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1 BIOGEOCHEMISTRY IN HIGHLY REDUCED MUSSEL FARM
2 SEDIMENTS DURING MACROFAUNAL RECOLONIZATION
3 BY *AMPHIURA FILIFORMIS* AND *NEPHTYS SP.*

4
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21
22 Running head: Macrofauna and biogeochemistry in mussel farm sediments

1 Abstract

2 Mussel farming is considered a viable means for reducing coastal eutrophication. This study
3 assessed the importance of bioturbation by recolonizing fauna for benthic solute fluxes and
4 porewater distributions in manipulated mussel farm sediments. Three consecutive time-series flux
5 incubations were performed during an experimental period of three weeks in sieved farm sediment
6 treated with the brittle star *Amphiura filiformis* and the polychaete *Nephtys sp.* The functional
7 behaviour of *Nephtys sp.* and interactions between *Nephtys sp.* and the spontaneously colonizing
8 spionid *Malacoceros fuliginosus* determined the biogeochemical response in the *Nephtys sp.*
9 treatment. For example, the oxic zone was restricted and benthic nitrate and silicate fluxes were
10 reduced compared to the brittle star treatment. *A. filiformis* seemed to enhance the bioadvective
11 solute transport, although an increased supply of oxygen was due to the highly reducing conditions
12 of the sediment mainly seen as secondary effects related to porewater distributions and benthic
13 nutrient fluxes.

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19 KEYWORDS: Bioturbation, Benthic fluxes, Nutrients, Porewater, *Amphiura filiformis*, *Nephtys sp.*,

20 *Malacoceros fuliginosus*

1 **1. Introduction**

2 During the last century, human activities have considerably increased the supply of organic material
3 and associated nutrients to coastal marine environments (Boesch et al., 2006; Nixon, 1995).

4 Enhanced mobilization of e.g. nitrogen and phosphorus is largely caused by land use, agricultural
5 fertilization, discharge of human waste, animal production and combustion of fossil fuels (Kemp et
6 al., 2005; Cloern, 2001). The fundamental regulatory mechanisms and overall ecological
7 consequences from perturbations are, however, often complex and not well understood.

8
9 In 2005, a large-scale project was launched on the west coast of Sweden with the overall aim to
10 reduce nutrient (particularly dissolved inorganic nitrogen; DIN) concentrations from coastal waters
11 by long-line mussel (*Mytilus edulis*, L) farming (Lindahl et al., 2005). In contrast to fish farms,
12 mussel farms need no external food source as mussels feed on seston suspended in the water. As a
13 direct consequence, several studies have shown that mussel beds may control the planktonic biomass
14 (Noren et al., 1999; Asmus and Asmus 1991). There are also, however, contrasting observations that
15 activities by mussels have the potential to promote local primary production (Nizzoli et al., 2005;
16 Asmus and Asmus, 1991). Since nutrients incorporated and focused in mussel tissues are removed
17 upon harvesting, mussel farming is considered an alternative and potentially ecologically sustainable
18 means of reducing coastal eutrophication (Haamer, 1996; Ryther et al., 1972). For example, by
19 harvesting one ton of mussels ~ 10 kg of nitrogen are removed from the system (c.f. Lindahl et al.,
20 2005). Additional consequences to mussel farming include enhanced light penetration caused by the
21 reduced amount of particles suspended in the water. This may favour the proliferation of
22 macroalgae, eelgrass and benthic micro algae. Retention of nutrients from the water is thereby
23 stimulated even further (Sundbäck et al., 2004; Christensen et al., 2003).

24

1 While mussel farms are generally assumed to decrease the integrated sedimentation immediately
2 adjacent to the farm area, sedimentation of organic matter (e.g. fecal- and pseudofecal material, live
3 and dead mussels, and predators) is often enhanced and focused towards surface sediments directly
4 beneath the farms (Hartstein and Stevens, 2005; Dahlbäck and Gunnarsson, 1981). An increased flux
5 of organic matter to the sediment surface, with an overall composition often different from that of
6 autochthonous planktonic material (Hartstein and Stevens, 2005), support fluxes of dissolved
7 oxidants (e.g. oxygen and nitrate) to the sediment from the overlying water (Christensen et al.,
8 2003). In combination with a limited capacity for bottom water renewal, the enhanced supply of
9 organic material may reduce bottom water oxygen concentrations to levels below which biomass and
10 diversity of benthic macrofauna are significantly reduced or even depleted in surface sediments
11 (Diaz and Rosenberg, 2008).

12

13 Burrowing and reworking activities by benthic macrofauna often regulate rates and pathways during
14 organic matter mineralization in bioturbated surface deposits (Aller, 2001). Ecosystem response
15 from macrofaunal activities is often exemplified and quantified in terms of transport of reactants and
16 metabolites across the sediment-water interface, as well as by multi-dimensional distributions of
17 solutes within the sediment (Norling et al., 2007; Hulth et al., 2002). Respiration and ventilation of
18 burrow constructions by benthic macrofauna not only support oxygen transport to anoxic regions of
19 the sediment, but repetitive successions between oxic and anoxic conditions are promoted through
20 periodic irrigation of burrows (Forster and Graf, 1995; Kristensen, 1981). Field and laboratory
21 investigations have demonstrated that repetitive oscillations between redox states stimulate an
22 efficient mineralization of organic material and thereby reduce organic matter accumulation in the
23 sedimentary record, compared to when the organic matter is exposed only to oxic or anoxic
24 conditions (Aller, 1994, Hulthe et al., 1998). In addition to rates and extent of organic matter
25 mineralization, cycling of e.g. C, N, P, Fe, Mn and S is directly related to the redox status and
26 oxygenation of the sediment (Thamdrup, 2000; Canfield, 1994). The overall importance of faunal

1 activities for system response is often related to distinct and specific activity patterns, frequently
2 determined by individual species traits and complex multi-species feedbacks (Norling et al., 2007;
3 Lohrer et al., 2004).

4
5 The overriding aim of this study was to experimentally quantify the biogeochemical response
6 associated with the introduction of bioturbating macrofauna to highly reduced sediments
7 immediately below a mussel farm. We focused our investigations on element cycling, evidenced by
8 porewater distributions and benthic fluxes of oxygen and nutrients, in sieved surface sediment.
9 Overall, limited information is available from experiments directly designed to investigate faunal
10 recolonization as a technique for remediation of organic rich sediments under active or abandoned
11 farms. The present contribution is an accompanying study to the large-scale experiment aimed to
12 reduce the DIN content from coastal waters by means of mussel farming.

13

14 **2. Materials and methods**

15 **2.1 Experimental overview**

16 Specimens of the brittle star *Amphiura filiformis* (O. F. Müller) and the polychaete *Nephtys* (*Nephtys*
17 *sp.*; hereafter called *Nephtys*) were added to sieved surface sediment from immediately below a
18 mussel farm, and incubated with overlying water in Plexiglas cores for benthic flux measurements.
19 Sediment-water incubations of the sieved sediment were made in a time-series of three consecutive
20 benthic flux incubations, performed 1, 9 and 22 days after adding the fauna, respectively (Fig. 1).
21 Incubations were made in the dark. To measure the progressive mobilization of solutes during
22 organic matter mineralization, porewater distributions of oxygen and nutrients were determined in
23 the control cores at day 0, 8 and 23. Due to practical considerations (oxygen) and a destructive
24 sampling protocol (nutrients), porewater distributions were only measured at the termination of
25 experiments (day 23) in cores with macrofauna. Prior to the macrofaunal recolonization experiment,

1 benthic fluxes and porewater composition were investigated in intact cores from the mussel farm site
2 for comparison with results obtained during the experimental manipulations.

3

4 **2.2 Sampling and macrofauna**

5 The long-line mussel farm (*Mytilus edulis*, L.), located south of the island of Orust on the Swedish
6 west coast, was investigated in February 2006 (Fig. 2, Table 1). It had at the time of sampling been
7 under the continuous use for the past 23 years, and constituted one of the oldest sites still utilized for
8 long-line mussel farming in Sweden. The annual production is about 200 tons of mussels.

9

10 Sediment was collected with an Olausson box corer. Whole sediment cores were sub-sampled from
11 the box corer by carefully inserting 30 cm long Plexiglas tubes ($\text{\O} = 10$ cm) into the sediment. The
12 intact cores were used for benthic flux measurements and porewater analysis of natural mussel farm
13 sediment. Porosity was measured in the top two centimetres by weight loss after drying the sediment
14 at 60 °C until constant weight. Surface sediment (0 - 2 cm) was retrieved separately from the box
15 corer and sieved through a 1 mm mesh to remove macrofauna, mussel shells and other debris. This
16 sediment, later used for the recolonization experiment with benthic macrofauna, was transferred to a
17 large plastic container (75 × 55 × 45 cm) and left to acclimatize in the dark under continuous laminar
18 flow of bottom water from the Gullmarsfjord (T = 8 °C, S = 34).

19

20 *In situ* bottom water was collected for benthic flux incubations of intact sediment by a membrane
21 pump system (Gardena). Bottom water was also retrieved for oxygen and nutrients; NH_4^+ , $\Sigma \text{NO}_3^- +$
22 NO_2^- (hereafter referred to as NO_3^-), HPO_4^{2-} and $\text{Si}(\text{OH})_4$. Oxygen was immediately precipitated and
23 titrated within 24 h according to Winkler, while samples for nutrients were filtered on-line (0.45 μm ,
24 cellulose acetate) and stored frozen until analysis by an autoanalyzer (Technicon TRAACS 800,
25 Bran and Luebbe, Germany) according to standard colorimetric methods (Strickland and Parson,
26 1972).

1
2 Benthic macrofauna was collected in the Gullmarsfjord on two occasions (7 and 13 days before the
3 experiment) with a Smith-McIntyre grab (Fig. 2, Table 1). *A. filiformis* was collected at a water
4 depth of 40 m just outside the Kristineberg Marine Research Station, while *Nephtys* was sampled in
5 sediments of the Lysekil fishing harbour at 4 - 6 m water depth, and in the shallow bay Bökevik near
6 the Research Station at 4 - 8 m depth. Until the start of experiments, fauna was acclimatized in
7 aquaria containing sieved sediment from the mussel farm under continuous water flow of bottom
8 water from the Gullmarsfjord.

9
10 The echinoderm *A. filiformis* (Amphiuridae) is a deposit- and suspension feeder with gallery-diffusor
11 and downward-conveyor reworking behaviour (Francois et al., 2001). It creates a burrow several (~
12 5) centimetres into the sediment and extends its arms in channels to the surface sediment and the
13 overlying bottom water (Buchanan, 1964). It normally remains stationary in the sediment. However,
14 if bottom water oxygen decreases below ~ 40 μM , specimens of *A. filiformis* will progressively
15 move towards the sediment surface (Rosenberg et al., 1991). *Nephtys* (Annelida, Polychaeta) creates
16 a gallery of burrows within a range of 0-10 cm in the sediment (Diaz and Schaffner, 1990) and has
17 been observed to feed on both detritus and small infauna (Caron et al., 2004).

18 The species of macrofauna used in this experiment were chosen for their specific bioturbating
19 functions and high densities observed in nature (Rosenberg, 1995; Sanders, 1960). Previous studies
20 using similar experimental set-up have demonstrated that nutrient and oxygen dynamics are
21 significantly affected by *A. filiformis* reworking activities (Vopel et al., 2003; Karlson, 2005). In
22 addition to a profound effect on overall biogeochemistry in surface sediments, *A. filiformis* is also
23 observed to survive in organic rich environments (Nilsson, 1998; Vistisen and Vismann, 1997).
24 *Nephtys* is a genus with several opportunistic species, commonly found under reducing sediment
25 conditions (Pearson and Rosenberg, 1978).

26

1 2.3 Benthic fluxes

2 The sieved and homogenized surface sediment from beneath the mussel farm was acclimatized for
3 32 days, after which 30 cm long Plexiglas tubes ($\text{\O} = 10$ cm) were inserted into the ~ 10 cm deep
4 sediment layer. The cores were connected to constant water flow of bottom water from the
5 Gullmarsfjord and individuals of macrofauna were carefully added to the surface sediment.
6 Macrobenthos were added at a final biomass of 1.0 ± 0.1 g wet weight core^{-1} (i.e. five *A. filiformis*
7 and twenty *Nephtys* core^{-1}). This corresponded to ~ 130 g wet weight m^{-2} and ~ 640 and 2500 ind. m^{-2} ,
8 respectively. Applied densities of *A. filiformis* and *Nephtys* are found in nature (Rosenberg, 1995;
9 Sanders, 1960), although densities of *Nephtys* are within the high range of natural abundances.

10

11 1, 9 and 22 days after additions of macrofauna, dark benthic flux incubations were initiated in that
12 the flow-through system was turned off and the cores were sealed with air-tight Plexiglas lids
13 (Karlson et al., 2005; Hulth et al, 1994). A Teflon-coated magnetic stirring bar was attached to the
14 top of the lid for mixing of the overlying water (40 rpm) during the incubations. During each period
15 of benthic flux measurements, samples were periodically removed (5-6 times) from the overlying
16 water for oxygen and nutrients (NH_4^+ , NO_3^- , HPO_4^{2-} and $\text{Si}(\text{OH})_4$). However, no phosphate data is
17 available due to malfunctioning of the phosphate channel during analysis. Samples were removed
18 from the overlying water by polypropylene syringes (nutrients), or directly through a tube connected
19 to the sample port (oxygen). The water removed during sampling was compensated for by a
20 simultaneous input of *in situ* water of known oxygen and nutrient concentrations. The sediment-
21 water incubations were terminated before the oxygen concentration in the overlying water decreased
22 by more than 20% from the initial concentration i.e. after ~ 4 h. Concentrations were corrected for
23 the volume removed and added during the sampling procedure prior to calculating benthic flux rates
24 from the initial linear concentration change in the overlying water (Hulth et al, 1994). Total oxygen
25 fluxes measured during the benthic flux incubations are referred to as J_{tot} .

26

1 The intact sediment cores from the sampling site were applied under constant flow of *in situ* bottom
2 water upon arrival at the research station. After approximately 24 h from time of sampling,
3 incubations for benthic fluxes in the intact sediment cores were made according to principles
4 previously described for the recolonization experiment.

5

6 **2.4 Sediment porewater**

7 Sediment from the recolonization experiment was sectioned (control cores on day 0, 8 and 23, cores
8 with macrofauna on day 23) into the 0-1, 1-2, 4-6 and 7-9 cm sediment layers. The intact cores were
9 immediately upon arrival at the research station sectioned for porewater analysis in 1 cm intervals
10 down to 10 cm. Porewater was separated from the sediment by refrigerated centrifugation (8 °C,
11 3000 rpm, 25 min, Beckman J2-21M/E Centrifuge, UK), and filtered on-line (0.45 µm, cellulose
12 acetate). Samples (NH_4^+ , HPO_4^{2-} and $\text{Si}(\text{OH})_4$) were stored frozen until analysis as described in 2.2.
13 Nitrate concentrations were not determined in the porewater as the highly reducing properties of the
14 sediment likely restricted the oxic zone, and rapidly removed additional nitrate supplied from the
15 overlying water. Thus, as we used a cm resolution during sediment sectioning, nitrate was likely
16 below detection throughout the sediment column.

17

18 Oxygen micro-distributions (Clark-type electrode, Unisense) in the sediment were determined in the
19 control cores on day 0, 8 and 23. At the last occasion (day 23), micro-distributions of oxygen were
20 determined also in the cores with macrofauna (25 µm resolution, $n = 2$ cores treatment⁻¹, 4 or 5
21 profiles core⁻¹). The depth of oxygen penetration in each core was estimated from the whole oxygen
22 profile (Hulth et al., 1994; Bouldin, 1968). Diffusive fluxes, J_{diff} , of oxygen between the overlying
23 water and the surface sediment were estimated using Fick's first law of diffusion adapted to
24 sediment conditions (Berner, 1980):

25

$$J_{\text{diff}} = -\phi \times D_s \times (\partial C / \partial z) \quad (1)$$

2

3 where ϕ is the porosity in the top layer (0–2 cm) of the sediment and $(\partial C / \partial z)$ are the linear
4 concentration gradients in the surface sediment layer immediately below the sediment surface. The
5 whole-sediment diffusion coefficient (D_s) was calculated from the diffusion coefficient in free
6 solution, D_o , corrected for temperature, salinity (viscosity), and tortuosity, using the Archie-type
7 relation: $D_s \sim \phi^2 D_o$ (Boudreau, 1996).

8

9 **2.5 Statistical analysis**

10 Total benthic flux rates, oxygen diffusive fluxes and depth of oxygen penetration were evaluated by
11 univariate analysis of variance (ANOVA) for differences between treatments at the termination of
12 experiments ($\alpha = 0.05$). Due to the progressive development of porewater distributions and benthic
13 flux rates, statistical evaluation of faunal treatments were only performed using data obtained at the
14 termination of the time-series experiment. Porewater distributions of nutrients were evaluated by
15 partly nested split plot RM (repeated measures) ANOVA, with the main factor “treatment” nested
16 within cores and the repeated factor “depth” within each core. Significance levels for the split-plot
17 ANOVA were made more conservative using adjusted degrees of freedom for the F -tests, calculated
18 from the Greenhouse-Geisser estimate (ϵ) based on the index of sphericity (Quinn and Keough
19 2002). If the interaction was significant ($\alpha = 0.05$), the main factors were only explored further if
20 they showed a strong main effect in the interaction plot. If significance was detected among
21 treatments in the one-way and RM ANOVA, Tukey HSD *post hoc* tests were performed to explore
22 which treatments that formed significantly different homogenous groups. All significant results were
23 further explored. Prior to analysis, graphical exploratory techniques were used to check data for
24 assumptions related to normal distribution and homogeneity of variances (box plots) (Quinn and

1 Keough, 2002). The standard error of the mean value is utilized for presenting the variance in the
2 data set.

3 **3. Results**

4 **3.1 In situ conditions of the mussel farm sediment**

5 Visual inspection revealed a smooth sediment surface at the sampling site, with conspicuous
6 filamentous sulphur oxidizing bacterial mats (*Beggiatoa* spp.) on the surface. Occasionally, surface
7 sediment was littered with dead fauna and debris directly derived from the mussel lines. In
8 accordance with a distinct smell of hydrogen sulphide during sediment handling, the surface
9 sediment was largely devoid of larger macrofauna. Spionids, dominated by *Malacoceros fuliginosus*
10 (Annelida, Polychaeta) was the only macro (>1 mm) infauna found during sieving of the surface
11 sediment, although a large (2.3 g) polychaete (*Nereis* sp.) was retrieved from one of the intact cores
12 after the benthic flux incubations were terminated.

13
14 Nutrient concentrations measured in the bottom water at the mussel farm site were low; ammonium
15 ($0.17 \pm 0.02 \mu\text{M}$), nitrate ($2.0 \pm 0.4 \mu\text{M}$), phosphate ($0.30 \pm 0.02 \mu\text{M}$), silicate ($1.6 \pm 0.5 \mu\text{M}$). The
16 oxygen concentration was close to saturation ($\sim 320 \mu\text{M}$) at the prevailing temperature and salinity
17 (Table 1). In the porewater, ammonium concentrations increased linearly with sediment depth to
18 well above 2 mM, phosphate concentrations increased from 35 at the surface sediment to 90 μM at
19 10 cm sediment depth, and silicate concentrations ranged between 200 and 275 μM (Fig. 3). There
20 was a net flux of oxygen ($-44 \pm 10 \text{ mmol m}^{-2} \text{ d}^{-1}$) and nitrate ($-0.95 \pm 0.24 \text{ mmol m}^{-2} \text{ d}^{-1}$) from the
21 overlying water into the sediment (Table 1). In contrast, ammonium ($0.80 \pm 0.24 \text{ mmol m}^{-2} \text{ d}^{-1}$) and
22 silicate ($6.3 \pm 3.7 \text{ mmol m}^{-2} \text{ d}^{-1}$) were supplied to the overlying bottom water from the sediment. The
23 phosphate flux was close to zero ($0.02 \pm 0.09 \text{ mmol m}^{-2} \text{ d}^{-1}$).

24

1 3.2 Benthic macrofauna and porewater

2 *Beggiatoa* spp. and orange-brown patches of likely Fe(III) and Mn(IV)- oxides were seen on the
3 sediment surface in the experimental cores. *Beggiatoa* was present in all cores throughout the entire
4 experiment, visible on the sediment surface and in a layer a few cm down in the sediment. The
5 distribution and extent depended on treatment, with the *Amphiura* cores having the least coverage of
6 *Beggiatoa*. In the control cores, the layer of *Beggiatoa* was thin and well-defined close to the
7 sediment surface (~ 1.5 - 2 cm). In the *Nephtys* cores, *Beggiatoa* was typically seen in the worm
8 burrows.

9
10 At the start of the recolonization experiment (Fig. 1), small spionid-tubes protruded ~0.5 cm from
11 the sediment surface. However, when terminating the experiment, no spionids were found in the
12 *Nephtys* cores while they were abundant in the other treatments. Once added, the brittle stars and the
13 polychaetes more or less immediately burrowed into the sediment. All individuals of *A. filiformis*
14 and most (~ 95 %) of *Nephtys* were found upon retrieving the macrofauna at the end of the
15 experiment. Specimens of *A. filiformis* were exclusively found above 4 cm in the sediment, whilst
16 individuals of *Nephtys* were more or less evenly distributed down to ~7 cm sediment depth (Fig. 4).
17 Fecal material was observed on the sediment surface in the *Nephtys* cores. In association, there were
18 black spots of likely Fe-sulfides.

19
20 At the end of the experiment, the narrowest penetration of oxygen ($170 \pm 10 \mu\text{m}$) was found in the
21 *Nephtys* cores. The oxic zone was $230 \pm 10 \mu\text{m}$ in the *A. filiformis* treatment, which was significantly
22 deeper compared with the *Nephtys* treatment ($p = 0.008$). The oxygen penetration was also
23 significantly deeper in the control treatment compared with the *Nephtys* treatment ($p = 0.005$).
24 During the experiment, the oxic zone (average \pm SE) in the control cores was $200 \pm 40 \mu\text{m}$ (day 0),
25 $170 \pm 30 \mu\text{m}$ (day 8) and $240 \pm 10 \mu\text{m}$ (day 23, Fig. 5). In accordance with measured depths of
26 penetration, gradients of oxygen immediately below the sediment surface ($\delta C / \delta x$)_{x=0} were most

1 pronounced for the *Nephtys* treatment, followed by the control and the *A. filiformis* treatments (Fig.
2 5). Calculated diffusive fluxes were $12 \pm 1 \text{ mmol m}^{-2} \text{ d}^{-1}$ (*Nephtys*), $9.0 \pm 1.0 \text{ mmol m}^{-2} \text{ d}^{-1}$ (control),
3 and $8.1 \pm 0.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ (*A. filiformis*). Thus, diffusive oxygen fluxes were ~ 40 % higher in the
4 *Nephtys*. than in the *A. filiformis* treatments. The difference between the two treatments was
5 significant ($p = 0.049$).

6
7 At ~ 8 cm depth, porewater concentrations of phosphate and silicate in the control cores at day 23
8 were in the same range as those in the intact cores ($70 \pm 4 \text{ } \mu\text{M}$ and $490 \pm 50 \text{ } \mu\text{M}$, respectively),
9 whereas ammonium concentrations were reduced by a factor of ~ 30 ($58 \pm 1 \text{ } \mu\text{M}$) (Fig. 3 and 6b). In
10 the recolonization experiment, *A. filiformis* appeared to reduce ammonium and phosphate in the
11 porewater compared with the control (Fig. 6a). Ammonium was reduced in the whole core (25 - 50
12 %) whereas for phosphate, the reduction was mainly observed in the top few centimetres (1-2 cm;
13 $1.2 \pm 0.1 \text{ } \mu\text{M}$ in the *A. filiformis* cores compared with $31 \pm 12 \text{ } \mu\text{M}$ in the control treatment). There
14 was a significant interaction between depth and treatment for ammonium ($p = 0.010$). The ANOVA
15 and subsequent post hoc tests suggested a significant difference in porewater ammonium between
16 cores with *A. filiformis* and the other treatments (*Nephtys*, $p = 0.007$; the control, $p = 0.004$). In
17 contrast, *Nephtys* increased concentrations of phosphate and silicate (particularly deeper in the core)
18 compared with the control. Porewater concentrations of silicate in the *Nephtys* treatment were
19 significantly higher compared with the control ($p = 0.037$) and the *A. filiformis* ($p = 0.036$)
20 treatments.

21

22 **3.3 Benthic fluxes**

23 Overall, measured total fluxes of oxygen during the recolonization experiment were slightly lower
24 but in the same range (-30 ± 5 to $-44 \pm 3 \text{ mmol m}^{-2} \text{ d}^{-1}$) as those measured in intact sediment from
25 the mussel farm ($-44 \pm 10 \text{ mmol m}^{-2} \text{ d}^{-1}$). In contrast, nutrient fluxes were generally higher in the

1 experimental setup with sieved surface sediment than in intact cores from the mussel farm site
2 (Table 1, Fig. 7).

3

4 Overlying water nitrate was in general terms consumed (-4.6 ± 0.3 to 1.1 ± 0.7 $\text{mmol m}^{-2} \text{d}^{-1}$), while
5 ammonium (1.0 ± 0.3 to 2.8 ± 0.4 $\text{mmol m}^{-2} \text{d}^{-1}$) and silicate (14 ± 2 to 34 ± 7 $\text{mmol m}^{-2} \text{d}^{-1}$) were
6 produced by the sediment. At the last benthic flux measurement, fluxes of nitrate ($p = 0.011$) and
7 silicate ($p = 0.029$) were significantly different in treatments with *Nephtys* compared with the *A.*
8 *filiformis* treatments.

9

10 Benthic fluxes of ammonium and silicate were highest in cores with *A. filiformis* (2.0 ± 0.4 and $34 \pm$
11 7 $\text{mmol m}^{-2} \text{d}^{-1}$, respectively). Rates of nitrate consumption were highest (-1.9 ± 0.2 $\text{mmol m}^{-2} \text{d}^{-1}$) in
12 the *A. filiformis* treatment while close to zero for the control (-0.5 ± 0.8 $\text{mmol m}^{-2} \text{d}^{-1}$). In the
13 *Nephtys* treatment, nitrate fluxes were zero or even positive (1.1 ± 0.7 $\text{mmol m}^{-2} \text{d}^{-1}$), i.e. nitrate was
14 supplied from the sediment to the overlying water (Fig. 7).

15

16 4. Discussion

17 4.1 Macrofauna

18 Small spionid polychaetes (*Malacoceros fuliginosus*) spontaneously colonized the sediment during
19 the acclimatization period were present in all cores at the beginning of the recolonization experiment
20 (day 0). *M. fuliginosus* is one of the dominating benthic species in organic rich sediments (Cardell et
21 al., 1999; Pearson and Rosenberg, 1978) and is also known to survive in sewage sludge under highly
22 reduced conditions (Costello and Read, 1994). Spionids are generally tentaculate (palp) surface
23 deposit or filter feeders (Dauer et al., 1981), and the tubes found in the cores of the recolonization
24 experiment indicated a filter feeding behaviour. In contrast to observations made in the control and

1 the *A. filiformis* cores, no evidence of spionids was found in the *Nephtys* cores when terminating the
2 time-series flux incubation (day 23). This removal was likely attributed to *Nephtys* feeding on the
3 tube-building polychaetes (Caron et al., 2004). Although total density and biomass of spionids (~10
4 individuals core⁻¹ larger than 1.5 cm) were small compared to those of the added fauna, in principle,
5 the experimental design encompassed “control” cores with spionids, cores with *A. filiformis* and
6 spionids, and cores with only *Nephtys*. Predation on the colonizing *M. fuliginosus* by *Nephtys* was
7 found important for the biogeochemical response of the *Nephtys* treatment throughout the
8 experiment. Furthermore, and from an ecological perspective, this experiment suggested *M.*
9 *fuliginosus* as an early colonizer of highly reducing sediments, with *Nephtys* as a potential successor
10 of species in such environments.

11

12 **4.2 Oxygen dynamics**

13 The extension of the oxic zone (i.e. oxygen penetration) normally directly reflects the complex
14 balance between reductants (e.g. organic material and reduced metabolites from organic matter
15 mineralization) in the sediment and oxidants (e.g. oxygen) supplied from the overlying water.
16 During the winter season it is quite common that oxygen only penetrates a few mm into coastal
17 marine sediments off the Swedish west coast (Hulth et al., *unpublished*; Rasmussen and Jørgensen,
18 1992). In conjunction with the distinct smell of hydrogen sulphide during sediment handling and
19 observations of *Beggiatoa* spp. in the surface sediment layer, the restricted layer of oxygenated
20 sediment (170 - 250 µm) after more than 60 days in direct contact with oxygenated bottom water
21 confirmed observations of intense organic matter mineralization and significant mobilization of
22 reductants to the porewater. Severely reducing conditions in mussel farm sediments have been
23 observed by others (Nizzoli et al., 2005; Christensen et al., 2003; Gilbert et al., 1997). In the Thau
24 lagoon (off the French coast in the Mediterranean Sea), for example, depth of oxygen penetration
25 was systematically shallower (0.1 – 1.7 mm) in sediments immediately below a mussel farm than at
26 the reference site (2.0 – 4.0 mm) (Gilbert et al., 1997).

1
2 Irrigation and reworking by benthic macrofauna change surface sediments from simple stratified
3 environments to systems having complex time-dependent, multi-dimensional geometries of transport
4 and reactions (Aller, 2001). Biogeochemical effects observed in bioturbated sediments are often
5 related to macrofaunal functionality and species specific traits, rather than abundance, biomass and
6 size structure of the macrofaunal community (Norling et al., 2007; Ieno et al., 2006; Mermillod-
7 Blondin et al., 2004). Despite a constant biomass of added macrofauna, reworking activities in this
8 experiment caused differences between treatments in the porewater as well as benthic fluxes (Fig. 6
9 and 7). At the end of experiments, the oxic zone was most narrow for the *Nephtys* treatment.
10 Activities by *Nephtys* therefore seemed to restrict the biological transport of oxygen from the
11 overlying water compared with the control and the *A. filiformis* cores. A restricted transport of
12 oxygen to the surface sediment was likely a direct consequence of the feeding mode of *Nephtys*,
13 actively removing irrigating meiofauna and spionids. Furthermore, *Nephtys* mobilized and focused
14 reactive organic material by the deposition of reactive fecal material on the sediment surface, further
15 enhancing rates of oxygen consumption.
16
17 Normally, oxygen consumption decreases during consecutive time-series flux incubations following
18 the progressive depletion of labile organic material (Karlson et al., 2007). In our study, however,
19 total oxygen consumption rates were more or less constant with time, suggesting an alternative main
20 route of oxygen reduction than oxidation of organic material. Due to the highly reducing
21 environment, oxygen was likely consumed by the large pool of reduced metabolites (e.g. hydrogen
22 sulphide) rather than through aerobic mineralization of recently deposited organic material. The
23 narrow penetration of oxygen (170 – 250 μm), confirmed a pronounced anaerobic metabolism and
24 mobilization of reduced compounds within the deposit.
25

1 There was no significant difference in total oxygen consumption between treatments at day 22 (Fig.
2 7). Oxygen consumption rates *per se* were therefore not considered a representative indicator of
3 faunal response in this experiment. A primary observation from quantification of benthic fluxes in
4 bioturbated sediments is that oxygen and solute transport between the overlying water and surficial
5 sediment is normally enhanced compared to predictions based on one-dimensional molecular
6 diffusion (Kristensen and Hansen, 1999). Included in the concept of macrofaunal functionality are
7 the geometric scaling and diffusive distance between burrows as important parameters for organic
8 matter mineralization and solute mobilization (Gilbert et al., 2003). While measured benthic fluxes
9 (J_{tot}) integrate respiration by fauna, effects from pore water irrigation and ventilation, as well as the
10 creation of a three-dimensional burrow system, such factors are normally not included in flux
11 estimations based on one-dimensional molecular diffusion (J_{diff} ; Hulth et al., 1994; Rasmussen and
12 Jørgensen, 1992). The ratio between the total transport of oxygen across the sediment water interface
13 (i.e. the measured benthic oxygen flux) and the diffusive oxygen flux, was significantly larger than 1
14 and varied between treatments. The ratio was largest for the *A. filiformis* ($J_{\text{tot}} / J_{\text{diff}} = 5.7 \pm 0.4$),
15 followed by the control ($J_{\text{tot}} / J_{\text{diff}} = 4.2 \pm 0.5$), and the *Nephtys* treatment ($J_{\text{tot}} / J_{\text{diff}} = 3.4 \pm 0.3$). A J_{tot}
16 / J_{diff} ratio of $\gg 1$ indicated a pronounced bioadvective transport of oxygen to surface sediments and
17 the formation of multi-dimensional diffusion geometries by *A. filiformis* reworking, feeding and
18 ventilation activities. Natural densities of *A. filiformis* ($\sim 1000 \text{ ind. m}^{-2}$) have been observed to
19 account for 80 % of the total flux of oxygen into Skagerrak sediments (Vopel et al., 2003).
20 Furthermore, although a comparably small biomass, spionids drastically affected overall
21 oxygenation of the surface sediments, as evidenced by the high $J_{\text{tot}} / J_{\text{diff}}$ ratios in the control and the
22 *A. filiformis* treatments

23

24

25

1 4.3 Nutrient cycling

2 At the sampling site, porewater distributions of phosphate and silicate indicated that the uppermost ~
3 three centimetres of the sediment were affected by recently deposited organic material (Fig. 3). In
4 addition to the supply of autochthonous planktonic material, a non-steady state situation of the
5 surface sediment could have been caused by a significant sedimentation event in association with the
6 mussel harvesting the week prior to sampling.

7

8 Ammonium measured in the porewater of the manipulated sediment was much lower compared with
9 the intact cores from the mussel farm site (Fig. 3 and 6). Observations of ~ 30 times lower
10 ammonium concentrations in the porewater of the recolonization experiment were possibly related to
11 the handling procedures of the sediment prior to experiments. For example, atmospheric oxygen may
12 have been introduced to the sediment during the homogenization procedure. Subsequent oxidation of
13 ammonium to nitrite and nitrate (nitrification) and a stimulated pathway of coupled
14 nitrification/denitrification, alternatively partial nitrification coupled to anaerobic ammonium
15 oxidation (anammox, Thamdrup and Dalsgaard, 2002), could have removed nitrogen from the
16 sediment-water system already prior to the time-series flux incubation. The lower absolute
17 concentrations of DIN in the porewater during the recolonization experiment, eventually caused by a
18 removal of DIN during sediment handling, should not, however, affect the overall conclusions made
19 from this experiment.

20

21 Concentrations and patterns of nutrient distributions in the porewater of the manipulated sediment
22 were directly associated with the progressive mineralization of organic material, faunal irrigation,
23 and the concentration-dependent diffusive transport of reactants and reaction products across the
24 sediment-water interface (Fig. 6). General patterns from the experimental cores, although more
25 evident from distributions of phosphate and silicate than of ammonium, confirmed the time-
26 dependent evolution of porewater solutes following the initial conditions of the sieved sediment. The

1 gradual stabilization of porewater gradients and acclimatization of macrofauna to the experimental
2 system directly affected measured benthic solute fluxes (Fig. 7).

3

4 In sieved and homogenized sediment systems, concentrations are by default constant with depth (i.e.
5 gradients are zero) at the beginning of experiments. Due to the time-dependent nature of porewater
6 distributions and benthic oxygen and solute fluxes, with patterns highly variable also between
7 solutes, it is essential to control and quantify the evolution of porewater solutes prior to benthic flux
8 incubations. For comparative reasons, this is particularly important in consecutive time-series
9 incubations as those performed in this study. For accurate quantification of benthic flux rates and to
10 avoid confounding non-steady state effects with treatments effects in manipulated systems,
11 porewater distributions should be determined in combination with benthic flux incubations. A site
12 dependent acclimatization period is recommended for the sediment porewater system as well as for
13 the fauna during macrofaunal recolonization using manipulated (sieved) sediment in experimental
14 systems.

15

16 Quite in contrast to observations that *A. filiformis* oxygenate a large volume of the surrounding
17 sediment (Vopel et al., 2003), mean depth of oxygen penetration in cores with *A. filiformis* was
18 similar to that in the control cores (Fig. 5). Additional oxygen supplied to the highly reducing
19 sediment by activities of *A. filiformis* (as evidenced e.g. by $J_{\text{tot}} / J_{\text{diff}}$ ratio $\gg 1$) was therefore likely
20 rapidly consumed during oxidation of reduced compounds (e.g. NH_4^+ , Fe^{2+} , Mn^{2+} , and HS^-) rather
21 than accumulated in the porewater. Oxidation of Fe^{2+} and the formation of Fe(III)-oxide surfaces
22 induced by reworking activities of the brittle star may have stimulated phosphate adsorption and the
23 reversible removal of phosphate from the porewater in the upper layer of the sediment where *A.*
24 *filiformis* was active (Thamdrup and Dalsgaard, 2000; Sundby et al., 1992, Fig. 6).

25

1 The reduced concentrations of phosphate (and ammonium) in the porewater of the *A. filiformis* cores
2 compared with the control and the *Nephtys* cores could also be an effect of a stimulated ventilation
3 of the porewater. The difference was most pronounced down to ~ 5 cm sediment depth, i.e. in the
4 uppermost surface layer where most individuals of *A. filiformis* were recovered after the experiment
5 and most pronounced irrigation therefore was to be expected (Fig. 4). Nitrate fluxes from the water
6 into the sediment are most often a consequence of denitrification of nitrate supplied from the
7 overlying water (Dw) (Nielsen, 1992). Additionally, in environments with a high organic content,
8 nitrate may be consumed by dissimilatory nitrate/nitrite reduction to ammonium (DNRA). Examples
9 of such extreme environments where DNRA has been found to be important for N cycling include
10 shellfish farm sediments (Nizzoli et al., 2006; Christensen et al., 2003; Gilbert et al., 1997), and
11 laminated sediments of the Baltic Sea (Karlson et al., 2005). Nitrate fluxes to the sediment were in
12 our study highest in the brittle star cores compared with the other treatments. In conjunction with the
13 high ammonium efflux measured in the *A. filiformis* treatment, this suggests a stimulation of DNRA
14 in the *A. filiformis* cores.

15
16 Due to the highly reducing conditions of the sediment, oxygen supplied to the surface sediment by *A.*
17 *filiformis* reworking and irrigation activities mainly affected distributions of redox sensitive nutrients
18 (e.g. ammonium and phosphate) in the porewater rather than oxygen distributions in the sediment. In
19 contrast to the *A. filiformis* treatment, nutrient fluxes were reduced by activities of *Nephtys* compared
20 with the control (Fig. 7). Although statistically not significant, *Nephtys* seemed to decrease the
21 ammonium flux and turn the sediment from a sink to a source of nitrate. Such observations indicate
22 fauna-mediated oxygenation of the surface sediment and a stimulated nitrification immediately
23 below the sediment-water interface in the *Nephtys* cores. Observations of higher porewater
24 concentrations of both silicate and phosphate (ammonium concentrations in the porewater were
25 similar to those observed in the control cores) at depth in the *Nephtys* treatment were likely caused
26 by the feeding mode of the polychaete, redistributing and reconfiguring particulate organic material

1 available for organic matter mineralization. This mode of particle reworking, in combination with
2 the predatory removal of irrigating meiofauna and spionids, as well as the deposition of fecal
3 material in the surface sediment layer, contributed to rates and patterns of benthic fluxes observed in
4 the *Nephtys* cores.

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1 5. Conclusion

2 Within the experimental restrictions (e.g. sieved surface sediment enclosed together with oxygenated
3 overlying water without a natural supply of organic material from the actual sampling site) we
4 conclude from our study that the same biomass of *A. filiformis* and *Nephtys* resulted in significantly
5 different benthic fluxes and solute porewater distributions in severely reduced surface sediment from
6 below a mussel farm.

7

8 Additions of *A. filiformis* enhanced the bioadvective transport of reactants and metabolites across the
9 sediment-water interface. An increased supply of oxygen, evidenced e.g. by a $J_{\text{tot}}/J_{\text{diff}}$ ratio of ~ 6 ,
10 was due to the highly reducing conditions of the sediment not mainly seen as an increase in oxygen
11 penetration, but rather as secondary effects related to distributions and fluxes of nutrients.

12

13 The functional behaviour of *Nephtys* and predation on the colonizing *M. fuliginosus* determined the
14 biogeochemical character of the *Nephtys* treatment. Deposition of fecal material on the sediment
15 surface, feeding on meiofauna and *M. fuliginosus*, and overall bioturbation patterns (gallery diffusor)
16 restricted the oxic zone and decreased benthic nutrient fluxes in the *Nephtys* treatment.

17

18 The measured biogeochemical response associated with the introduction of bioturbating macrofauna
19 to the reduced sediment environment highlighted the potential of recolonization as a remediation
20 technique of organically enriched sediments. Tolerant species such as *A. filiformis* and *Nephtys*
21 survived and affected oxygen and nutrient cycling in mussel farm sediments following
22 manipulations. As effects from faunal additions were different for the two species investigated, our
23 study emphasized the importance of functional behaviour of fauna, not only related to particle
24 reworking but also to irrigation and transport of solutes during studies designed for the
25 biogeochemical fate of organic material in benthic ecosystems.

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1 Table 1. Sampling positions and *in situ* conditions at the mussel farm sampling site (average \pm
 2 SE where appropriate).

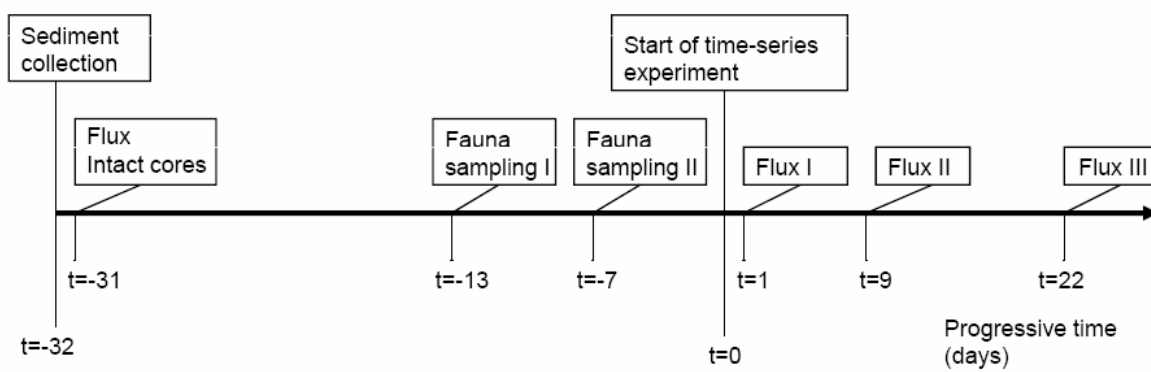
3	Sediment sampling site	N 58°04,21' E 11°35,86'
4	<i>Nephtys</i> sampling sites	N 58°16,47' E 11°26,50'
5		N 58°14,94' E 11°26,91'
6	<i>A. filiformis</i> sampling site	N 58°15,56' E 11°27,71'
7		
8	Air temperature (°C)	5.3
9	Water depth (m)	12
10	Water temperature (12 m, °C)	3.0
11	Bottom water salinity (12 m)	29
12		
13	O ₂ (μM, n=3)	320 \pm 5
14	NH ₄ ⁺ (μM, n=4)	0.17 \pm 0.02
15	NO ₃ ⁻ (μM, n=4)	2.0 \pm 0.4
16	HPO ₄ ²⁻ (μM, n=4)	0.30 \pm 0.02
17	Si(OH) ₄ (μM, n=4)	1.6 \pm 0.5
18		
19	Porosity (0-1 cm, n=6)	0.80 \pm 0.02
20	Porosity (1-2 cm, n=4)	0.82 \pm 0.02
21		
22	<i>Benthic fluxes</i>	
23	O ₂ (mmol m ⁻² d ⁻¹ , n=5)	-44 \pm 10
24	NH ₄ ⁺ (mmol m ⁻² d ⁻¹ , n=5)	0.80 \pm 0.24
25	NO ₃ ⁻ (mmol m ⁻² d ⁻¹ , n=5)	-0.95 \pm 0.24
26	HPO ₄ ²⁻ (mmol m ⁻² d ⁻¹ , n=5)	0.02 \pm 0.09
27	Si(OH) ₄ (mmol m ⁻² d ⁻¹ , n=5)	6.3 \pm 3.7

- 1 Figure 1 Schematic description of the experimental design and an overview of procedures
2 performed during the experiment.
3
- 4 Figure 2 Map of the Swedish west coast including the sampling area (enlarged). Indicated are
5 sites for sampling of sediment (●), *Amphiura filiformis* (■) and *Nephtys* (•).
6
- 7 Figure 3 Porewater distributions (average \pm SE, n = 2) of ammonium (A), phosphate (B) and
8 silicate (C) in intact sediment cores from beneath the mussel farm. Arrows indicate
9 bottom water concentrations.
10
- 11 Figure 4 Schematic illustration of general bioturbation patterns of *Amphiura filiformis*,
12 *Malacoceros fuliginosus* and *Nephtys* (left panel). Depth distributions of fauna in
13 percentage of total individuals recovered for *A. filiformis* (n = 2 cores), *M. fuliginosus*
14 (n = 5 cores) and *Nephtys* (n = 3 cores) (right panel).
15
- 16 Figure 5 Distribution of oxygen in the porewater of the control (⊗), *Amphiura filiformis* (■), and
17 *Nephtys* (Δ) treatments at the termination of experiments.
18 The cores were sampled every 25 μ m (two cores per treatment; average \pm SE for each
19 core, n = 4 or 5 core⁻¹).
20
- 21 Figure 6 Ammonium, phosphate and silicate concentrations (average \pm SE, n = 2) in the
22 porewater of the control (●), *Amphiura filiformis* (■), and *Nephtys* (▲) treatments at
23 the termination of experiments (A). Concentrations in the control cores 0 (○), 8 (⊗) and
24 23 (●) days from additions of fauna (B). Porewater was separated from the sediment at
25 the intervals 0-1, 1-2, 4, 6 and 7-9 cm. Symbols are displaced from the mean depth of

1 sampling for clarity. Arrows indicate bottom water concentrations (no data for
2 phosphate). ´

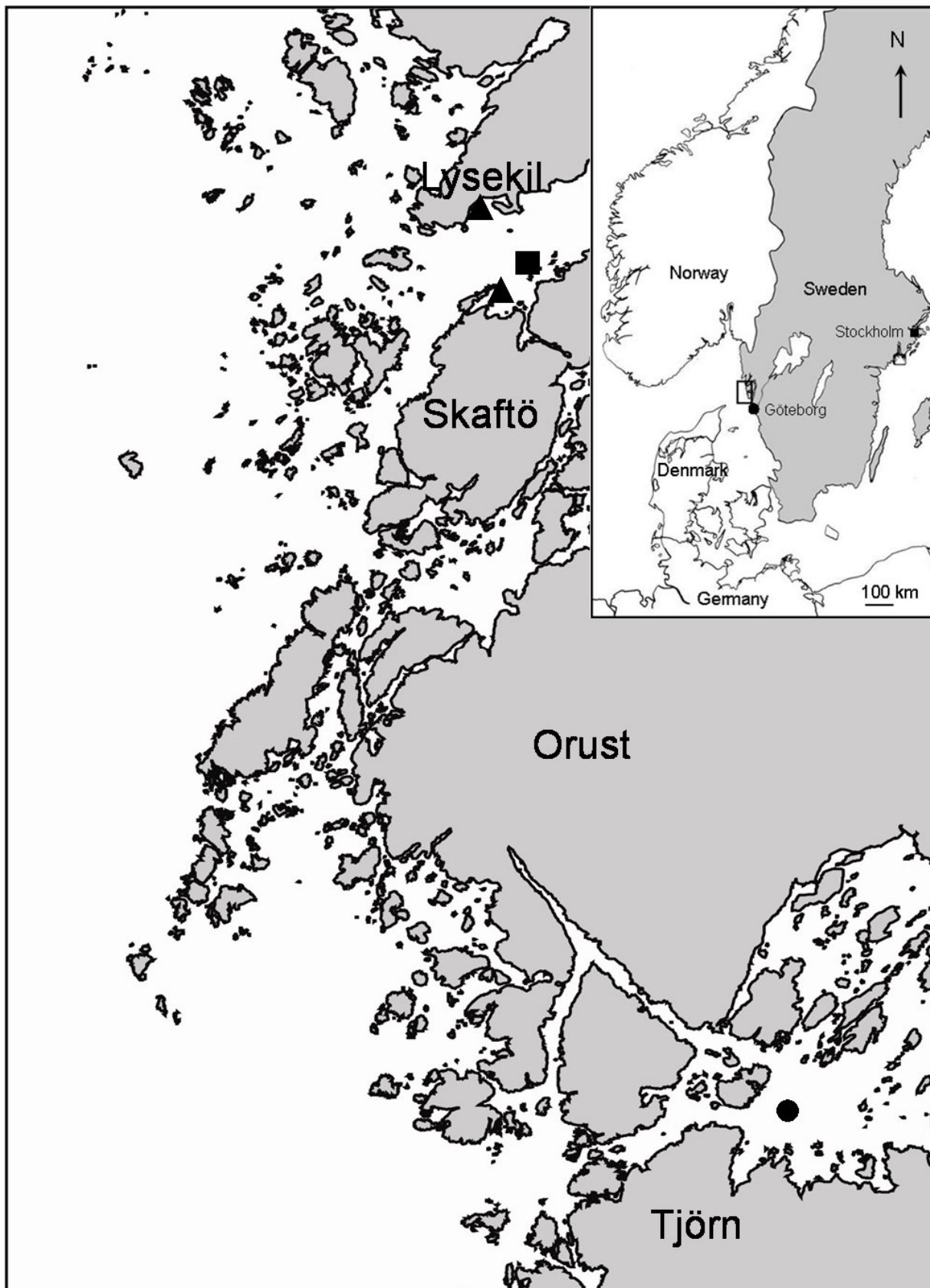
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4 Figure 7 Benthic fluxes of oxygen (A), ammonium (B), nitrate (nitrite+nitrate) (C) and silicate
5 (D) for the control cores and cores with *Amphiura filiformis* and *Nephtys*. The filled
6 circle (●) is the mean flux (n = 5) obtained during the incubations, the box represents
7 the standard error and the whiskers indicate the standard deviation for the mean flux
8 measured. Sediment-water incubations were performed 1, 9 and 22 days after adding
9 the fauna to the sediment cores.

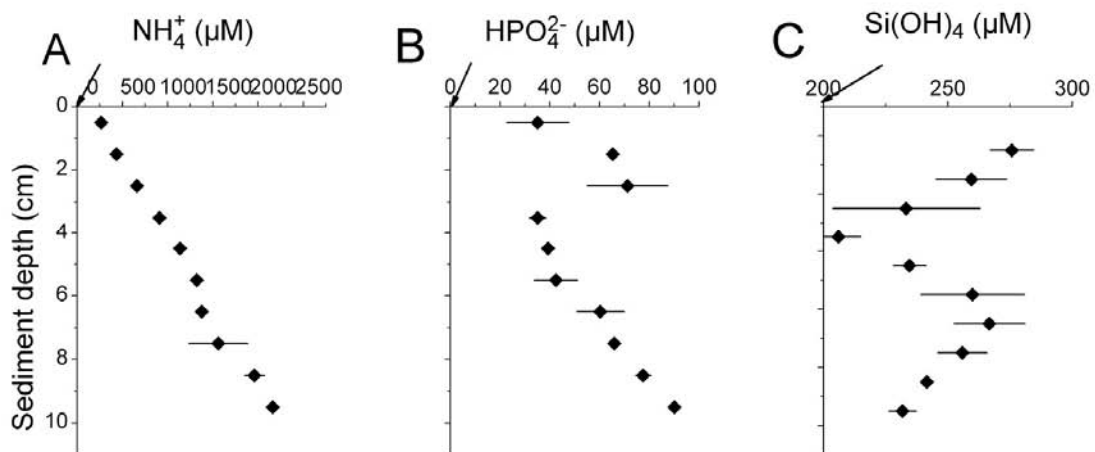


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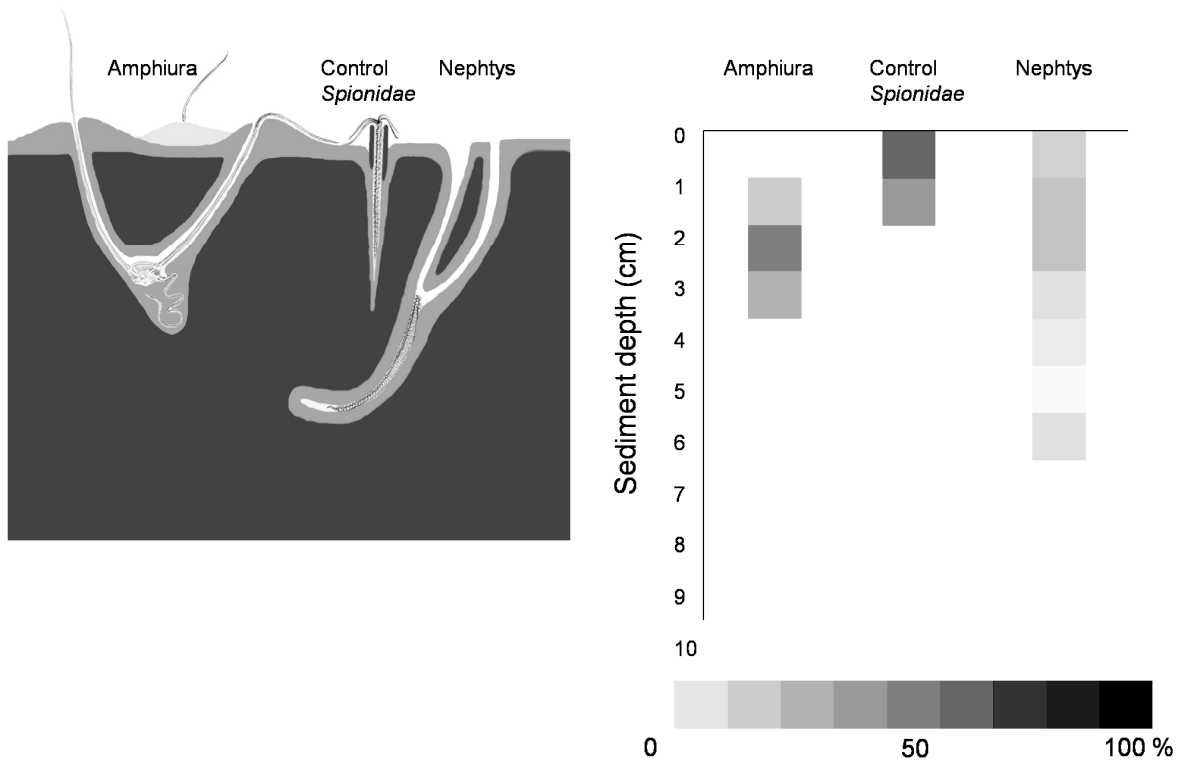


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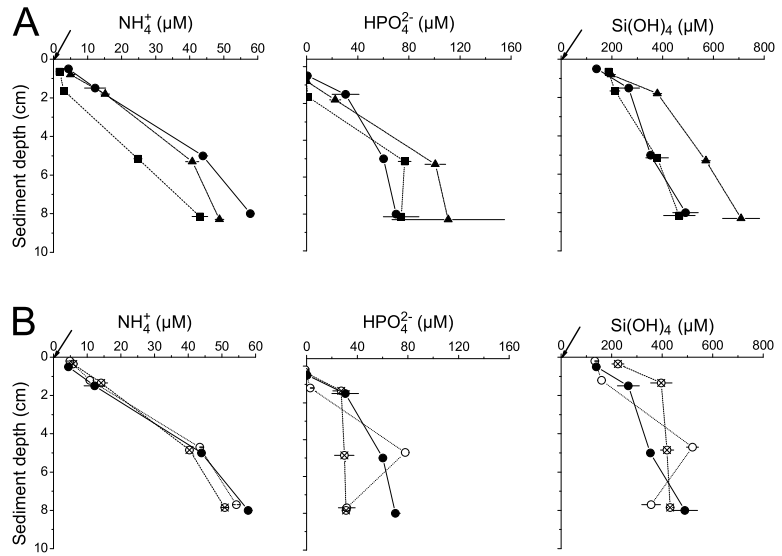
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