Biogeochemistry in highly reduced mussel farm sediments during macrofaunal recolonization by

Stina Lindqvist, Karl Norling, Stefan Hulth

► To cite this version:

Stina Lindqvist, Karl Norling, Stefan Hulth. Biogeochemistry in highly reduced mussel farm sediments during macrofaunal recolonization by. *Marine Environmental Research*, 2009, 67 (3), pp.136. 10.1016/j.marenvres.2008.12.005 . hal-00501990

HAL Id: hal-00501990

<https://hal.science/hal-00501990>

Submitted on 13 Jul 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Biogeochemistry in highly reduced mussel farm sediments during macrofaunal recolonization by *Amphiura Filiformis* and *Nephtys Sp.*

Stina Lindqvist, Karl Norling, Stefan Hulth

PII: S0141-1136(08)00260-2

DOI: [10.1016/j.marenvres.2008.12.005](https://doi.org/10.1016/j.marenvres.2008.12.005)

Reference: MERE 3309

To appear in: *Marine Environmental Research*

Received Date: 23 May 2008

Revised Date: 12 December 2008

Accepted Date: 15 December 2008

Please cite this article as: Lindqvist, S., Norling, K., Hulth, S., Biogeochemistry in highly reduced mussel farm sediments during macrofaunal recolonization by *Amphiura Filiformis* and *Nephtys Sp.*, *Marine Environmental Research* (2008), doi: [10.1016/j.marenvres.2008.12.005](https://doi.org/10.1016/j.marenvres.2008.12.005)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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

Stina Lindqvist^{1*}, Karl Norling^{2, §}, and Stefan Hulth¹

¹ Department of Chemistry, University of Gothenburg, 412 96 Göteborg, Sweden

² Department of Marine Ecology, University of Gothenburg, Kristineberg 566, 450 34 Fiskebäckskil,
Sweden

* Corresponding author

E-mail: stina.lindqvist@chem.gu.se

Phone: +46(0)31-772 2777

Fax: +46(0)31-772 2785

§ present address: Norwegian Institute for Water Research, Gau stadalléen 21, NO-0349 Oslo,
Norway

Running head: Macrofauna and biogeochemistry in mussel farm sediments

Abstract

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

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

1. Introduction

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

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

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

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

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

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

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

2. Materials and methods

2.1 Experimental overview

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

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

2.2 Sampling and macrofauna

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

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

In situ bottom water was collected for benthic flux incubations of intact sediment by a membrane pump system (Gardena). Bottom water was also retrieved for oxygen and nutrients; NH_4^+ , $\Sigma \text{NO}_3^- + \text{NO}_2^-$ (hereafter referred to as NO_3^-), HPO_4^{2-} and $\text{Si}(\text{OH})_4$. Oxygen was immediately precipitated and titrated within 24 h according to Winkler, while samples for nutrients were filtered on-line (0.45 μm , cellulose acetate) and stored frozen until analysis by an autoanalyzer (Technicon TRAACS 800, Bran and Luebbe, Germany) according to standard colorimetric methods (Strickland and Parson, 1972).

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

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

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

2.3 Benthic fluxes

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

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

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

2.4 Sediment porewater

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

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

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

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

2.5 Statistical analysis

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

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

3. Results

3.1 In situ conditions of the mussel farm sediment

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

Nutrient concentrations measured in the bottom water at the mussel farm site were low; ammonium ($0.17 \pm 0.02 \mu\text{M}$), 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 oxygen concentration was close to saturation ($\sim 320 \mu\text{M}$) at the prevailing temperature and salinity (Table 1). In the porewater, ammonium concentrations increased linearly with sediment depth to well above 2 mM, phosphate concentrations increased from 35 at the surface sediment to 90 μM at 10 cm sediment depth, and silicate concentrations ranged between 200 and 275 μM (Fig. 3). There 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 overlying water into the sediment (Table 1). In contrast, ammonium ($0.80 \pm 0.24 \text{ mmol m}^{-2} \text{ d}^{-1}$) and silicate ($6.3 \pm 3.7 \text{ mmol m}^{-2} \text{ d}^{-1}$) were supplied to the overlying bottom water from the sediment. The phosphate flux was close to zero ($0.02 \pm 0.09 \text{ mmol m}^{-2} \text{ d}^{-1}$).

3.2 Benthic macrofauna and porewater

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

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

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

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

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

3.3 Benthic fluxes

Overall, measured total fluxes of oxygen during the recolonization experiment were slightly lower 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 the mussel farm ($-44 \pm 10 \text{ mmol m}^{-2} \text{ d}^{-1}$). In contrast, nutrient fluxes were generally higher in the

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

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 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 produced by the sediment. At the last benthic flux measurement, fluxes of nitrate ($p = 0.011$) and silicate ($p = 0.029$) were significantly different in treatments with *Nephtys* compared with the *A. filiformis* treatments.

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

4. Discussion

4.1 Macrofauna

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

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

4.2 Oxygen dynamics

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

Irrigation and reworking by benthic macrofauna change surface sediments from simple stratified environments to systems having complex time-dependent, multi-dimensional geometries of transport and reactions (Aller, 2001). Biogeochemical effects observed in bioturbated sediments are often related to macrofaunal functionality and species specific traits, rather than abundance, biomass and size structure of the macrofaunal community (Norling et al., 2007; Ieno et al., 2006; Mermillod-Blondin et al., 2004). Despite a constant biomass of added macrofauna, reworking activities in this experiment caused differences between treatments in the porewater as well as benthic fluxes (Fig. 6 and 7). At the end of experiments, the oxic zone was most narrow for the *Nephtys* treatment. Activities by *Nephtys* therefore seemed to restrict the biological transport of oxygen from the overlying water compared with the control and the *A. filiformis* cores. A restricted transport of oxygen to the surface sediment was likely a direct consequence of the feeding mode of *Nephtys*, actively removing irrigating meiofauna and spionids. Furthermore, *Nephtys* mobilized and focused reactive organic material by the deposition of reactive fecal material on the sediment surface, further enhancing rates of oxygen consumption.

Normally, oxygen consumption decreases during consecutive time-series flux incubations following the progressive depletion of labile organic material (Karlson et al., 2007). In our study, however, total oxygen consumption rates were more or less constant with time, suggesting an alternative main route of oxygen reduction than oxidation of organic material. Due to the highly reducing environment, oxygen was likely consumed by the large pool of reduced metabolites (e.g. hydrogen sulphide) rather than through aerobic mineralization of recently deposited organic material. The narrow penetration of oxygen (170 – 250 μm), confirmed a pronounced anaerobic metabolism and mobilization of reduced compounds within the deposit.

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

4.3 Nutrient cycling

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

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

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

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

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

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

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

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

ACCEPTED MANUSCRIPT

5. Conclusion

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

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

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

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

1 Acknowledgements

2 O. Bäckman, P. Engström, A. Granhed, and L. Ljungqvist assisted during sampling and sample
3 preparation. R. Rosenberg provided comments to the manuscript. Financial support was granted by
4 the Swedish Research Council (VR), the Foundation for Strategic Environmental Research
5 (MISTRA), the Swedish Research Council for Environment, Agricultural Sciences and Spatial
6 Planning (FORMAS), the Swedish Marine Research Centre (GMF) and the Wilhelm and Martina
7 Lundgrens Vetenskapsfond.

References

- Aller, R.C., 1994. Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. *Chemical Geology*, 114(3-4): 331-345.
- Aller, R.C., 2001. Transport and reactions in the bioirrigated zone. In: B. Boudreau and B.B. Jørgensen (Editors), *The benthic boundary layer: transport processes and biogeochemistry*. Oxford University Press, Oxford, pp. 269–301.
- Asmus, R.M. and Asmus, H., 1991. Mussel beds - limiting or promoting phytoplankton. *Journal of Experimental Marine Biology and Ecology*, 148(2): 215-232.
- Berner, R.A., 1980. *Early diagenesis: A theoretical approach*. Princeton University Press, Princeton, USA, 241 pp.
- Boesch, D.F., Hecky, R.E., O'Melia, C.R., Schindler, D.W. and Seitzinger, S., 2006. Eutrophication of Swedish seas. 5509, Swedish Environmental Protection Agency.
- Boudreau, B.P., 1996. The diffusive tortuosity of fine-grained unlithified sediments. *Geochimica et Cosmochimica Acta*, 60(16): 3139-3142.
- Bouldin, D.R., 1968. Models for describing the diffusion of oxygen and other mobile constituents across the mud-water interface. *Journal of Ecology*, 56: 77-87.
- Buchanan, J.B., 1964. A comparative study of some features of the biology of *Amphiura filiformis* and *Amphiura chiajei* (Ophiuroidea) considered in relation to their distribution. *Journal of Marine Biology Association UK*, 44: 565-576.
- Canfield, D.E., 1994. Factors influencing organic carbon preservation in marine sediments. *Chemical Geology*, 114: 315-329.
- Cardell, M.J., Sarda, R. and Romero, J., 1999. Spatial changes in sublittoral soft-bottom polychaete assemblages due to river inputs and sewage discharges. *Acta Oecologica-International Journal of Ecology*, 20(4): 343-351.

- 1 Caron, A., Desrosiers, G., Olive, P.J.W., Retière, C. and Nozais, C., 2004. Comparison of diet and
2 feeding activity of two polychaetes, *Nephtys caeca* (Fabricius) and *Nereis virens* (Sars), in an
3 estuarine intertidal environment in Québec, Canada. *Journal of Experimental Marine Biology*
4 *and Ecology*, 304(2): 225-242.
- 5 Christensen, P.B., Glud, R.N., Dalsgaard, T. and Gillespie, P., 2003. Impacts of longline mussel
6 farming on oxygen and nitrogen dynamics and biological communities of coastal sediments.
7 *Aquaculture*, 218(1-4): 567-588.
- 8 Cloern, J.E., 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine*
9 *Ecology-Progress Series*, 210: 223-253.
- 10 Costello, M.J. and Read, P., 1994. Toxicity of sewage-sludge to marine organisms - a review.
11 *Marine Environmental Research*, 37(1): 23-46.
- 12 Dahlbäck, B. and Gunnarsson, L.Å.H., 1981. Sedimentation and sulfate reduction under a mussel
13 culture. *Marine Biology*, 63: 269-275.
- 14 Dauer, D.M., Maybury, C. and Ewing, R.M., 1981. Feeding behaviour and general ecology of
15 several spionid polychaetes from the Chesapeake Bay. *Journal of Experimental Marine*
16 *Biology and Ecology*, 54: 21-38.
- 17 Diaz, R.J. and Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems.
18 *Science*, 321: 926-929.
- 19 Diaz, R.J. and Schaffner, L.C., 1990. The functional role of estuarine benthos. In: M. Haire and E.C.
20 Krome (Editors), *Perspectives on the Chesapeake Bay. Advances in Estuarine Sciences*.
21 *Chesapeake Research Consortium, Gloucester Pt., Virginia*, pp. 25-56.
- 22 Forster, S. and Graf, G., 1995. Impact of irrigation on oxygen flux into the sediment - intermittent
23 pumping by *Callianassa subterranea* and piston-pumping by *Lanice conchilega*. *Marine*
24 *Biology*, 123(2): 335-346.
- 25 Francois, F., Poggiale, J.-C., Durbec, J.-P. and Stora, G., 2001. A new model of bioturbation for
26 functional approach to sediment reworking resulting from macrobenthic communities. In: J.Y.

- 1 Aller, S.A. Woodin and R.C. Aller (Editors), Organism-sediment interactions. The Belle W.
2 Baruch Library in Marine Science. University of South Carolina Press, Columbia, pp. 73-86.
- 3 Gilbert, F., Aller, R.C. and Hulth, S., 2003. The influence of macrofaunal burrow spacing and
4 diffusive scaling on sedimentary nitrification and denitrification: An experimental simulation
5 and model approach. *Journal of Marine Research*, 61(1): 101-125.
- 6 Gilbert, F., Souchu, P., Bianchi, M. and Bonin, P., 1997. Influence of shellfish farming activities on
7 nitrification, nitrate reduction to ammonium and denitrification at the water-sediment interface
8 of the Thau lagoon, France. *Marine Ecology-Progress Series*, 151(1-3): 143-153.
- 9 Haamer, J., 1996. Improving water quality in a eutrophied fjord system with mussel farming. *Ambio*,
10 25(5): 356-362.
- 11 Hartstein, N.D. and Stevens, C.L., 2005. Deposition beneath long-line mussel farms. *Aquacultural*
12 *Engineering*, 33(3): 192-213.
- 13 Hulth, S., Aller, R.C., Engström, P. and Selander, E., 2002. A pH plate fluorosensor (optode) for
14 early diagenetic studies of marine sediments. *Limnology and Oceanography*, 47(1): 212-220.
- 15 Hulth, S., Blackburn, T.H. and Hall, P.O.J., 1994. Arctic sediments (Svalbard) - consumption and
16 microdistribution of oxygen. *Marine Chemistry*, 46(3): 293-316.
- 17 Hulthe, G., Hulth, S., and Hall, P.O.J., 1998. Effect of oxygen on degradation rate of refractory and
18 labile organic matter in continental margin sediments. *Geochimica et Cosmochimica Acta*,
19 62(8): 1319-1328.
- 20 Ieno, E.N, Solan, M., Batty, P. and Pierce, G.J., 2006. How biodiversity affects ecosystem
21 functioning: roles of infaunal species richness, identity and density in the marine benthos.
22 *Marine Ecology-Progress Series*, 311: 263-271.
- 23 Karlson, K., 2005. Impact of benthic macrofauna on sediment biogeochemistry - the importance of
24 bottom water oxygen concentrations. Ph.D. thesis, Göteborg University, Göteborg, pp. 489.

- 1 Karlson, K., Hulth, S., Ringdahl, K. and Rosenberg, R., 2005. Experimental recolonisation of Baltic
2 Sea reduced sediments: survival of benthic macrofauna and effects on nutrient cycling. *Marine*
3 *Ecology-Progress Series*, 294: 35-49.
- 4 Karlson, K., Hulth, S. and Rosenberg, R., 2007. Density of *Monoporeia affinis* and biogeochemistry
5 in Baltic Sea sediments. *Journal of Experimental Marine Biology and Ecology*, 344: 123–135.
- 6 Kemp, W.M., Boynton, W.R, Adolf, J.E., Boesch, D.F., Boicourt, W.C., Brush, G., Cornwell, J.C.,
7 Fisher, T.R., Glibert, P.M., Hagy, J.D., Harding, L.W., Houde, E.D., Kimmel, D.G., Miller,
8 W.D., Newell, R.I.E., Roman, M.R., Smith, E.M. and Stevenson, J.C., 2005. Eutrophication of
9 Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology-Progress*
10 *Series*, 303: 1-29.
- 11 Kristensen, E., 1981. Direct measurement of ventilation and oxygen uptake in three species of
12 tubicolous polychaetes (*Nereis* spp.). *Journal of Comparative Physiology B* 145: 45-50.
- 13 Kristensen, E. and Hansen, K., 1999. Transport of carbon dioxide and ammonium in bioturbated
14 (*Nereis diversicolor*) coastal, marine sediments. *Biogeochemistry*, 45(2): 147-168.
- 15 Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.O., Olrog, L., Rehnstam-Holm, A.S.,
16 Svensson, J., Svensson, S. and Syversen, U., 2005. Improving marine water quality by mussel
17 farming: A profitable solution for Swedish society. *Ambio*, 34(2): 131-138.
- 18 Lohrer, A.M., Thrush, S.F. and Gibbs, M.M., 2004. Bioturbators enhance ecosystem function
19 through complex biogeochemical interactions. *Nature*, 431(7012): 1092-1095.
- 20 Mermillod-Blondin, F., Rosenberg, R., François-Carcaillet, F., Norling, K. and Mauclaire, L., 2004.
21 Influence of bioturbation by three benthic infaunal species on microbial communities and
22 biogeochemical processes in marine sediment. *Aquatic Microbial Ecology*, 36(3): 271-284.
- 23 Nielsen, L.P., 1992. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS*
24 *Microbiology Letters*, 86: 357-362

- 1 Nilsson, H.C., 1998. Effects of hypoxia and organic enrichment on growth of the brittle stars
2 *Amphiura filiformis* (O.F. Müller) and *Amphiura chiajei* Forbes. Journal of Experimental
3 Marine Biology and Ecology, 237: 11-30
- 4 Nixon, S.W., 1995. Coastal marine eutrophication: A definition, social causes, and future concerns.
5 *Ophelia*, 41: 199-219.
- 6 Nizzoli, D., Welsh, D.T., Bartoli, M. and Viaroli, P., 2005. Impacts of mussel (*Mytilus*
7 *galloprovincialis*) farming on oxygen consumption and nutrient recycling in a eutrophic
8 coastal lagoon. *Hydrobiologia*, 550: 183-198.
- 9 Nizzoli, D., Welsh, D.T., Fano, E.A. and Viaroli, P., 2006. Impact of clam and mussel farming on
10 benthic metabolism and nitrogen cycling, with emphasis on nitrate reduction pathways. Marine
11 Ecology-Progress Series, 315: 151-165.
- 12 Noren, F., Haamer, J. and Lindahl, O., 1999. Changes in the plankton community passing a *Mytilus*
13 *edulis* mussel bed. Marine Ecology-Progress Series, 191: 187-194.
- 14 Norling, K., Rosenberg, R., Hulth, S., Grémare, A. and Bonsdorff, E., 2007. Importance of
15 functional biodiversity and species specific traits of benthic fauna for ecosystem functions in
16 marine sediments. Marine Ecology-Progress Series, 332: 11-23.
- 17 Pearson, T.H. and Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment
18 and pollution of the marine environment. *Oceanography and Marine Biology: an Annual*
19 *Review*, 16: 229-311.
- 20 Quinn, G.P. and Keough, M.J., 2002. Experimental design and data analysis for biologists,
21 Cambridge, UK.
- 22 Rasmussen, H. and Jørgensen, B.B., 1992. Microelectrode studies of seasonal oxygen-uptake in a
23 coastal sediment - role of molecular-diffusion. Marine Ecology-Progress Series, 81(3): 289-
24 303.
- 25 Rosenberg, R., 1995. Benthic marine fauna structured by hydrodynamic processes and food
26 availability. *Netherland Journal of Sea Research*, 34: 303-317.

- 1 Rosenberg, R., Hellman, B. and Johansson, B., 1991. Hypoxic tolerance of marine benthic fauna.
2 Marine Ecology-Progress Series, 79: 127-131.
- 3 Ryther, J.H., Dunstan, W.M., Tenore, K.R. and Huguenin, J.E., 1972. Controlled eutrophication:
4 Increasing food production from the sea by recycling human wastes 22(3): 144-152.
- 5 Sanders, H.L., 1960. Benthic studies in Buzzards bay. III. The structure of the soft-bottom
6 community. Limnology and Oceanography, 5(2): 138-153.
- 7 Strickland, J.D.H. and Parson, T.R., 1972. A practical handbook of seawater analysis, Bulletin 167
8 2:nd ed. revised. Fisheries Research Board of Canada, Ottawa, pp. 310.
- 9 Sundby, B., Gobeil, C., Silverberg, N. and Mucci, A., 1992. The phosphorus cycle in coastal marine-
10 sediments. Limnology and Oceanography, 37(6): 1129-1145.
- 11 Sundbäck, K., Linares, F., Larson, F., Wulff, A. and Engelsen, A., 2004. Benthic nitrogen fluxes
12 along a depth gradient in a microtidal fjord: The role of denitrification and microphytobenthos.
13 Limnology and Oceanography, 49(4): 1095-1107.
- 14 Thamdrup, B., 2000. Bacterial manganese and iron reduction in aquatic sediments. Advances in
15 Microbial Ecology 16: 41-84.
- 16 Thamdrup, B. and Dalsgaard, T., 2002. Production of N₂ through anaerobic ammonium oxidation
17 coupled to nitrate reduction in marine sediments. Applied and Environmental Microbiology,
18 68: 1312-1318.
- 19 Vistisen, B. and Vismann, B., 1997. Tolerance to low oxygen and sulfide in *Amphiura filiformis* and
20 *Ophiura albida* (Echinodermata: Ophiuroidea). Marine Biology, 128: 241-246.
- 21 Vopel, K., Thistle, D. and Rosenberg, R., 2003. Effect of brittle star *Amphiura filiformis*
22 (Echinodermata: Ophiuroidea) on oxygen flux into the sediment. Limnology and
23 Oceanography, 48: 2034-2045.

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

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

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

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

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

1

1

1

1