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

2 **nutrient dynamics**

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19

1 **Abstract**

2

3 The effects of the polychaetes _Marenzelleria_ sp. (Polychaeta, Spionidae), nonindigenous,

4 rapidly increasing species in the Baltic Sea, on benthic nutrient fluxes, denitrification and

5 sediment pore water nutrient concentration were studied in laboratory experiments using

6 a flow-through setup with muddy sediment from coastal regions of the Gulf of Finland.

7 In addition, different forms of sediment phosphorus (P), separated by chemical

8 fractionation, were studied in three sediment layers. At a population density

9 corresponding to about half the highest measured in the northern Baltic Sea,

10 _Marenzelleria_ sp. increased the fluxes of P and ammonium to the water column. No

11 effect could be recorded for denitrification. Since the previously dominant species of the

12 area, _Monoporeia affinis_, can enhance denitrification and lower the amount of dissolved

13 P in the pore water, the replacement of _M. affinis_ with _Marenzelleria_ spp. may lead to

14 increased P flux to the water column and decreased denitrification, further increasing the

15 ammonium flux to the water column. However, sediment reworking by _Marenzelleria_

16 spp. also oxidizes the surface sediment in the long run, improving its ability to retain P

17 and support nitrification. Therefore, the impact of _Marenzelleria_ spp. on sediment

18 nutrient release may not be as drastic as the initial reactions seen in our experiments

19 suggest.

20

21 **Keywords**

24

1 **Introduction**

2

3   Mineralization of the organic matter in shallow bodies of water mainly occurs in

4   sediments. Environmental conditions at the bottom, such as temperature and oxygen (O_2)

5   concentration, vary seasonally and yearly, affecting the structure of microbial

6   communities, the rate at which mineralization occurs and the pathways that are used.

7   Both nitrogen (N) and phosphorus (P) mineralization and the fate of the mineralized

8   nutrients are dependent largely on the O_2 conditions. Microbial denitrification is an

9   anaerobic process converting nitrite and nitrate ($NO_X = NO_2^- + NO_3^-$) to gaseous

10 dinitrogen (N_2), thereby decreasing the amount of fixed N in the water ecosystem. In

11 recent years, another natural process removing fixed N from the water ecosystem,

12 anaerobic ammonium oxidation (anammox), was discovered in marine sediments

13 (Dalsgaard and Thamdrup, 2002; Thamdrup and Dalsgaard, 2002; Trimmer et al., 2003).

14 In this process, microbes oxidize ammonium (NH_4^+) with NO_2^- to form N_2. The NO_x

15 used in the processes can originate either from the water column, or from an aerobic

16 nitrification process in the oxic layer of the sediment. Since nitrification is dependent on

17 the supply of O_2, anoxic denitrification can also proceed only as long as there is enough

18 O_2 for the nitrifiers to produce NO_x. During anoxia, NH_4^+ accumulates in the bottom

19 water, from which it can return to the productive water layers in mixing or upwelling

20 events. The P release from the sediment is also commonly associated with the depletion

21 of bottom water O_2 (e.g. Mortimer, 1941, 1942). Anoxia leads to reduction of iron (Fe)

22 compounds (hydrated oxides) in sediments and in suspended particles, and to consequent

23 release of Fe-bound P to the bottom water. In addition to the Fe compounds, P in the

24 sediment also has several other binding forms, the binding strengths and reactivities of

25 which differ (e.g. Boström et al., 1982).

26

1   Benthic animals enhance microbial activities in several ways. They mix newly

2   sedimented material to the deeper sediment layers, transport oxidized and reduced

3   compounds in their burrows, concentrate organic matter in faecal pellets and reduce the

4   size of particles, thereby increasing particle surface area for microbial colonization (e.g.

5   Kristensen, 1988). The animals mix oxic bottom water into the surface sediment,

6   increasing the volume of oxygenated sediments. In addition, the channels of the

7   burrowing animals increase transport of oxic bottom water, both by diffusion and

8   physical transport by the animals, to the deeper anoxic sediment layers. The

9   mineralization products and the metabolites of the burrowing animals are effectively

10 removed from the channels by irrigation, increasing the fluxes out of the sediment by

11 keeping the concentration gradient between the sediment pore water and burrow water

12 high. The shape, location and ventilation frequency of the channels in the sediment vary

13 according to the species responsible for bioturbation and have major implications for the

14 nutrient dynamics within and around the burrows (e.g. Welsh, 2003).

15

16 Bioturbation enhances nitrification activity by increasing surfaces with access to both

17 NH_4^+ and O_2 (Pelegri and Blackburn, 1994; Tuominen et al., 1999; Svensson et al.,

18 2001), although reduction in nitrification, caused by partial digestion of nitrifiers by

19 insect larvae in organic-poor (but not organic-rich) sediment, has also been reported

20 (Altmann et al., 2004). Due to enhanced nitrification, the denitrification rate also

21 generally increases in the presence of benthic animals (Pelegri and Blackburn, 1994;

22 Svensson and Leonardson, 1996; Hansen and Kristensen, 1998; Bartoli et al., 2000;

23 Svensson et al., 2001), although this effect is not always clear (Tuominen et al., 1999).

24

25 Bioturbation enhances P binding to the sediment, since Fe compounds in oxygenated

26 sediments retain P more effectively (e.g. Andersen et al., 1991; Tuominen et al., 1999).

27 However, bioturbation can also temporarily increase sediment P release when

1   bioturbation reaches deeper, reduced sediment layers with dissolved P in the pore water

2   (e.g. Hansen et al., 1998). If the oxic sediment layer is thin, or the binding sites for P are

3   already highly saturated, there may not be enough binding sites for all the released P. In

4   larger channels, P can also be transported by advection in addition to diffusion (e.g.

5   Kristensen, 1988), and the flux of pore water P can occur so quickly that some of the P

6   released will reach the bottom water. The compounds, excreted by the benthic animals,

7   that line the walls of the burrow channels, may also hinder the adsorption of dissolved P

8   to the oxides of Fe and aluminium (Al) on sediment particles. In addition, the general

9   increase in microbial activity caused by bioturbation leads to increased release of P in

10   mineralization of organic matter (e.g. Andersen and Jensen, 1991; Hansen et al., 1998).

11   However, if the bottom water is oxic, the P released is trapped back on the suspended

12   particles and the sediment surface.

13

14   The invasive polychaete worm, identified as the North American *Marenzelleria viridis*

15   (Verrill) (Polychaeta, Spionidae), was first recorded in the southern Baltic Sea in 1985

16   (Bick and Burckhardt, 1989), in 1990 in the coastal Gulf of Finland (Stigzelius et al.,

17   1997), and in the northernmost arm of the Baltic, the Gulf of Bothnia, in 1996 (Stigzelius

18   et al., 1997). Later the taxonomy of *Marenzelleria* species was revised by Sikorski and

19   Bick (2004) who described a new species, *M. neglecta,* in the Baltic Sea. More recently,

20   the occurrence of three different species in the Baltic Sea, namely *M. viridis*, *M. neglecta*

21   and *M. arctia* (Chamberlin) was confirmed by molecular methods (Bastrop and Blank,

22   2006). The species are morphologically very similar and likely to have only minor, if

23   any, functional (e.g. bioturbation) differences. The species status in previous Baltic Sea

24   studies cannot be fully confirmed and their current distribution is under study (Blank et

25   al., in prep.).

26

1   *Marenzelleria* spp. now commonly occur in the coastal northern Baltic Sea (Laine et al.,

2   2003a; 2003b; Perus and Bonsdorff, 2004), and high abundances and biomasses were

3   recorded both in the southern Baltic (Kube et al., 1996; Zettler, 1996) and in the Gulf of

4   Riga (Cederwall et al., 1999). Recently, high densities and biomass values of up to 4000

5   ind. m^{-2} and 80 g m^{-2} wet weight, respectively, have been observed in the deep open Gulf

6   of Bothnia (Finnish Institute of Marine Research (FIMR), unpublished data). The

7   previously abundant and dominant amphipod (*Monoporeia affinis* (Lindström))

8   populations have declined strongly during recent decades in the coastal areas of the

9   northern Baltic Sea (Laine et al., 2003a, 2003b; Perus and Bonsdorff, 2004) and also in

10   the open Bothnian Sea (Norkko et al., 2007). Concomitantly, the importance of

11   *Marenzelleria* spp. is expected to increase in the soft-bottom ecosystem. *Marenzelleria*

12   *viridis* is an infaunal species, forming highly branched burrow networks, that feeds with

13   palps in the sediment-water interface by collecting either material deposited on the

14   sediment surface or suspended particles in the near-bottom water (Dauer et al., 1981). In

15   the southern Baltic, the L- or J-shaped burrows of *Marenzelleria* spp. extend downwards

16   25-35 cm into the sediment (Zettler et al., 1994), which is much deeper than for any of

17   the native Baltic soft-bottom macrofaunal species. Thus, in the Baltic Sea ecosystem

18   these species appear to have occupied an open niche and have the potential for affecting

19   the sediment nutrient dynamics through deep-reaching bioturbation (Olenin and

20   Leppäkoski, 1999). Despite the small, slim morphology of the animals, they have the

21   potential for affecting conditions on the seafloor, due to the large number of animals, the

22   deep burrows they dig and their clear resilience to challenging environmental conditions,

23   such as low O_2 concentrations (Schiedek, 1997).

24

25   We studied the effect of *Marenzelleria* sp. (most probably *M. arctia*, see Material and

26   methods) population on its new environment. Laboratory experiments were performed

27   using a flow-through setup with coastal Gulf of Finland muddy sediment. We measured

1 benthic nutrient fluxes, denitrification, O_2 penetration depth in the sediment and sediment

2 pore water nutrient concentration at low and high densities of _Marenzelleria_ sp. as well

3 as in control sediments without animals. In addition, different forms of sediment P,

4 separated by chemical fractionation, were studied in three sediment layers with and

5 without high densities of _Marenzelleria_ sp.

6

7 **Materials and methods**

8

9 **Experimental setup**

10 The _Marenzelleria_ sp. individuals were collected on October 18, 2004 in the Åland Sea

11 at a 285-m-deep station (F64; 60º18.0' N, 19º15.0' E). This area hosts a dense

12 _Marenzelleria_ sp. population, e.g. in June 2004 a density of 4000 ind. m^{-2} was recorded

13 at this site. At the time of sampling the species was assumed to be _M. viridis_ but samples

14 taken at the same site in 2005 and identified with molecular methods revealed that the

15 population consisted of _M. arctia_ only (Blank et al., submitted). Thus, the worms used in

16 this study most probably belong to _M. arctia_ but since the species identity in the present

17 material was not confirmed, we refer to them as _Marenzelleria_ sp. Sediment was

18 collected using a van Veen grab and gently mixed with water. Swimming _Marenzelleria_

19 spp. were caught in a 1-mm sieve. The animals were then placed in aerated transport

20 boxes with 5 cm of sieved local sediment and kept at +5 °C until transport to the

21 laboratory.

22

23 The sediment used in the experiments was collected from a coastal station (Tvärminne,

24 Storfjärden, northern Gulf of Finland, 59º51.3' N, 23º15.8' E), representing a

25 characteristic, outer archipelago accumulation bottom consisting of soft mud. The water

26 depth at the sampling station is 33 m. A box corer was used to collect the deeper anoxic

27 sediment and an Ockelman sledge to collect the oxic surface sediment. The mud was

1   gently sieved (1 mm for the deeper sediment and 0.5 mm for the surface slurry) to

2   remove macrozoobenthos. The experiment was conducted at +5 °C in a coldroom,

3   corresponding to *in situ* temperature. Round polyacrylic aquariums (30 aquariums with

4   inner diameter 14 cm, height 16 cm) were packed with 8 cm of deeper mud that was

5   covered with 2 cm of surface mud. The aquariums were filled with filtered seawater of

6   the same salinity (6.1) and temperature as the sampling station, taken at the beginning of

7   the experiment from a nearby location, filtered (0.2 μm) and kept in the coldroom for the

8   length of the experiment. The aquariums were covered with 0.5-mm wire mesh covers.

9   After letting the aquariums stabilize for 12 days, the first samples (time zero

10   measurements) were taken and animals were distributed in the aquariums so that nine

11   aquariums received no worms (control units, C), nine received five worms each (low-

12   density units, LOW, corresponding to 325 ind. m^{-2}) and nine received 30 worms each

13   (high-density units, HIGH, corresponding to 1950 ind. m^{-2}). The mesh covers were

14   equipped with hypodermic needles that were attached to tubing, bringing filtered water

15   from the container through a peristaltic pump at a rate of 1.5 ml min^{-1}. Excess water was

16   allowed to overflow.

17

18   **Sampling and analyses**

19   Samples were taken at time zero (before adding the worms) and then on days 2, 6 and 14

20   after connecting the flow-through system. The sampling was randomized and three

21   aquariums of each treatment were sacrificed at each sampling time (except on day 14,

22   when one LOW, one HIGH and two C units were discarded due to problems in

23   waterflow).

24

25   The samples for nutrient fluxes (NH_4^+, NO_x, phosphate (PO_4^{3-}) and Fe) were collected

26   from the aquariums using a peristaltic pump, collecting outflowing water at the same rate

27   as water was pumped into the aquariums (1.5 ml min^{-1}). In addition, the inflowing water

1   from the container was sampled and the fluxes were calculated from the difference in

2   concentrations, using the residence time and sediment area. All nutrients were analysed

3   using standard methods (Koroleff, 1983). All units were photographed prior to sediment

4   sampling. The sediment samples for all the different measurements were taken

5   simultaneously so that all the tube cores needed were inserted in the aquarium at the

6   same time, ensuring undisturbed samples.

7

8   Denitrification was measured using the isotope pairing technique (Nielsen, 1992). Three

9   replicate samples were taken in clear plastic cores (diameter 2.6 cm, height 9 cm) so that

10   about half of the core was filled with the sediment and half with the water from above.

11   The samples were enriched with $K^{15}NO_3$ (98% labelling, Cambridge Isotope

12   Laboratories, Andover, MA, USA) to a final concentration of 100 µM of $^{15}NO_3^-$ in the

13   overlying water and incubated, with a magnetic stirrer on the lid of the cores, at *in situ*

14   temperature in darkness for 3-4 hours. Incubation was terminated with $ZnCl_2$, samples

15   were mixed and subsamples were sent in gastight 12-ml vials (Exetainer; Labco, High

16   Wycombe, Buckinghamshire, UK) to the National Research Institute, Silkeborg,

17   Denmark, for analysis of N_2 isotopic composition.

18

19   The O_2 profiles were measured in undisturbed sample cores, similar to those used in the

20   denitrification measurements, using Clark-type oxygen microelectrodes (100-µm tips,

21   OX-100; Unisense, Aarhus, Denmark) that gave a spatial resolution of about 200 µm. For

22   pore water nutrient analyses, one sample per aquarium was taken using a corer with an 8-

23   cm inner diameter. The sample was sliced in 2-cm strata from the surface to and 8-cm

24   depth in the sediment, and slices from the same depth from replicate aquariums (three of

25   each treatment) were combined. The sediment redox potential was measured in each

26   combined slice with an electrode (SenTixORP), corrected by temperature and converted

27   to an E_h value. The pore water was then extracted by squeezing the samples with a

1   Millipore Zero Headspace Extractor, (Millipore, Billerica, MA, USA) using 0.45-µm

2   filter (Nuclepore; Whatman), under N_2 atmosphere.

3

4   Two replicate sediment samples for P fractionation studies were collected from two C

5   units and two HIGH units on days 2 and 14. The samples were taken into small plastic

6   corers (see denitrification) and the water above the sediment was removed (siphoned)

7   immediately. The samples were kept in the corers at +5 ºC, capped and protected from

8   light. Each sediment core was subsampled the following day in an N_2 atmosphere (O_2

9   content below 5%) in a glove box, separating three sediment layers: 0-2 cm, 2-4 cm and

10   4-6 cm. The subsamples were stored in plastic bottles at +5 ºC until analyses (for about 3

11   weeks). The various chemical forms of sediment P were determined using a P

12   fractionation method slightly modified from that described in Jensen and Thamdrup

13   (1993) (detailed description in Lukkari et al., submitted). The method separates six P

14   pools (Jensen et al., 1995): loosely adsorbed and pore water P (extracted with sodium

15   chloride, NaCl; referred to as NaCl-iP), a redox-sensitive fraction of P bound to hydrated

16   oxides of reducible metals (mainly those of Fe) (sodium dithionite, $Na_2S_2O_4$, in

17   bicarbonate buffer, $NaHCO_3$, at pH 7; NaBD-iP), P bound to oxides of Al and

18   nonreducible Fe (sodium hydroxide, NaOH; NaOH-iP), apatite-P (hydrochloric acid,

19   HCl; HCl-iP) and residual, mainly organic, P (extracted with HCl after combustion; Res-

20   iP). In addition to these five fractions, the pool of mobile organic P (nonreactive P, NRP)

21   was determined as the difference between total P (TP) and dissolved inorganic P (iP),

22   summarized from the first three steps. The iP was determined from filtered (Nuclepore

23   polycarbonate membranes, pore size 0.4 µm) extracts with a UV-VIS spectrophotometer

24   (Genesys 10uv Thermo Spectronic; Genesys, Daly City, CA, USA) with a 50-mm flow-

25   injection cuvette and TP with a spectrophotometer after acid persulphate digestion

26   (Koroleff, 1983).

27

1   The sediment dry matter (DM) content was first determined using a moisture analyser (a

2   balance equipped with a halogen lamp dryer, Ohaus MB45; Ohaus, Pine Brook, NJ,

3   USA), and the amount of fresh sediment extracted was determined as a fresh weight

4   corresponding to 0.3 g dry mass. The volume of the extracts was always 30.0 ml yielding

5   a sediment DM-to-solution ratio of 1:100. The sediment subsamples were not sieved, but

6   visible animals or their remains were removed.

7

8   The effects of _Marenzelleria_ spp. and time on denitrification and benthic nutrient fluxes

9   were tested using two-factor analysis of variance (ANOVA) and when significant

10   differences ($\alpha = 0.05$, $p < 0.01$) were found, treatments were further tested against the C

11   units, using Dunnett's test ($\alpha = 0.05$). Differences in pore water nutrient profiles between

12   the treatments and sampling occasions, based on concentrations in different layers, were

13   tested using the multivariate analysis of similarities (ANOSIM) procedure (Clarke and

14   Warwick, 2001). The differences in sediment P fractions between the C and HIGH

15   treatments were tested using t-tests assuming unequal variances ($\alpha = 0.05$, $p < 0.05$) and

16   the effects of time within treatment were tested using paired t-tests ($\alpha = 0.05$, $p < 0.05$).

17   The significances of all correlations (denitrification, fluxes, concentrations of dissolved

18   O_2 and nutrients) were analysed using Pearson's correlation test ($\alpha = 0.05$, $p < 0.01$).

19

20   **Results**

21

22   In the C units, the two-layer structure basically persisted until the end of the experiment.

23   By day 6 some darkening of the initially light brown colour in the lower parts of the

24   surface layer was observed. By day 14, there was an even more clear difference and only

25   the uppermost 5 mm in the C units had retained the original colour, whereas the lower

26   part of the surface layer was clearly darker, with black spots, indicating reduction and

1 sulphide formation. Microbial growth on the sediment surface was indicated by light-

2 coloured patches and increase in surface roughness by day 14.

3

4 The worms began to burrow immediately after they had been transferred to the

5 aquariums. Burrows were observed from day 2 onwards, both in the LOW and HIGH

6 units. Sometimes worm heads projected out of the sediment and the worms were lashing

7 with their palps to feed but disappeared quickly when disturbed. The burrows formed a

8 dense network in the vertical and horizontal directions, especially in the HIGH density

9 units. Active burrowing was observed in the uppermost 5 cm and single burrows

10 penetrated to more than 8-cm depths in the sediment. Occasionally a thin and transparent

11 membrane lining was observed in the burrows. The deeper parts of the upper sediment

12 layer became darker in the same way as in the C units. However, the actively used worm

13 burrows could be distinguished even in the deep sediment by a thin light-coloured,

14 apparently oxidized layer that was similar to the colour of the sediment surface.

15

16 Burrow openings and faecal pellets were visible on the sediment surface. The faecal

17 pellets were up to 7 mm in length and formed radial cones around the openings. In the

18 HIGH units the sediment surface was almost completely covered by pellets by day 14,

19 whereas in the LOW units roughly half of the surface was covered by pellets and the

20 other half resembled the surface of the C units. After day 14 some additional units that

21 were not used for the experiment were sieved. The worms in these units were all alive

22 and actively swimming when transferred to the water.

23

24 The O_2 penetration depth ranged from 0.3 to 1.5 mm and was 0.5 mm at 17 out of the 28

25 times measured, with no differences between the treatments. The O_2 consumption

26 likewise did not differ between the treatments, since the O_2 concentration 0.5 cm above

27 the sediment surface was similar in all aquariums. There were no clear differences in

1   redox profiles between the different units. However, the redox potential decreased in the

2   surface layer (0-2 cm) of all units during the experiment from an initial level of -10 to

3   +105 mV to a level of -130 to -30 mV in the end. In the deeper sediment the redox

4   potential varied less and was mostly between -150 and -100 mV. Despite the dense

5   burrow networks that developed especially in the HIGH units, no obvious change was

6   detected in porosity between the treatments. However, the surface sediments could be

7   distinguished from the deeper sediments by the porosity difference.

8

9   **Nutrient fluxes**

10 The nutrient fluxes at the sediment-water interface fluctuated considerably during the

11 experiment (Figure 1). The NO_x flux was out of the sediment, except on the last sampling

12 day when it was directed into the sediment in all treatments. There were significant

13 differences between the C and HIGH (but not the C and LOW) units on day 2, when the

14 C units released nine times more NO_x out of the sediment than did the HIGH units. No

15 differences between the treatments were found on day 6, and the significance of the

16 differences on day 14 could not be tested due to the lack of replicates of the C treatment.

17 The NH_4^+ fluxes increased throughout the experiment, were always directed out of the

18 sediment and were always highest in the HIGH, and lowest in the C units. Significant

19 differences between treatments were found on day 2, when the HIGH units released nine

20 times more NH_4^+ than did the C units, but not on day 6. On day 14 the difference was

21 even greater, but the significance could not be tested (see above). The PO_4^{3-} flux was

22 likewise directed out of the sediment throughout the experiment and the highest values

23 were always recorded in the HIGH units and lowest in the C units. The differences

24 between the HIGH (but not the LOW) and C units were significant on days 2 and 6. They

25 were even higher on day 14, but the significance could not be tested (see above). The Fe

26 flux was always out of the sediment and increased in the course of the experiment. On

1   day 2, the flux in the HIGH (but not the LOW) units was significantly higher than in the

2   C units.

3

4   The NO_3^- flux correlated positively (Pearson correlation, Table 1) with the O_2 penetration

5   depth, NO_3^- concentration and denitrification, and negatively with the denitrification

6   potential (D15). The NH_4^+ flux was negatively correlated with total denitrification (Dtot)

7   and coupled nitrification-denitrification (Dn), and positively with D15. The PO_4^{3-} flux

8   was positively correlated only with the Fe flux, which was negatively correlated with the

9   O_2 penetration depth.

10

11   **Denitrification**

12   No effect of _Marenzelleria_ spp. was detected on the denitrification rates in the

13   experiment. The Dtot and Dn values decreased significantly in all aquariums in the

14   course of the experiment, independent of the treatment (Figure 2). The percentage of

15   denitrification that was based on water column NO_x (Dw) likewise differed significantly

16   between sampling days, but instead of a general decrease, it showed very high values on

17   day 6 in all treatments, decreasing again to very low values by day 14. No interactions

18   between the treatments and time were found. In contrast to the ^{14}N based natural

19   denitrification (Dtot), the denitrification potential (D15), based on the added ^{15}N, tended

20   to increase throughout the experiment in all treatments (Figure 2). In addition to the time,

21   the treatments also significantly affected the D15 rates. On days 2 and 6, the D15 rates in

22   the HIGH, but not in the LOW, units were significantly higher than in the C units. The

23   effect disappeared by day 14, when no differences between units with and without

24   animals were found.

25

26   The Dtot rates correlated positively (Table 1) with Dn, O_2 penetration depth and NO_3^-

27   concentration and flux, and negatively with the D15 rate and NH_4^+ flux. The Dn level

1 was positively correlated with the O_2 penetration depth and negatively with the D15 rate

2 and NH_4^+ flux. The Dw level correlated positively with the NO_3^- concentration and flux.

3 The D15 rate was positively correlated with the NH_4^+ flux and negatively with the NO_3^-

4 concentration.

5

6 **Pore water nutrient profiles**

7 In all units, the pore water NH_4^+ concentration increased almost linearly with sediment

8 depth (Figure 3). The concentrations in the surface layer (0-2 cm) varied from about 220

9 to 380 µmol l^{-1} at the beginning of the experiment and increased to about 300-425 µmol

10 l^{-1} on day 14. In the bottom layer (6-8 cm) the concentration was more stable, about 820-

11 1050 µmol l^{-1}. No differences were found between the C, LOW and HIGH units. Only on

12 day 14 were the NH_4^+ concentrations in all layers of the HIGH units slightly lower than

13 in the C and LOW units. The sediment pore water NO_x concentration was measured only

14 in the surface layer (0-2 cm) and was very low throughout the experiment (mean 1.9

15 µmol l^{-1}).

16

17 The PO_4^{3-} profiles were the most variable of the measured pore water parameters (Figure

18 3). In the surface layer, the concentrations were below 65 µmol l^{-1} except on day 14 in

19 the C unit. The maximum PO_4^{3-} concentrations in the deeper layers exceeded 200 µmol l^{-}

20 ^{1}. On days 6 and 14, the PO_4^{3-} concentrations in the deeper layers of the HIGH units were

21 lower than in the C and LOW units. The Fe concentration varied only in the uppermost

22 layer (Figure 3). In the C units the surface concentration of Fe always exceeded 15 µmol

23 l^{-1}. In all units the concentrations at 2-8-cm depths were very low, < 5 µmol l^{-1}, and did

24 not vary during the experiment. In the HIGH units the surface layer Fe concentrations fell

25 to low levels, similar to those observed in the deep sediments on days 6 and 14. No

26 significant differences were detected in any of the pore water nutrient profiles between

27 the treatments or sampling occasions.

1

2 **Sediment P fractions**

3 The difference in P fractions between the treatments and as a function of time could not

4 be tested statistically at each data point, because there were only two replicate samples in

5 the C units on day 14. The concentration of total extractable P (TP_{extr}) was about 60 and

6 40 µmol g^{-1} DM in the surface (0-2 cm) and bottom (2-6 cm) sediment layers,

7 respectively, with NaBD-iP and HCl-iP forming the major fractions. NaCl-iP and NaBD-

8 iP were the fractions most affected by the treatment, while Res-P was the most stable P

9 form (Figure 4).

10

11 NaCl-iP constituted only a minor percentage of TP_{extr} (< 1%). Its concentration was

12 highest in the surface layer (0-2 cm) and did not change markedly in the C units between

13 days 2 and 14. The increase in NaCl-iP in the surface layer from day 2 to day 14 in the

14 HIGH units was significant. On day 2, the HIGH units had significantly lower NaCl-iP at

15 0-2 cm than the C units. On day 14, no marked differences between the treatments were

16 found.

17

18 NaBD-iP formed 11-35% of the TP_{extr}. Its percentage of the sediment P was higher in the

19 0-2-cm layer than in the other two layers and increased during the experiment, especially

20 in the surface layers and in the HIGH units. NaBD-iP in the HIGH units was significantly

21 higher on day 14 than on day 2 in the two uppermost layers. The evident increase with

22 time in the C units (Figure 4) could not be tested statistically (only two replicates on day

23 14). On day 2, NaBD-iP was lower in the HIGH units than in the C units at 0-2 cm and 2-

24 4 cm; the concentration difference was larger in the former, although statistically

25 significant only in the latter. On day 14, the HIGH units had lower NaBD-iP

26 concentrations at 2-4 cm and 4-6 cm than the C units.

27

1   NRP constituted 20-31% of the TP_{extr} and its percentage of the sediment P decreased in

2   the C units and increased in the HIGH units (not significantly) during the experiment.

3   The decrease in NRP in the surface layer of the C units is evident (Figure 4), while that in

4   the 2-4-cm and 4-6-cm layers is within standard deviation of the replicate results. On day

5   2, NRP was lower in all depth layers in the HIGH units compared with those in the C

6   units, but significantly only in the surface layer. On day 14, the NRP concentration was

7   higher in the two deepest layers of the HIGH units than in the C units (Figure 4).

8

9   NaOH-iP formed 4-7% of the TP_{extr} in the sediment and its percentage did not change

10   during the experiment. However, possibly due to the very small deviation in the results,

11   the relatively small (< 1 µmol g^{-1} DM) changes in NaOH-iP concentrations were

12   statistically significant. NaOH-iP decreased in the 0-2-cm layer of the C units between

13   days 2 and 14. On day 2, the NaOH-iP concentration was lower in the 4-6-cm layer of the

14   C units than of the HIGH units, and on day 14 it was higher in the 2-4-cm layer of the C

15   units compared with the HIGH units.

16

17   The percentage of HCl-iP was 17-35% of the TP_{extr}. No statistically significant

18   differences were found in the concentrations of HCl-iP between days and treatments. The

19   Res-P fraction formed 15-27% of the TP_{extr}. Its percentage of TP increased slightly in the

20   C units and decreased in the HIGH units during the experiment. None of the changes

21   were statistically significant, but the increase in Res-P in the surface layer of the C units

22   from day 2 to day 14 is evident (Figure 4). In addition, despite the high deviation in the

23   HIGH units (especially on day 14), the results suggest a decrease in Res-P from day 2 to

24   day 14. On day 2, Res-P was higher in the HIGH units compared with the C units and

25   vice versa on day 14.

26

27

1   **Discussion**

2

3   The redox conditions in the surface layer of the aquariums gradually deteriorated in all

4   treatments during the experiment. This could be seen both visually, as a darkening of the

5   light brown surface layer from below, and as a decreased redox potential (Mortimer

6   1941). Reduced substances diffused from the deep sediment layer upwards and at the

7   same time the mineralization processes in the upper sediment layer used up the O_2

8   storage, resulting in a decreasing volume of the oxidized sediment. Despite active burrow

9   formation, the _Marenzelleria_ spp. did not counteract the decrease in redox potential by

10 transporting more O_2 to the sediment, when the entire sediment volume was considered.

11 They also did not affect the porosity, which may have been caused by different water and

12 sediment relocation in the HIGH units in comparison to C units. Burrow formation could

13 have caused sediment packing in the interspace between the burrows with no overall

14 change in the water content. The coarse sampling procedure may also have failed to

15 detect small-scale differences in the loose surface layer where the water content was

16 highest.

17

18 The animals affected the nutrient fluxes shortly after they were introduced to the

19 sediment, but the effect may have been transient, since no significant differences were

20 found in the rates on day 6 (except for the PO_4^{3-} flux). The differences could not be

21 tested on day 14, but appeared to increase from day 6 for NH_4^+ and PO_4^{3-}, although there

22 were wide variations. No significant differences were detected in the pore water nutrient

23 profiles, although some effects could be seen at the higher population density. Similarly,

24 Karlson et al. (2005) found no significant effect of _M. viridis_ (or _Macoma balthica_ (L.) or

25 _Monoporeia affinis_) on pore water nutrients in their recolonization experiment, using

26 reduced natural Baltic Sea sediments. The general increase in the surface layer NH_4^+

27 concentration indicates diffusion from the deeper layers. The slightly lower NH_4^+

1   concentrations in the HIGH units, compared with the C and LOW units, at the end of the

2   experiment is in accordance with the observed higher efflux of NH_4^+ that could have

3   been caused by the more intensive burrow formation at the higher worm density.

4

5   **Nitrification and denitrification**

6   The high flux of NO_3^- out of the sediment confirms that the nitrification rate was rapid in

7   the sediment. On day 2, the HIGH units released significantly less NO_3^- in the water than

8   did the C units. The Dn rate was, however, similar in all treatments, indicating that

9   nitrification saturated the NO_3^- demand of the denitrifiers in all treatments. At the same

10   time the NH_4^+ flux was significantly higher in the HIGH units, suggesting either a lower

11   nitrification rate in the HIGH units, simultaneous dissimilatory NO_3^- reduction to NH_4^+

12   (DNRA) or simply more rapid transport of NH_4^+ out of the sediment. A lower

13   nitrification rate was not likely, because the animals enhance nitrification by increasing

14   the oxic-anoxic interfaces in the sediment (Pelegri and Blackburn, 1994; Tuominen et al.,

15   1999; Svensson et al., 2001). DNRA is usually considered to be of minor importance in

16   natural sediments and to occur mainly in organically enriched environments, such as fish

17   farm sediments (Hattori, 1983; Christensen et al., 2000). However, Karlson et al. (2005)

18   found that DNRA represented a major pathway of NO_3^- removal in laboratory

19   experiments similar to ours, in which reduced Baltic Sea sediments were used. Yet

20   another explanation for the low NO_3^- and high NH_4^+ fluxes is the shorter residence time

21   of NH_4^+, produced in the mineralization of organic matter in the sediments with animals,

22   due to the enhanced transport from the burrows into the overlying water. Similarly,

23   increase in NH_4^+ flux was observed in deep-burrowing polychaete _Nereis virens_ (Sars)

24   (Henriksen et al., 1980). The situation on day 14, when the NO_3^- concentration in the

25   water was very low and the flux was towards the sediments, represents typical steady-

26   state conditions in which denitrification consumes NO_3^- at the rate it is produced. That

27   the Dn rate did not increase as the flux turned into the sediments suggests that other

1   processes, such as DNRA, contributed to the NO_3^- reduction as well. This is also

2   supported by the high NH_4^+ flux out of the sediments.

3

4   Macrofauna stimulated denitrification in several studies (e.g. Kristensen and Blackburn,

5   1987; Hansen and Kristensen, 1998; Pelegri and Blackburn, 1994; Svensson and

6   Leonardson, 1996; Gilbert et al., 1998; Bartoli et al., 2000; Svensson et al., 2001).

7   However, in the only study using _M. viridis_, no significant effects on denitrification rate

8   were found (Karlson et al., 2005). Similarly, in our study the _Marenzelleria_ spp. did not

9   affect the denitrification rates that decreased in all aquariums during the experiment,

10   independent of the treatment. No explanation was found for the decline. The

11   denitrification process is generally regulated by temperature, O_2 concentration (both

12   directly and indirectly via nitrification), NO_3^- concentration and the availability of

13   organic carbon. In these experiments, the temperature was stable and the concentration of

14   O_2 dropped from 75% to 50% saturation from day 0 to day 2, after which it remained

15   constant in all aquariums. The O_2 penetration depth likewise did not change. The NO_3^-

16   availability clearly regulated the Dw rate that fluctuated together with the NO_3^-

17   concentration, but the Dn rate was independent of the water column NO_3^- concentration.

18   Dn is controlled by the rate of nitrification, which in turn is regulated by the availability

19   of O_2 and NH_4^+, neither of which were in short supply in the aquariums, although the

20   availability of NH_4^+ increased during the experiment. The availability of organic carbon

21   in the aquariums could have been too low to support the heterotrophic denitrifying

22   community. However, both the denitrification rates and the concentrations of labile

23   organic carbon peak in October-December in the study area (Hietanen and Kuparinen, in

24   press). In addition, the D15 rates nearly doubled from day 2 to day 14 in all aquariums,

25   indicating that enough carbon was available for the denitrifiers. The increasing D15 rates

26   also verify that the denitrifying bacteria were not grazed faster than they grew. While the

27   animals did not affect D14 (denitrification based on the natural $^{14}NO_3^-$), the D15 rate was

1 significantly enhanced on days 2 and 6 in the HIGH units, although the effect

2 disappeared by day 14. The D15 rate reflects Dw in unlimited NO_3^- concentrations. The

3 increase in D15 in the HIGH units could have been caused by expansion of the

4 denitrifying zone, since the animals create oxic-anoxic interfaces in their burrows below

5 the primary oxidized layer. That this effect was not significant in the Dw rates could be

6 explained by the slightly lower NO_3^- concentration in the HIGH units – the Dw rates

7 were similar in all treatments, although less NO_3^- was available in the HIGH units.

8 However, time was also the most significant factor affecting the D15 rate, since no

9 differences between the rates could be seen on day 14 any more. The increase in D15 in

10 the C units also indicates that the sieved and repacked sediment layers had not fully

11 stabilized by the beginning of the experiments, despite the 12-day interval between filling

12 the aquariums and the first measurements. The same effect could also be seen in the NO_x

13 flux that reached the typical steady-state pattern only by day 14. In experiments using a

14 similar setup and northern Baltic Sea sediments, Tuominen et al. (1999) found that the

15 pore water NO_x profile already resembled that of the intact sediment 2 days after

16 sediment repacking, indicating rapid recovery of the sensitive nitrifiers after sediment

17 mixing. In these experiments the cores were fully stabilized after 2 weeks. However, due

18 to the slow growth of the nitrifying bacteria compared with the denitrifying bacteria, it

19 may also take considerably longer for the system to stabilize (Welsh, 2003).

20

21 **Dissolved phosphorus**

22 The PO_4^{3-} profiles in the C units reflect the different redox states of the two sediment

23 layers. The oxidized surface layer retains PO_4^{3-} in the Fe compounds, decreasing its

24 concentration in the pore water, while in the more reduced bottom layer PO_4^{3-} remains in

25 the pore water in dissolved form, diffusing upwards driven by the concentration

26 difference between the layers (Mortimer 1941). The maximum formed at the 2-4-cm

27 layer, immediately below the interface between the surface and bottom layers (see also

1   Lewandowski and Hupfer, 2005). The differences in the pore water PO_4^{3-} between the

2   treatments already appeared on day 2 and by day 14 the PO_4^{3-} concentration in the HIGH

3   treatment was clearly lower than in the C and LOW treatments. This may have resulted

4   from the more intensive mixing and subsequent higher initial release of PO_4^{3-} into the

5   pore water in the HIGH treatments, also seen as the higher flux of PO_4^{3-} out of the

6   sediment on all sampling occasions. A similar effect was noted by Lewandowski and

7   Hupfer (2005) in experiments using chironomids (*Chironomus plumosus* (L.)) and

8   oligochaetes (*Tubifex tubifex* (Müller) and *Limnodrilus hoffmeisteri* (Claparède)). In

9   addition, organic substances excreted by burrowing animals (e.g. Kristensen, 1988) and

10   the organic molecules produced in the enhanced degradation or transformation of organic

11   matter may to some extent block the binding surfaces for P in the burrow channels,

12   leading to higher efflux out of the sediment. In the LOW treatments, the PO_4^{3-} flux

13   increased to the same level as in the HIGH units on the last sampling occasion,

14   suggesting that the less extensive burrow formation at the lower animal density delayed

15   the release of the dissolved P from the bottom layer. The high and increasing

16   concentration of Fe in the pore water at the surface of the C units is related to the

17   reduction and upward diffusion in the deeper layers (Mortimer 1941). In contrast, the

18   surface pore water Fe concentration decreased throughout the experiment, in the presence

19   of *Marenzelleria* spp., especially at the high density, indicating higher release from the

20   sediment (seen also in the Fe flux to the overlying water), or precipitation as

21   oxyhydroxides to the surface sediment. Similar observations were made by Lewandowski

22   and Hupfer (2005) in experiments using chironomids and oligochaetes.

23

24   **Sediment P fractions**

25   The high density of *Marenzelleria* sp. clearly affected the distribution of P into different

26   forms in the sediment. At the beginning of the experiment, the activity of *Marenzelleria*

27   sp. released loosely bound or pore water P (NaCl-iP) to the overlying water from the

1   surface sediment, which was reflected in the high PO_4^{3-} flux out of the sediment in the

2   HIGH units. Although small, such changes in NaCl-iP may be important, because even P

3   concentrations near the detection limit of the common analytical methods used can affect

4   algal growth (Baldwin et al., 2003). The effect of _Marenzelleria_ sp. was most

5   pronounced in the redox-sensitive P fraction (NaBD-iP). The increase in NaBD-iP in the

6   surface sediment of the HIGH units was probably related to sediment oxidation by

7   animal reworking, as was also noted by Lewandowski and Hupfer (2005). The increase

8   in redox-sensitive P in the surface layer of the C units may have been caused by a slow

9   reduction of the bottom sediment and consequent release, upward diffusion and

10   entrapment of P to the Fe compounds in the oxic sediment.

11

12   Part of the transformable organic P (NRP) may have been degraded in the surface

13   sediment layer during the 12-day preincubation, since NRP also contains easily

14   hydrolyzable compounds (Ahlgren et al., 2005). The lower percentage of NRP in the

15   bioturbated units 2 days after introducing the animals may have resulted from enhanced

16   mineralization of the easily degradable organic molecules (Hansen et al., 1998), although

17   it is not clear whether this could have occurred within such a short time. Pure physical

18   mixing of the surface and bottom sediment layers by the animals does not solely explain

19   the lower NRP levels, because the same effect was also seen in the two deeper layers.

20   The increase in NRP towards the end of the experiment in the HIGH treatment, on the

21   other hand, may have resulted from the enhanced degradation of the more resistant

22   organic matter affected by sediment reworking, and the congruent decrease in the Res-P

23   fraction supports this conclusion. Kristensen et al. (1992) reported that bioturbation

24   caused loss of detritus and that the relatively refractory organic matter was affected more

25   than the labile material. Since NRP extracted with NaOH also includes inorganic P

26   compounds, e.g. polyphosphates and pyrophosphates that are produced in biological

27   transformation processes (degradation of organic matter), these probably also increased

1   the percentage of this fraction (Hupfer et al., 1995; Ahlgren et al., 2005). Defecation of

2   *Marenzelleria* sp., visible as piles of faecal pellets on the sediment surface, may also

3   have increased the share of NRP.

4

5   As expected, the P bound to oxides of nonreducible metals (NaOH-iP) was not greatly

6   affected during the experiment, since release from this fraction requires increase in pH or

7   presence of competing anions (Scheffer and Scheffer, 1984; Ryden et al., 1987). Benthic

8   animals can indirectly liberate NaOH-iP by mixing the surface sediment with bottom

9   water of higher pH (Drake and Heaney, 1987; Lewandowski et al., 2005). In our

10   experiment, the sediment probably acted as a buffer against pH changes, but some

11   microscale changes may have occurred, explaining the minor changes observed. These

12   can also be artifacts, despite the statistical significance, since the experimental units had

13   not fully stabilized by the beginning of the experiments.

14

15   Apatite P (HCl-iP), which is mainly detrital, is a resistant form of sediment P and did not

16   change markedly during this experiment. Res-P, however, showed marked changes

17   despite its theoretically resistant nature. Res-P contains primarily organic P that is

18   resistant to degradation, but may release P under favourable conditions, e.g. with

19   enhanced microbial degradation. This may explain the decrease in Res-P in the

20   *Marenzelleria* sp.-treated units during the experiment. The total organic P also decreased

21   in the bioturbated units during the experiment, suggesting that the high density of

22   *Marenzelleria* spp. enhanced degradation of the organic P in the sediment (Hansen et al.,

23   1998).

24

25   **Ecological aspects of *Marenzelleria* spp. invasion**

26   Introduced species alter the community structure and may also cause significant large-

27   scale changes in the function of the recipient ecosystem (e.g. Carlton, 1996; Leppäkoski

1   et al., 2002; Vanderploeg et al., 2002). The soft-bottom macrozoobenthos in the Baltic

2   Sea is characterized by very low species richness and a low number of functional groups

3   (Bonsdorff and Pearson, 1999; Laine, 2003). Different and even contrasting effects by

4   macrofauna on nutrient fluxes were explained by species-specific differences in the mode

5   of sediment mixing and structure building, irrigation behaviour and burrowing depth

6   (Welsh, 2003; Mermillod-Blondin et al., 2004; Michaud et al., 2006). Thus, invasions by

7   nonindigenous species, if they result in a change in the community structure, also have a

8   high potential for causing changes in biogeochemical cycles.

9

10 The recent invasion of _Marenzelleria_ spp. in the northern Baltic Sea now also extends to

11 open sea areas in the Gulf of Bothnia. This change has also caused a complete shift in

12 community dominance. Some areas that previously were highly dominated by the

13 amphipod _Monoporeia affinis_ (e.g. Andersin et al., 1977) are currently dominated by

14 corresponding numbers of _Marenzelleria_ spp. At several sites, densities corresponding to

15 and exceeding (up to 4000 ind. m^{-2}) the densities used in our experiment developed in

16 only 1-2 years after the first occurrence of the species (FIMR unpublished data). This

17 could also have caused profound changes in the nutrient fluxes, since these two species

18 may affect nutrient cycling in different ways. _Monoporeia affinis_ can stimulate

19 denitrification and decrease the amount of dissolved P in the pore water of sediments in

20 the Gulf of Finland (Gran and Pitkänen, 1999; Tuominen et al., 1999). Karlson et al.

21 (2005) also found higher denitrification rates in sediments affected by _M. affinis_,

22 compared with _M. viridis,_ but no difference in the P fluxes. However, in the less

23 eutrophied Gulf of Bothnia, no increase in the denitrification rate due to bioturbation of

24 the amphipods could be detected (Tuominen et al., 2003), probably because the oxidized

25 sediment layer is thicker than in the Gulf of Finland and bioturbation by _M. affinis_,

26 therefore, does not reach deep enough to increase the volume of the oxidized sediment.

27 The different bioturbation effects of _Monoporeia affinis_ and _Marenzelleria viridis_ are

1   probably caused by their functional differences. *Monoporeia affinis* is a surface and

2   subsurface deposit feeder that dwells mostly in the uppermost sediment surface layer

3   (Hill and Elmgren, 1987; Karlson et al., 2005). Its active sediment mixing at high

4   densities causes homogenization of sediment without permanent burrow structures,

5   whereas *Marenzelleria* builds more permanent burrows that may enhance the solute

6   fluxes between the deeper sediment layers and the overlying water. However, in natural

7   communities with mixed species compositions, the patterns identified experimentally for

8   single species may not emerge similarly, due to the complex interactions in the

9   mineralization processes (Welsh, 2003). Therefore, *in situ* studies are needed to predict

10   the actual impact of *Marenzelleria* in the benthic processes of the Baltic Sea.

11

12   **Conclusions**

13

14   In our 2-week experiments *Marenzelleria* sp. increased the fluxes of P and NH_4^+ to the

15   water column. No effect could be recorded for denitrification. Since the previously

16   dominant species of the area, *Monoporeia affinis*, can enhance denitrification and lower

17   the amount of dissolved P in the pore water, the replacement of *M. affinis* with

18   *Marenzelleria* spp. may lead to increased P flux to the water column and decreased

19   denitrification, further increasing the NH_4^+ flux to the water column. Nutrient release to

20   the bottom water instead of burial and removal from the water ecosystem by

21   denitrification further accelerates eutrophication, the main problem in the Baltic Sea.

22   However, in the long run sediment reworking by *Marenzelleria* spp. also oxidizes the

23   surface sediment, improving its ability to retain P and support nitrification. Therefore, the

24   impact of *Marenzelleria* spp. on sediment nutrient release may not be as drastic as the

25   initial reactions seen in our experiments suggest.

26

27

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9

10 **References**

11

12 Ahlgren, J., Tranvik, L., Gogoll, A., Waldebäck, M., Markides, K., Rydin, E., 2005.

13   Sediment depth attenuation of biogenic phosphorus compounds measured by ^{31}P

14   NMR. Environ. Sci. Technol. 39:867-872.

15 Altmann, D., Stief, P., Amann, R., de Beer, D., 2004. Nitrification in freshwater

16   sediments as influenced by insenct larvae: Quantification by microsensors and

17   fluorescence *in situ* hybridization. Microb. Ecol. 48, 145-153.

18 Andersen F.Ø., Jensen, H.S., 1991. The influence of chironomids on decomposition of

19   organic matter and nutrient exchange in a lake sediment. Verh. Internat. Verein.

20   Limnol. 24, 3051-3055.

21 Andersin A-B., Lassig, J., Sandler, H., 1977. Community structures of soft-bottom

22   macrofauna in different parts of the Baltic. In: B.F. Keegan, P.O. Ceidigh and

23   P.J.S. Boaden (Eds.), Biology of benthic organisms. Pergamon Press, New York,

24   pp. 8-20.

25 Baldwin, D.S., Whittington, J., Oliver, R., 2003. Temporal variability of dissolved P

26   speciation in a eutrophic reservoir? Implications for predicating algal growth.

27   Wat. Res. 37, 4595-4598.

1 Bartoli, M., Nizzoli, D., Welsh, D.T., Viaroli, P., 2000. Short-term influence of

2   recolonization by the polychaete worm *Nereis succinea* on oxygen and nitrogen

3   fluxes and denitrification: a microcosm simulation. Hydrobiologia 431, 165-174.

4 Bastrop, R., Blank, M., 2006. Multiple invasions – a polychaete genus enters the Baltic

5   Sea. Biological Invasions 8, 1195-1200.

6 Bick, A., Burckhardt, R., 1989. Erstnachweis von *Marenzelleria viridis* (Polychaeta,

7   Spionidae) für den Ostseeraum, mit einem Bestimmungsschlüssel der Spioniden

8   der Ostsee. Mitt. Zool. Mus. Berl. 65, 237-247.

9 Bonsdorff, E., Pearson, T.H., 1999. Variation in sublittoral macrozoobenthos of the

10   Baltic Sea along environmental gradients: A functional-group approach.

11   Australian J. Ecol. 24, 312-326.

12 Boström, B., Jansson, M., Forsberg, C., 1982. Phosphorus release from lake sediments.

13   Arch. Hydrobiol. Beih. Ergebn. Limnol. 18, 5-59.

14 Carlton, J.T., 1996. Marine bioinvasions: the alteration of marine ecosystems by

15   nonindigenous species. Ocenography 9, 36-43.

16 Cederwall, H., Jermakovs, V., Lagzdins, G. 1999. Long-term changes in the soft-bottom

17   macrofauna of the Gulf of Riga. ICES J. Mar. Sci., Suppl. 56, 41-48.

18 Christensen, P.B., Rysgaard, S., Sloth, N.P., Dalsgaard, T., Schwærter, S., 2000.

19   Sediment remineralization, nutrient fluxes, denitrification and dissimilatory

20   nitrate reduction in an estuarine fjord with sea cage trout farms. Aquat. Microb.

21   Ecol. 21, 73-84.

22 Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: an approach to

23   statistical analysis and interpretation, 2nd edition. PRIMER-E, Plymouth.

24 Dalsgaard, T., Thamdrup, B., 2002. Factors controlling anaerobic ammonium oxidation

25   with nitrite in marine sediments. Appl. Environ. Microbiol. 68, 3802-2808.

1 Dauer, D.M., Maybury, C.A., Ewing, R.M., 1981. Feeding behaviour and general

2   ecology of several spionid polychaetes from the Chesapeake Bay. J. Exp. Mar.

3   Biol. Ecol. 54, 21-38.

4 Drake, J.C., Heaney, S.I., 1987. Occurrence of phosphorus and its potential

5   remobilization in the littoral sediments of a productive English lake. Freshwat.

6   Biol. 17:513-523.

7 Gilbert, F., Stora, G., Bonin, P., 1998. Influence of bioturbation on denitrification activity

8   in Mediterranean coastal sediments: an *in situ* experimental approach. Mar. Ecol.

9   Prog. Ser. 163, 99-107.

10 Gran, V., Pitkänen, H., 1999. Denitrification in estuarine sediments in the eastern Gulf of

11   Finland, Baltic Sea. Hydrobiologia 393, 107-115.

12 Hansen, K., Kristensen, E., 1998. The impact of the polychaete *Nereis diversicolor* and

13   enrichment with macroalgal (*Chaetomorpha linum*) detritus on benthic

14   metabolism and nutrient dynamics in organic-poor and organic-rich sediment. J.

15   Experim. Mar. Biol. Ecol. 231, 201-223.

16 Hansen, K., Mouridsen, S., Kristensen, E., 1998. The impact of *Chironomus plumosus*

17   larvae on organic matter decay and nutrient (N, P) exchange in a shallow

18   eutrophic lake sediment following a phytoplankton sedimentation. Hydrobiologia

19   364, 65-74.

20 Hattori, A., 1983. Denitrification and dissimilatory nitrate reduction. In: Carpenter, E.,

21   Capone, D.B., (Eds.) Nitrogen in the marine environment. Academic Press, San

22   Diego, CA, p 191-232

23 Henriksen, K., Hansen, J.I., Blackburn, T.H., 1980. The influence of benthic infauna on

24   exchange rates of inorganic nitrogen between sediment and water. Ophelia,

25   Suppl. 1, 249-256.

1   Hietanen, S., Kuparinen, J., in press. Seasonal and short-term variation in denitrification

2   and anammox at a coastal station on the Gulf of Finland, Baltic Sea.

3   Hydrobiologia.

4   Hill, C., Elmgren, R., 1987. Vertical distribution in the sediment in the co-occurring

5   benthic amphipods *Pontoporeia affinis* and *P. femorata*. Oikos 49, 221-229.

6   Hupfer, M., Gächter, R., Rüegger, H., 1995. Polyphosphate in lake sediments – ^{31}P NMR

7   spectroscopy as a tool for its identification. Limnol. Oceanogr. 40, 610-617.

8   Jensen, H.S., Mortensen, P.B., Andersen, F.Ø., Rasmussen, E., Jensen, A., 1995.

9   Phosphorus cycling in a coastal marine sediment, Aarhus Bay, Denmark. Limnol.

10   Oceanogr. 40, 908-917.

11 Jensen, H.S., Thamdrup, B., 1993. Iron-bound phosphorus in marine sediments as

12   measured by bicarbonate-dithionite extraction. Hydrobiologia 253, 47-59.

13 Karlson, K., Hulth, S., Ringdahl, K., Rosenberg., R., 2005. Experimental recolonisation

14   of Baltic Sea reduced sediments: survival of benthic macrofauna and effects on

15   nutrient cycling. Mar. Ecol. Prog. Ser. 294, 35-49.

16 Koroleff, F., 1983. Determination of phosphorus. In: Grasshoff, K., Ehrhardt, M.,

17   Kremling, K. (Eds.), Methods of seawater analysis, 2nd ed. Verlag Chemie

18   Gmbh, Weinheim, pp. 125-142.

19 Kristensen, E., 1988. Benthic fauna and biogeochemical processes in marine sediments:

20   Microbial activities and fluxes. In: Blackburn, T.H., Sørensen, J. (Eds.), Nitrogen

21   cycling in coastal marine environments. John Wiley & Sons Ltd, Chichester, pp.

22   275-299.

23 Kristensen, E., Andersen, F.Ø., Blackburn, T.H., 1992. Effects of benthic macrofauna

24   and temperature on degradation of macroalgal detritus: The fate of organic

25   carbon. Limnol. Oceanogr. 37, 1404-1419.

1   Kristensen, E., Blackburn, H., 1987. The fate of organic carbon and nitrogen in

2   experimental marine sediment systems: Influence of bioturbation and hypoxia. J.

3   Mar. Res. 45, 231-257.

4   Kube, J., Zettler, M.L., Gosselck, F., Ossig, S., Powilleit, M., 1996. Distribution of

5   Marenzelleria viridis (Polychaeta, Spionidae) in the southwestern Baltic Sea in

6   1993/94 – ten years after introduction. Sarsia 81, 131-142.

7   Laine, A.O., 2003. Distribution of soft-bottom macrofauna in the deep open Baltic Sea in

8   relation to environmental variability. Est. Coast. Shelf Sci 57, 87-97.

9   Laine, A.O., Luodekari, K., Poikonen M., Viitasalo, M., 2003a. A comparison between

10   1928 and 2000 indicates major changes in macrozoobenthos species composition

11   and abundance on the SW coast of Finland (Baltic Sea). Proc. Estonian Acad. Sci.

12   Biol. Ecol. 52, 3-16.

13 Laine, A.O., Pesonen, L., Myllynen, K., Norha, T., 2003b. The effects of the changes in

14   water quality on the benthic fauna in the sea areas of Helsinki and Espoo between

15   1973 and 2001. Publications by City of Helsinki Environment Centre 10/2003, 47

16   p (In Finnish with English summary).

17 Leppäkoski, E., Gollasch, S., Olenin, S. (eds), 2002. Invasive aquatic species of Europe.

18   Distribution, impacts and management. Kluwer Academic Publishers, Dordrecht.

19 Lewandowski, J., Hupfer, M., 2005. Effect of macrozoobenthos on two-dimensional

20   small-scale heterogeneity of pore water phosphorus concentrations in lake

21   sediments: A laboratory study. Limnol. Oceanogr. 50, 1106-1118.

22 Lewandowski, J., Schadach, M., Hupfer, M., 2005. Impact of macrozoobenthos on two-

23   dimensional small-scale heterogeneity of pore water phosphorus concentrations:

24   *in situ* study in lake Arendsee (Germany). Hydrobiologia 549, 43-55.

25 Mermillod-Blondin, F., Rosenberg, R., Francois-Carcaillet, F., Norling, K., Mauclaire,

26   L., 2004. Influence of bioturbation by three benthic infaunal species on microbial

communities and biogeochemical processes in marine sediments. Aquat. Microb. Ecol. 36, 271-284.

Michaud, E., Desrosiers, G., Mermillod-Blondin, F., Sundby, B., Stora, G. 2006. The functional group approach to bioturbation: II. The effects of *Macoma balthica* community on fluxes of nutrients and dissolved organic carbon across the sediment-water interface. J. Exp. Mar. Biol. Ecol. 337, 178-189.

Mortimer, C.H., 1941. The exchange of dissolved substances between mud and water in lakes. J. Ecol. 29, 280-329.

Mortimer, C.H., 1942. The exchange of dissolved substances between mud and water in lakes. J. Ecol. 30, 147-201.

Nielsen, L.P., 1992. Denitrification in sediment determined from nitrogen isotope pairing. FEMS Microb. Ecol. 86, 357-362.

Norkko, A., Laakkonen, T., Laine, A., 2007. Trends in soft-sediment macrobenthic communities in the open sea areas of the Baltic Sea. In: Olsonen, R. (ed.) FIMR monitoring of the Baltic Sea environment – annual report 2006. Meri 59, 59-64.

Olenin, S., Leppäkoski, E., 1999. Non-native animals in the Baltic Sea: alteration of benthic habitats in coastal inlets and lagoons. Hydrobiologia 393, 233–243.

Pelegri, S.P., Blackburn, T.H., 1994. Bioturbation effects of the amphipod *Corophium volutator* on microbial nitrogen transformations in marine sediments. Mar. Biol. 121, 253-258.

Perus, J., Bonsdorff, E., 2004. Long-term changes in macrozoobenthos in the Åland archipelago, northern Baltic Sea. J. Sea Res. 52, 45-56.

Ryden, J.C., Syers, J.K., Tillman, R.W., 1987. Inorganic anion sorption and interactions with phosphate sorption by hydrous ferric oxide gel. J. Soil Sci. 38, 211-217.

Scheffer, B., Scheffer, K., 1984. Der Einfluß von Kalk und Kieselsäure auf die Phosphatmobilität (CAL). Böden Landwirtsch Forschung. 37, 1-8.

Schiedek, D., 1997. *Marenzelleria* cf. *viridis* (Polychaeta: Spionidae) - ecophysiological adaptations to a life in the coastal waters of the Baltic Sea. Aquat. Ecol. 31, 199-210.

Sikorski, A.V., Bick, A., 2004. Revision of *Marenzelleria* Mesnil, 1896 (Spionidae, Polychaeta). Sarsia 89, 253-275.

Stigzelius, J., Laine, A., Rissanen, J., Andersin, A.-B., Ilus, E., 1997. The introduction of *Marenzelleria viridis* (Polychaeta, Spionidae) into the Gulf of Finland and the Gulf of Bothnia (northern Baltic Sea). Ann. Zool. Fenn. 34, 205-212.

Svensson, J.M., Enrich-Prast, A., Leonardson, L., 2001. Nitrification and denitrification in a eutrophic lake sediment bioturbated by oligochaetes. Aquat. Microb. Ecol. 23, 177-186.

Svensson, J.M., Leonardson, L., 1996. Effects of bioturbation by tube-dwelling chironomid larvae on oxygen uptake and denitrification in eutrophic lake sediments. Freshw. Biol. 35, 289-300.

Thamdrup, B., Dalsgaard, T., 2002. Production of N_2 throught anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Appl. Environ. Microbiol. 68, 1312-1318.

Trimmer, M., Nicholls, J.C., Deflandre, B., 2003. Anaerobic ammonium oxidation measured in sediments along the Thames estuary. Appl. Environ. Microbiol. 69, 6447-6454.

Tuominen, L., Mäkelä, K., Lehtonen, K.K., Haahti, H., Hietanen, S., Kuparinen, J., 1999. Nutrient fluxes, pore water profiles and denitrification in sediment influenced by algal sedimentation and bioturbation by *Monoporeia affinis*. Est. Coast. Shelf Sci. 49, 83-97.

Tuominen, L., Mäkelä, K., Lehtonen, K.K., Kuparinen, J., Haahti, H., 2003. Effect of algal sedimentation and *Monoporeia affinis* on nutrient fluxes, pore water profiles and denitrification in sediment microcosms. Bor. Env. Res. 8, 229-243.

1   Vanderploeg, H.A., Nalepa, T.F., Jude, D.J., Mills, E.L., Holeck, K.T., Liebig, J.R.,

2   Grigorovich, I.A., Ojaveer, H., 2002. Dispersal and emerging ecological impacts

3   of Ponto-Caspian species in the Laurentian Great Lakes. Can. J. Fish. Aquat. Sci.

4   59, 1209-1228.

5   Welsh, D.T., 2003. It's a dirty job but someone has to do it: The role of marine benthic

6   macrofauna in organic matter turnover and nutrient recycling to the water

7   column. Chem. Ecol. 19, 321-342.

8   Zettler, M.L., Bochert, R., Bick, A., 1994. Röhrenbau und Vertikalverteilung von

9   *Marenzelleria viridis* (Polychaeta, Spionidae) in einem inneren Küstengewässer

10   der südlichen Ostsee. Rostock. Meeresbiolog. Beitr. (1994) 2, 215-225.

11 Zettler, M.L., 1996. Successful establishment of the spionid polychaete, *Marenzelleria*

12   *viridis* (Verrill, 1873), in the Darss-Zingst estuary (southern Baltic) and its

13   influence on the indigenous macrozoobenthos. Arch. Fish. Mar. Res. 43, 273-284.

14

1 **Tables**

2

3 Table 1. Pearson correlation matrix ($\alpha = 0.05$, $p < 0.01$). Dtot total denitrification, Dw

4 denitrification based on water column NO_x, Dn coupled nitrification-denitrification, D15

5 denitrification potential, O_2 depth O_2 penetration depth into the sediment, Conc

6 concentrations and Flux fluxes of O_2 and nutrients. Significant correlations marked in

7 bold.

8

	Dtot	Dw	Dn	D15	O_2 depth	Conc NO_x	Conc O_2	Flux NH_4^+	Flux NO_x	Flux PO_4^{3-}
Dw	0.51									
Dn	**0.88**	0.04								
D15	**-0.66**	-0.05	**-0.74**							
O_2 depth	**0.72**	0.50	**0.56**	-0.41						
Conc NO_x	**0.77**	**0.79**	0.46	**-0.52**	**0.81**					
Conc O_2	0.25	0.10	0.23	0.01	0.44	0.26				
Flux NH_4^+	**-0.70**	-0.36	**-0.61**	**0.68**	-0.38	-0.52	0.11			
Flux NO_x	**0.72**	**0.80**	0.40	-0.47	**0.82**	**1.00**	0.26	-0.48		
Flux PO_4^{3-}	-0.35	-0.37	-0.21	0.20	-0.07	-0.32	0.29	0.48	-0.28	
Flux Fe	-0.51	-0.41	-0.36	0.37	**-0.39**	-0.55	0.00	0.52	-0.52	**0.81**

9

10

11

1 **Figure legends**

2

3 Figure 1. Nutrient fluxes between sediment and water (mmol m^{-2} d^{-1}, avg ± sd).

4 Significant differences from the control treatment are marked with an asterisk.

5

6 Figure 2. A) Coupled nitrification-denitrification (Dn, white columns) and denitrification

7 based on water column nitrate (Dw, dark columns) (mmol N m^{-2} d^{-1}, avg ± sd) B)

8 Denitrification potential (D15) (mmol N m^{-2} d^{-1}, avg ± sd). Significant differences from

9 the control treatment are marked with an asterisk.

10

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

12 HIGH treatments.

13

14 Figure 4. P fractions (µmol P g^{-1} DM, avg ± sd) in C and HIGH units on days 2 and 14.

15 White bars 0-2 cm, grey bars 2-4 cm, black bars 4-6 cm.