The complex effects of the invasive polychaetes *Marenzelleria* spp. on benthic

nutrient dynamics

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Abstract

The effects of the polychaetes *Marenzelleria* sp. (Polychaeta, Spionidae), nonindigenous, rapidly increasing species in the Baltic Sea, on benthic nutrient fluxes, denitrification and sediment pore water nutrient concentration were studied in laboratory experiments using a flow-through setup with muddy sediment from coastal regions of the Gulf of Finland. In addition, different forms of sediment phosphorus (P), separated by chemical fractionation, were studied in three sediment layers. At a population density corresponding to about half the highest measured in the northern Baltic Sea, *Marenzelleria* sp. increased the fluxes of P and ammonium to the water column. No effect could be recorded for denitrification. Since the previously dominant species of the area, *Monoporeia affinis*, can enhance denitrification and lower the amount of dissolved P in the pore water, the replacement of *M. affinis* with *Marenzelleria* spp. may lead to increased P flux to the water column and decreased denitrification, further increasing the ammonium flux to the water column. However, sediment reworking by *Marenzelleria* spp. also oxidizes the surface sediment in the long run, improving its ability to retain P and support nitrification. Therefore, the impact of *Marenzelleria* spp. on sediment nutrient release may not be as drastic as the initial reactions seen in our experiments suggest.

Keywords

Introduction

Mineralization of the organic matter in shallow bodies of water mainly occurs in sediments. Environmental conditions at the bottom, such as temperature and oxygen (O$_2$) concentration, vary seasonally and yearly, affecting the structure of microbial communities, the rate at which mineralization occurs and the pathways that are used. Both nitrogen (N) and phosphorus (P) mineralization and the fate of the mineralized nutrients are dependent largely on the O$_2$ conditions. Microbial denitrification is an anaerobic process converting nitrite and nitrate (NO$_X$ = NO$_2^-$ + NO$_3^-$) to gaseous dinitrogen (N$_2$), thereby decreasing the amount of fixed N in the water ecosystem. In recent years, another natural process removing fixed N from the water ecosystem, anaerobic ammonium oxidation (anammox), was discovered in marine sediments (Dalsgaard and Thamdrup, 2002; Thamdrup and Dalsgaard, 2002; Trimmer et al., 2003). In this process, microbes oxidize ammonium (NH$_4^+$) with NO$_2^-$ to form N$_2$. The NO$_X$ used in the processes can originate either from the water column, or from an aerobic nitrification process in the oxic layer of the sediment. Since nitrification is dependent on the supply of O$_2$, anoxic denitrification can also proceed only as long as there is enough O$_2$ for the nitrifiers to produce NO$_X$. During anoxia, NH$_4^+$ accumulates in the bottom water, from which it can return to the productive water layers in mixing or upwelling events. The P release from the sediment is also commonly associated with the depletion of bottom water O$_2$ (e.g. Mortimer, 1941, 1942). Anoxia leads to reduction of iron (Fe) compounds (hydrated oxides) in sediments and in suspended particles, and to consequent release of Fe-bound P to the bottom water. In addition to the Fe compounds, P in the sediment also has several other binding forms, the binding strengths and reactivities of which differ (e.g. Boström et al., 1982).
Benthic animals enhance microbial activities in several ways. They mix newly sedimented material to the deeper sediment layers, transport oxidized and reduced compounds in their burrows, concentrate organic matter in faecal pellets and reduce the size of particles, thereby increasing particle surface area for microbial colonization (e.g. Kristensen, 1988). The animals mix oxic bottom water into the surface sediment, increasing the volume of oxygenated sediments. In addition, the channels of the burrowing animals increase transport of oxic bottom water, both by diffusion and physical transport by the animals, to the deeper anoxic sediment layers. The mineralization products and the metabolites of the burrowing animals are effectively removed from the channels by irrigation, increasing the fluxes out of the sediment by keeping the concentration gradient between the sediment pore water and burrow water high. The shape, location and ventilation frequency of the channels in the sediment vary according to the species responsible for bioturbation and have major implications for the nutrient dynamics within and around the burrows (e.g. Welsh, 2003).

Bioturbation enhances nitrification activity by increasing surfaces with access to both NH$_4^+$ and O$_2$ (Pelegri and Blackburn, 1994; Tuominen et al., 1999; Svensson et al., 2001), although reduction in nitrification, caused by partial digestion of nitrifiers by insect larvae in organic-poor (but not organic-rich) sediment, has also been reported (Altmann et al., 2004). Due to enhanced nitrification, the denitrification rate also generally increases in the presence of benthic animals (Pelegri and Blackburn, 1994; Svensson and Leonardson, 1996; Hansen and Kristensen, 1998; Bartoli et al., 2000; Svensson et al., 2001), although this effect is not always clear (Tuominen et al., 1999).

Bioturbation enhances P binding to the sediment, since Fe compounds in oxygenated sediments retain P more effectively (e.g. Andersen et al., 1991; Tuominen et al., 1999). However, bioturbation can also temporarily increase sediment P release when
bioturbation reaches deeper, reduced sediment layers with dissolved P in the pore water (e.g. Hansen et al., 1998). If the oxic sediment layer is thin, or the binding sites for P are already highly saturated, there may not be enough binding sites for all the released P. In larger channels, P can also be transported by advection in addition to diffusion (e.g. Kristensen, 1988), and the flux of pore water P can occur so quickly that some of the P released will reach the bottom water. The compounds, excreted by the benthic animals, that line the walls of the burrow channels, may also hinder the adsorption of dissolved P to the oxides of Fe and aluminium (Al) on sediment particles. In addition, the general increase in microbial activity caused by bioturbation leads to increased release of P in mineralization of organic matter (e.g. Andersen and Jensen, 1991; Hansen et al., 1998). However, if the bottom water is oxic, the P released is trapped back on the suspended particles and the sediment surface.

The invasive polychaete worm, identified as the North American *Marenzelleria viridis* (Verrill) (Polychaeta, Spionidae), was first recorded in the southern Baltic Sea in 1985 (Bick and Burckhardt, 1989), in 1990 in the coastal Gulf of Finland (Stigzelius et al., 1997), and in the northernmost arm of the Baltic, the Gulf of Bothnia, in 1996 (Stigzelius et al., 1997). Later the taxonomy of *Marenzelleria* species was revised by Sikorski and Bick (2004) who described a new species, *M. neglecta*, in the Baltic Sea. More recently, the occurrence of three different species in the Baltic Sea, namely *M. viridis*, *M. neglecta* and *M. arctica* (Chamberlin) was confirmed by molecular methods (Bastrop and Blank, 2006). The species are morphologically very similar and likely to have only minor, if any, functional (e.g. bioturbation) differences. The species status in previous Baltic Sea studies cannot be fully confirmed and their current distribution is under study (Blank et al., in prep.).
**Marenzelleria** spp. now commonly occur in the coastal northern Baltic Sea (Laine et al., 2003a; 2003b; Perus and Bonsdorff, 2004), and high abundances and biomasses were recorded both in the southern Baltic (Kube et al., 1996; Zettler, 1996) and in the Gulf of Riga (Cederwall et al., 1999). Recently, high densities and biomass values of up to 4000 ind. m\(^{-2}\) and 80 g m\(^{-2}\) wet weight, respectively, have been observed in the deep open Gulf of Bothnia (Finnish Institute of Marine Research (FIMR), unpublished data). The previously abundant and dominant amphipod (*Monoporeia affinis* (Lindström)) populations have declined strongly during recent decades in the coastal areas of the northern Baltic Sea (Laine et al., 2003a, 2003b; Perus and Bonsdorff, 2004) and also in the open Bothnian Sea (Norkko et al., 2007). Concomitantly, the importance of **Marenzelleria** spp. is expected to increase in the soft-bottom ecosystem. **Marenzelleria** *viridis* is an infaunal species, forming highly branched burrow networks, that feeds with palps in the sediment-water interface by collecting either material deposited on the sediment surface or suspended particles in the near-bottom water (Dauer et al., 1981). In the southern Baltic, the L- or J-shaped burrows of **Marenzelleria** spp. extend downwards 25-35 cm into the sediment (Zettler et al., 1994), which is much deeper than for any of the native Baltic soft-bottom macrofaunal species. Thus, in the Baltic Sea ecosystem these species appear to have occupied an open niche and have the potential for affecting the sediment nutrient dynamics through deep-reaching bioturbation (Olenin and Leppäkoski, 1999). Despite the small, slim morphology of the animals, they have the potential for affecting conditions on the seafloor, due to the large number of animals, the deep burrows they dig and their clear resilience to challenging environmental conditions, such as low O\(_2\) concentrations (Schiedek, 1997).

We studied the effect of **Marenzelleria** sp. (most probably *M. arctica*, see Material and methods) population on its new environment. Laboratory experiments were performed using a flow-through setup with coastal Gulf of Finland muddy sediment. We measured
benthic nutrient fluxes, denitrification, O\textsubscript{2} penetration depth in the sediment and sediment pore water nutrient concentration at low and high densities of \textit{Marenzelleria} sp. as well as in control sediments without animals. In addition, different forms of sediment P, separated by chemical fractionation, were studied in three sediment layers with and without high densities of \textit{Marenzelleria} sp.

**Materials and methods**

**Experimental setup**

The \textit{Marenzelleria} sp. individuals were collected on October 18, 2004 in the Åland Sea at a 285-m-deep station (F64; 60°18.0’ N, 19°15.0’ E). This area hosts a dense \textit{Marenzelleria} sp. population, e.g. in June 2004 a density of 4000 ind. m\textsuperscript{-2} was recorded at this site. At the time of sampling the species was assumed to be \textit{M. viridis} but samples taken at the same site in 2005 and identified with molecular methods revealed that the population consisted of \textit{M. arctica} only (Blank et al., submitted). Thus, the worms used in this study most probably belong to \textit{M. arctica} but since the species identity in the present material was not confirmed, we refer to them as \textit{Marenzelleria} sp. Sediment was collected using a van Veen grab and gently mixed with water. Swimming \textit{Marenzelleria} spp. were caught in a 1-mm sieve. The animals were then placed in aerated transport boxes with 5 cm of sieved local sediment and kept at +5 °C until transport to the laboratory.

The sediment used in the experiments was collected from a coastal station (Tvärminne, Storfjärden, northern Gulf of Finland, 59°51.3’ N, 23°15.8’ E), representing a characteristic, outer archipelago accumulation bottom consisting of soft mud. The water depth at the sampling station is 33 m. A box corer was used to collect the deeper anoxic sediment and an Ockelman sledge to collect the oxic surface sediment. The mud was
gently sieved (1 mm for the deeper sediment and 0.5 mm for the surface slurry) to
remove macrozoobenthos. The experiment was conducted at +5 °C in a coldroom,
corresponding to in situ temperature. Round polyacrylic aquariums (30 aquariums with
inner diameter 14 cm, height 16 cm) were packed with 8 cm of deeper mud that was
covered with 2 cm of surface mud. The aquariums were filled with filtered seawater of
the same salinity (6.1) and temperature as the sampling station, taken at the beginning of
the experiment from a nearby location, filtered (0.2 µm) and kept in the coldroom for the
length of the experiment. The aquariums were covered with 0.5-mm wire mesh covers.

After letting the aquariums stabilize for 12 days, the first samples (time zero
measurements) were taken and animals were distributed in the aquariums so that nine
aquariums received no worms (control units, C), nine received five worms each (low-
density units, LOW, corresponding to 325 ind. m\(^{-2}\)) and nine received 30 worms each
(high-density units, HIGH, corresponding to 1950 ind. m\(^{-2}\)). The mesh covers were
equipped with hypodermic needles that were attached to tubing, bringing filtered water
from the container through a peristaltic pump at a rate of 1.5 ml min\(^{-1}\). Excess water was
allowed to overflow.

### Sampling and analyses

Samples were taken at time zero (before adding the worms) and then on days 2, 6 and 14
after connecting the flow-through system. The sampling was randomized and three
aquariums of each treatment were sacrificed at each sampling time (except on day 14,
when one LOW, one HIGH and two C units were discarded due to problems in
waterflow).

The samples for nutrient fluxes (NH\(_4^+\), NO\(_x\), phosphate (PO\(_4^{3-}\)) and Fe) were collected
from the aquariums using a peristaltic pump, collecting outflowing water at the same rate
as water was pumped into the aquariums (1.5 ml min\(^{-1}\)). In addition, the inflowing water
from the container was sampled and the fluxes were calculated from the difference in concentrations, using the residence time and sediment area. All nutrients were analysed using standard methods (Koroleff, 1983). All units were photographed prior to sediment sampling. The sediment samples for all the different measurements were taken simultaneously so that all the tube cores needed were inserted in the aquarium at the same time, ensuring undisturbed samples.

Denitrification was measured using the isotope pairing technique (Nielsen, 1992). Three replicate samples were taken in clear plastic cores (diameter 2.6 cm, height 9 cm) so that about half of the core was filled with the sediment and half with the water from above. The samples were enriched with K$_{15}$NO$_3$ (98% labelling, Cambridge Isotope Laboratories, Andover, MA, USA) to a final concentration of 100 µM of $^{15}$NO$_3^-$ in the overlying water and incubated, with a magnetic stirrer on the lid of the cores, at in situ temperature in darkness for 3-4 hours. Incubation was terminated with ZnCl$_2$, samples were mixed and subsamples were sent in gastight 12-ml vials (Exetainer; Labco, High Wycombe, Buckinghamshire, UK) to the National Research Institute, Silkeborg, Denmark, for analysis of N$_2$ isotopic composition.

The O$_2$ profiles were measured in undisturbed sample cores, similar to those used in the denitrification measurements, using Clark-type oxygen microelectrodes (100-µm tips, OX-100; Unisense, Aarhus, Denmark) that gave a spatial resolution of about 200 µm. For pore water nutrient analyses, one sample per aquarium was taken using a corer with an 8-cm inner diameter. The sample was sliced in 2-cm strata from the surface to and 8-cm depth in the sediment, and slices from the same depth from replicate aquariums (three of each treatment) were combined. The sediment redox potential was measured in each combined slice with an electrode (SenTixORP), corrected by temperature and converted to an E$_{h}$ value. The pore water was then extracted by squeezing the samples with a
Millipore Zero Headspace Extractor, (Millipore, Billerica, MA, USA) using 0.45-µm filter (Nuclepore; Whatman), under N\textsubscript{2} atmosphere.

Two replicate sediment samples for P fractionation studies were collected from two C units and two HIGH units on days 2 and 14. The samples were taken into small plastic corers (see denitrification) and the water above the sediment was removed (siphoned) immediately. The samples were kept in the corers at +5 \degree C, capped and protected from light. Each sediment core was subsampled the following day in an N\textsubscript{2} atmosphere (O\textsubscript{2} content below 5\%) in a glove box, separating three sediment layers: 0-2 cm, 2-4 cm and 4-6 cm. The subsamples were stored in plastic bottles at +5 \degree C until analyses (for about 3 weeks). The various chemical forms of sediment P were determined using a P fractionation method slightly modified from that described in Jensen and Thamdrup (1993) (detailed description in Lukkari et al., submitted). The method separates six P pools (Jensen et al., 1995): loosely adsorbed and pore water P (extracted with sodium chloride, NaCl; referred to as NaCl-iP), a redox-sensitive fraction of P bound to hydrated oxides of reducible metals (mainly those of Fe) (sodium dithionite, Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4}, in bicarbonate buffer, NaHCO\textsubscript{3}, at pH 7; NaBD-iP), P bound to oxides of Al and nonreducible Fe (sodium hydroxide, NaOH; NaOH-iP), apatite-P (hydrochloric acid, HCl; HCl-iP) and residual, mainly organic, P (extracted with HCl after combustion; Res-iP). In addition to these five fractions, the pool of mobile organic P (nonreactive P, NRP) was determined as the difference between total P (TP) and dissolved inorganic P (iP), summarized from the first three steps. The iP was determined from filtered (Nuclepore polycarbonate membranes, pore size 0.4 µm) extracts with a UV-VIS spectrophotometer (Genesys 10uv Thermo Spectronic; Genesys, Daly City, CA, USA) with a 50-mm flow-injection cuvette and TP with a spectrophotometer after acid persulphate digestion (Koroleff, 1983).
The sediment dry matter (DM) content was first determined using a moisture analyser (a balance equipped with a halogen lamp dryer, Ohaus MB45; Ohaus, Pine Brook, NJ, USA), and the amount of fresh sediment extracted was determined as a fresh weight corresponding to 0.3 g dry mass. The volume of the extracts was always 30.0 ml yielding a sediment DM-to-solution ratio of 1:100. The sediment subsamples were not sieved, but visible animals or their remains were removed.

The effects of *Marenzelleria* spp. and time on denitrification and benthic nutrient fluxes were tested using two-factor analysis of variance (ANOVA) and when significant differences ($\alpha = 0.05, p < 0.01$) were found, treatments were further tested against the C units, using Dunnett’s test ($\alpha = 0.05$). Differences in pore water nutrient profiles between the treatments and sampling occasions, based on concentrations in different layers, were tested using the multivariate analysis of similarities (ANOSIM) procedure (Clarke and Warwick, 2001). The differences in sediment P fractions between the C and HIGH treatments were tested using t-tests assuming unequal variances ($\alpha = 0.05, p < 0.05$) and the effects of time within treatment were tested using paired t-tests ($\alpha = 0.05, p < 0.05$). The significances of all correlations (denitrification, fluxes, concentrations of dissolved $O_2$ and nutrients) were analysed using Pearson’s correlation test ($\alpha = 0.05, p < 0.01$).

**Results**

In the C units, the two-layer structure basically persisted until the end of the experiment. By day 6 some darkening of the initially light brown colour in the lower parts of the surface layer was observed. By day 14, there was an even more clear difference and only the uppermost 5 mm in the C units had retained the original colour, whereas the lower part of the surface layer was clearly darker, with black spots, indicating reduction and
sulphide formation. Microbial growth on the sediment surface was indicated by light-
coloured patches and increase in surface roughness by day 14.

The worms began to burrow immediately after they had been transferred to the
aquariums. Burrows were observed from day 2 onwards, both in the LOW and HIGH
units. Sometimes worm heads projected out of the sediment and the worms were lashing
with their palps to feed but disappeared quickly when disturbed. The burrows formed a
dense network in the vertical and horizontal directions, especially in the HIGH density
units. Active burrowing was observed in the uppermost 5 cm and single burrows
penetrated to more than 8-cm depths in the sediment. Occasionally a thin and transparent
membrane lining was observed in the burrows. The deeper parts of the upper sediment
layer became darker in the same way as in the C units. However, the actively used worm
burrows could be distinguished even in the deep sediment by a thin light-coloured,
apparently oxidized layer that was similar to the colour of the sediment surface.

Burrow openings and faecal pellets were visible on the sediment surface. The faecal
pellets were up to 7 mm in length and formed radial cones around the openings. In the
HIGH units the sediment surface was almost completely covered by pellets by day 14,
whereas in the LOW units roughly half of the surface was covered by pellets and the
other half resembled the surface of the C units. After day 14 some additional units that
were not used for the experiment were sieved. The worms in these units were all alive
and actively swimming when transferred to the water.

The O₂ penetration depth ranged from 0.3 to 1.5 mm and was 0.5 mm at 17 out of the 28
times measured, with no differences between the treatments. The O₂ consumption
likewise did not differ between the treatments, since the O₂ concentration 0.5 cm above
the sediment surface was similar in all aquariums. There were no clear differences in
redox profiles between the different units. However, the redox potential decreased in the surface layer (0-2 cm) of all units during the experiment from an initial level of -10 to +105 mV to a level of -130 to -30 mV in the end. In the deeper sediment the redox potential varied less and was mostly between -150 and -100 mV. Despite the dense burrow networks that developed especially in the HIGH units, no obvious change was detected in porosity between the treatments. However, the surface sediments could be distinguished from the deeper sediments by the porosity difference.

**Nutrient fluxes**

The nutrient fluxes at the sediment-water interface fluctuated considerably during the experiment (Figure 1). The NO$_x$ flux was out of the sediment, except on the last sampling day when it was directed into the sediment in all treatments. There were significant differences between the C and HIGH (but not the C and LOW) units on day 2, when the C units released nine times more NO$_x$ out of the sediment than did the HIGH units. No differences between the treatments were found on day 6, and the significance of the differences on day 14 could not be tested due to the lack of replicates of the C treatment.

The NH$_4^+$ fluxes increased throughout the experiment, were always directed out of the sediment and were always highest in the HIGH, and lowest in the C units. Significant differences between treatments were found on day 2, when the HIGH units released nine times more NH$_4^+$ than did the C units, but not on day 6. On day 14 the difference was even greater, but the significance could not be tested (see above). The PO$_4^{3-}$ flux was likewise directed out of the sediment throughout the experiment and the highest values were always recorded in the HIGH units and lowest in the C units. The differences between the HIGH (but not the LOW) and C units were significant on days 2 and 6. They were even higher on day 14, but the significance could not be tested (see above). The Fe flux was always out of the sediment and increased in the course of the experiment. On
day 2, the flux in the HIGH (but not the LOW) units was significantly higher than in the C units.

The NO$_3^-$ flux correlated positively (Pearson correlation, Table 1) with the O$_2$ penetration depth, NO$_3^-$ concentration and denitrification, and negatively with the denitrification potential (D15). The NH$_4^+$ flux was negatively correlated with total denitrification (Dtot) and coupled nitrification-denitrification (Dn), and positively with D15. The PO$_4^{3-}$ flux was positively correlated only with the Fe flux, which was negatively correlated with the O$_2$ penetration depth.

**Denitrification**

No effect of *Marenzelleria* spp. was detected on the denitrification rates in the experiment. The Dtot and Dn values decreased significantly in all aquariums in the course of the experiment, independent of the treatment (Figure 2). The percentage of denitrification that was based on water column NO$_x$ (Dw) likewise differed significantly between sampling days, but instead of a general decrease, it showed very high values on day 6 in all treatments, decreasing again to very low values by day 14. No interactions between the treatments and time were found. In contrast to the $^{14}$N based natural denitrification (Dtot), the denitrification potential (D15), based on the added $^{15}$N, tended to increase throughout the experiment in all treatments (Figure 2). In addition to the time, the treatments also significantly affected the D15 rates. On days 2 and 6, the D15 rates in the HIGH, but not in the LOW, units were significantly higher than in the C units. The effect disappeared by day 14, when no differences between units with and without animals were found.

The Dtot rates correlated positively (Table 1) with Dn, O$_2$ penetration depth and NO$_3^-$ concentration and flux, and negatively with the D15 rate and NH$_4^+$ flux. The Dn level...
was positively correlated with the O$_2$ penetration depth and negatively with the D15 rate
and NH$_4^+$ flux. The Dw level correlated positively with the NO$_3^-$ concentration and flux.
The D15 rate was positively correlated with the NH$_4^+$ flux and negatively with the NO$_3^-$
concentration.

**Pore water nutrient profiles**

In all units, the pore water NH$_4^+$ concentration increased almost linearly with sediment
depth (Figure 3). The concentrations in the surface layer (0-2 cm) varied from about 220
to 380 µmol l$^{-1}$ at the beginning of the experiment and increased to about 300-425 µmol
l$^{-1}$ on day 14. In the bottom layer (6-8 cm) the concentration was more stable, about 820-
1050 µmol l$^{-1}$. No differences were found between the C, LOW and HIGH units. Only on
day 14 were the NH$_4^+$ concentrations in all layers of the HIGH units slightly lower than
in the C and LOW units. The sediment pore water NO$_x$ concentration was measured only
in the surface layer (0-2 cm) and was very low throughout the experiment (mean 1.9
µmol l$^{-1}$).

The PO$_4^{3-}$ profiles were the most variable of the measured pore water parameters (Figure
3). In the surface layer, the concentrations were below 65 µmol l$^{-1}$ except on day 14 in
the C unit. The maximum PO$_4^{3-}$ concentrations in the deeper layers exceeded 200 µmol l$^{-1}$.
On days 6 and 14, the PO$_4^{3-}$ concentrations in the deeper layers of the HIGH units were
lower than in the C and LOW units. The Fe concentration varied only in the uppermost
layer (Figure 3). In the C units the surface concentration of Fe always exceeded 15 µmol
l$^{-1}$. In all units the concentrations at 2-8-cm depths were very low, < 5 µmol l$^{-1}$, and did
not vary during the experiment. In the HIGH units the surface layer Fe concentrations fell
to low levels, similar to those observed in the deep sediments on days 6 and 14. No
significant differences were detected in any of the pore water nutrient profiles between
the treatments or sampling occasions.
Sediment P fractions

The difference in P fractions between the treatments and as a function of time could not be tested statistically at each data point, because there were only two replicate samples in the C units on day 14. The concentration of total extractable P (TP\textsubscript{extr}) was about 60 and 40 µmol g\textsuperscript{-1} DM in the surface (0-2 cm) and bottom (2-6 cm) sediment layers, respectively, with NaBD-iP and HCl-iP forming the major fractions. NaCl-iP and NaBD-iP were the fractions most affected by the treatment, while Res-P was the most stable P form (Figure 4).

NaCl-iP constituted only a minor percentage of TP\textsubscript{extr} (< 1%). Its concentration was highest in the surface layer (0-2 cm) and did not change markedly in the C units between days 2 and 14. The increase in NaCl-iP in the surface layer from day 2 to day 14 in the HIGH units was significant. On day 2, the HIGH units had significantly lower NaCl-iP at 0-2 cm than the C units. On day 14, no marked differences between the treatments were found.

NaBD-iP formed 11-35% of the TP\textsubscript{extr}. Its percentage of the sediment P was higher in the 0-2-cm layer than in the other two layers and increased during the experiment, especially in the surface layers and in the HIGH units. NaBD-iP in the HIGH units was significantly higher on day 14 than on day 2 in the two uppermost layers. The evident increase with time in the C units (Figure 4) could not be tested statistically (only two replicates on day 14). On day 2, NaBD-iP was lower in the HIGH units than in the C units at 0-2 cm and 2-4 cm; the concentration difference was larger in the former, although statistically significant only in the latter. On day 14, the HIGH units had lower NaBD-iP concentrations at 2-4 cm and 4-6 cm than the C units.
NRP constituted 20-31% of the TP$_{ext}$ and its percentage of the sediment P decreased in the C units and increased in the HIGH units (not significantly) during the experiment. The decrease in NRP in the surface layer of the C units is evident (Figure 4), while that in the 2-4-cm and 4-6-cm layers is within standard deviation of the replicate results. On day 2, NRP was lower in all depth layers in the HIGH units compared with those in the C units, but significantly only in the surface layer. On day 14, the NRP concentration was higher in the two deepest layers of the HIGH units than in the C units (Figure 4).

NaOH-iP formed 4-7% of the TP$_{ext}$ in the sediment and its percentage did not change during the experiment. However, possibly due to the very small deviation in the results, the relatively small (< 1 µmol g$^{-1}$ DM) changes in NaOH-iP concentrations were statistically significant. NaOH-iP decreased in the 0-2-cm layer of the C units between days 2 and 14. On day 2, the NaOH-iP concentration was lower in the 4-6-cm layer of the C units than of the HIGH units, and on day 14 it was higher in the 2-4-cm layer of the C units compared with the HIGH units.

The percentage of HCl-iP was 17-35% of the TP$_{ext}$. No statistically significant differences were found in the concentrations of HCl-iP between days and treatments. The Res-P fraction formed 15-27% of the TP$_{ext}$. Its percentage of TP increased slightly in the C units and decreased in the HIGH units during the experiment. None of the changes were statistically significant, but the increase in Res-P in the surface layer of the C units from day 2 to day 14 is evident (Figure 4). In addition, despite the high deviation in the HIGH units (especially on day 14), the results suggest a decrease in Res-P from day 2 to day 14. On day 2, Res-P was higher in the HIGH units compared with the C units and vice versa on day 14.
Discussion

The redox conditions in the surface layer of the aquariums gradually deteriorated in all treatments during the experiment. This could be seen both visually, as a darkening of the light brown surface layer from below, and as a decreased redox potential (Mortimer 1941). Reduced substances diffused from the deep sediment layer upwards and at the same time the mineralization processes in the upper sediment layer used up the O$_2$ storage, resulting in a decreasing volume of the oxidized sediment. Despite active burrow formation, the *Marenzelleria* spp. did not counteract the decrease in redox potential by transporting more O$_2$ to the sediment, when the entire sediment volume was considered. They also did not affect the porosity, which may have been caused by different water and sediment relocation in the HIGH units in comparison to C units. Burrow formation could have caused sediment packing in the interspace between the burrows with no overall change in the water content. The coarse sampling procedure may also have failed to detect small-scale differences in the loose surface layer where the water content was highest.

The animals affected the nutrient fluxes shortly after they were introduced to the sediment, but the effect may have been transient, since no significant differences were found in the rates on day 6 (except for the PO$_4^{3-}$ flux ). The differences could not be tested on day 14, but appeared to increase from day 6 for NH$_4^+$ and PO$_4^{3-}$, although there were wide variations. No significant differences were detected in the pore water nutrient profiles, although some effects could be seen at the higher population density. Similarly, Karlson et al. (2005) found no significant effect of *M. viridis* (or *Macoma balthica* (L.) or *Monoporeia affinis*) on pore water nutrients in their recolonization experiment, using reduced natural Baltic Sea sediments. The general increase in the surface layer NH$_4^+$ concentration indicates diffusion from the deeper layers. The slightly lower NH$_4^+$
concentrations in the HIGH units, compared with the C and LOW units, at the end of the experiment is in accordance with the observed higher efflux of NH$_4^+$ that could have been caused by the more intensive burrow formation at the higher worm density.

**Nitrification and denitrification**

The high flux of NO$_3^-$ out of the sediment confirms that the nitrification rate was rapid in the sediment. On day 2, the HIGH units released significantly less NO$_3^-$ in the water than did the C units. The Dn rate was, however, similar in all treatments, indicating that nitrification saturated the NO$_3^-$ demand of the denitrifiers in all treatments. At the same time the NH$_4^+$ flux was significantly higher in the HIGH units, suggesting either a lower nitrification rate in the HIGH units, simultaneous dissimilatory NO$_3^-$ reduction to NH$_4^+$ (DNRA) or simply more rapid transport of NH$_4^+$ out of the sediment. A lower nitrification rate was not likely, because the animals enhance nitrification by increasing the oxic-anoxic interfaces in the sediment (Pelegri and Blackburn, 1994; Tuominen et al., 1999; Svensson et al., 2001). DNRA is usually considered to be of minor importance in natural sediments and to occur mainly in organically enriched environments, such as fish farm sediments (Hattori, 1983; Christensen et al., 2000). However, Karlson et al. (2005) found that DNRA represented a major pathway of NO$_3^-$ removal in laboratory experiments similar to ours, in which reduced Baltic Sea sediments were used. Yet another explanation for the low NO$_3^-$ and high NH$_4^+$ fluxes is the shorter residence time of NH$_4^+$, produced in the mineralization of organic matter in the sediments with animals, due to the enhanced transport from the burrows into the overlying water. Similarly, increase in NH$_4^+$ flux was observed in deep-burrowing polychaete *Nereis virens* (Sars) (Henriksen et al., 1980). The situation on day 14, when the NO$_3^-$ concentration in the water was very low and the flux was towards the sediments, represents typical steady-state conditions in which denitrification consumes NO$_3^-$ at the rate it is produced. That the Dn rate did not increase as the flux turned into the sediments suggests that other
processes, such as DNRA, contributed to the NO$_3^-$ reduction as well. This is also supported by the high NH$_4^+$ flux out of the sediments.

Macrofauna stimulated denitrification in several studies (e.g. Kristensen and Blackburn, 1987; Hansen and Kristensen, 1998; Pelegri and Blackburn, 1994; Svensson and Leonardson, 1996; Gilbert et al., 1998; Bartoli et al., 2000; Svensson et al., 2001). However, in the only study using *M. viridis*, no significant effects on denitrification rate were found (Karlson et al., 2005). Similarly, in our study the *Marenzelleria* spp. did not affect the denitrification rates that decreased in all aquariums during the experiment, independent of the treatment. No explanation was found for the decline. The denitrification process is generally regulated by temperature, O$_2$ concentration (both directly and indirectly via nitrification), NO$_3^-$ concentration and the availability of organic carbon. In these experiments, the temperature was stable and the concentration of O$_2$ dropped from 75% to 50% saturation from day 0 to day 2, after which it remained constant in all aquariums. The O$_2$ penetration depth likewise did not change. The NO$_3^-$ availability clearly regulated the Dw rate that fluctuated together with the NO$_3^-$ concentration, but the Dn rate was independent of the water column NO$_3^-$ concentration. Dn is controlled by the rate of nitrification, which in turn is regulated by the availability of O$_2$ and NH$_4^+$, neither of which were in short supply in the aquariums, although the availability of NH$_4^+$ increased during the experiment. The availability of organic carbon in the aquariums could have been too low to support the heterotrophic denitrifying community. However, both the denitrification rates and the concentrations of labile organic carbon peak in October-December in the study area (Hietanen and Kuparinen, in press). In addition, the D15 rates nearly doubled from day 2 to day 14 in all aquariums, indicating that enough carbon was available for the denitrifiers. The increasing D15 rates also verify that the denitrifying bacteria were not grazed faster than they grew. While the animals did not affect D14 (denitrification based on the natural $^{14}$NO$_3^-$), the D15 rate was
significantly enhanced on days 2 and 6 in the HIGH units, although the effect
disappeared by day 14. The D15 rate reflects Dw in unlimited NO$_3^-$ concentrations. The
increase in D15 in the HIGH units could have been caused by expansion of the
denitrifying zone, since the animals create oxic-anoxic interfaces in their burrows below
the primary oxidized layer. That this effect was not significant in the Dw rates could be
explained by the slightly lower NO$_3^-$ concentration in the HIGH units – the Dw rates
were similar in all treatments, although less NO$_3^-$ was available in the HIGH units.
However, time was also the most significant factor affecting the D15 rate, since no
differences between the rates could be seen on day 14 any more. The increase in D15 in
the C units also indicates that the sieved and repacked sediment layers had not fully
stabilized by the beginning of the experiments, despite the 12-day interval between filling
the aquariums and the first measurements. The same effect could also be seen in the NO$_x$
flux that reached the typical steady-state pattern only by day 14. In experiments using a
similar setup and northern Baltic Sea sediments, Tuominen et al. (1999) found that the
pore water NO$_x$ profile already resembled that of the intact sediment 2 days after
sediment repacking, indicating rapid recovery of the sensitive nitrifiers after sediment
mixing. In these experiments the cores were fully stabilized after 2 weeks. However, due
to the slow growth of the nitrifying bacteria compared with the denitrifying bacteria, it
may also take considerably longer for the system to stabilize (Welsh, 2003).

**Dissolved phosphorus**

The PO$_4^{3-}$ profiles in the C units reflect the different redox states of the two sediment
layers. The oxidized surface layer retains PO$_4^{3-}$ in the Fe compounds, decreasing its
concentration in the pore water, while in the more reduced bottom layer PO$_4^{3-}$ remains in
the pore water in dissolved form, diffusing upwards driven by the concentration
difference between the layers (Mortimer 1941). The maximum formed at the 2-4-cm
layer, immediately below the interface between the surface and bottom layers (see also
Lewandowski and Hupfer, 2005). The differences in the pore water PO$_4^{3-}$ between the
treatments already appeared on day 2 and by day 14 the PO$_4^{3-}$ concentration in the HIGH
treatment was clearly lower than in the C and LOW treatments. This may have resulted
from the more intensive mixing and subsequent higher initial release of PO$_4^{3-}$ into the
pore water in the HIGH treatments, also seen as the higher flux of PO$_4^{3-}$ out of the
sediment on all sampling occasions. A similar effect was noted by Lewandowski and
Hupfer (2005) in experiments using chironomids (*Chironomus plumosus* (L.)) and
oligochaetes (*Tubifex tubifex* (Müller) and *Limnodrilus hoffmeisteri* (Claparède)). In
addition, organic substances excreted by burrowing animals (e.g. Kristensen, 1988) and
the organic molecules produced in the enhanced degradation or transformation of organic
matter may to some extent block the binding surfaces for P in the burrow channels,
leading to higher efflux out of the sediment. In the LOW treatments, the PO$_4^{3-}$ flux
increased to the same level as in the HIGH units on the last sampling occasion,
suggesting that the less extensive burrow formation at the lower animal density delayed
the release of the dissolved P from the bottom layer. The high and increasing
concentration of Fe in the pore water at the surface of the C units is related to the
reduction and upward diffusion in the deeper layers (Mortimer 1941). In contrast, the
surface pore water Fe concentration decreased throughout the experiment, in the presence
of *Marenzelleria* spp., especially at the high density, indicating higher release from the
sediment (seen also in the Fe flux to the overlying water), or precipitation as
oxyhydroxides to the surface sediment. Similar observations were made by Lewandowski
and Hupfer (2005) in experiments using chironomids and oligochaetes.

### Sediment P fractions

The high density of *Marenzelleria* sp. clearly affected the distribution of P into different
forms in the sediment. At the beginning of the experiment, the activity of *Marenzelleria*
sp. released loosely bound or pore water P (NaCl-iP) to the overlying water from the
surface sediment, which was reflected in the high $\text{PO}_4^{3-}$ flux out of the sediment in the HIGH units. Although small, such changes in NaCl-iP may be important, because even P concentrations near the detection limit of the common analytical methods used can affect algal growth (Baldwin et al., 2003). The effect of *Marenzelleria* sp. was most pronounced in the redox-sensitive P fraction (NaBD-iP). The increase in NaBD-iP in the surface sediment of the HIGH units was probably related to sediment oxidation by animal reworking, as was also noted by Lewandowski and Hupfer (2005). The increase in redox-sensitive P in the surface layer of the C units may have been caused by a slow reduction of the bottom sediment and consequent release, upward diffusion and entrapment of P to the Fe compounds in the oxic sediment.

Part of the transformable organic P (NRP) may have been degraded in the surface sediment layer during the 12-day preincubation, since NRP also contains easily hydrolyzable compounds (Ahlgren et al., 2005). The lower percentage of NRP in the bioturbated units 2 days after introducing the animals may have resulted from enhanced mineralization of the easily degradable organic molecules (Hansen et al., 1998), although it is not clear whether this could have occurred within such a short time. Pure physical mixing of the surface and bottom sediment layers by the animals does not solely explain the lower NRP levels, because the same effect was also seen in the two deeper layers. The increase in NRP towards the end of the experiment in the HIGH treatment, on the other hand, may have resulted from the enhanced degradation of the more resistant organic matter affected by sediment reworking, and the congruent decrease in the Res-P fraction supports this conclusion. Kristensen et al. (1992) reported that bioturbation caused loss of detritus and that the relatively refractory organic matter was affected more than the labile material. Since NRP extracted with NaOH also includes inorganic P compounds, e.g. polyphosphates and pyrophosphates that are produced in biological transformation processes (degradation of organic matter), these probably also increased
the percentage of this fraction (Hupfer et al., 1995; Ahlgren et al., 2005). Defecation of
Marenzelleria sp., visible as piles of faecal pellets on the sediment surface, may also
have increased the share of NRP.

As expected, the P bound to oxides of nonreducible metals (NaOH-iP) was not greatly
affected during the experiment, since release from this fraction requires increase in pH or
presence of competing anions (Scheffer and Scheffer, 1984; Ryden et al., 1987). Benthic
animals can indirectly liberate NaOH-iP by mixing the surface sediment with bottom
water of higher pH (Drake and Heaney, 1987; Lewandowski et al., 2005). In our
experiment, the sediment probably acted as a buffer against pH changes, but some
microscale changes may have occurred, explaining the minor changes observed. These
can also be artifacts, despite the statistical significance, since the experimental units had
not fully stabilized by the beginning of the experiments.

Apatite P (HCl-iP), which is mainly detrital, is a resistant form of sediment P and did not
change markedly during this experiment. Res-P, however, showed marked changes
despite its theoretically resistant nature. Res-P contains primarily organic P that is
resistant to degradation, but may release P under favourable conditions, e.g. with
enhanced microbial degradation. This may explain the decrease in Res-P in the
Marenzelleria sp.-treated units during the experiment. The total organic P also decreased
in the bioturbated units during the experiment, suggesting that the high density of
Marenzelleria spp. enhanced degradation of the organic P in the sediment (Hansen et al.,
1998).

Ecological aspects of Marenzelleria spp. invasion

Introduced species alter the community structure and may also cause significant large-
scale changes in the function of the recipient ecosystem (e.g. Carlton, 1996; Leppäkoski
et al., 2002; Vanderploeg et al., 2002). The soft-bottom macrozoobenthos in the Baltic Sea is characterized by very low species richness and a low number of functional groups (Bonsdorff and Pearson, 1999; Laine, 2003). Different and even contrasting effects by macrofauna on nutrient fluxes were explained by species-specific differences in the mode of sediment mixing and structure building, irrigation behaviour and burrowing depth (Welsh, 2003; Mermillod-Blondin et al., 2004; Michaud et al., 2006). Thus, invasions by nonindigenous species, if they result in a change in the community structure, also have a high potential for causing changes in biogeochemical cycles.

The recent invasion of *Marenzelleria* spp. in the northern Baltic Sea now also extends to open sea areas in the Gulf of Bothnia. This change has also caused a complete shift in community dominance. Some areas that previously were highly dominated by the amphipod *Monoporeia affinis* (e.g. Andersin et al., 1977) are currently dominated by corresponding numbers of *Marenzelleria* spp. At several sites, densities corresponding to and exceeding (up to 4000 ind. m\(^{-2}\)) the densities used in our experiment developed in only 1-2 years after the first occurrence of the species (FIMR unpublished data). This could also have caused profound changes in the nutrient fluxes, since these two species may affect nutrient cycling in different ways. *Monoporeia affinis* can stimulate denitrification and decrease the amount of dissolved P in the pore water of sediments in the Gulf of Finland (Gran and Pitkänen, 1999; Tuominen et al., 1999). Karlson et al. (2005) also found higher denitrification rates in sediments affected by *M. affinis*, compared with *M. viridis*, but no difference in the P fluxes. However, in the less eutrophied Gulf of Bothnia, no increase in the denitrification rate due to bioturbation of the amphipods could be detected (Tuominen et al., 2003), probably because the oxidized sediment layer is thicker than in the Gulf of Finland and bioturbation by *M. affinis*, therefore, does not reach deep enough to increase the volume of the oxidized sediment. The different bioturbation effects of *Monoporeia affinis* and *Marenzelleria viridis* are
probably caused by their functional differences. *Monoporeia affinis* is a surface and subsurface deposit feeder that dwells mostly in the uppermost sediment surface layer (Hill and Elmgren, 1987; Karlson et al., 2005). Its active sediment mixing at high densities causes homogenization of sediment without permanent burrow structures, whereas *Marenzelleria* builds more permanent burrows that may enhance the solute fluxes between the deeper sediment layers and the overlying water. However, in natural communities with mixed species compositions, the patterns identified experimentally for single species may not emerge similarly, due to the complex interactions in the mineralization processes (Welsh, 2003). Therefore, *in situ* studies are needed to predict the actual impact of *Marenzelleria* in the benthic processes of the Baltic Sea.

12 **Conclusions**

14 In our 2-week experiments *Marenzelleria* sp. increased the fluxes of P and NH$_4^+$ to the water column. No effect could be recorded for denitrification. Since the previously dominant species of the area, *Monoporeia affinis*, can enhance denitrification and lower the amount of dissolved P in the pore water, the replacement of *M. affinis* with *Marenzelleria* spp. may lead to increased P flux to the water column and decreased denitrification, further increasing the NH$_4^+$flux to the water column. Nutrient release to the bottom water instead of burial and removal from the water ecosystem by denitrification further accelerates eutrophication, the main problem in the Baltic Sea. However, in the long run sediment reworking by *Marenzelleria* spp. also oxidizes the surface sediment, improving its ability to retain P and support nitrification. Therefore, the impact of *Marenzelleria* spp. on sediment nutrient release may not be as drastic as the initial reactions seen in our experiments suggest.
Acknowledgements

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References


Table 1. Pearson correlation matrix ($\alpha = 0.05$, $p < 0.01$). Dtot total denitrification, Dw denitrification based on water column NO$_x$, Dn coupled nitrification-denitrification, D15 denitrification potential, O$_2$ depth O$_2$ penetration depth into the sediment, Conc concentrations and Flux fluxes of O$_2$ and nutrients. Significant correlations marked in bold.

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<th>Dtot</th>
<th>Dw</th>
<th>Dn</th>
<th>D15</th>
<th>O$_2$ depth</th>
<th>Conc NO$_x$</th>
<th>Conc O$_2$</th>
<th>Flux NH$_4^+$</th>
<th>Flux NO$_x$</th>
<th>Flux PO$_4^{3-}$</th>
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Figure legends

Figure 1. Nutrient fluxes between sediment and water (mmol m$^{-2}$ d$^{-1}$, avg ± sd). Significant differences from the control treatment are marked with an asterisk.

Figure 2. A) Coupled nitrification-denitrification (Dn, white columns) and denitrification based on water column nitrate (Dw, dark columns) (mmol N m$^{-2}$ d$^{-1}$, avg ± sd) B) Denitrification potential (D15) (mmol N m$^{-2}$ d$^{-1}$, avg ± sd). Significant differences from the control treatment are marked with an asterisk.

Figure 3. Pore water nutrient concentrations (µM). Diamonds C, squares LOW, triangles HIGH treatments.

Figure 4. P fractions (µmol P g$^{-1}$ DM, avg ± sd) in C and HIGH units on days 2 and 14. White bars 0-2 cm, grey bars 2-4 cm, black bars 4-6 cm.