Nutrient fluxes from reduced Baltic Sea sediment: effects of oxygenation and macrobenthos

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ABSTRACT: Effects of bottom water oxygenation and macrofaunal colonisation on benthic fluxes of nitrogen (N), phosphorus (P) and silicon (Si) from long-term anoxic Baltic Sea bottom sediment were investigated. Sediment boxcosms from an anoxic site at 150 m depth in the open Baltic proper were incubated in the laboratory to follow the development of benthic nutrient fluxes during 74 d exposure to flow-through of oxygen-rich water. In contrast to traditional end-point experimental designs, our repeated measurement approach allowed for separation of transient and long-term effects of oxygenation and bioturbation on benthic nutrient recycling. The composition, but not the rate, of the benthic total dissolved N efflux changed by oxygenation from being dominated by NH_4 *in situ* to being mostly composed of $NO_2 + NO_3$ and dissolved organic N (DON) under oxic conditions. Oxygenation in the boxcosms decreased the benthic efflux of dissolved silicate (DSi) and essentially shut off the *in situ* flux of dissolved inorganic phosphorus (DIP). After 20 d of oxygenation, 2 bottom macrofauna taxa, the polychaete *Marenzelleria* spp. and the amphipod *Monoporeia affinis*, were introduced to a subset of the boxcosms. Bioturbation by either taxa increased the efflux of dissolved inorganic N (DIN), DON and DSi to the overlying water. The P-rich benthic flux under *in situ* anoxic conditions roughly approached Redfield N:P stoichiometry after oxygenation in the sediment boxcosms. Upon addition of macrofauna, bioturbation generated even higher N:P flux ratios.

KEY WORDS: Bioturbation · Oxygenation · Benthic nutrient fluxes · Baltic Sea · Bottom sediment

INTRODUCTION

The high productivity of many coastal marine and estuarine systems is partly due to their relatively shallow depth, which enables efficient coupling of nutrients between the benthic and pelagic domains (Rowe et al. 1975). Particulate nutrients deposited in costal sediments may either recycle back into the water column or become permanently retained or transformed in the seabed. Their fate depends on several physicochemical and biological factors, particularly the oxygen gas (O_2) concentration in the bottom water (Kemp et al. 2005, Viktorsson et al. 2012, 2013, Bonaglia et al. 2014).

The expansion of hypoxic and anoxic bottom water conditions (Diaz & Rosenberg 2008) has altered benthic nutrient cycling in coastal marine and estuarine systems worldwide (Kemp et al. 2005, Vahtera et al. 2007, Friedrich et al. 2014). For example, conversion of bioavailable nitrogen (N) into gaseous forms via canonical (heterotrophic) denitrification and anaerobic ammonium oxidation (anammox) are major sink mechanisms in the nitrogen cycle (Galloway et al. 2004, Dalsgaard et al. 2005). However, in organic-rich sediments overlain by hypoxic–anoxic but nitrate-containing bottom water, these processes are often replaced by dissimilatory nitrate–nitrite reduction to ammonium (DNRA), in which bioavailable ammo-

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nium (NH_4), rather than gaseous forms of N, is the metabolic end product (McCarthy et al. 2008, Thamdrup & Dalsgaard 2008 and references therein, Jäntti & Hietanen 2012, Bonaglia et al. 2014). Furthermore, oxidation of NH_4 to NO_3 via nitrification does not take place in anoxic systems (Thamdrup & Dalsgaard 2000). Thus, bottom water oxygen depletion often increases net N recycling rates to the water column by stimulation of benthic NH_4 fluxes (Hall et al. 1992, Joye & Anderson 2008, McCarthy et al. 2008, Bonaglia et al. 2014), and oxygenation of anoxic bottom water has been demonstrated to reduce the benthic dissolved inorganic N (DIN) release in a marine fjord by roughly half (De Brabandere et al. 2015).

Oxygen deficiency also reduces the capacity of the sediment to retain dissolved inorganic phosphorus (DIP), due to inactivation of DIP-scavenging mechanisms that retain phosphorus (P) when surficial sediments are overlain by oxic bottom water (Steenbergh 2012), such as DIP accumulation by bacteria (Gächter & Meyer 1993) and DIP immobilisation by ferric iron (oxyhydr)oxides (e.g. Mortimer, 1941, 1942, Gunnars & Blomqvist 1997) (hereafter called 'iron oxides'). It has been demonstrated experimentally that certain iron oxides (ferrihydrite, goethite) are efficient adsorbents for dissolved silicate ($Si(OH)_4$; hereafter called DSi) (Sigg & Stumm 1981, Anderson & Benjamin 1985). Actually, the adsorption affinity may be higher for DSi than for DIP when both ions are present in freshwater lake solution at 'natural' concentrations (Kato 1969, Brinkman 1993). Thus, similarly to the benthic DIP flux (Sundby et al. 1986, Gunnars & Blomqvist 1997), the benthic DSi flux would be expected to decrease under oxygenated conditions, due to adsorption of DSi on iron oxide surfaces (cf. Danielsson 2014). Also, in parallel to what is commonly observed for phosphate (Sundby et al. 1986), drastically increased benthic DSi fluxes are foreseen due to reductive dissolution of the iron–silicate complexes upon deoxygenation of the bottom water (cf. Mortimer 1941, Ekeroth et al. 2016). However, the most common view on benthic DSi regeneration in coastal marine environments is that the benthic DSi flux rate is not strongly redox dependent, but directly proportional to the dissolution of biogenic silica in the sediment (Rutgers van der Loeff et al. 1984, Yamada & D'Elia 1984, Testa et al. 2013), which in turn is positively related to temperature and pH (Lewin 1961) as well as the degree of saturation of DSi in the sediment pore water (van Cappellen & Qiu 1997).

Oxygen availability fundamentally influences the structure and function of the benthic macrofauna community (Diaz & Rosenberg 1995). Benthic animals have potential to augment organic matter degradation (e.g. Aller 1988, Hulthe et al. 1998) and influence nutrient remineralization pathways, as well as flux rates of dissolved and particle-bound nutrients across the sediment–water interface (e.g. Aller 1980, Rutgers van der Loeff et al. 1984, Ekeroth et al. 2012). Therefore, by alteration of macrofauna communities, changing oxygen conditions might indirectly alter sediment nutrient cycling, e.g. Karlson et al. (2007a).

The wide spread of anoxia in the Baltic Sea has largely eradicated bioturbating macrofauna from sub-halocline sediments (Karlson et al. 2002). The macrobenthos community adjacent to this azoic zone consists of a relatively species-poor mixture of marine and freshwater taxa adapted to a bottom water salinity of ca. 6–10‰. The deposit-feeding pontoporeid amphipod *Monoporeia affinis* is of freshwater origin but can also cope with the brackish conditions in all but the southwesternmost parts of the Baltic Sea (Bonsdorff 2006). It resides in the upper few cm of soft bottom sediments during the day (Hill & Elmgren 1987, Karlson et al. 2005) but can swim in the pelagic zone during the night (Lindström & Lindström 1980). *M. affinis* was the numerically dominant macrobenthos species in the Baltic proper (Ankar & Elmgren 1976, Ankar 1977) before an invasion by (3 sibling) spionid polychaete species of the genus *Marenzelleria*, which started in the 1980s (Żmudziński et al. 1996). *Marenzelleria* spp. was well established some decade later (Żmudziński et al. 1996, Bastrop & Blank 2006, Blank et al. 2008). The rapid growth of *Marenzelleria* populations in the Baltic Sea can be explained in part by their high tolerance towards low oxygen conditions (Schiedek 1997), which lets them colonize semipermanently hypoxic areas (Maximov 2011). *Marenzelleria* spp. create U-, L- or J-shaped, narrow (ø ca. 2 mm) burrows which typically extend 6 to 25 cm into the sediment (Zettler et al. 1994, Karlson et al. 2005, Quintana et al. 2011, Renz & Forster 2013, 2014), which is deeper than other Baltic Sea macrobenthos. The worms reach a maximum length of ~10 cm (Essink & Kleef 1988) and are likely able to alternate between deposit and suspension feeding near the sediment surface (Dauer et al. 1981).

The spread of anoxic conditions have also increased eutrophication in the Baltic Sea by weakening the sedimentary sink mechanisms for N and P (Vahtera et al. 2007, Jäntti & Hietanen 2012, Viktorsson et al. 2012, 2013). For example, deoxygenation of oxic sediment areas in the Baltic proper in the 1990s mobilised some 160 000 t of P to the water column (Savchuk 2005). In turn, by promoting cyanobacterial

N-fixation in the Baltic proper, higher P bioavailability results in higher inputs of fixed N to the ecosystem (e.g. Vahtera et al. 2007). Consequently, and due to land-based emissions, winter water concentrations of DIN and DIP in the Baltic proper (Gotland Sea) have at least doubled since 1950 (Gustafsson et al. 2012). In contrast, most studies indicate that DSi concentrations declined between 1970 and 2001 (Papush & Danielsson 2006), and it has been proposed that the currently N-limited spring bloom, which is dominated by diatoms, may become DSi limited if DSi availability continues to decline (Conley et al. 2008). However, this risk appears small, as DSi concentrations have increased considerably in the 21st century (Larsson 2014).

Our aim was to study the effects of oxygenation of anoxic Baltic Sea sediment on benthic key nutrient (N, P and Si) recycling to provide empirical information regarding biogeochemical responses of anoxic and azoic bottom sediment when exposed to improving redox conditions and macrofaunal colonization. Soft bottom sediment boxcosms were collected from a long-term anoxic site in the northwestern Baltic proper, and benthic nutrient flux measurements were conducted during exposure of these sediments to either oxygenated, but macrofauna-free (azoic), or oxygenated and bioturbated conditions. Benthic nutrient flux measurements were also done *in situ* under anoxic ambient conditions by means of a benthic chamber lander at the station where the boxcosm sediment was collected. This experimental design allowed for separation of initial effects of oxygenation and effects on longer time scales due to animal colonization. The relatively long duration (74 d), repeated measurements design, and benthic flux measurements of both inorganic and organic N and P fractions, constitute novel aspects of our experiment.

MATERIALS AND METHODS

Study area

The Baltic Sea is a semi-enclosed, brackish sea in northern Europe (Fig. 1). It is boarded by 9 countries, and ~85 million people inhabit its drainage area (Sweitzer et al. 1996). The southernmost and largest main basin, the Baltic proper, is heavily loaded by nutrients, which has led to eutrophication and bottom water oxygen depletion (Karlson et al. 2002, Gustafsson et al. 2012). In 2011, almost a quarter of the total bottom area in the Baltic proper and the adjoining gulfs of Finland and Riga below the perma-
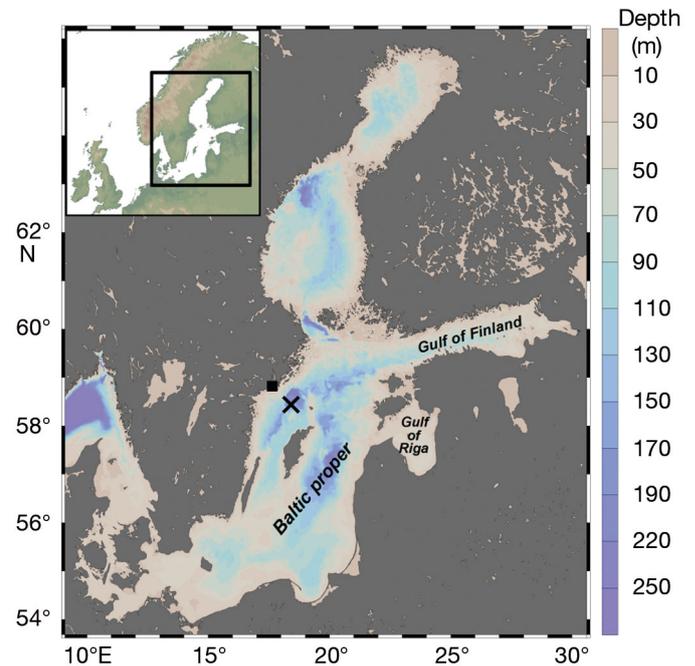

Fig. 1. Baltic Sea area and the sub-basins the Baltic proper, the Gulf of Finland and the Gulf of Riga. ×: position of the lander deployment and box core sampling; ■: Askö Laboratory

nent halocline at 60–80 m water depth were covered by year-round anoxic sediment (Hansson et al. 2013).

In situ benthic nutrient flux measurements

The small 'Göteborg' chamber lander was deployed at the study site in the north-western Baltic proper (58° 26.2095' N, 18° 25.3751' E, WGS84, 149 m water depth, Fig. 1) on 2 August, 2012. The device has 2 open-bottom quadratic polycarbonate chambers, each enclosing a volume of bottom water overlying 400 cm^2 of seabed sediment. This device has 10 attached syringes and a paddle wheel that mixes the water inside the chambers (Ståhl et al. 2004). Oxygen concentrations were measured in each chamber using oxygen optodes (model 3830; Aanderaa Data Instruments), while conductivity measurements (for salinity determination) were made with sensors (model 3919A; Aanderaa Data Instruments), each at 1 min intervals. For comparison, another pair of sensors, attached to the frame of the lander, recorded ambient bottom water oxygen and conductivity conditions 0.5 m above the seafloor.

The lander was gently lowered with a rope by hand to the bottom. Six trawl balls (each 10 dm^3) were attached to the rope to make the instrument slightly

negatively buoyant. During the last few meters, the descent speed was very slow (~1 m min^{-1}) to minimise disturbance of the sediment surface by the downward bow-wave formed in front of the lander. Each chamber had a lid that was open during descent and closed ~4 h after the lander was deployed on the sea floor. During this pre-incubation period, the stirring of the chamber was switched on, and any remaining upper pelagic water in the chambers was exchanged with ambient bottom water through the lid. Also, any oxygen that was dissolved in the polycarbonate walls of the chambers diffused into the water and was ventilated out of the chambers during the pre-incubation.

The lander was programmed to function autonomously. After the pre-incubation, the lid was closed and the nutrient flux incubation started. After 15 min, a known volume (~60 ml) of de-ionized water was injected into each chamber. The resulting slight decrease in salinity was used to calculate the volume of the enclosed water mass. During the incubation, the lander was programmed to withdraw samples in the remaining 9 syringes of each chamber at pre-set times. However, 8 of the total 18 sample syringes failed to trigger. Thus, 4 samples were recovered from one of the chambers and 6 samples were recovered from the other. Collected sample water was replaced by ambient bottom water sucked in through a coil on top of the chamber. The duration of the deployment was ~22 h, including 4 h of pre-incubation.

Collection of sediment boxcosms

The box corer used for collecting sediments has been documented *in situ* to collect high quality soft sediment boxcores (Blomqvist et al. 2015). During the lander deployment, 9 boxcosms were collected at 149 m water depth close to the lander station. The content (% dry wt) of total C (TC) and total N (TN) in the upper 1 cm of collected sediment was 8% TC and 0.8% TN. The polycarbonate liners of the box corer had an inner area of 841 cm^2 (29 × 29 cm) and were about three-quarters filled with sediment (~34 cm sediment height), with one-quarter (~14 l) of supernatant bottom water. On recovery, the liners were placed on PVC bottom plates. A polycarbonate lid was placed on top of each liner, and the whole setup was held together by a ratchet strap. To minimise resuspension of the sediment during transport, a floating plastic foam insert was placed under the lid and was removed after installation of the boxcosms in the laboratory. Still, some sediment resuspension

was unavoidable during transport. No signs of bioturbation could be seen in the sampled sediment boxcosms.

Laboratory experimental set-up

The boxcosms were transported to a dark, temperature-controlled room at the Stockholm University Askö Laboratory (Fig. 1), in the Trosa Archipelago, and partially submerged in water to dampen spatial and temporal temperature variations. Well-oxygenated and sand-filtered brackish seawater from 15 m depth outside the Askö Laboratory was cooled to 5°C, and supplied at a rate of ~30 ml min^{-1} via hoses connected to the lids of the boxcosms. DIN, DIP and DSi concentrations in the inflowing water were factors of approximately 2, 6, and 3, respectively, lower than in the bottom water at the sediment collection site, while dissolved organic N and P (DON and DOP) concentrations were similar *in situ* and in the sand-filtered water. A water outlet on the side of each boxcosm permitted flow-through of oxygenated water. Aquarium pumps recirculated the water in the boxcosms via netted polycarbonate dispersers, which prevented animals from being sucked into the circulation system. In most of the boxcosms, dissolved oxygen concentration and temperature was measured continuously by lid-mounted sensors (models 3830 and 4835; Aanderaa Data Instruments), and the data was logged by a computer outside of the cold room. Due to technical problems, some oxygen and temperature data were lost (see 'Results').

The set-up of all boxcosms was completed on 4 August 2012 (Day 0). Nutrient flux measurements were made on Days 4, 17, 33, 60 and 74 of the experiment (see 'Nutrient flux measurements in boxcosms').

Sampling and introduction of animals

Macrofauna were collected from 20–28 m depth by means of a benthic sled (Blomqvist & Lundgren 1996) in the Yttre Hållsfjärden bay, near the Askö Laboratory. Collected animals and sediment were stored in 50 l plastic boxes, filled with brackish seawater, and gently stirred. The water and suspended sediment was then poured through a sieving net (mesh size 0.5 mm), and the amphipod *Monoporiea affinis* and the polychaete *Marenzelleria* spp. (hereafter *Marenzelleria*) retained on the net were carefully picked out with a pair of tweezers.

There are 3 sibling species of *Marenzelleria* in the Baltic sea which are morphologically very similar. The collected *Marenzelleria* were not identified to species level. Still, it can be assumed that *M. arctia* dominated the sampled population, as the other 2 species have been found only very sporadically (*M. neglecta*), or not at all (*M. viridis*) at 20–30 m depth in the Askö area (Bastrop & Blank 2006, Blank et al. 2008).

The selected macrofauna were added to triplicate boxcosms on Day 20, shortly after the second flux measurement. The boxcosms received either 170 *Marenzelleria* or 181 *M. affinis* individuals, corresponding to abundances of 2020 and 2150 ind. m^{-2}, respectively, which are realistic field abundances in the Baltic Sea (Villnäs & Norkko 2011). A mixture of animal sizes was added with the aim of mimicking the natural size distributions in the sampled area. The remaining 3 boxcosms served as oxygenated macrofauna-free control systems. The sediment surface in control boxcosms remained without any signs of bioturbation throughout the experiment.

Chemical analyses and definition of nutrient fractions

Three water samples per sampling occasion were collected from each chamber and each boxcosm during lander and boxcosm incubations. For the boxcosms, an additional unfiltered sample was collected upon every sampling. The samples were then filtered through 0.45 µm pore size cellulose acetate filters (Filtropur S 0.45; Sarstedt), pre-soaked and pre-rinsed with deionised water. The set of unfiltered samples were analysed for total P (TP) and TN after digestion with acid-persulphate at high temperature (modified after Valderrama 1981). Using the same digestion technique, one set of filtered (filtrate) samples were analysed for operationally defined total dissolved N and P (TDN and TDP, respectively). The concentration differences between the unfiltered and filtered total samples were used to calculate the concentrations of particulate N (PN) and P (PP) (i.e. PN = TN − TDN, PP = TP − TDP).

A second set of filtered samples were analysed for NH$_4$, $\sum$(NO$_2$ + NO$_3$) and DSi. DIN was defined as the sum of NH$_4$ and (NO$_2$ + NO$_3$) concentrations. The third set of 10 ml filtrate samples were acidified by addition of 0.5 ml blank-tested H$_2$SO$_4$ (5%) within 1 h after filtration and analysed for soluble reactive P (SRP). The acid preservation avoids oxidation artefacts of phosphate in the filtrate when exposed to oxic conditions in the test tube, which otherwise could have resulted in underestimation, particularly for the anoxic samples from the lander. The SRP concentration was assumed to represent the sum of orthophosphate, pyrophosphate and polyphosphate, i.e. DIP. As to the dissolved organic species, the concentration of DON was calculated as the concentration difference between TDN and DIN, and the DOP concentration by subtracting the SRP concentration from the TDP value.

All nutrient analyses were performed by the Chemistry Laboratory at the Department of Ecology, Environment and Plant Sciences, Stockholm University, using segmented flow colorimetric analysis, i.e. slightly modified ALPKEM O I Analytical Flow Solution IV Methods #319527 (NO$_2$ + NO$_3$), #319526 (NH$_4$), #319528 (SRP), and #319529 (DSi), all based on standard analytical procedures described by Koroleff (1983).

Nutrient flux measurements in boxcosms

The flow-through of water in each boxcosm was shut off during flux incubations, while the internal circulation systems remained switched on. Each flux incubation lasted for ~24 h, during which the supernatant water mass of every boxcosm was sampled 6 times in each boxcosm. Water samples were collected with a syringe through a hole in the boxcosm lid, tightly plugged between the flux measurements.

Calculation of benthic nutrient fluxes

Benthic fluxes were determined from least square linear regressions of the concentration change versus time in the enclosed water mass within the chambers of the lander or boxcosms, respectively. Fluxes were corrected for the decreasing volume (boxcosms) or exchange inflows of ambient bottom water (lander), due to withdrawal of sample water. In the correction of the lander fluxes, ambient bottom water concentrations were calculated as the mean value of the initial sample from each chamber, collected 20 min after the start of incubation.

Statistics

Since flux measurements were conducted several times in each boxcosm, linear mixed effects modelling was chosen as the most suitable statistical method for

the statistical analysis of the non-independent data (Quinn & Keough 2002). To assess the effects of bio-turbation on nutrient fluxes, linear mixed (between/within subject) effects modelling was used with independent categorical, fixed between subjects variable 'Species' (3 levels: unbioturbated control, *M. affinis*, *Marenzelleria*) and within subjects variable 'Day' (3 levels: Days 33, 60, 74). Also, the interaction term 'Species × Day' was included in the analysis. Similarly, a between–within subjects analysis was performed to test for significant differences in fluxes during the first 2 flux measurements (Days 4 and 17) between the 3 groups of triplicate boxcosms that belonged to different treatments during Days 20–74.

Model formulation and model selection followed the rationale given by Logan (2010). Several models with different correlation matrixes, and versions of these assuming heterocascadicy for each nutrient fraction, were tested against each other by ANOVA. Model selection was based on log likelihood (p < 0.05) or Akaike information criterion (AIC) values when log likelihood values could not be determined. In the latter cases, a more complex model was re-garded as superior to a simpler model if AIC values differed by 2 or more units. Between subjects, treatment effects (i.e. effects of bioturbation) were tested by ANOVA analysis of the most appropriate linear mixed effects model. The Tukey post hoc test was used for post hoc analysis of the 'Species' factor. In cases when there was a significant interaction between the main variables, simple main effects for each day were determined by ANOVA and post hoc analysis conducted with pairwise *t*-tests (Bonferroni p-value adjusted for multiple comparisons). Two-sample *t*-tests were used to compare *in situ* flux values to flux values in the oxygenated, azoic control boxcosms. The assumption of homogeneity of variances was checked with *F*-tests. Statistical analysis was done using the software R 2.15.2 (R Core Team 2012), with the 'nlme' package for model formulation (Pinheiro et al. 2014).

RESULTS

Examples of representative linear regressions used for the determination of fluxes are provided in Fig. 2. Temperature and oxygen saturation in the water of the boxcosms were logged with 1 min resolution but not always simultaneously in all boxcosms. Available data, covering ~50% of the total experimental period, showed that the oxygen saturation was typically between 70 and 80% (ranging 65–90%), and
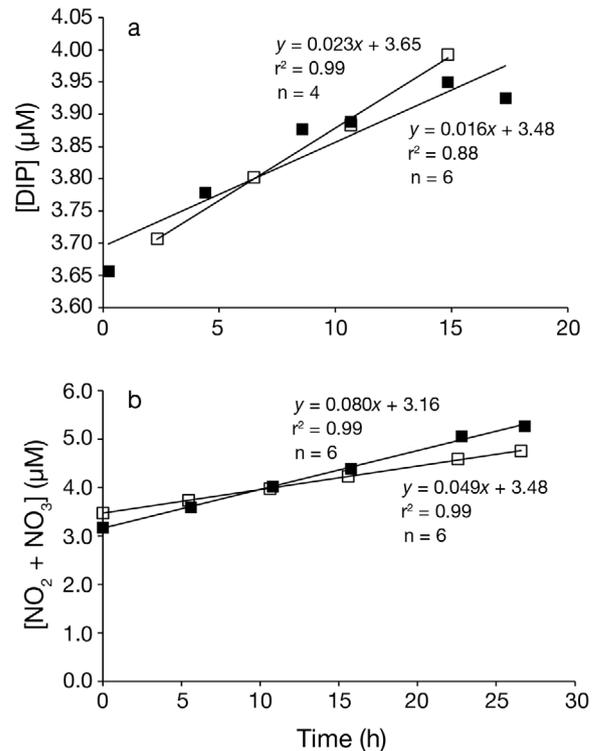

Fig. 2. (a) Dissolved inorganic phosphorus (DIP) concentration vs. time in the chambers of the *in situ* lander; (b) concentration of $NO_2 + NO_3$ vs. time in boxcosms during the flux measurement at Day 60. Open and filled symbols denote duplicate lander chambers or boxcosms

temperature was maintained at 3–8°C, except during the initial 5 h when the water in the boxcosms was cooling.

In situ flux measurements

During the lander incubation, the ambient bottom water oxygen concentration (~0.5 m above the sediment) ranged between 0 and 7.8 µM O_2 (0–0.2 mg O_2 l^{-1}). Bottom water salinity and temperature were stable at 10.5 and 5.6°C, respectively. The injection of deionised water into the chambers for volume determination lowered the salinity by less than 0.1 (i.e. to about 10.4) and raised the oxygen concentration of the previously anoxic water to 4–5 µM O_2 (0.1–0.2 mg O_2 l^{-1}). The introduced oxygen was consumed within 10 h and was considered to have negligible effect on the solute fluxes measured in the chambers.

Dissolved inorganic P, NH_4 and DSi were released from the sediment (Fig. 3a−c, Table 1), while average fluxes of DOP and DON were very small with relatively large standard deviations (Fig. 4a,b, Table 1). The small amounts of $NO_2 + NO_3$ in the chamber

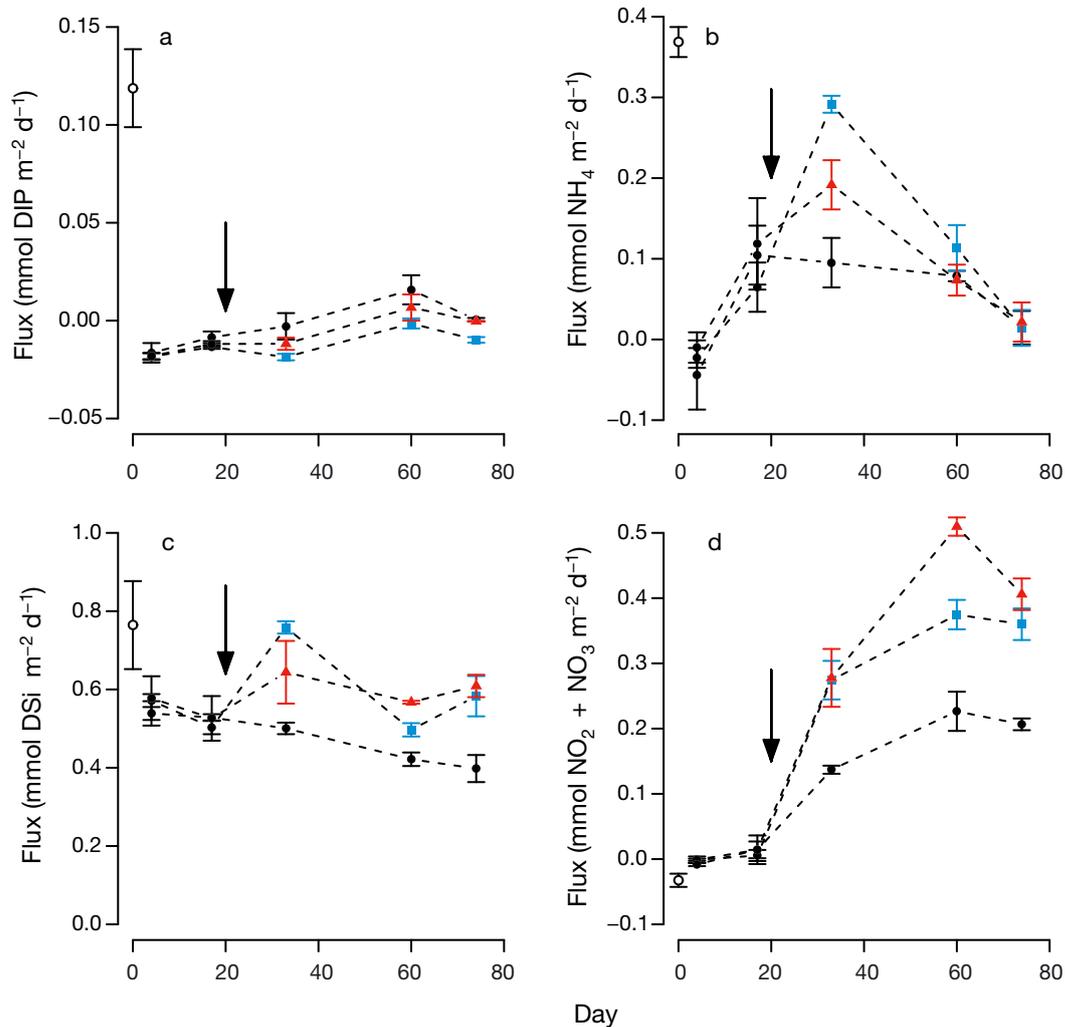Fig. 3. Benthic fluxes of (a) dissolved inorganic P (DIP), (b) NH_4, (c) dissolved silicate (DSi), and (d) $NO_2 + NO_3$. *In situ* flux values (○), control boxcosms (●), *Monoporiea affinis* boxcosms (■), and *Marenzelleria* boxcosms (▲). Animals were introduced on Day 20 (indicated with arrow). Error bars denote SE of the mean for *in situ* fluxes (n = 2) and for boxcosm fluxes (n = 3)

water decreased quickly during the first half of the incubation, but then levelled off at ~0.05 μM NO_2 + NO_3 in both chambers. The non-linear concentration change of $NO_2 + NO_3$ makes it difficult to calculate a reliable value of the flux into the sediment. The average $NO_2 + NO_3$ flux in Table 1 and Fig. 3d is based on regression of all available data points from the incubation and provides a minimum estimate of the true *in situ* update flux. Bottom water nutrient concentrations are reported in Table 1.

Ex situ flux measurements

During the first 2 flux measurements, made before the additions of fauna, no significant differences

were found between average nutrient fluxes in the 3 groups of triplicate boxcosms that later belonged to different treatments (ANOVA, p < 0.05, Figs. 3–5).

After addition of animals, DSi fluxes were consistently and significantly higher in bioturbated boxcosms than in the controls (ANOVA, $F_{2,6} = 16.52$, p = 0.0036, Tukey contrasts, p < 0.05, Fig. 3c). Similarly, DIN (i.e. the sum of NH_4 and $NO_2 + NO_3$) effluxes in both bioturbated treatments were roughly double those in the azoic controls (ANOVA, $F_{2,6} = 24.08$, p = 0.0014, Tukey contrasts, p > 0.05, Fig. 5). This was mainly a result of higher $NO_2 + NO_3$ fluxes in treatments with added animals (ANOVA, $F_{2,6} = 26.42$, p = 0.0011, Tukey contrasts, p < 0.05, Fig. 3d). Both animal species also increased the efflux of DON to the overlying water over that in the controls (ANOVA,

Table 1. Bottom water nutrient concentrations and benthic nutrient fluxes (mean ± SD, n = 2) measured during the *in situ* lander incubation. Negative fluxes denote sediment uptake. DON: dissolved organic N; TDN: total dissolved N; DIP: dissolved inorganic P; DOP: dissolved organic P; TDP: total dissolved P; DSi: dissolved silicate

Nutrient species	Bottom water concentration (µM)	Benthic flux (mmol m^{-2} d^{-1})
NH$_4$	9.37	0.37 ± 0.026
NO$_2$ + NO$_3$	0.14	−0.032 ± 0.014[a]
DON	16.36	−0.0050 ± 0.28
TDN	25.87	0.33 ± 0.24
DIP	3.66	0.12 ± 0.028
DOP	0.25	0.0025 ± 0.0042
TDP	3.91	0.12 ± 0.024
DSi	60.41	0.76 ± 0.16

[a]Concentration changes for NO$_2$ + NO$_3$ in the chambers were non-linear; *in situ* uptake was therefore likely underestimated. For further details, see '*In situ* flux measurements'

$F_{2,6} = 23.11$, p = 0.0015, Fig. 4b), but *Monoporiea affinis* caused a larger DON release than *Marenzelleria* (Tukey contrasts, p < 0.05).

Ammonium fluxes on Day 33 (third flux measurement) were significantly different between levels of the 'Species' factor (ANOVA, $F_{2,6} = 17.35$, p = 0.0032), with flux rates in the *M. affinis* treatment significantly higher than in the controls (pairwise *t*-test, p = 0.005, Fig. 3b). However, as indicated by the significant Species × Day interaction term (ANOVA, $F_{4,12} = 5.50$, p = 0.0094), the initially high NH$_4$ fluxes in bioturbated boxcosms decreased over time and converged near those in the control boxcosms in the end of the experiment (Fig. 3b). A similar pattern was recorded for DOP fluxes in the *M. affinis* boxcosms (ANOVA, Species × Day interaction, $F_{4,12} = 5.55$, p = 0.0092, Fig. 4a).

DIP fluxes were low and generally negative, i.e. directed into the sediment (Fig. 3a). The rates of DIP
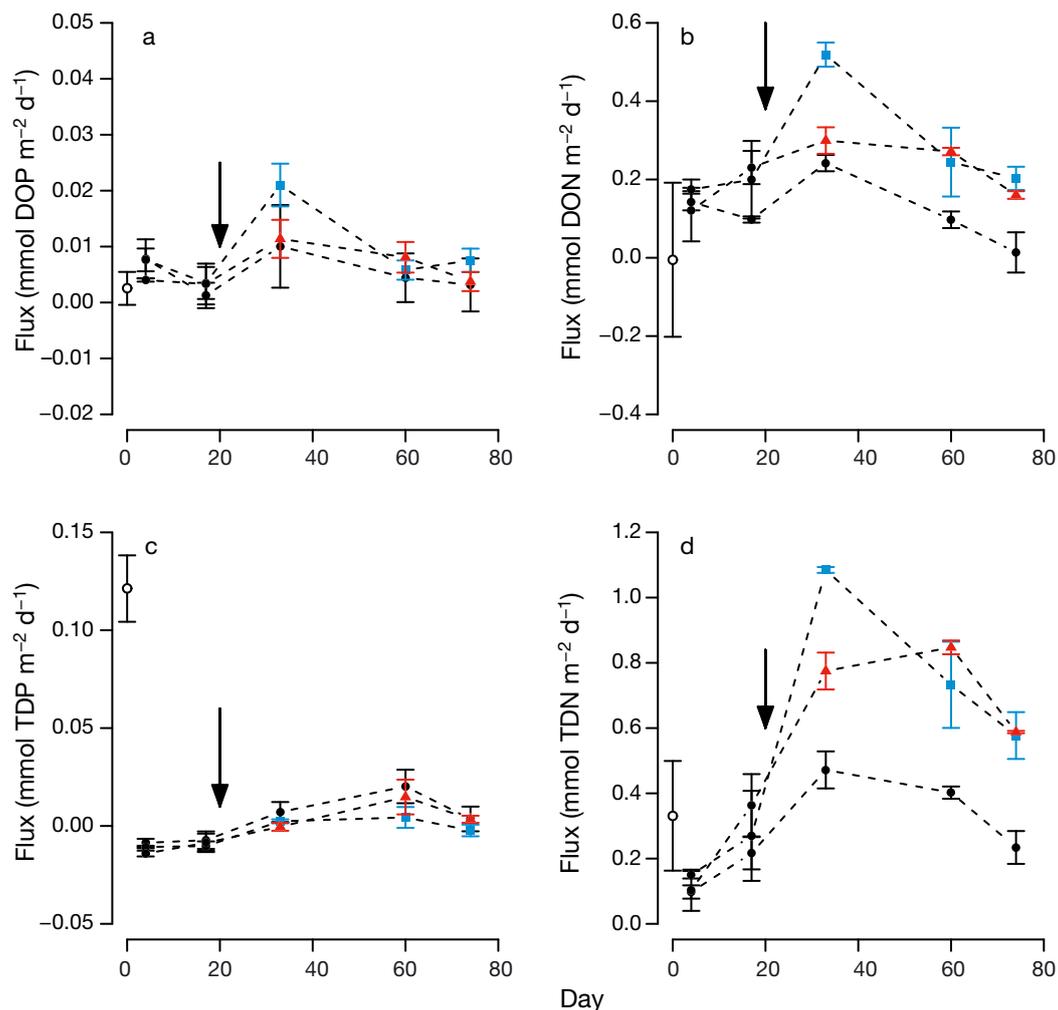

Fig. 4. Benthic fluxes of (a) dissolved organic P (DOP), (b) dissolved organic N (DON), (c) total dissolved P (TDP), and (d) total dissolved N (TDN). *In situ* flux values (○), control boxcosms (●), *Monoporiea affinis* boxcosms (■), and *Marenzelleria* boxcosms (▲). Animals were introduced on Day 20 (indicated with arrow). Error bars denote SE of the mean for *in situ* fluxes (n = 2) and for boxcosm fluxes (n = 3)
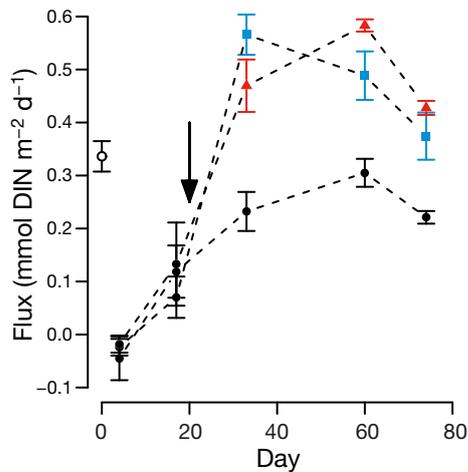
Fig. 5. Benthic dissolved inorganic N (DIN) fluxes. *In situ* flux values (○), control boxcosms (●), *Monoporiea affinis* boxcosms (■), and *Marenzelleria* boxcosms (▲). Animals were introduced on Day 20 (indicated with arrow). Error bars denote SE of the mean for *in situ* fluxes (n = 2) and for boxcosm fluxes (n = 3)

uptake differed among treatments (ANOVA, $F_{2,6}$ = 8.00, p = 0.02), and were significantly higher for *M. affinis* than for *Marenzelleria* and the azoic control treatments (Tukey contrasts, p < 0.05, Fig. 3a). Average particulate fluxes (PP and PN) were unaffected by bioturbation, and ranged between −0.01 and 0.024 mmol PP m^{-2} d^{-1} and −0.11 to 0.34 mmol PN m^{-2} d^{-1}.

DISCUSSION

Our experimental design with repeated measurements before and after oxygenation and animal addition provides valuable information on transient and longer-term changes of nutrient dynamics in chemically reduced sediment exposed to oxygenated conditions. This approach allows for separation of effects due to bottom water oxygenation per se from the effects related to bioturbation by the 2 investigated bottom fauna species.

Some nutrient fluxes varied considerably throughout our 2.5 mo experiment (Figs. 3–5). Such temporal variability is rather common in manipulative experimental studies (e.g. Tuominen et al. 1999, Michaud et al. 2006). For example, Michaud et al. (2005) noted significantly different oxygen uptake rates 20 and 28 d after the addition of the polychaete *Nereis virens* to experimental sediment microcosms. This illustrates that temporal variability can be a methodological source of discrepancy of experimental results

derived from studies of different duration, especially those involving only a single flux measurement (e.g. Karlson et al. 2005, Bonaglia et al. 2013, Urban-Malinga et al. 2013). Another drawback in such end-point experimental designs is that the homogeneity of response variables (e.g. nutrient fluxes) among experimental units is unknown before they are grouped into different treatments, e.g. exposed to bioturbation by different species of macrofauna. This paucity of information can make it practically impossible to unequivocally separate treatment effects from large random differences beyond the control of the investigator between experimental units.

Altered nutrient fluxes by bottom water oxygenation

Fluxes of DIP, DSi and DIN measured under anoxic conditions changed considerably upon bottom water oxygenation in the laboratory. Here, nutrient fluxes in azoic, oxygenated boxcosms are related to those measured under anoxic *in situ* conditions, with the aim to identify direct effects of bottom water oxygenation per se on benthic nutrient exchange. Later, effects related to bioturbation by *Marenzelleria* and *M. affinis*, as well as the environmental implications of our results, will be discussed.

Similar responses of DIP and DSi fluxes to oxygenated conditions

Oxygenation of the bottom water caused rapid changes in nutrient fluxes across the sediment–water interface. For instance, the high benthic DIP efflux from the sediment during anoxic conditions ceased soon after oxygenation and then remained very low throughout the experiment. For most of the experimental period, the net DIP flux was even directed into the sediment (Fig. 3a). Similarly, the DSi flux measured during anoxic conditions had decreased sharply by ~0.2 mmol m^{-2} d^{-1}, or by 30% (2-sample *t*-tests: t = 3.327, df = 9, p < 0.01) already 4 d into the experiment (Fig. 3c). A similar relative difference between DSi fluxes under oxygenated and hypoxic conditions has been reported from seasonally hypoxic sediment at 28 m water depth off the coast of Romania, in the Black Sea (Friedrich et al. 2014).

In contrast to DIP, fluxes of DSi in the azoic boxcosms continued to decrease throughout the experimental period (Fig. 3c). This decline was likely influ-

enced by a gradual depletion of labile biogenic silica in the boxcosms sediments, and thereby unrelated to the oxygenation per se. Actually, the supply of biogenic silica to the system via the inflowing water was likely very low, as the experiment was conducted in late summer to late autumn, when the production of pelagic diatoms is negligible in the Askö area of the north-western Baltic proper (Hobro 1979).

The present experimental study corroborates the hypothesis that oxic bottom water conditions reduce benthic release of DSi in the Baltic Sea (Danielsson 2014), while previous reports focusing on redox-dependent benthic DSi cycling are few and contradictive (e.g. Kamp-Nielsen 1974, Belias et al. 2007, Ekeroth et al. 2016, Siipola et al. 2016). The mechanistic explanation for the lower DSi flux after oxygenation may be the same as for the lower DIP flux, namely scavenging by iron oxides sorbents in the surficial sediment (e.g. Mortimer 1941, 1942, Sundby et al. 1986, Gunnars & Blomqvist 1997). Silicon-rich ferric particles are evidently abundant in some freshwater environments (Carlson & Schwertmann 1981) and are readily formed under laboratory conditions (Anderson & Benjamin 1985, Mayer & Jarrell 2000). Direct quantification of iron-bound Si (Fe–Si) in sediments is currently not analytically possible, but certain chemical extraction schemes indicate a considerable Fe–Si pool in the Baltic (Tallberg et al. 2009) and in other coastal marine sediments (Tallberg et al. 2008), as well as in freshwater lakes (Hartikainen et al. 1996). Geohistorically, it is also worth noting that adsorption of DSi onto iron oxides has been proposed as a key process in the formation of iron and silicon-rich laminated sedimentary rocks in the Neoarchean–Paleoproterozoic transition (ca. 2500 million years ago) (Fisher & Knoll 2009).

Nitrogen flux

Our *in situ* benthic N flux was dominated by NH_4 (Table 1, Fig. 3b). This flux decreased rapidly already after 4 d of oxygenation and remained below ~0.1 mmol NH_4 m^{-2} d^{-1} in the control systems throughout the experimental period (Fig. 3b). In contrast, the $NO_2 + NO_3$ flux increased over time during oxic conditions in the boxcosms, stabilizing at ~0.20 mmol $NO_2 + NO_3$ m^{-2} d^{-1} from Day 33 onwards (Fig. 3d). The average DIN efflux during Days 33–74 in the control boxcosms was 0.08 mmol DIN m^{-2} d^{-1} below the *in situ* flux (Fig. 5). Assuming that the ammonification rate was constant, this value is an estimate of how much the denitrification and/or anam-

mox rate increased by the increased availability of $NO_2 + NO_3$ after oxygenation, suggesting that approximately 30% of the NH_4 efflux was nitrified and subsequently denitrified/reduced to gaseous N after oxygenation. Artificial oxygenation of an anoxic marine fjord on the Swedish west coast resulted in a comparable decrease in the net benthic DIN release (De Brabandere et al. 2015).

Our DON fluxes ranged from 0 to 0.2 mmol DON m^{-2} d^{-1} in the experiment. Large variability under anoxic conditions makes it impossible to assess whether or not oxygenation influenced DON fluxes. However, in the oxygenated azoic boxcosms, the DON fluxes were relatively large (Fig. 4b) in comparison with DIN fluxes during the first half of the experiment (Fig. 5). Benthic DON fluxes of similar or higher magnitudes than benthic DIN fluxes have been reported previously (see compilation by Bronk & Steinberg 2008), especially in coastal sediments rich in reactive organic matter (Hansen & Blackburn 1992, Enoksson 1993, Blackburn et al. 1996, Alkhatib et al. 2013), and DON can clearly be a crucial component of coastal marine N budgets (cf. Alkhatib et al. 2012, 2013).

Bioturbation by *M. affinis* and *Marenzelleria*

The 2 experimentally studied macrofauna species had similar influence on benthic nutrient flux rates. Common effects were stimulation of benthic fluxes of $NO_2 + NO_3$, DON and DSi (Figs. 3c,d & 4b), and these are of critical ecological relevance.

Stimulation of benthic N and DSi fluxes

The $NO_2 + NO_3$ and DIN efflux was at least a factor of 2 higher in the bioturbated boxcosms than in the control systems (Figs. 3d & 5). This indicates that the animals stimulated the nitrification rate in the sediment and that most of the produced $NO_2 + NO_3$ fluxed out of the sediment rather than being denitrified or reduced by anammox. Presumably, this is an effect of bioirrigation by the animals facilitating rapid outflow of pore water $NO_2 + NO_3$ to the supernatant water (Welsh 2003). Also, given the high oxygen availability in the boxcosms, the high fluxes of $NO_2 + NO_3$ may have been caused by a dominance of aerobic respiration over denitrification (Joye & Anderson 2008). Our results do not contradict previous findings that *M. affinis* may stimulate benthic denitrification (Tuominen et al. 1999, Karlson 2007, Karlson et al.

2005, 2007a). Rather, the higher $NO_2 + NO_3$ (and DIN) fluxes in the presence of *M. affinis* compared to controls show that their potential influence on denitrification cannot compensate for their simultaneous stimulation of overall N mineralisation. *Marenzelleria* is known to have a minimal effect on benthic denitrification (Karlson et al. 2005, Hietanen et al. 2007, Kristensen et al. 2011) or to even diminish it (Bonaglia et al. 2013).

Both studied macrofauna taxa increased DON fluxes significantly in the boxcosms (Fig. 4b). This may both be an effect of the excretion of urea and other DON compounds by the animals (Lomstein et al. 1989, Tupas & Koike 1990) and due to intensified solute exchange of DON already present in the pore water of the bioturbated sediment (cf. Burdige 2001). Previously, it has been suggested that benthic macrofauna may stimulate benthic DON fluxes by bioturbation (Burdige & Zheng 1998, Landén & Hall 2000), but to our knowledge, this hypothesis has so far not been validated experimentally.

Recently, it has become apparent that the DON fraction in marine environments is more bioreactive than previously thought (e.g. Bronk et al. 2007, Alkhatib et al. 2012). For example, bacterial regrowth bioassays showed that about one-third of the riverine DON inputs to the Baltic Sea were bioavailable (Stepanauskas et al. 2002). Thus, the stimulation of benthic DON fluxes by the animals may have environmental consequences, particularly as the ability of DON utilisation appears widespread among nuisance phytoplankton groups, such as toxic bloom-forming species and cyanobacteria (Berg et al. 2003, Glibert et al. 2004, Kim & Kim 2013). Clearly, in light of the potentially substantial influence of DON bioavailability on pelagic productivity (cf. Eilola & Stigebrandt 1999, Korth et al. 2012), mobilisation of DON by bioturbating macrofauna appears to be an essential topic for further studies.

The DSi flux out of the oxygenated sediment was likewise stimulated by both *M. affinis* and *Marenzelleria* by approximately 50 % (Fig. 3c), indicating that bioturbating fauna might compensate for the initial decrease in the anoxic benthic DSi flux following bottom water oxygenation (see 'Altered nutrient fluxes by bottom water oxygenation'). Bonaglia et al. (2013) found a similar relative increase in the benthic DSi flux due to *Marenzelleria* bioturbation using an abundance of 2000 ind. m^{-2}. Assuming a linear relationship between animal abundance and DSi flux stimulation for *M. affinis* (Karlson et al. 2007b), our results also comply with experimental results showing a doubling of the benthic DSi recycling rate at

approximately twice the *M. affinis* abundance used in the present study (Karlson 2007). However, at lower abundances (1500 ind. m^{-2}), *M. affinis* is reported to have marginal influence on the benthic flux of DSi from Baltic sediments (Tuominen et al. 1999).

Low influence on P fluxes

In contrast to rates of N and DSi exchange, the effects of bioturbation on benthic DIP fluxes were minor (*M. affinis*) or non-existent (*Marenzelleria*). The reduction of benthic DIP fluxes by *M. affinis* in the present study was similar to previous records (Tuominen et al. 1999, Karlson et al. 2005, Karlson 2007), but was very small in comparison with the initial decrease in the DIP flux upon oxygenation of the bottom water (Fig. 3a).

A recent modelling study calculated that bioturbation in muddy sediments by *Marenzelleria* at abundances of up to 3000 ind. m^{-2} should have a slight stimulatory effect on benthic P release, whereas at abundances above 3500 ind. m^{-2} P immobilisation would be greatly increased (Norkko et al. 2012). For the abundance of *Marenzelleria* investigated here (~2000 ind. m^{-2}), their model implies an increased P efflux of ~0.08 mol P m^{-2} over a 5 yr period, equivalent to a flux difference between control and *Marenzelleria* systems in our experiment of roughly 0.05 mmol P m^{-2} d^{-1}, which we did not find (Fig. 3a). On the contrary, our flux record suggests that DIP retention was consistently slightly higher (although not statistically significant) in the *Marenzelleria* systems, compared to controls (Fig. 3a). Thus, our experiment does not confirm the model result of Norkko et al. (2012) but corroborates other experimental studies showing insignificant effects of bioturbation by *Marenzelleria* on benthic DIP exchange (Karlson et al. 2005, Urban-Malinga et al. 2013).

It should be noted that bioirrigation efficiency and reworking potential may differ considerably between the *Marenzelleria* sibling species (Renz & Forster 2013), yielding diverging effects on sediment nutrient dynamics (Renz & Forster 2014). The lack of species identification in this and most related previous studies, therefore, introduces uncertainty in the comparison of results and may explain diverging experimental findings on the effects of *Marenzelleria* bioturbation on benthic DIP exchange rates (Viitasalo-Frösén et al. 2009, Bonaglia et al. 2013). Our results are most likely representative for *M. arctia* (see 'Sampling and introduction of animals').

DOP

Our DOP fluxes were mostly unaffected by bioturbation, as over the full experimental period, there was no statistically significant effect of bioturbation on the benthic DOP fluxes (Fig. 4a). In a related laboratory experiment with homogenised sediment from the same location as in the present study, *M. affinis* at an abundance of 6500 ind. m^{-2} mobilised DOP and particulate bound P (PP) from the sediment at rates of ~0.02 mmol DOP m^{-2} d^{-1} and 0.04 mmol PP m^{-2} d^{-1}. These releases were probably driven by bioresuspension which rendered the supernatant water turbid (Ekeroth et al. 2012). In the present study, no resuspension was observed, and small fluxes of particulate nutrients unrelated to bioturbation were recorded. As resuspension by *M. affinis* is positively related to animal abundance (Viitasalo-Frösén et al. 2009), the relatively low animal abundance in the present study is a plausible explanation for the insignificant effect of *M. affinis* on benthic DOP and PP fluxes.

On Day 33, DOP fluxes in the *M. affinis* boxcosms were significantly elevated above fluxes in control boxcosms and in *Marenzelleria* boxcosms (Fig. 4a). This DOP release, shortly after *M. affinis* were added to the experimental systems, coincided with an increased NH_4 flux rate. Also, the latter was observed in the *Marenzelleria* boxcosms (Fig. 3b). This suggests that the animal additions might produce an immediate transient pulse of dissolved substances out of the sediment (cf. Kristensen & Blackburn 1987). Thus, DOP mobilisation by *M. affinis* may be a transient effect following animal colonization (Welsh 2003). Further studies appear needed to clarify the influence of animal colonisation on benthic DOP fluxes.

Environmental implications

The very low DIN:DIP molar ratio (2.8) of the flux measured *in situ* (Fig. 6) indicate preferential regeneration of DIP, a common feature under anoxic bottom water conditions (e.g. Steenbergh 2012 and references therein) and a key driver for sustained high DIP bioavailability in the Baltic Sea (Viktorsson et al. 2013). For the oxygenated azoic sediments, average DIN and DIP fluxes were linearly correlated (Fig. 6). Major axis regression of DIN and DIP fluxes under these conditions results in a molar slope value of 11.9 (Fig. 6). Thus, DIN and DIP was exchanged across the sediment–water interface at slightly below the
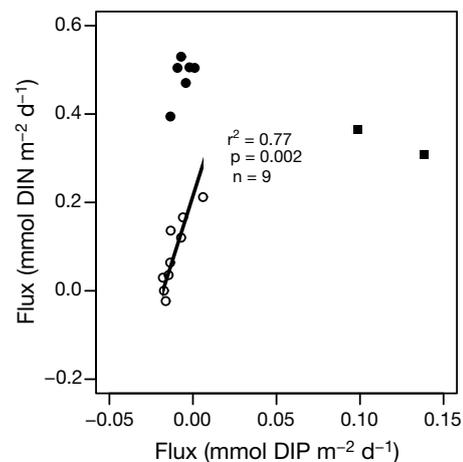

Fig. 6. Dissolved inorganic N (DIN) vs. dissolved inorganic P (DIP) fluxes. Average values per boxcosm during oxygenated azoic (O) or oxygenated bioturbated (●) conditions. Squares: values for the 2 chambers of the benthic lander *in situ* measurements during ambient anoxic conditions. Line: major axis regression for oxygenated azoic boxcosms. Regression slope value is 11.9

Redfield ratio (DIN:DIP = 16: Redfield 1958) during oxic–azoic conditions. This is in stark contrast to the *in situ* nutrient fluxes under anoxic conditions, which were much more DIP-rich (Fig. 6). Hence, our experimental results suggest that an oxygenation event of the Baltic proper deep water would result in a rapidly decreased DIP flux, increasing the DIN:DIP ratio in the sub-halocline water mass. Higher DIN:DIP ratios would disfavour diazotrophic cyanobacteria (Kahru et al. 2000), whose extensive production during the summer is considered a major and now worsening environmental problem in the Baltic Sea (Kahru & Elmgren 2014).

Average DIN:DIP ratios increased even more in bioturbated boxcosms (Fig. 6), mostly due to the stimulation of $NO_2 + NO_3$ efflux by the animals (Fig. 3d). Also, considerably more DON was recycled to the water column in the presence of either *M. affinis* or *Marenzelleria*, compared to during azoic oxygenated or anoxic conditions (Fig. 4b). Actually, the approximately doubled TDN flux in bioturbated boxcosms compared with azoic controls (Fig. 4d) suggests that the macrofauna reduced the net N sink of the sediment by half. Thus, intensified N regeneration would be expected if oxic conditions prevail long enough to allow for colonisation by *Marenzelleria* and *M. affinis*.

The most likely environmental consequence of an approximately doubled TDN flux is higher export production of the N-limited spring bloom (Granéli et al. 1990), increasing the deep water oxygen con-

sumption in the Baltic proper. This contrasts to the common view of *M. affinis* and *Marenzelleria* as drivers for improvements of environmental conditions in the Baltic Sea (cf. Karlson et al. 2007a, Norkko et al. 2012), which is based primarily on the animals' influence on benthic P sequestration. The present study indicates that the marginal (at most) influence by these species on the benthic DIP and DOP flux is negligible compared with the strong regulation by benthic oxygen conditions.

Also, our results suggest that bottom water oxygenation rapidly decreases benthic DSi recycling but that this is counteracted by bioturbation. Thus, it is unlikely that improved redox conditions in the Baltic Sea would persistently increase benthic Si retention and thereby lower DSi availability in the water column, which was suggested by Danielsson (2014).

Finally, the substantial stimulation of benthic DIN, DON and DSi fluxes in the presence of macrofauna are notable and demonstrate the need to integrate environmental biogeochemistry with macrobenthic dynamics to provide a reliable and ecologically relevant model of nutrient cycling in the Baltic Sea.

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