediment accumulation rates originated from (Mattila *et al.*, 2006).

^f Average sediment accumulation rate of the sampling site from 1995 to 2003.

^g Average sediment accumulation rates of the Gulf of Finland were used, since sediment accumulation rate of the sampling sites was not available.

^h App. 1 m above sediment.

ⁱ Calculated from the 0–1-cm depths.

Table 3. Sediment samples used (I,II).

			Depth (cm below the seafloor)								
Area	Sampling site	Time	0-1	1-2	4-5	6-7	9-10	14-15	19-20	20-25	Publication
Estuary	Paila10	Sept 2003	x			x		x	x	x	I
	Paila14		x			x		x	x	x	
	AS5		x			x		x	x	x	
	AS3	Aug 2004	x			x		x	x	x	II
	Ahla2		x	x	x		x	x		x	
	Ahla6		x	x	x		x	x		x	
	Ahla9		x	x	x		x	x		x	
Coast	AS2	Sept 2003	x			x		x	x	x	I
	C63	Apr 2004	x	x	x		x				I, II
	Bisa1	Aug 2004	x	x	x		x	x		x	II
	XV1		x	x	x		x ^a	x			
	BZ1		x	x	x		x	x		x	
Open sea	AS7	Sept 2003	x					x	x	x	I, II
	JML		x			x		x		x	
	GF1	Apr 2004	x			x		x	x	x	I
	E3		x	x	x		x	x		x	
	GF2F		x	x	x		x	x		x	
	LL3A			x	x						

^aExceptionally, the 8-9-cm depth layer was sampled instead of the 9-10-cm depth.

Table 4. Methods used in the publications I to III.

Method	Publication
Sediment sampling	I, II, III
Molecular microbiological methods:	
DNA extraction	I, II, III
PCR amplification	I, II, III
Terminal restriction fragment length polymorphism (T-RFLP) analysis	I, II, III
Cloning and sequencing	I, II, III
Identification of terminal restriction fragments (T-RFs)	I, II, III
Phylogenetics	
Neighbour-joining tree	III
Sediment composition, geochemical analysis and dating	III
Multivariate statistical analyses	
Constrained analysis of principal coordinates (CAP)	I, II, III
Discriminant analysis	II, III
Homogeneity of group variances	II, III
Piecewise Mantel correlogram	III
Multivariate multiple regression	I, II, III
Variance partitioning	I, II, III

Table 5. Data types used in multivariate statistical analysis with molecular microbiological T-RFLP data.

Termed data type	Sediment parameter	Publication
Chemical	Various chemical forms of P ^a	I, II
	Elements from P extraction solutions: P-binding elements (Fe, Mn, Al, Ca, Si) or related to its cycle (Mg)	I, II
	Elements of the sediment solid phase (e.g. P, N, C, Fe, Mn, Al, Ca, Mg, Si)	I, II, III
Geochemical	Trace elements (e.g. Sr, U, Cr, Pb) and total C, N as well as P of the sediment solid phase	III
Dating	AMS- ¹⁴ C dates	III
Spatial	Sediment depth, geographic location	I, II, III
Sediment composition	Character of the sediment layers: laminated, bioturbated and homogeneous	III
Data type	Water column parameter	Publication
Site-specific properties ^b	Sediment accumulation rate (SAR), water depth	I, II

^a The different chemical forms of phosphorus are presented in Table 1. The different fractions were determined previously (Lukkari *et al.*, 2008; Lukkari *et al.*, 2009a, 2009b), using the sequential extraction method of Jensen and Thamdrup (Jensen & Thamdrup, 1993), slightly modified by Lukkari *et al.* (2007a, 2007b).

^b Defined as environmental (I).

4 RESULTS

4.1 Bacterial community composition of modern and historical Baltic Sea sediments

This section summarizes the most important results of the multivariate statistical analyses of bacterial T-RFs and the various chemical, spatial and temporal factors, as well as other site-specific properties in the northeastern Baltic Sea sediments covering the last 8000 years. It also combines the 16S rRNA gene sequence information from the surface (I, II) and deep subsurface sediments (III). The results showed that sediment phosphorus chemistry and organic content as well as past water column oxygen and salinity changes shaped the present and past bacterial communities, respectively. However, the horizontal and vertical variation, not only in the bacterial communities, but also in the chemical and environmental parameters, such as redox conditions, indicated that spatial and temporal factors impacted the bacterial variation, either via the environment or per se.

4.1.1 Bacterial communities of modern sediments varied horizontally and vertically by chemistry

This study showed that the entire bacterial community composition of the recently deposited sediments (1–25 cm), as determined by T-RFs, varied along local and regional scales (Fig. 1) and vertically with depth (Table 3), mainly by the sediment chemistry. The CAP analyses showed that the bacterial T-RFs varied along three transects (Fig. 1), mainly by the increasing concentrations of organic carbon, nitrogen and phosphorus, which are a sign of organic matter (Figure 2A in I and Figure 3A in II). The concentration of organic carbon, nitrogen and phosphorus increased from two estuaries (Paimionlahti Bay and Ahvenkoskenlahti Bay, Fig. 1) towards the coastal and open GOF and from the less organic-rich Baltic Proper towards the most organic-rich sites in the eastern GOF (Figure 2A and Dataset 2 in I, Figure 3A and Dataset 2 in II).

In addition, the bacterial communities varied along the gradients of different forms of phosphorus and elements related to its cycling from Paimionlahti Bay towards the open-sea areas of the Baltic Proper and the western GOF (Figure 2A in I). Paimionlahti Bay was rich in Fe-bound (redox-sensitive) phosphorus (Figure 1C in I) and NaBD-extractable (redox-sensitive) Fe as well as total Fe (Figure 2A and Dataset 2 in I), whereas in the western GOF and the Baltic Proper labile organic phosphorus was predominated.

The CAP analyses also revealed vertically structured bacterial community compositions (Figure 2A in I and Figure 3A in II). The communities varied downcore mainly in the decreasing concentrations of organic carbon, nitrogen and phosphorus (Figure 3A in II), as well as mobile phosphorus fractions, such as redox-sensitive or labile organic phosphorus (Figure 2A in I), indicating progressive mineralization processes and decrease in redox potential (Table 2).

The redox conditions also appeared to impact the bacterial communities on horizontal scales. Throughout the study area, hypoxia in the near-bottom water increased with the

depth of the water column, indicated by the high negative correlation between O₂ and the water column depth (Spearman's rho: -0.720, $p = 0.01$).

Based on the CAP analyses, the chemical parameters apparently governed the variation in bacterial communities. The variance partition analyses specified that the pure chemical parameters explained 24–25% of the variation in the bacterial communities (Figure 4 in I and II). The pure spatial factors (sediment depth and geographical location) explained from 9% to 11% of the variation in bacterial communities. In addition, up to 14% of the bacterial variation was explained by the shared proportion of spatial and chemical factors, which demonstrated that some of the chemical factors were spatially structured. The pure site-specific properties (sediment accumulation rate and water column depth) explained 5–6% of the variation in bacterial communities (Figure 4 in I and II).

In the discriminant analysis, which included no chemical constraints, the communities of the estuary, coast and open sea grouped more tightly together than in the CAP, with a correct classification of 95% ($p = 0.0001$) (Figure 5A in II). The communities also discriminated by *a priori* depth classes (0–2, 4–8, 9–15 and 19–25 cm), although the correct classification was lower (64%, Figure 5B in II) than in the classification of estuary, coast and open-sea communities. The firm groupings, particularly on the horizontal scale, demonstrated that in addition to the chemical parameters, other factors in local sediment environments also shaped the bacterial communities.

4.1.1.1 Bacteria-chemistry interactions and the regional distribution of bacterial taxa

The horizontal distribution of the various bacterial taxa and the individual bacterial taxa-chemistry associations were specified by the discriminant analysis (Figure 5A in II) and CAP analysis, respectively (Figures 2A and 2B in I and Figure 3B in II), as well as by the 16S rRNA sequence libraries (Figure 5 in I and Figure 6 in II). In addition, the deltaproteobacterial taxa were investigated by plotting their T-RFs along the transect from Paimionlahti Bay to the Baltic Proper (Figure 6 in I). All bacteria-chemistry interactions (positive correlations between bacterial taxa and chemical parameters) are listed in Table 6.

Deltaproteobacteria, including the sulphate and iron/sulphur reducers, were common throughout the study area. However, iron/sulphur-reducing deltaproteobacterial taxa, such as *Desulfovibrio*, were most abundant in the Paimionlahti Bay estuary (Figure 6 in I), whereas sulphate-reducing taxa predominated in the open-sea sediments of the GOF (Figure 6 in I and Figure 5A in II). In the surface sediments, sulphate reducing genera, particularly the genus *Desulfobacula* (T-RFs 271/272 and 423 base pairs (bp)), correlated positively with organic nitrogen in the coastal and open sea of the GOF and Archipelago Sea (Figures 2A and 2B in I and Figure 3B in II), with labile organic phosphorus in the western GOF and Baltic Proper (Figures 2A and 2B in I), and with total organic phosphorus in the central and eastern GOF (Figure 3B in II). They were also associated with elevated correlations of NaBD-extractable (redox-sensitive) and NaOH-extractable

Mn (Figure 3B in II). In the Paimionlahti Bay estuary, the order *Desulfuromonadales* (T-RF 217 bp) was associated with high concentrations of redox-sensitive phosphorus and Fe (Figures 2A and 2B in I).

Based on the discriminant analysis (Figure 5A in II), the classes *Flavo-* and *Sphingobacteria* (phylum *Bacteroidetes*) as well as the classes *Alphaproteo-* and *Gammaproteobacteria* prevailed in the coastal sediments. The T-RF 30 bp representing these bacteria showed strong positive correlation with organic carbon, nitrogen and phosphorus, particularly in the surface sediments of the eastern coast.

The class *Betaproteobacteria* (phylum *Proteobacteria*) was common in both estuaries (Paimionlahti Bay, Ahvenkoskenlahti Bay) (Figure 5 in I, Figure 6 in II and Figure 5A in II). Interestingly, the family *Anaerolineaceae* of phylum *Chloroflexi* was abundant in the organic-rich Ahvenkoskenlahti Bay estuary (Figure 5A in II).

4.1.1.2 Vertical distribution of the bacterial taxa and their interactions with chemistry

The vertical differentiation of bacterial T-RFs was examined in the GOF and Ahvenkoskenlahti Bay estuary (Figure 5B in II). In addition, the associations of the bacterial taxa and chemical parameters in subsurface layers were determined by the CAP analyses throughout the study area (Table 6) (Figures 2A and 2B in I and Figure 3B in II).

Based on the discriminant analysis, the surface layers (0–2 and 4–8 cm) were clearly distinguished from the deepest layers (9–15 and 19–25 cm) (Figure 5B in II). Below the surface layers, where the sulphate reducers and *Flavo-*, *Sphingo-*, *Alphaproteo-* and *Gammaproteobacteria* were abundant (see the previous paragraph), sulphate-reducing taxa occurred commonly until their proportion dropped in the deepest depth class (19–25 cm below the seafloor, Figure 5B in II). In contrast, T-RFs of the family *Anaerolineaceae* (*Chloroflexi*), the phyla *Planctomycetes* and *Firmicutes*, as well as the class *Betaproteobacteria* increased downcore, and in the 19–25-cm depth *Anaerolineaceae* predominated (Figure 5B in II).

Sulphate-reducing taxa (T-RFs 62 and 203 bp), such as the genus *Desulfovibrio* correlated positively with NaOH-extractable (Al oxide-bound) phosphorus and total Fe in the subsurface of Paimionlahti Bay (Figure 2A and 2B in I). In Ahvenkoskenlahti Bay, *Anaerolineaceae* correlated positively with Al oxide-bound phosphorus in the deepest sediments (Figure 3B in II).

4.1.1.3 Main chemical parameters driving the bacterial communities

The effects of the individual chemical parameters used in the CAP models on the bacterial communities depended on the area studied. Along the organic matter gradient, organic carbon, nitrogen and phosphorus as well as NaOH-extractable phosphorus were the most important parameters and explained 36% of the variation in bacterial communities (Table 2 in II). In turn, along the gradients of different forms of phosphorus and elements related

to phosphorus cycling, Al oxide-bound phosphorus and silicon (Si) as well as NaBD-extractable redox-sensitive Fe explained the largest proportions of the variation in bacterial communities (Figure 3 in I).

4.1.2 Bacterial communities of the deep subsurface from the central Gulf of Finland

4.1.2.1 Bacteria-geochemistry interactions

CAP analysis of the bacterial T-RFs and the chemical data of the laminated sediment layers spanning the entire history of the Baltic Sea showed that elevated concentrations of elements (Cr, lead (Pb), Sr, sodium (Na), phosphorus and U), particularly trace elements Sr and U, were tightly associated with the bacterial communities of the Litorina Sea laminae (Figure 1 in III). In contrast, the bacterial communities of the youngest laminae of the Late Litorina and Litorina Sea were positively correlated with total phosphorus (as well as total carbon and nitrogen, III, supporting material). Based on the bacteria-chemistry associations in CAP, the Litorina Sea phase separated from the Early and late Litorina sea phases, which grouped together (Figure 1 in III). The variance partition analysis determined that the chemical parameters accounted for most of the bacterial variation (37.7%) in the deep laminae sediment layers (III).

4.1.2.2 Vertical structure of bacterial communities spanning 8000 years

The bacterial community composition also varied with depth (time) (Figure 3 in II). The piecewise Mantel correlogram, which discovers the nonlinearities, found a discontinuous bacterial community structure. From the surface down to 167 cm approximately 2000 years ago, the bacterial community heterogeneity, which was based on the Bray-Curtis dissimilarity of the T-RFs and their abundance in the various sediment samples, gradually decreased until it reached a plateau at a depth of 306 cm approximately 4500 years ago. Below the plateau, from 388 to 423 cm, the bacterial community heterogeneity suddenly increased in the Litorina Sea phase approximately 6200–6600 years ago (Figure 3 in III).

4.1.2.3 Bacterial communities discriminated based on the three Litorina Sea phases

The discriminant analysis of the T-RFs from laminated, bioturbated and homogeneous sediments determined that *a priori* bacterial communities could be differentiated based on the three Litorina Sea phases (Figure 2A in III). *A priori* groups were formed, based on the heterogeneity trends seen in the piecewise Mantel correlogram (Figure 3 in III). The *a priori* bacterial communities represented the present Late Litorina Sea, the Litorina Sea, and the Early Litorina Sea phases with a correct classification of 90% ($p = 0.001$). The bacterial communities also discriminated between the different depth classes, although with a lower correct classification (78%, $p = 0.001$) than between the sea phases. In contrast, *a priori* bacterial communities that were derived from the homogeneous, bioturbated and laminated sediments were not clearly discriminated (Figure 2C in III).

4.1.2.4 Bacterial taxa in the deep subsurface

The neighbour-joining tree, which was used to analyse the partial 16S rRNA gene sequences, showed that the phyla *Chloroflexi* and *Planctomycetes* were abundant in the deep subsurface sediments (91–533 cm), spanning the last 8000 years. In addition, the

large number of sequences of the deep subsurface was related to the putative candidate division bacteria JS1 (Figure 4 in III). The discriminant analysis showed that the members of candidate division JS1 were equally abundant in the three historical Litorina Sea phases (observed T-RFs 221 and 223 bp in Figure S6 in III). The discriminant analyses also demonstrated that the nitrogen-fixing cyanobacteria such as *Nodularia* became abundant in the Late Litorina Sea phase, whereas the cyanobacteria unable to fix nitrogen were abundant earlier, in the middle Litorina Sea phase (Table 7) (Figure S6 in III). Other bacterial taxa characteristic of the deep sediment layers, such as cyanobacteria, are presented in table 6.

4.1.3 Comparison of present and past 16S rRNA gene sequence libraries

To compare the bacterial taxa from the surface (0–1 cm) and deep subsurface sediments (91–533 cm), over 1200 16S rRNA gene sequences were identified (Fig. 3.). The 16S rRNA gene libraries clearly showed that the phylum *Chloroflexi* was abundant from the surface to the deepest layer and covered up to approximately 30% in the various libraries (Fig. 3). The majority of the *Chloroflexi* sequences represented the family *Anaerolineaceae*.

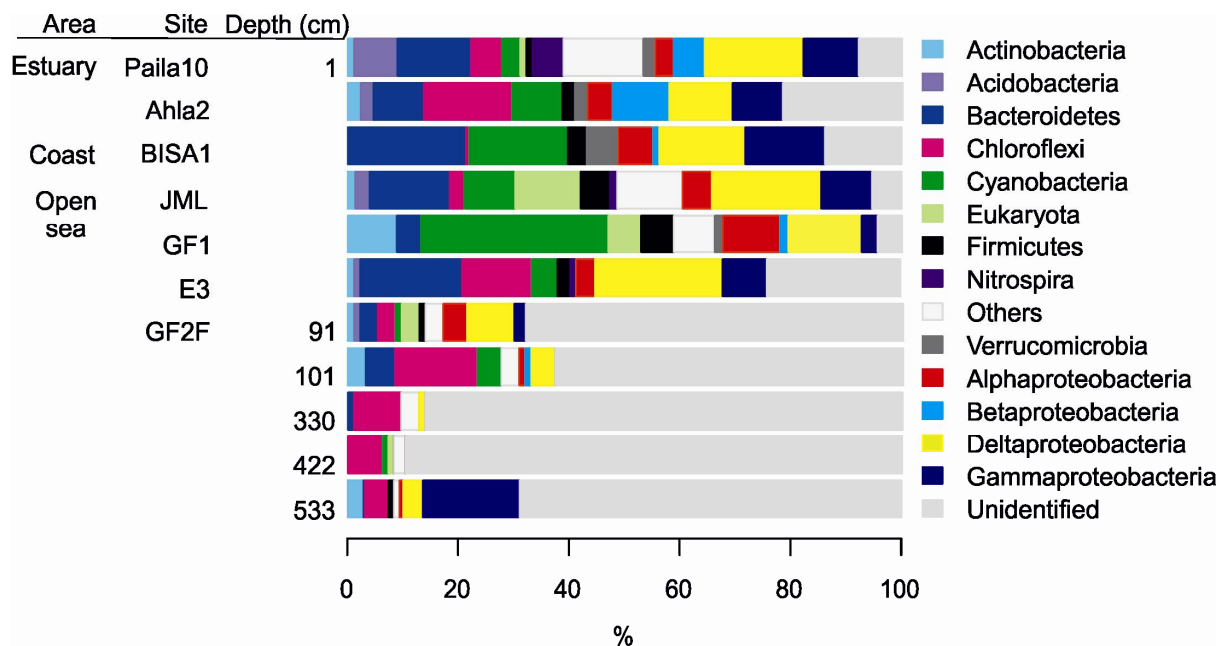


Fig. 3. The 16S rRNA gene sequences of the surface sediments (0–1 cm) from the Paimionlahti Bay (P10) and Ahvenkoskenlahti Bay (Ahla2) estuaries, coastal and open-sea sites and from the deep sediment layers of the central Gulf of Finland (GF2F).

The striking difference in bacterial community composition between the surface and deep subsurface was the number of unidentified sequences, which covered approximately 5–25% and 65–85% of all sequences detected in the surface and deep sediments, respectively. However, a large part of the unidentified sequences of the deep layers potentially represented the candidate division JS1 and the phylum *Planctomycetes* (Figure 4 in III).

In the surface sediments, the classes *Alpha-*, *Delta-*, and *Gammaproteobacteria* and the phylum *Bacteroidetes*, including mainly the classes *Flavo-* and *Sphingobacteria*, as well as the phylum Cyanobacteria were common and covered approximately 50–75% of all sequences (Fig.3) (Figure 5 in I and Figure 6 in II). In contrast, these bacterial taxa were found in the deep layers only coincidentally and their proportions were usually lower than at the surface.

To summarize, the 16S rRNA sequence libraries suggest that the phylum *Chloroflexi* is characteristic of present and past Baltic Sea sediments. The phylum *Planctomycetes* and the candidate division bacteria JS1 thrived in the deep biosphere, whereas the classes of phyla *Proteobacteria* and *Bacteroidetes* preferred the sediment surface.

Table 6. Most significant HaeIII-digested T-RFs of bacterial 16S rRNA genes in the sediment samples, their assignment to taxonomical groups and potential function, and statistically significant chemical parameters that correlated with T-RFs, as well as probable mechanisms of potential bacteria-chemistry interactions in nutrient fluxes. Data summarized from I (Figures 2A and 2B) and II (Figure 3B).

T-RF (bp)	Identified taxon	Bacterial function	Chemical parameter	Potential mechanism/Relevance of interaction in nutrient fluxes	Area	Sediment depth ^a
30	<i>Flavobacteria</i> (c) <i>Sphingobacteria</i> (c)	Initial organic matter mineralization	Organic P ^b Organic N ^c Organic C ^c	Increase in hypoxia-induced and organic matter-derived fluxes of P and N by degradation of organic matter	Coast /open sea, central/eastern GOF	Surface
61	<i>Desulfobacterium</i> (g)	Sulphate reduction, terminal organic matter mineralization	HClCa ^d	No potential effect on nutrient fluxes, reflection of aged state of organic matter	Coast /open sea, from western to eastern GOF	Middle to deep
62	<i>Desulfobacterium</i> (g)		NaOHIP ^e Fe	No potential effect on nutrient fluxes Increase in Fe-bound P flux via production of sulphides, which capture Fe	Estuary, Paimionlahti Bay	Surface to deep
191	<i>Desulforhopalus</i> (g)		Organic P ^b Organic N ^c Organic C ^c	Increase in Fe-bound P flux via organic matter mineralization and concomitant production of sulphides, which capture Fe	Coast/ open sea, central /eastern GOF	Surface
191	<i>Desulforhopalus</i> (g)		NaOHMn ^f NaBDMn ^g	Mn reduction via sulphide production, possibly Mn-associated P flux by Mn reduction	Coast/open sea western/ central GOF Coast/open sea, from western to eastern GOF	Surface
203	<i>Desulfovibrio</i> (g)	Fe reduction	NaOHIP ^e Fe	Increase in Fe-bound P flux via bacterial Fe reduction since NaOH-extractable fraction ^e may have included some NaBDiP ^h Increase in Fe-bound P flux via bacterial Fe reduction	Estuary, Paimionlahti Bay	Deep
206	<i>Desulfobacteraceae</i> (f) <i>Desulfobulbaceae</i> (f)	Sulphate reduction, terminal organic matter mineralization	HClCa ^d	No potential effect on nutrient fluxes, reflection of aged state of organic matter	Open sea, western/ central GOF	Deep

T-RF (bp)	Identified taxon	Bacterial function	Chemical parameter	Potential mechanism/Relevance of interaction in nutrient fluxes	Area	Sediment depth ^a
217	<i>Desulfuromonadales</i> (o)	Fe/S reduction	NaBDiP ^h NaBDFe ⁱ	Increase in iron-bound P flux via bacterial Fe reduction	Estuary, Paimionlahti Bay	Surface to middle
255	<i>Desulfuromusa</i> (g)		NaOHIP ^e	Increase in Fe-bound P flux via bacterial Fe reduction since NaOH-extractable fraction ^e may have included some NaBDiP ^h	Estuary, Paimionlahti Bay	Surface to Deep
228	<i>Chloroflexi</i> (p): <i>Anaerolineaceae</i> (f)	Fermentation, terminal organic matter mineralization	NaOHIP ^j	Probable effect on methane fluxes via possible syntrophy with methanogens in degradation of terrestrial organic matter, included in NaOH-extractable fraction ^j	Estuary, Ahvenkoskenlahti Bay	Deep
257	<i>Bacteroidetes</i> (p): e.g. <i>Sphingobacteria</i> (c), <i>Clostridia</i> (p)	Aerobic/facultative anaerobic initial organic matter mineralization	NaOHIP ^j	Increase in hypoxia-induced and organic-matter derived fluxes of P and N by degradation of terrestrial organic matter included in NaOH-extractable fraction ^j		Middle
271	<i>Desulfobacula</i> (g)	Sulphate reduction, terminal organic matter mineralization	NRP ^k Organic N ^c Organic C ^c	Increase in Fe-bound P flux via organic matter mineralization and concomitant production of sulphides, which capture Fe	Coast/open sea, western GOF	Surface
272	<i>Desulfobacula</i> (g)		Organic P ^b Organic N ^c Organic C ^c		Coast/open sea, central/eastern GOF	
272	<i>Desulfobacula</i> (g)		NaOHMn ^f NaBDMn ^g	Mn reduction via sulphide production, possibly Mn-associated P flux by Mn reduction	Coast/open sea western/central GOF Coast/open sea, from western to eastern GOF	

T-RF (bp)	Identified taxon	Bacterial function	Chemical parameter	Potential mechanism/Relevance of interaction in nutrient fluxes	Area	Sediment depth ^a
423	<i>Desulfobacula</i> (g)	Sulphate reduction, terminal organic matter mineralization	Organic P ^b Organic N ^c Organic C ^c	Increase in Fe-bound P flux via organic matter mineralization and concomitant production of sulphides, which capture Fe	Coast/open sea, central/eastern GOF	Surface
423	<i>Desulfobacula</i> (g)		NaOHMn ^f NaBDMn ^g	Mn reduction via sulphide production, possibly Mn-associated P flux by Mn reduction	Coast/ open sea western/central GOF	
					Coast/ open sea from western to eastern GOF	

bp = base pairs

c = class, g = genus, f = family, o = order, p = phylum

C = carbon, N = nitrogen, P = phosphorus, Fe = iron

^a From surface (0–1 cm) to the deepest sediment layer (24–25 cm).

^b Includes labile and recalcitrant phosphorus and is an indication of organic matter.

^c Indicates presence of organic matter. Is relatively fresh, particularly in the eastern part of the GOF.

^d HCl-extractable calcium (Ca), Ca dissolved with acid, e.g. from apatite minerals.

^e Alkali-extractable phosphorus, which is bound to aluminium (Al) oxides in sediment and is relatively immobile.

^f Alkali-extractable manganese (Mn), potentially associated with organic matter and alkali-extractable phosphorus.

^g Reducible Mn, from Mn-oxides, associated with NaBD-extractable phosphorus.

^h NaBD-extractable phosphorus, which is bound to Fe oxyhydroxides in sediment. Redox-sensitive and mobile.

ⁱ Redox-sensitive Fe, from Fe oxyhydroxides.

^j Alkali-extractable phosphorus, which is bound to Al oxides in sediment and is relatively immobile. Indicates presence of terrestrial organic matter in Ahvenkoskenlahti Bay.

^k Labile organic phosphorus, which also includes degradation products and indicates presence of organic matter.

Table 7. Examples of bacterial taxa found in the deep sediments and their potential biogeochemical role in the deep biosphere. Data were summarized from the 16S rRNA gene libraries (Fig. 3), the neighbour-joining tree (Figure 4 in III) and from the discriminant analyses of the T-RFs of the accumulation basin in the central GOF (18–534 cm, site GF2, Figure S6 in III) and of the deepest layers (19–25-cm depth class) of the recently deposited modern sediments (Figure 5B in II).

Bacterial taxa	Expected/observed T-RFs (bp)	Prevalence in this study	Typical growth environments	Type of metabolism	Potential role in deep anoxic sediments
<i>Chloroflexi</i> : mainly <i>Anaerolineaceae</i> (f)	227, 235/227,235 261,262/260,262 267/266,267	From 19–25-cm depth down to 533–534 cm	Organic- and/or methane-rich environments, e.g. sediments ² or activated sludges ³	Anaerobic fermenter using simple sugars or amino acids ³	Possibly participates in terminal anoxic mineralization/methane processes
<i>Planctomycetes</i>	233/232,233 295/295,296	From 19–25-cm depth down to 533–534 cm	Diverse ⁴ , e.g. methane-rich environments such as deep sediments ²	Diverse, carry genes for C1 transfer ⁴	Possibly participates in methane processes/ carbon cycling
Candidate division JS1	223–225/ 221, 223	From the 18-cm ¹ depth down to 534 cm	Deep methane-rich sediments ⁵	Not known, probably anaerobic ⁸	Possibly participates in anaerobic oxidation of methane ¹¹
<i>Gammaproteobacteria</i> : <i>Pseudomonas</i> (g)	Not identified	Early Litorina Sea phase (533–534-cm depth layer)	Diverse, common in soil, water column and sediments ⁶	Aerobe/anaerobe ⁹ , versatile physiological capacity ¹⁰	Probably inactive, not common in deep sediments
<i>Cyanobacteria</i> : <i>Synechococcus</i> (g)	128/126, 128 137/136, 137, 139	Middle Litorina Sea phase (Sediment layers from 318 cm down to 422 cm)	Water column, phytoplankton	Usually non-nitrogen fixing oxygenic photoautotroph	Probably inactive
<i>Cyanobacteria</i> : <i>Nodularia</i> (g)	291/289, 291, 293	Late Litorina Sea phase (Sediment layers from 18 cm down to 306 cm)	Water column, phytoplankton	Nitrogen-fixing oxygenic photoautotroph	Probably inactive
<i>Arthrobacter</i> (g)	Not identified	Early Litorina Sea phase (533–534-cm depth layer)	Soil ⁷	Usually aerobe ⁷	Probably inactive

f = family, g = genus

¹As in Figure S1 in II.

²Inagaki *et al.*, 2006; Edlund *et al.*, 2008; Harrison *et al.*, 2009; Blazejak & Schippers, 2010.

³Yamada *et al.*, 2005, 2006, 2007; Yamada & Sekiguchi, 2009.

⁴Fuerst & Sagulenko, 2011.

⁵Webster *et al.*, 2004; Inagaki *et al.*, 2006; Harrison *et al.*, 2009; Webster *et al.*, 2011.

⁶Silby *et al.*, 2011; Wu *et al.*, 2011.

⁷Eschbach *et al.*, 2003.

⁸Webster *et al.*, 2004.

⁹Macy *et al.*, 1989; Altenschmidt & Fuchs, 1991.

¹⁰Spiers *et al.*, 2000; Silby *et al.*, 2011.

¹¹Chevalier *et al.*, 2013.

5 DISCUSSION

5.1 Sediment bacteria reflected the present and historical environments of the Baltic Sea and participated in nutrient cycling

The sediment chemistry appeared to be the main driver of the variation in the bacterial community composition. However, the chemical parameters were interlinked with the sediment spatial and temporal factors as well as physical properties of the water column, which also affected the sediment bacterial communities. The sediment bacterial communities signalled the severe eutrophic state of the present Baltic Sea and suggested that the bacterial communities actively participated in the biogeochemical nutrient cycling by contributing to the release of nutrients such as phosphorus from the sediment to the water column. The bacterial community composition also reflected past environmental changes of the Baltic Sea water column, such as salinity and bottom-water oxygen levels, as well as the mineralization of organic matter on short and long time scales. Moreover, the bacterial communities were grouped, based on the known historical Baltic Sea phases: the Early Litorina Sea, Litorina Sea and the Late Litorina Sea. The study demonstrates a novel way to utilize all the bacterial community data as a palaeomicrobiological tool in reconstructing the history of a sea basin.

5.1.1 Sediment bacterial communities signalled the eutrophic state of the Baltic Sea

The CAP analyses illustrated that the bacterial community composition of the recently deposited sediments (0–25 cm below the seafloor) changed horizontally and vertically along the gradients of organic carbon, nitrogen and phosphorus, as well as other chemical forms of phosphorus and elements related to its cycling. The chemical parameters accounted for most of the bacterial community variation (24–25%). The horizontal and vertical structure of the chemistry-driven bacterial communities can be explained by the somewhat spatially structured chemical parameters (11–14%) and pure spatial factors (distance and depth, i.e. time, 9–11%). The variation in the bacterial communities along the chemical gradients indicated that nutrient loading, the high amount of organic matter and its quality, as well as the consequent hypoxia, drove the sediment bacterial community composition, which in turn probably affected nutrient cycling.

The sediment accumulation rate and the water depth, which both affect the bottom water hypoxia and the quantity and quality of organic matter, accounted for up to 6% of the variation in the bacterial communities. The eastern coastal sediments, particularly BISA1 with high sediment accumulation rate, were the most organic-rich and organic matter was less degraded than in sediments located elsewhere (C:N and C:P ratios in Table 2). Generally, the sediment accumulation rate was considerably high at several sites, where the near-bottom water was widely hypoxic or barely oxic (Table 2). The near-bottom water oxygen concentration correlated negatively with the depth of the water column (Spearman's rho correlation -0.72), which indicated that water depth contributed to the hypoxia, most likely due to presence of a halocline. Hypoxia in the near-bottom water

impacted the sediment redox conditions (Table 2), which also affected the bacterial community composition vertically. In the most hypoxic open-sea area, the bacterial community composition was dominated by potential anaerobic bacteria, even at the sediment surface.

The strong effect of the local environments, which in coastal areas and the open sea were organic-rich and widely hypoxic, could be seen in the discriminant analysis, which showed that bacterial communities from the estuary, coastal and open-sea sediments were clearly differentiated. The differentiation suggested that these areas were divergent ecosystems, although they were dependent on each other to a certain extent, e.g. due to riverine transport, water circulation, production and transport of organic matter.

5.1.2 Sediment bacteria in present-day nutrient cycling

The nutrient loading and consequent eutrophication alters the biogeochemical cycling of nutrients such as phosphorus and nitrogen (Kemp *et al.*, 2005). Here, the associations between individual bacterial taxa and sediment chemical parameters also indicated that certain bacteria contributed to the nutrient cycling, such as phosphorus and nitrogen, in several ways, as summarized in Table 6. The most important interactions are discussed in the following sections.

5.1.2.1 Presumptive initial degraders of organic matter

The classes *Flavo-* and *Sphingobacteria* as well as the *Alphaproteo*, and *Gammaproteobacteria* predominated in the coastal surface sediments and showed strong positive correlation with the organic carbon, nitrogen and phosphorus, particularly at the eastern sites overlain by oxic bottom water. Given that organic carbon, nitrogen and phosphorus are a sign of organic matter and that oxygen usually penetrates only a few millimetres in organic-rich sediments (Revsbech *et al.*, 1980), the correlation suggests that these bacteria benefited from the high amount of organic matter under oxic or only slightly hypoxic conditions.

Consistently, several previous studies showed that the classes *Flavo-* and *Sphingobacteria* as well as the *Alpha-*, and *Gammaproteobacteria* include a large number of aerobes and facultative anaerobes (Ivanova & Mikhailov, 2001; Kirchman, 2002; Urios *et al.*, 2008; Choi *et al.*, 2010; Declerck, 2010) that benefited from the oxic conditions and thrived in organic-rich environments, such as fish farm sediments (Bissett *et al.*, 2006), shrimp faeces (Beardsley *et al.*, 2011) or activated sludge (Wong *et al.*, 2004). In addition, bacteria of the phylum *Bacteroidetes*, such as class *Sphingobacteria* and especially *Flavobacteria*, are central to the initial biopolymer degradation of sedimentary organic matter (Kirchman, 2002; Bauer *et al.*, 2006; Bissett *et al.*, 2008; Gomez-Pereira *et al.*, 2012), such as high-molecular-weight organics (Cottrell & Kirchman, 2000).

These bacteria most likely contributed to the release of nutrients such as phosphorus and nitrogen from sediment directly by degrading organic matter and indirectly by consuming oxygen or other oxidants, such as nitrate, which alters the nitrogen and phosphorus cycling

(Kemp *et al.*, 2005). For instance, ammonium can be accumulated and released from the sediment under the reduced conditions, which can limit nitrification and accelerate DNRA. Phosphorus, in turn, is released from ferric Fe oxyhydroxides when they are reduced to ferrous form in the absence of oxygen or nitrate (Mortimer, 1941, 1942; Froelich *et al.*, 1979). The phosphorus flux from organic compounds can be accelerated, since phosphate released from organic compounds by mineralization can likewise not be bound to ferrous oxyhydroxides (Mortimer, 1971).

As a sign of either mineralization-derived or hypoxia-induced nutrient release, elevated concentrations of ammonium and phosphate were measured from the near-bottom water in the GOF, even at the most oxic sites (Table 2) (Lukkari *et al.*, 2009a, 2009b), which indicated a high level of mineralization. A mineralization-derived phosphate flux was detected in sediments with C:P ratio approaching 400 (Andersen & Jensen, 1992). Therefore, it was likely that mineralization contributed considerably to the phosphate flux in the oxic coastal area, where the organic C:P ratio was around 150 (Figure 2A in II). Furthermore, as an indication of hypoxia-induced nitrogen cycling, DNRA predominated in the area with low oxygen conditions of the GOF (Jäntti & Hietanen, 2012)

5.1.2.2 Sulphate-reducing and sulphur/iron-reducing taxa of *Deltaproteobacteria*

Another abundant bacterial group, the sulphate reducers, which are mainly anaerobic (Muyzer & Stams, 2008) and other taxa of the class *Deltaproteobacteria*, such as potential Fe reducers, varied horizontally throughout the study area but also vertically with depth. Sulphate reducers, particularly the family *Desulfobacteraceae* and its genus *Desulfobacula*, predominated in the hypoxic sediments of the open GOF, even at the sediment surface, and correlated positively, e.g. with organic nitrogen and phosphorus as well as redox-sensitive Mn (Table 6). Interestingly, in Paimionlahti Bay, sulphur/iron reducing taxa such as *Desulfuromonadales* (Kuever *et al.*, 2005) and sulphate-reducing taxa that are linked to Fe reduction, such as *Desulfovibrio*, prevailed (Park *et al.*, 2008; Byrne *et al.*, 2010). These bacteria were associated with elevated concentrations of redox-sensitive Fe and phosphorus, as well as total Fe.

The distribution and correlations of sulphate reducers with several chemical parameters (Table 6) suggested that these bacteria benefited from high amounts of organic matter and participated in mineralization of organic matter by reducing sulphate, Fe and possibly Mn, and consequently affected phosphorus cycling. Organic matter is essential for sulphate reducers (Pallud & Van Cappellen, 2006) and they can mineralize half of the organic matter deposited in the coastal sediments (Jørgensen, 1982). Moreover, a recent study showed that particularly the family *Desulfobacteraceae*, which thrives in the organic-rich coastal and open GOF, benefits from the organic-rich environment (Kondo *et al.*, 2012).

Sulphate reducers most likely contributed to the release of Fe-bound phosphorus from the sediment to the water column for several different ways (Table 6). For instance, by producing sulphide, they inhibit the binding of phosphorus to Fe, since sulphide forms

insoluble ferrosulphides with ferrous Fe under anoxic conditions (Berner, 1970), and thus ferric Fe is not available for binding phosphorus. The effect of abundant and active sulphate reducers on Fe cycling in the Baltic Sea sediment was also shown by the existence of black laminae, rich in ferrosulphides, below the surface layer of several open-sea and coastal sites (e.g. Figure 7 in I) (Lukkari *et al.*, 2009a, 2009b). Another mechanism that releases Fe-bound phosphorus could be Fe reduction, e.g. by *Desulfovibrio*, which leads to the dissolution of ferric oxyhydroxides and subsequently to the release of phosphorus from Fe. Both mechanisms may potentially enable for phosphorus fluxes in areas with oxic sediment surfaces, if the conditions shift to anoxic.

5.1.2.3 Bacteria in terminal mineralization processes

Planctomycetes and *Chloroflexi*, of which the latter represented mainly the family *Anaerolineaceae*, were discovered principally in the deepest depth class (19–25 cm). *Anaerolineaceae* was highly abundant and correlated with Al oxide-bound phosphorus, which indicated the presence of terrestrial organic matter in the deepest layers of Ahvenkoskenlahti Bay. The correlation and the increased occurrence of *Planctomycetes* and *Chloroflexi* in the subsurface sediments suggested that these bacteria are crucial to terminal mineralization and carbon cycling (see paragraph 5.1.4).

5.1.2.4 Phosphate-accumulating bacteria

Polyphosphate-accumulating bacteria, such as *Actinobacteria* (Kong *et al.*, 2005) and *Thioploca/Beggiatoa*-like bacteria, which form growth on the top of the nutrient- and sulphide-rich sediments (Schulz *et al.*, 1999), even in cold environments (Jørgensen *et al.*, 2010), are common in sediments. These bacteria, which accumulate phosphate under anoxic conditions and release phosphate under oxic conditions, can considerably affect phosphorus effluxes (Hupfer *et al.*, 2007; Dale *et al.*, 2013).

The 16S rRNA sequences of potential polyphosphate-accumulating *Actinobacteria* were abundant in the surface sediment of hypoxic site GF1, where the phosphate concentration of the near-bottom water and incubation-derived phosphorus flux were elevated (Table 2). In addition, several hypoxic or barely oxic open-sea and coastal sediments were covered by a microbial mat (see paragraph 3.1.), possible including the *Thioploca/Beggiatoa*-related bacteria. However, the potential phosphorus-accumulating and -releasing microbial growth could at least partly explain the considerably high incubation-derived phosphate fluxes and phosphorus release into the oxic near-bottom water at sites BISA1 and XV1 (Table 2), where the redox-conditions could have fluctuated due to high levels of sedimentation from the productive and shallow water column. Although the importance of phosphate-accumulating bacteria in this study remained unclear, the study suggests that the contribution of *Thioploca/Beggiatoa*-related bacteria to nutrient releases should be assessed in the eutrophic GOF.

5.1.3 Sediment bacterial community composition reflected historical environments

5.1.3.1 Vertical structure of bacterial communities and bacteria-geochemistry highlighted the salinity and oxygen changes

The CAP analysis showed the positive correlation of the bacterial communities with elements (Cr, Pb, Sr, Na, U and phosphorus), particularly trace elements Sr and U, in the laminae layers of the middle Litorina Sea phase. Since increased concentrations of Sr and U in the sediment are used as an indicator of the palaeosalinity of seawater (Lopez-Buendia *et al.*, 1999; Vincent *et al.*, 2006), the association could be a sign of increased water salinity and decreased oxygen concentrations in the bottom areas. In addition, accumulation of U is a sign of oxygen depletion in bottom waters (Nath *et al.*, 1997; Zheng *et al.*, 2003). In this respect, the positive correlations of bacterial communities with Sr and U indicated that the Litorina Sea phase has been more saline and less oxic than the Early and Late Litorina Sea phases.

Furthermore, enrichment of Cr and (vanadium) V in sediments is increased by the high organic content (Sohlenius *et al.*, 1996; Sternbeck *et al.*, 2000). The correlation of bacterial communities with Cr and V, which was excluded from the final CAP since it was collinear with Cr (Figure S4 in III), also suggested that the laminae of the middle Litorina Sea has also been organic-rich. Indeed, organic-rich laminae layers were developed at that time (app. 400 cm, Figure S1 in III), which consistently indicated the accumulation of organic matter due to oxygen deficiency (Zillen *et al.*, 2008).

The piecewise Mantel correlogram (Figure 3 in III) showed that bacterial community structure changed discontinuously with sediment depth (time), spanning the entire Baltic Sea history. The linear decrease and the subsequent plateau in bacterial community heterogeneity continued from the surface down to 306 cm (app. 4500 years ago). Below 306 cm, community heterogeneity suddenly increased from 388 to 423 cm. The change in community composition to a more heterogeneous (increase in Bray-Curtis dissimilarity) may have been due to environmental changes such as salinity. The probable increase in salinity resulted in halocline formation and consequently hypoxia, as well as the accumulation of organic matter, approximately 6200–6600 years ago in the saline Litorina Sea phase.

Increased salinity has been estimated in different parts of the Baltic from ca. 8000 to 5000 cal yr. BP (Sohlenius *et al.*, 1996; Emeis *et al.*, 2003; Berglund *et al.*, 2005), with the peaked levels approximately 6800–6200 cal yr. BP (Berglund *et al.*, 2005). A previous study from the Baltic Proper (Sohlenius & Westman, 1998) showed that increased salinity in the Early Litorina Sea phase contributed to formation of a halocline, which caused nutrient enrichment in the bottom water. Later, due to replacement of the bottom water by brackish water via the Danish Straits, nutrients rose to the photic zone and accelerated primary production, which caused the accumulation of organic carbon and anoxia in the sediment during the Litorina Sea, 6500–4500 years ago. However, detailed information on past salinity changes from the central GOF is limited. Our study suggests that in the

central GOF, the salinity maximum of the Litorina Sea phase was approximately 6600–6200 years ago and led to the preservation of organic matter, which contributed to the increase in heterogeneity in bacterial community composition.

5.1.3.2 Bacterial community composition supported the historical Baltic Sea phases

In the discriminant analysis, the bacterial communities differentiated, based on the Litorina Sea phases: the present and brackish Late Litorina Sea, the most saline Litorina Sea, and the less saline Early Litorina Sea, with a correct classification of 90% ($p = 0.001$). A previous report from the southern Baltic estimated that the middle Litorina Sea phase, with the salinity maximum, ended ca. 4000–3000 cal yr. BP (Berglund *et al.*, 2005) and was followed by the brackish and present Late Litorina Sea phase (Zillen *et al.*, 2008). The Early Litorina Sea phase, which was the transition from the Ancylus Lake to the saline Litorina Sea, was estimated to have ended ca. 8500–7400 cal yr. BP (Andren *et al.*, 2000; Berglund *et al.*, 2005). Our study thus supported the three Litorina Sea phases.

In contrast, the bacterial communities were not clearly discriminated between homogeneous, bioturbated and laminated sediments. This was partly due to bioturbation by benthic animals, which can mix the adjacent layers (Kristensen, 2000), or the quality and chemical composition of the organic matter may have been more similar within a sea phase than between the sea phases with the different salinities. Therefore, this study concluded that palaeosalinity was the central parameter driving the bacterial community composition.

5.1.3.3 Characteristic bacteria of the historical sea phases

The elevated concentrations of nitrogen and pigment data have previously been considered as an indication of increased numbers of nitrogen-fixing cyanobacteria in the Early Litorina Sea (Westman *et al.*, 2003). In contrast, the discriminant analyses determined that the nitrogen-fixing cyanobacteria became abundant in the Late Litorina Sea phase, while those cyanobacteria unable to fix nitrogen were more abundant in the middle Litorina Sea phase (Table 7).

The nitrogen-fixing cyanobacteria may have been favoured by the internal loading of phosphorus (Zillen & Conley, 2010). During the last two millennia of the Late Litorina Sea, the high productivity was due to the increase in population and land use (Zillen & Conley, 2010), which has resulted in the increased bottom-water hypoxia. This led to the release of Fe-bound phosphorus from the sediments to the water column (Mortimer, 1941, 1942; Froelich *et al.*, 1979), which may have been at a relatively high level during the last two millennia and could have boosted the primary production by cyanobacteria, which can fix nitrogen (Zillen & Conley, 2010).

Based on the 16S rRNA gene sequence data, a deep layer of the Early Litorina Sea phase was rich in *Pseudomonas* bacteria, with relatively abundant sequences of the genus *Arthrobacter*. The large numbers of these genera, which are ubiquitous in soil (Eschbach *et al.*, 2003; Silby *et al.*, 2011; Wu *et al.*, 2011), indicate terrestrial input in the Early Litorina Sea phase.

5.1.3.4 Bacterial community composition as a palaeomicrobiological tool

This study investigated whether all the DNA-based bacterial community composition data could be utilized as palaeomicrobiological tool, e.g. to study the history of a sea basin. The heterogeneity in the bacterial communities, which reflected historical environmental changes such as salinity, also mirrored the mineralization shifts of organic matter (from the surface down to 306 cm). The study concluded that the layers, at least below the plateau of linear mineralization, were comparable from the palaeoenvironmental standpoint and could be used to detect the past environmental changes separately from the mineralization shifts.

It is known that extracellular DNA is well preserved in sediments, particularly under anoxic conditions (Corinaldesi *et al.*, 2011) and that most of the bacteria in subsurface sediments are inactive, dormant or dead (Langerhuus *et al.*, 2012; Lomstein *et al.*, 2012). Thus, it was assumed here that sedimentary microbial activities may be very slow and the community structure may not have changed to a large extent since the original deposition in these deeper layers with high preservation potential.

5.1.4 Bacteria commonly found in present and past Baltic Sea sediments

The eutrophic conditions of the present Baltic Sea and the past organic-rich periods may be a common factor in the abundance of the phyla *Planctomycetes* and *Chloroflexi*, as well as putative candidate division JS1, in all the Litorina Sea phases detected. These bacteria have previously been found in deep biospheres (Webster *et al.*, 2004; Blazejak & Schippers, 2010), e.g. from methane hydrate-bearing sediments (Inagaki *et al.*, 2006) or the sulphate-methane transition zone (Harrison *et al.*, 2009; Webster *et al.*, 2011). The abundance of these bacteria throughout the 5-m-long sediment core could have been due to a diffuse methane flux and suggests that these bacteria can be essential in terminal mineralization and carbon cycling. Nevertheless, the biogeochemical roles of *Planctomycetes*, *Chloroflexi* and putative candidate division JS1 in sediments are not wellknown.

Interestingly, *Planctomycetes*, which under suboxic conditions possessed C1 transfer genes (Woebken *et al.*, 2007), were only present in methanogenic *Archaea* or methylotrophic *Proteobacteria*, and their metabolic functions are debated (Fuerst & Sagulenko, 2011). In addition, marine *Planctomycetes* encodes large numbers of sulphatases, possible for the degradation of sulphated polysaccharides, which are abundant in marine systems (Woebken *et al.*, 2007). Thus, the previous studies (Woebken *et al.*, 2007; Fuerst & Sagulenko, 2011) and this study together demonstrated that *Planctomycetes* can be vital in sulphur and methane cycling.

Also noteworthy is the potential discovery of lipid biomarkers that may indicate the anaerobic oxidation of methane (AOM) by the bacteria of candidate division JS1

(Chevalier *et al.*, 2013). This study together with Chevalier *et al.* (2013) suggest that candidate division JS1 may play an essential role in microbial methane turnover.

The phylum *Chloroflexi*, especially *Anaerolineaceae*, seems to be favoured by high amounts of organic matter and is found in organic-rich sediments (Inagaki *et al.*, 2006; Edlund *et al.*, 2008; Blazejak & Schippers, 2010) and in methanogenic activated sludges (Yamada *et al.*, 2005, 2007; Yamada & Sekiguchi, 2009). The cultured strains of *Anaerolineaceae* are anaerobic fermenters (Yamada *et al.*, 2006, 2007; Yamada & Sekiguchi, 2009), which may explain why they thrive in deeper layers than sulphate reducers, which often need sulphate or other external electron acceptors (Muyzer & Stams, 2008). Strains of *Anaerolineaceae*, which produce hydrogen, often grew more rapidly in the presence of hydrogenotrophic methanogens (Yamada *et al.*, 2007; Yamada & Sekiguchi, 2009). Therefore, the abundance of *Anaerolineaceae* in this study may have been due to hydrogenotrophic methanogenesis, which is common in the Baltic Sea (Whiticar, 2002; Thiessen *et al.*, 2006; Pimenov *et al.*, 2010, 2012).

5.1.5 Bacterial DNA in sediments: signs of active mineralizers or an inactive palaeome?

DNA in sediments is mostly extracellular and represents a large reserve of prokaryotic DNA sequences, even in recently deposited surface sediments, but particularly in deeper layers (Corinaldesi *et al.*, 2011), and can be used to study present as well as past bacterial communities. However, a drawback of total DNA-based techniques is an inability to distinguish the active bacteria, such as organic matter decomposers, from the inactive remnants of the preceding active bacterial communities, the palaeome (Inagaki *et al.*, 2005). Whether the 16S rRNA gene sequences represented the palaeome or potentially active bacteria in this study was assessed, based on the existing knowledge of the bacteria in aquatic systems.

Cyanobacteria were relatively abundant in certain surface sediments (Fig.3), but rare in the deeper sediments. Since cyanobacteria are not generally capable of heterogeneous growth, except for some species (Stal & Moezelaar, 1997) and occur in the sediment in a resting stage (as akinetes) (Baker & Bellifemine, 2000), they were considered to represent the palaeome and thus could be used to characterize the past phases of the Baltic Sea. The abundance of cyanobacteria in the surface sediments was interpreted as the sign of a bloom in the water column, which has been settled.

Other possible representatives of the palaeome in the deep sediment layers included the 16S rRNA sequences related to the genera *Pseudomonas* and *Arthrobacter*. Although the genus *Pseudomonas* possesses versatile physiological capacity (Spiers *et al.*, 2000) and is also found in anoxic environments, it was abundant in only one 16S rRNA gene library of the Early Litorina Sea, in which the genus *Arthrobacter* also occurred. Thus these bacteria, which are ubiquitous in soil (Eschbach *et al.*, 2003; Silby *et al.*, 2011; Wu *et al.*, 2011),

were considered an indication of terrestrial input and reflected the depositional history of the Baltic Sea.

Bacteria settling into the sediment can also reflect the current and recent conditions of the water column. For instance, here, the bacterial community composition of the surface sediments (Fig.3 and Figure 5 in II), including mainly *Alpha*-, *Delta*- and *Gammaproteobacteria*, *Bacteroidetes*, and occasionally *Verrucomicrobia* and *Actinobacteria*, resembled the community composition of the Baltic water column (Riemann *et al.*, 2008; Herlemann *et al.*, 2011). In the water column, the proportion of *Alpha*- and *Gammaproteobacteria*, *Bacteroidetes*, *Verrucomicrobia* and *Actinobacteria* decreased downwards with the decreasing oxygen concentrations, whereas that of *Deltaproteobacteria* increased (Herlemann *et al.*, 2011).

Here, the proportion of *Alpha*- and *Gammaproteobacteria*, as well as the phylum *Bacteroidetes* decreased below the 8-cm depth, which may signify that these bacteria reflected the communities of the oxic water column. These bacteria were also presumably active in oxic sediment, since their proportion increased in the surface sediments overlain by the oxic near-bottom water, indicating that they benefited from oxygen. *Actinobacteria* and particularly *Verrucomicrobia* were abundant only in certain 16S rRNA gene libraries of the surface sediment (Fig. 3) and their T-RFs were relatively rare, indicating that these bacteria may be a reflection of the water column. However, *Actinobacteria* may have maintained their viability under the hypoxic sediment conditions via intracellular polyphosphate accumulation (Kong *et al.*, 2005) and may have played a role in the release of phosphorus in sediments with fluctuating redox conditions.

Deltaproteobacteria, including mainly sulphate reducing bacteria, were abundant in the uppermost 20 cm of sediment but also occurred in the deepest layers (Fig.3). Sulphate reduction can occur even below the sulphate-methane transition zone (Holmkvist *et al.*, 2011a, 2011b) and thus this study presumed that some of the sulphate reducers could also be active below 20 cm.

5.1.6 Contribution of sediment bacterial communities to long-term nutrient fluxes

Although the proportion of sulphate reducers generally decreased below 20 cm, the family *Desulfobacteraceae* was associated with elevated concentrations of HCl-extractable Ca in the deep sediment layers (Table 6), where acid-extractable Ca is a sign of detrital apatite or other Ca minerals (Lukkari *et al.*, 2007a). Although the association may only indirectly reflect the aged stage of organic matter, it can also signal the activity of sulphate reducers, since roughly half of the organic phosphorus buried in these layers with high preservation potential (Figure 2A in II) was labile, which indicated the presence of degradation products (Lukkari *et al.*, 2009b).

The potential activity of sulphate-reducing bacteria, even at depths of metres (Holmkvist *et al.*, 2011a, 2011b), may inhibit the binding of mineralization-derived phosphate to Fe, since sulphide forms insoluble ferrosulphides with Fe (Berner, 1970). This may be significant in the Baltic Sea, which has been relatively organic-rich throughout the last two

millennia (Zillen & Conley, 2010). Evidently, sulphate reducers in the deep biosphere may potentially enable the long-term release of phosphorus.

5.1.7 Remarks of T-RFLP of 16S rRNA genes as methods for studying bacterial community composition

In this study, the entire bacterial community compositions of the sediment samples were compared, mainly by semiquantitative T-RFLP of 16S rRNA genes. Bias and artefacts in the T-RF data may have resulted e.g. from PCR (Hartmann & Widmer, 2008). For instance, template reannealing (Suzuki & Giovannoni, 1996; Suzuki *et al.*, 1998) or varying numbers of genes in a single cell coding for 16S rRNA (Pei *et al.*, 2010), and artefacts, such as chimeras, can produce a higher diversity of 16S rRNA genes *in vitro* than in the environment, and can skew the 16S rRNA gene frequencies of different bacterial phylogenetic groups. The downstream analyses, enzymatic restriction as well as capillary gel electrophoresis, can also result in variation that can impair the detection of community structures.

However, T-RFLP was reproducible, the normalization procedure for the removal of background 'noise' was carefully selected and four different restriction enzymes were used to increase the accuracy of the T-RF data, since a single T-RF can represent more than one bacterium (Kitts, 2001; Engebretson & Moyer, 2003). A previous study (Hartmann & Widmer, 2008) and this study suggest that T-RFLP is a reliable method for investigating bacterial community structures and for monitoring.

5.2 Conclusions and future prospective

In this study, DNA-based molecular methods were used to identify and compare the bacterial communities of the sediments, which covered the entire depositional history of the Baltic Sea back to 8000 years ago. The study demonstrated that the high organic content and bioavailability of different chemical forms of phosphorus as well as elements involved in phosphorus cycling shaped the bacterial communities of the recently deposited sediments. In addition, the past and present water column hypoxia substantially affected the bacterial communities.

The bacterial community composition reflected the current eutrophic and hypoxic state of the Baltic Sea. The dominance of anaerobic sulphate reducers, particularly the obligate anaerobe *Desulfobacula*, and the family *Anaerolineaceae* of the phylum *Chloroflexi* in the present and recently deposited sediments, signalled that hypoxia has progressed to the level at which most of the benthic energy of the aquatic food webs is directed down to the sediment (Diaz & Rosenberg, 2008).

The bacteria-chemistry correlations could indicate that the sediment bacterial communities actively participated in nutrient, e.g. phosphorus and nitrogen, recycling. For instance, the mineralization of organic matter can be a considerable source of nutrients in the organic-rich Baltic Sea sediments. Bacteria also indirectly contribute to the nutrient cycling, since they consume oxygen in the sediment, and consequently nutrients sensitive to hypoxia,

such as Fe-bound phosphorus, are recycled back to the water column. This vicious internal feedback mechanism reinforces the eutrophication.

The bacterial community composition of the deep sediment core from the central GOF formed three distinct groups that supported the known historical sea phases: the Early (brackish), middle (most saline) and the Late (brackish) Litorina Sea. This study also provides new information on the past salinity changes in the GOF. The sudden increase in the bacterial community heterogeneity suggests that salinity, which contributed to the preservation of organic matter, was highest approximately 6200–6600 years ago. In addition, it was shown that the DNA-based bacterial community data can be used as a palaeomicrobiological tool to detect historical environmental changes in the sedimentary record.

The abundance of *Chloroflexi*, the organic indicator bacteria, in all sea phases detected demonstrated that the Baltic Sea has been relatively organic-rich through its 8000-year-old history. However, the organic-rich nature of the Baltic Sea during the Early and middle Litorina Sea has been due to climatic and oceanographic changes that have led to sea-level rises and consequently increases in salinity and hypoxia (Zillen *et al.*, 2008).

For the recovery of the eutrophic Baltic Sea, it would be necessary to identify and quantify various bacteria-mediated mechanisms in phosphorus and nitrogen cycling so that conservation and nutrient management could be targeted as effectively as possible. Such investigations have recently been performed, e.g. by Steenberg *et al.* (2011) and Dale *et al.* (2013). Although the effect of phosphate-accumulating *Beggiatoa* on phosphorus cycling has, thus, been modelled (Dale *et al.*, 2013), there is still a great need for studies focusing on *Beggiatoa*-like bacteria in the Baltic Sea, where these bacteria are apparently common.

Another important point for the recovery of the Baltic Sea is the resilience of the bacterial communities (Allison & Martiny, 2008; Shade *et al.*, 2011). If a bacterial community is resilient, it will regain the state it had under less eutrophic conditions. It is not known whether the communities of the Baltic will recover, and if they do, how long it will take, and how much it will affect internal loading after the external nutrient loading into the Baltic Sea is remarkably reduced.

Perhaps most importantly, researchers should develop tools that hinder the runoff and loading into aquatic systems from agriculture-intensive areas. Although the unnecessary use of chemical fertilizers and manure in agriculture should be discontinued, devices and techniques that remove nutrients from aquatic systems are also needed. For instance, techniques that remove nutrients from the flowing water and recycle them, e.g. by reusing them as fertilizer in agriculture (NRM Natural resource management, s.a.), would reduce the need for external nutrients.

The study of the deep biosphere aids in understanding the history of the Baltic Sea from the microbiological standpoint and enables us to predict the future development of eutrophication and nutrient recycling. It would be essential to determine whether the phyla

Chloroflexi and *Planctomycetes* as well as candidate division JS1 are sources or sinks of methane, i.e. do they facilitate methane fluxes or oxidize methane anaerobically (AOM)? The organic- and methane-rich Baltic Sea would be an ideal place to determine the biogeochemical roles of these bacteria in the deep biosphere and how they may affect current nutrient cycling.

The information linked to biogeochemical cycling is needed globally to widen our understanding of nutrient cycling in continental seas where dead zones are spreading worldwide (Diaz & Rosenberg, 2008). For instance, the biogeochemical models used in nutrient management often lack the information of sediment microbial processes that influence nutrient fluxes. This study provided information on the eutrophication-related changes in bacterial community composition and assessed the role of sediment bacteria in nutrient fluxes, based on bacteria-chemistry correlations. The data suggest that the cycle of Fe-bound phosphorus regulated the bacteria-mediated nutrient recycling in the Archipelago Sea, whereas in the GOF, organic nutrients such as nitrogen affected recycling more. The data enable the proper planning of future studies and suggest that the scale of microbial sulphate and Fe reduction, as well as anaerobic processes related to nitrogen cycling, would be vitally important to detect in the GOF and could be included in biogeochemical models (e.g. Vanhatalo 2013). In addition, the study provides a novel palaeomicrobiological tool for investigating the historical environments recorded in the sediment, e.g. in ocean-drilling projects.

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A handwritten signature in black ink, appearing to read "Hanna". The signature is written in a cursive, flowing style.

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