



Digging worms for remediation of sediments impacted by mussel farms

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Dansk resumé

Opdræt af muslinger vil påvirke bunden under opdrætsanlæggene gennem den organiske berigelse fra sedimenterende fækaliier og muslinger, der falder af. Denne uønskede berigelse af sedimenterne resulterer i forøget iltforbrug, udsivning af næringssalte som ammonium og fosfat samt akkumulering af sulfid (Carlsson et al. 2009). Følgelig er biodiversiteten i sediment under opdrætsanlæg ofte lav og lavere end i kontrolområder. Gravende og dermed bioturberende børsteorme har potentiale til at stimulere omsætningen af de sedimenterende muslingefækaliier på samme vis, som det er konstateret under fiskefarme (Heilskov et al. 2006, Sanz-Lazaro et al. 2011). Dermed forbedres sedimenternes assimilative kapacitet (Hargrave et al. 2008) og forhindrer eller reducerer forringelsen af sedimentforholdene. I dette studie har vi tilsat børsteormene *Capitella* sp., som er lille og tolerant overfor organisk berigelse, og *Hediste diversicolor*, som er større, men mindre tolerant overfor organisk berigelse, til sediment belastet med organisk materiale fra muslinge anlæg. Begge orme er arter hjemmehørende i Skandinavien og *H. diversicolor* er potentielt en kommercielt interessant art, da den bruges af lystfiskere som agn (Bischoff et al. 2009). I undersøgelsen blev der tilsat orme i forskellig tæthed til sediment i felten henholdsvis under og udenfor et opdrætsanlæg, og efter 5 uger blev sedimentets iltoptagelse, fluxe ind og ud af næringssalte og sulfidpuljerne målt. Desuden blev sediment fra felten indsamlet og inkuberet i laboratoriet. Her blev der tilsat varierende mængde muslingefækaliier og antal orme, og efterfølgende blev der målt iltoptagelse og næringssaltfluxe på en tidsskala fra timer til få dage.

Resultaterne viste, at *H. diversicolor* har potentiale til at remediere sediment under opdrætsanlæg, da ormen stimulerer nedbrydningen af organisk materiale fra muslingeopdrættet i højere grad end andre typer af organisk materiale (tørret gær, affald fra fiskefarme). Denne effekt er sandsynligvis et resultat af både ormens direkte optagelse af muslingefækaliierne og nedblandingen i sedimentet af ormens bioturbering. Ved bioturberingen bliver sedimenterne samtidig iltet, hvorved akkumulering af såvel partikulært bundet som jebundet sulfid (AVS puljen) bliver reduceret. Derved bliver sedimentforholdene generelt forbedret, hvilket kan gavne den generelle rekolonisering af sedimenterne. I laboratorie-forsøgene viste effekten på iltoptagelse af tilsætning af *H. diversicolor* sig indenfor den første dag efter tilsætning og aftog derefter hurtigt, når der ikke blev tilsat yderligere organisk materiale i form af muslingefækaliier. Det kan antages, at effekten fastholdes ved kontinuerlig tilførsel af fækaliier som det også blev observeret i feltforsøgene, hvor raterne var højere under opdrætsanlægget sammenlignet med en referencestation. Effekten af tilsætning af *H. diversicolor* var mere kompleks hvad angår næringssaltfluxe. I en vis udstrækning var der forøgede fluxe ud af sedimentet, som et respons på den øgede omsætning af fækaliierne, og især umiddelbart efter tilsætning af fækaliier, men fx fluxe af fosfat var primært styret af den generelle

sedimentomsætning og der blev fundet optagelse af fosfat i alle behandlinger. Der var en tendens til, at øget tæthed af orme resulterede i øget omsætning og da disse forsøg blev gennemført med relativt lave tætheder (91-444 m⁻²) er det sandsynligt, at tætheder af orme på op til 1000 m⁻² kan øge omsætningen af fækcalier yderligere. Sammenlignet med *H. diversicolor* var effekten af tilsætning af *Capitella* sp. meget begrænset og ved maximale tætheder af *Capitella* svarende til lave tætheder af *H. diversicolor*. Dette resultat indikerer, at *Capitella* har begrænset betydning for omsætningen af muslingefækcalier, sandsynligvis som følge af forskelle i de to arters bioturbering. Imidlertid bør der for at få et fuldstændigt billede, gennemføres supplerende forsøg med større tætheder af *Capitella* end det var muligt i denne undersøgelse.

Digging worms for remediation of sediments impacted by mussel farms

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Executive summary

Mussel farms affect the biogeochemical conditions in the sediments underneath the farms due to organic enrichment by mussel biodeposits and by mussels falling of the long-lines, which is an unwanted effect of mussel farming (Carlsson et al. 2009). Organically-enriched sediments have high oxygen consumption rates contributing to oxygen depletion events. High efflux rates of nutrients such as ammonium and phosphate, contributing to eutrophication, are also observed together with accumulation of sulphides, which prevent benthic infauna from colonizing and surviving in the sediments. Thus, a low biodiversity is in general observed in this environment. Burrowing polychaetes have the potential to stimulate the decomposition of mussel biodeposits in a similar way as has been observed underneath fish farm (Heilskov et al. 2006, Sanz-Lazaro et al. 2011). This increases the assimilative capacity of the sediments (Hargrave et al. 2008) and avoids deterioration of the sediment conditions. In this study, the prediction that polychaetes counteract the negative effects of organic enrichment in mussel farms are addressed by conducting a number of field and laboratory studies using the organic tolerant polychaete, *Capitella* sp., and the less tolerant but larger polychaete *Hediste diversicolor*, which are added to sediments affected by organic enrichment from mussel farms. Both polychaetes are native in the study area and *Hediste diversicolor* has commercial potential as fish feed and as bait for anglers (Bischoff et al. 2009). Sediment oxygen uptake, nutrient fluxes and pools of sulphides were measured in the field 5 weeks after the two polychaetes were added in different densities to mussel farm sediment and compared to reference sediment. In the lab experiment, sediments were collected from a reference site and manipulated with different densities of polychaetes and doses of mussel biodeposits. Short-term (hours, days) dynamics in sediment oxygen uptake and nutrient fluxes were measured.

The results showed that *H. diversicolor* may be a strong candidate for remediation of mussel farm sediments, as it enhances the decomposition of organic matter to a larger extent than observed for other types of organic matter (dried yeast, fish farm waste products). This is probably due to direct consumption of the fecal pellets by the worms as well as mixing of the fecal pellets into the sediments by bioturbation. At the same time, the sediments were oxidized which reduced the accumulation of particular pore water sulphides as well as the iron-bound sulphides (the AVS pool). This may create favourable sediment conditions for recolonization of other infaunal species. In the lab experiment, the positive effects of *H. diversicolor* occurred within the first day of deposition of organic matter and then rapidly declined, when no further organic matter (fecal pellets) was added. It can be expected that the rates will remain high upon continuous addition as observed in the field, where rates were generally higher in mussel farm compared to reference sediments over the study period. The effects of addition of *H. diversicolor* on nutrient fluxes were

more complex. It did to some extent follow the enhanced decomposition with enhanced release of nutrients from the sediments, in particular right after the organic matter was added, but fluxes of phosphate were more controlled by the biogeochemistry of the sediments, where an uptake was observed in all treatments, probably due to a reoxidation event at the study site. In these experiments *H. diversicolor* was added at relatively low densities (91-444 m⁻²) and the trends with increasing density suggests that larger densities, e.g. up to 1000 m² may enhance the decomposition of biodeposits even further. Compared to *H. diversicolor*, the effects of *Capitella* sp. were limited. In the highest densities, the effects on sediment oxygen uptake and nutrient fluxes were similar to the low density of *H. diversicolor* and there were no apparent effects on the organic matter decomposition under enriched conditions by *Capitella*. This suggests that *Capitella* may have limited effects on the decomposition of mussel biodeposits, as compared to *H. diversicolor*, probably due to the difference in bioturbation activity between the two species. However, to further evaluate the potential of this species, higher densities of *Capitella* compared to what was used in this experiment should be used.

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Introduction

Studies have shown that the presence of infauna in the sediment enhances the oxic and suboxic zones and promote organic matter decomposition compared to defaunated sediment (Heilskov and Holmer 2003, Valdemarsen et al. 2010). Adding infauna to the sediments may thus remediate organic rich sediments as observed underneath a finfish farm where the polychaete *Capitella* sp. was added (Kinoshita et al. 2008). In this study the decomposition of organic matter was markedly enhanced by addition of *Capitella*, and thus seems to be a promising method to remediate affected sediments. *Capitella* spp. is relatively easy to cultivate in the laboratory as they have a short life cycle (4-6 weeks) and a rapid population growth (Bridges et al. 1994, Tsutsumi 1987). *Capitella* has, however, been found to maintain the sediments in a reduced stage with high sulfide pools, preventing other less sulfide tolerant species to colonize the sediments (Heilskov and Holmer 2001). Another potential bioremediator is *Hediste diversicolor*, as it shows high bioturbation and bioirrigation resulting in enhanced decomposition of organic matter and reoxidation of the sediments (Banta et al. 1999, Kristensen et al. 2012). *H. diversicolor* is a widespread polychaete under temperate conditions and can be found in high densities in organic rich sediments (Delefosse et al. 2012).

The aim of this study was to investigate the potential of remediation of sediments affected by mussel farms by adding burrowing polychaetes to the sediments. Mussel farms affect the biogeochemical conditions due to organic enrichment by mussel biodeposits in the sediments (Carlsson et al. 2009). It is not known whether burrowing polychaetes can stimulate the decomposition of mussel biodeposits in a similar way as found for fish farm waste products (Heilskov et al. 2006, Sanz-Lazaro et al. 2011) and thereby increase the capacity of the sediments to mineralise organic matter from external sources (Hargrave et al. 2008) and avoid deterioration of the sediment conditions. In addition to low biodiversity, organically enriched sediments have relatively high oxygen consumption rates contributing to oxygen depletion events, increased efflux rates of nutrients such as ammonium and phosphate contributing to eutrophication, and accumulate sulphides, which prevent benthic infauna from establishment in the sediments. These questions are addressed by conducting a number of field and laboratory studies where the organic tolerant polychaete, *Capitella* sp., and the less tolerant but larger polychaete *Hediste diversicolor*, are added to organically-enriched sediments from mussel farms. Both polychaete species are native in the study area and *Hediste diversicolor* has commercial potential as fish feed and as bait for anglers (Bischoff et al 2009).

Materials and Methods

Collection and cultivation of burrowing polychaetes

Laboratory cultures of *Capitella* sp. were reared in aerated aquaria (10 L, 30‰ S) at 21 °C on sediment with regular additions of a mixture of ground commercial fish food (Tetra Min[®]), baby cereal (Milpo[®]) and dried spinach in equal proportions as a supplementary food. To maintain the culture the polychaetes were fed with 250 µm sieved sediment every week. Adult *Hediste diversicolor* were bought from local fishermen 1-2 days before start of experiments and kept in aerated aquaria (20 L, 30 ‰ S, 21° C) with bottom sediment.

Field experiment

Addition of polychaetes to mussel sediments. Polychaetes were added to sediments underlying a mussel farm (MF) in Lysen, Limfjorden (Fig. 1) and at a reference station (REF) ~80 meters outside the farm at a water depth of 5 m in June 2011. The mussel farm has an area extent of 12.5 ha and annual production of 150 tons of live blue mussels (*Mytilus edulis*). Five worm densities were deployed at each station: 1) No fauna (no polychaetes), 2) *Capitella* sp (C40, 40 ind/146 m²), 3) *H. diversicolor* (H25, 25 ind/91 m²), 4) *H. diversicolor* (H100, 100 ind/366 m²) and 5) *H. diversicolor* (H200, 200 ind/732 m²). Ten circular PVC frames (i.d. 59 cm; h 30 cm) equipped with nets to cover the surface were deployed to keep the polychaetes in the sediment. Polychaete densities were prepared on land in plastic containers, and added to the sediment by a diver. The frames were deployed to a steady position minimum 20 cm into the sediment. The polychaetes were added through a hole in the net which was immediately closed afterwards to avoid that polychaetes escaped or were forced upwards before they had buried into the sediment. The nets were removed after 2 days. Additionally, three sediment cores (HAPS sampler, size: 0.25 m²) were sampled from each station to evaluate the present macrofauna population and abundance.

Sediment sampling. 5 weeks after the polychaetes were added to the sediments, in mid July 2011, three sediment cores were sampled from each frame. These cores were pre-incubated overnight in *in situ* bottom water at 21°C with constant air supply and continuous stirring by a Teflon coated magnet. Afterwards, fluxes of O₂ and nutrients (NH₄⁺, NO₃⁻ and PO₄³⁻) across the sediment-water interface were measured by taking initial water samples from all cores and subsequently closing the cores with stoppers. Cores were incubated for 2-3 hours in darkness before taking the final samples. The O₂-concentrations were measured by Winkler titrating on a 5 ml subsample and sediment O₂ consumption (SOC) determined as the difference between initial and final concentrations of O₂ and corrected for the incubation time and water volume. Nutrients were analysed on a flow-injection

system (QuickChem 8500 Series, Lachat Instruments) and the flux calculated as described for O₂.

After incubation, the cores were unsealed and left for 6-8 hours before they were sectioned in 2 cm intervals to 10 cm depth for determination of sediment characteristics and sulfide pools. Sediment density was obtained by weighing a known volume of the sediment slices. Organic matter contents were analysed by elemental analysis for C and N on a Carlo Erba 1100EA and total P pools were assessed by boiling under acidic conditions and measured spectrophotometric (Koroleff 1983). Sediments for analysis of sulphides were fixed in 1 M zinc acetate and stored frozen until analysed. Separation of reduced sulphur compounds were performed by the two-step distillation procedure (Fossing and Jorgensen 1989). After distillation, acid volatile sulphide (AVS) and chromium reducible sulphur (CRS) was trapped as ZnS and analysed spectrophotometric (Cline 1969).

The fauna samples were sieved through a 500 µm sieve. Very few, < 5 individuals, were present and data are not included here.

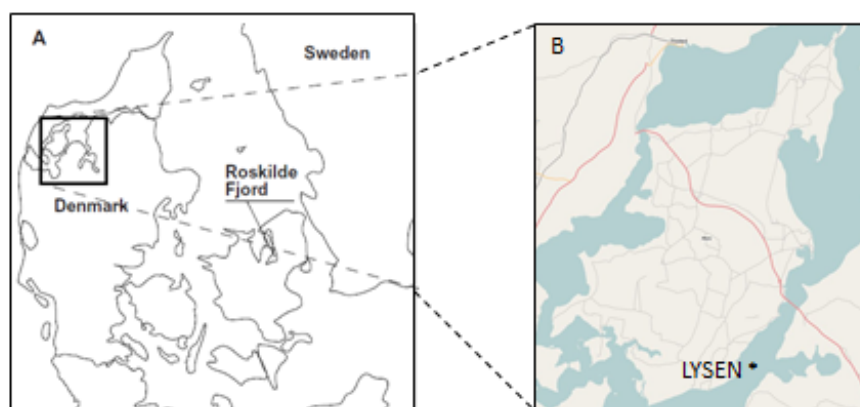


Fig. 1. Location of the sampling area in Lysen where the mussel farm was located

Laboratory experiment

Collection of sediment and mussels. Sediment was collected in October 2011 at the reference station (see above) to maintain conditions similar to the field experiment but without impacts of the mussel farm. Sediment was collected with a crab corer, and brought to the experimental facility in 20 L plastic buckets. All visible shells, stones, gravel and animals were removed from the sediment before use. *Mytilus edulis* were collected from the mussel farm and kept in tanks with continuous water flow and salinity of 30 at 18°C up to 4 days. Prior to fecal pellet collection, mussels were transferred to large aquaria with continuous aeration. Fecal pellets were regularly collected from the bottom of the aquaria into smaller plastic containers. Only pellets less than 24 h old were used for the experiment.

Additions of polychaetes and mussel fecal pellets. The sediment was filled into 27 small aquaria (cores) (4.72 L; 15x15x19 cm) to a sediment depth of 14 cm. The aquaria were submerged overnight in a large tank with continuous water flow at 18°C. The next day, polychaetes were added to the aquaria in three different treatments: (1) control (without polychaetes), (2) 10 individuals of *H. diversicolor* (444 m⁻²) and (3) 10 individuals of *Capitella* sp. (444 m⁻²). For each treatment, nine aquaria were used (n=9). After the polychaetes were added, a lid was placed on the cores to avoid that polychaetes escaped before they had buried in to the sediment. The lid was removed when the polychaetes were inside the sediment. The polychaetes were allowed to acclimatize for a few hours before the first addition of mussel fecal pellets. Within each treatment, 3 cores were left as controls (without added fecal pellets), 3 cores were added a low dose of mussel fecal pellets (~750 pellets) and 3 were added a high dose of fecal pellets (~1000 pellets). Both doses were within the range of previously measured daily sedimentation of mussel biodeposits at mussel farms in Limfjorden (Carlsson et al. 2010).

Sediment incubation. The following day, one sediment core (i.d. 5.2 cm) was subsampled from each aquarium. The cores were pre-incubated for 6 hours (see above), and prior to incubation start, initial samples were taken for measurement of O₂ and nutrients (see above) and fecal pellets were added to the cores in a similar manner as described for the aquaria. Due to the smaller area of the cores compared to the aquaria, low and high doses corresponding to ~100 and ~150 fecal pellets were added. The cores were then immediately sealed by rubber stoppers and incubated for ~6 hours. Final samples were taken and flux of O₂ and nutrients analysed and calculated as described above. Flux measurements continued for 5 days until oxygen consumption in the high-dose treated cores were at the same level as the control cores (without polychaetes or fecal pellets). The cores were equipped with a flow-through system and the overlying water column was continuously renewed during this period to ensure full oxygen saturation.

Results

Field experiment

Sediment oxygen consumption (SOC) in all treatments was higher at the Mussel farm compared to the Reference station. Especially high densities of *H. diversicolor* (H100 and H200) had a stimulating effect and SOC reached 182 ± 62 $\text{mmol m}^{-2} \text{d}^{-1}$ compared to 42 ± 24 $\text{mmol m}^{-2} \text{d}^{-1}$ in the Control sediments. Ammonium fluxes were consistently higher at the mussel farm, whereas there were no major differences in the phosphate and nitrate fluxes between stations. The addition of polychaetes did not significantly affect the fluxes due to the large variability in the measurements, but there was a consistent trend with larger SOC and ammonium efflux with increasing density of *H. diversicolor* in the Mussel farm sediment, whereas no stimulation was found at the Reference station. There was no significant effect of the addition of *Capitella* to the sediments.



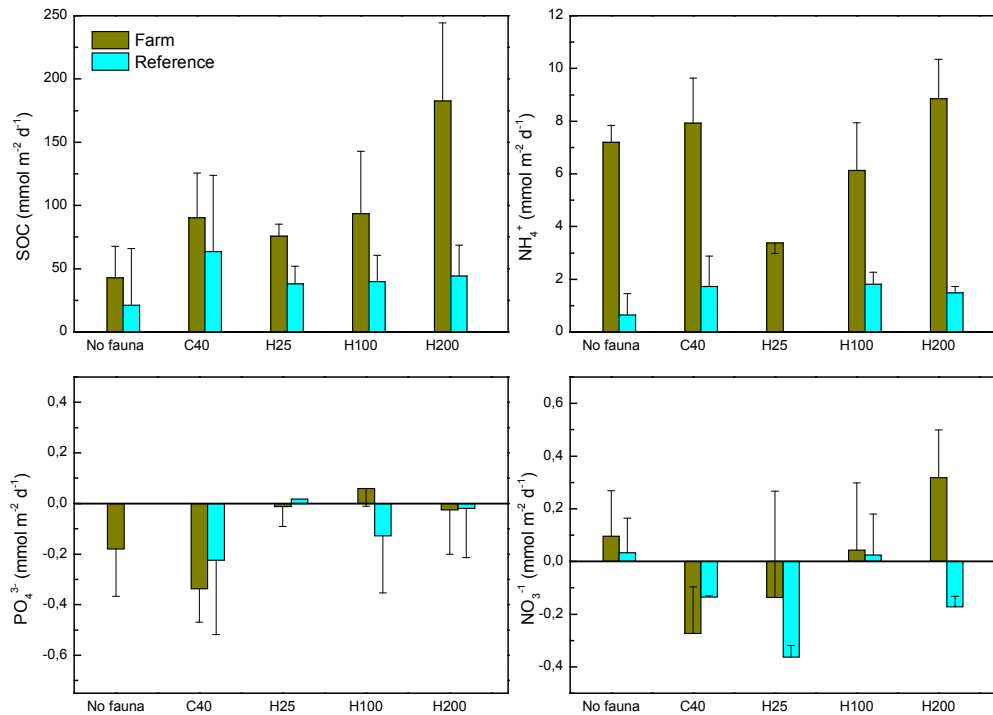


Fig. 2. Field experiment: Sediment oxygen consumption and nutrient fluxes at the Mussel farm station and Reference station 5 weeks after adding polychaetes to the underlying sediment. (n=3±STDEV)

The AVS pools were significant higher at the Mussel farm compared to the Reference station (2-way ANOVA, $p < 0.001$, Table 1), whereas it is opposite for the CRS pools, which were higher at the Reference station (2-way ANOVA, $p < 0.001$, Table 1). The addition of *H. diversicolor* resulted in lower AVS pools at the Mussel farm, in particular for the depth integrated pools (0-10 cm). There was no significant effect on the AVS pools of the addition of *Capitella*. A trend towards increased depth-integrated CRS pools by addition of *H. diversicolor* was observed in the Mussel farm, whereas no change was observed with addition of *Capitella*, and no effects of polychaetes addition was found at the Reference station except for H200 showing a decreasing trend.

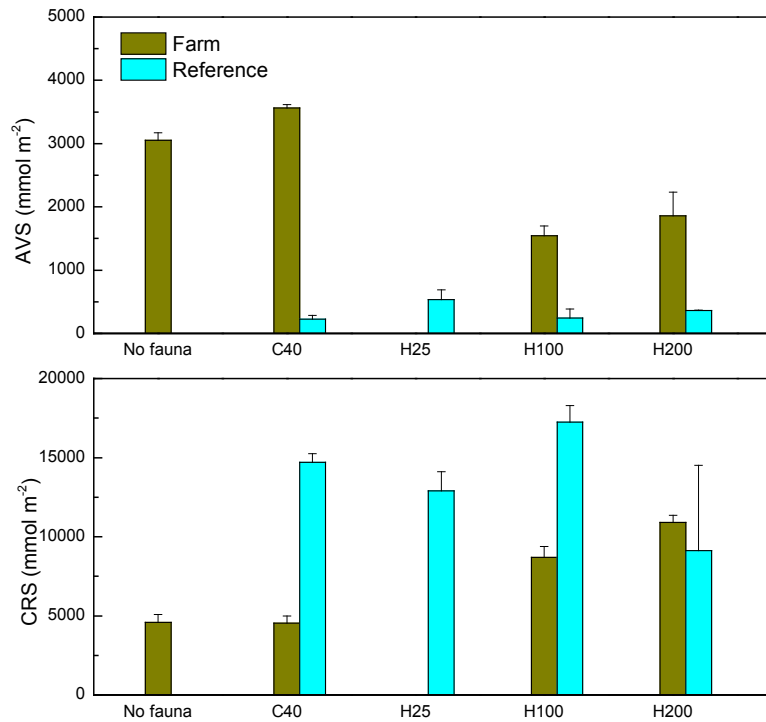


Fig. 3 Field experiment: Sediment sulphides (AVS and CRS) at the Mussel farm station and Reference station 5 weeks after adding polychaetes to the underlying sediment. (n=3±STDEV)

Table 1. Results of 2-way ANOVA on surface pools of AVS and CRS (0-2 cm)

| Response Variable | R ² | df | SS | F | P | Tukey HSD |
|---------------------|----------------|----|----------|--------|---------|---------------|
| AVS 2cm model | 0.78 | 9 | 2307.33 | 19.92 | <.0001* | |
| <i>station</i> | | 1 | 1770.27 | 89.23 | <.0001* | REF<MF |
| <i>worm</i> | | 4 | 272.13 | 3.44 | 0.0273* | H25<C40,H200 |
| <i>station*worm</i> | | 4 | 264.91 | 3.33 | 0.0301* | Suppl.table 1 |
| Error | | 20 | 396.77 | | | |
| CRS 2cm model | 0.87 | 9 | 26363.12 | 24.31 | <.0001* | |
| <i>station</i> | | 1 | 17926.18 | 148.81 | <.0001* | REF>MF |
| <i>worm</i> | | 4 | 4125.67 | 8.56 | 0.0003* | Suppl.table 1 |
| <i>station*worm</i> | | 4 | 4311.26 | 8.94 | 0.0003* | Suppl.table 1 |
| Error | | 20 | 2409.25 | | | |

The sediment nutrient contents were assessed at the Mussel farm in the control (without polychaetes), C40 and H25 (Table 2). There were no significant differences between the treatments (data not shown), only the POC content was increased in H100 and resulting in relatively high C:N ratios compared to control cores.

Table 2. Sediment nutrient contents (0-1 cm) at 3 stations at the mussel farm 5 weeks after polychaetes were added. Given is organic carbon (POC), organic nitrogen (PON) and total phosphorus (TP). (n=3±STD). The C:N ratio of the average POC and PON content is given.

| Station | POC (%DW) | PON(%DW) | C:N (molar) | TP ($\mu\text{mol gDW}^{-1}$) |
|---------|-----------|-----------|-------------|---------------------------------|
| Control | 5.14±0.84 | 0.48±0.10 | 12.5 | 23.3±2.2 |
| C40 | 5.69±1.70 | 0.44±0.07 | 15.0 | 22.0±1.3 |
| H100 | 6.89±0.30 | 0.51±0.10 | 15.7 | 19.4±5.5 |

Laboratory experiment

The addition of fecal pellets and polychaetes affected the SOC and nutrient fluxes already one day after the additions (Fig. 4, Table 3). The effect of fecal pellets on the SOC in control sediments was not significant, whereas the addition of polychaetes decreased the SOC. The combination of fecal pellets and *Capitella* increased the SOC, whereas SOC was only enhanced at the high dose with *H. diversicolor*. Addition of fecal pellets did not affect the flux of ammonium in control treatments, whereas addition of polychaetes enhanced the efflux but with interaction between species and fecal pellets. The efflux of phosphate was high in controls and no organic matter (OM) addition and decreased with higher OM addition of polychaetes, whereas there was no effect of OM in the treatments with polychaetes. The nitrate flux was enhanced with addition of polychaetes, whereas there was no effect of OM.

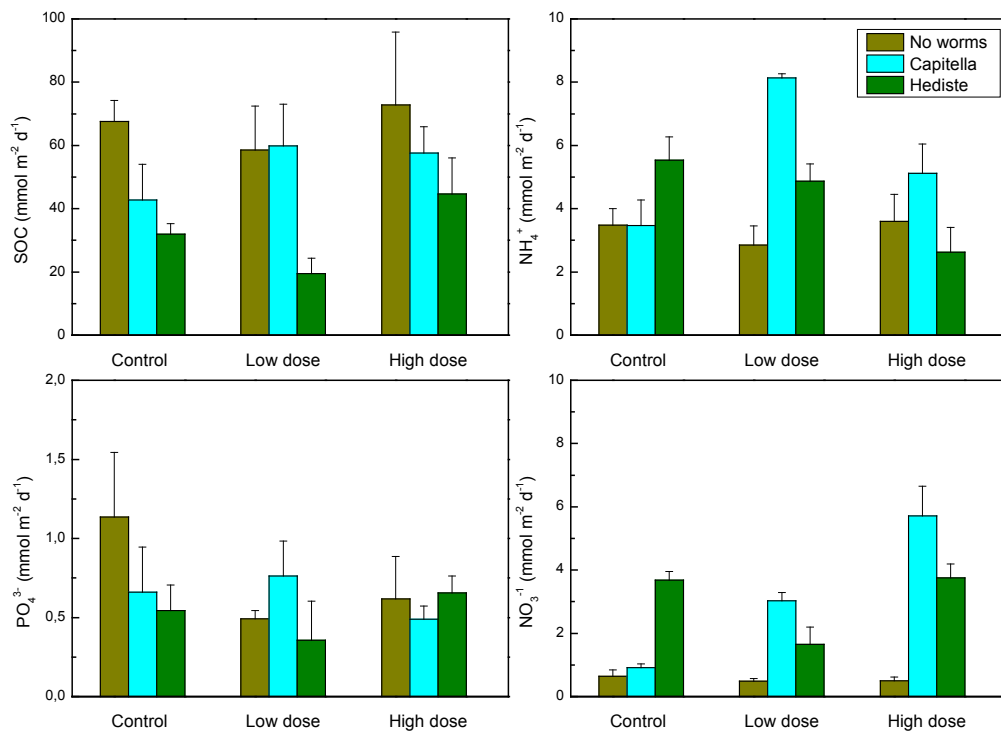


Fig. 4 Sediment oxygen consumption and nutrient fluxes 1 day after adding different doses of mussel fecal pellets (Low or High) to sediment cores in Controls (without polychaetes) and with *Capitella* or *Hediste* added. (n=3±STDEV)

Table 3. Results of 2-way ANOVA of fluxes in Fig. 4. Supplementary statistics in Suppl. Table 2

| Response Variable | df | SS | F | P | Tukey HSD |
|-------------------------------------|----|-----------|---------|---------|------------|
| Oxygen fluxes | | | | | |
| <i>dose</i> | 2 | 1996316.2 | 4.3230 | 0.0293* | L<H |
| <i>worm</i> | 2 | 8508073.0 | 18.4241 | <.0001* | Hed<Cap |
| <i>dose*worm</i> | 4 | 2018597.4 | 2.1856 | 0.1118 | |
| Error | 18 | 4156121 | | | |
| NH ₄ ⁺ fluxes | | | | | |
| <i>dose</i> | 2 | 19127.1 | 11.5266 | 0.0006* | L>H |
| <i>worm</i> | 2 | 40082.9 | 24.1552 | <.0001* | No<Hed<Cap |
| <i>dose*worm</i> | 4 | 64997.8 | 19.5848 | <.0001* | |
| NO ₃ ⁻ fluxes | | | | | |

| | | | | | |
|------------------|---|---------|----------|---------|------------|
| <i>dose</i> | 2 | 26127.4 | 42.7475 | <.0001* | L<H |
| <i>worm</i> | 2 | 69585.7 | 113.8504 | <.0001* | Cap,Hed>No |
| <i>dose*worm</i> | 4 | 48925.2 | 40.0237 | <.0001* | |
| PO42- fluxes | | | | | |
| <i>dose</i> | 2 | 512.9 | 2.7791 | 0.0887 | |
| <i>worm</i> | 2 | 411 | 2.2271 | 0.1367 | |
| <i>dose*worm</i> | 4 | 1134.7 | 3.0740 | 0.0429* | |
| | | | | | |

There were no consistent effects of mussel fecal pellets on the temporal development of SOC over 3 days after the OM addition. There was a significant effect of time ($p < 0.0005$), but there was significant interaction with both fauna and fecal treatment ($p < 0.0001$). In the No fauna treatments SOC increased with time, whereas with the polychaetes there was a tendency that maximum was reached after 4-20 h and decreasing afterwards. The ammonium flux showed no significant differences among times, but significant effects of fauna and fecal treatment ($p < 0.03$) and significant interaction between them ($p = 0.009$) were observed. In the control (No fauna) cores the ammonium flux was higher in the High Dose treatments, whereas an opposite pattern was observed for the *H. diversicolor* with lower efflux in High dose and decreasing with time. There was no effect of OM or time for the *Capitella* treatment. Phosphate was taken up by the sediments, with highest uptake in *H. diversicolor* with OM addition and in Control with no OM, although this was not significant. There were no effects of OM or time in *Capitella*. Addition of *H. diversicolor* and OM increased the efflux of nitrate, but rates declined with time. The opposite pattern was found in the Control with no OM, and there was no effect of OM or time for the *Capitella*. Statistical results are shown in Supplementary Table 2.

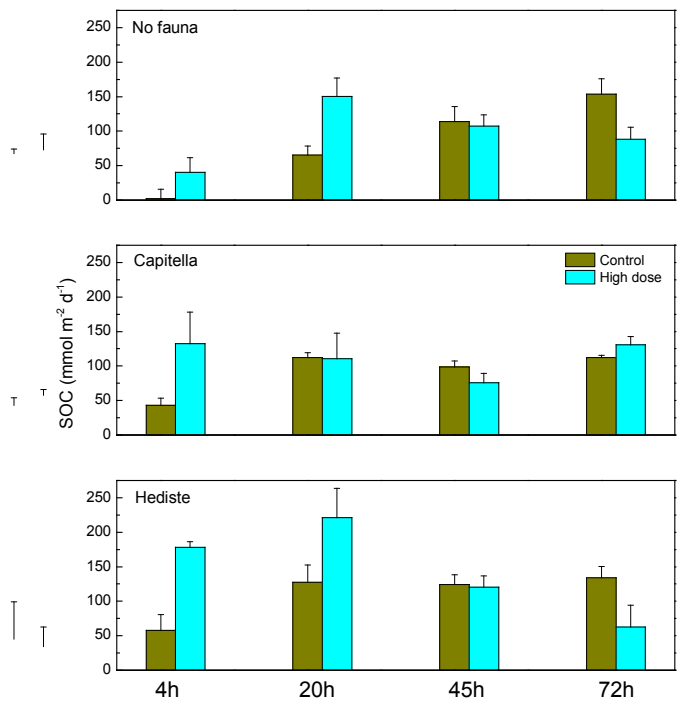


Fig. 5 Temporal changes in sediment oxygen consumption in treatments with high dose of mussel fecal pellets compared to no addition in sediment cores without polychaetes (Control) and with *Capitella* or *Hediste* added. (n=3±STDEV)

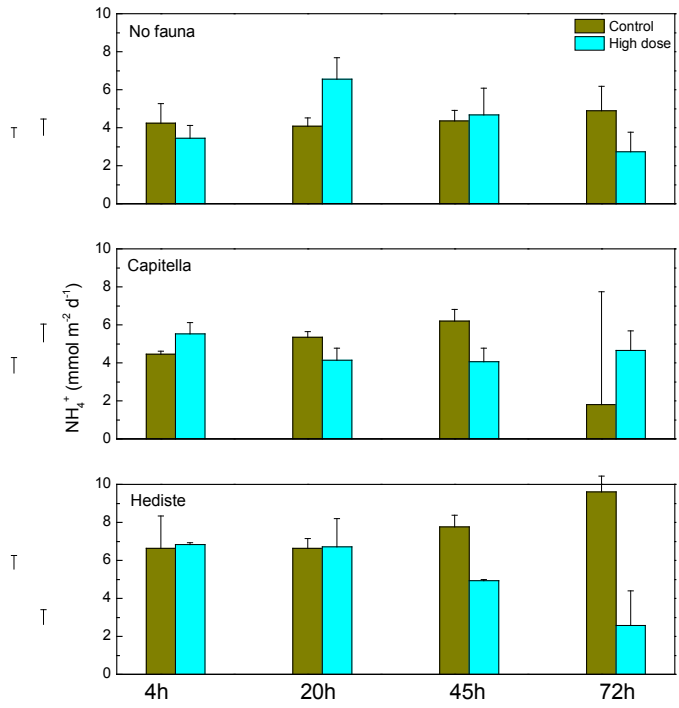


Fig. 6 Temporal changes in ammonium flux in treatments with high dose of mussel fecal pellets compared to no addition in sediment cores without polychaetes (Control) and with *Capitella* or *Hediste* added. ($n=3 \pm \text{STDEV}$)

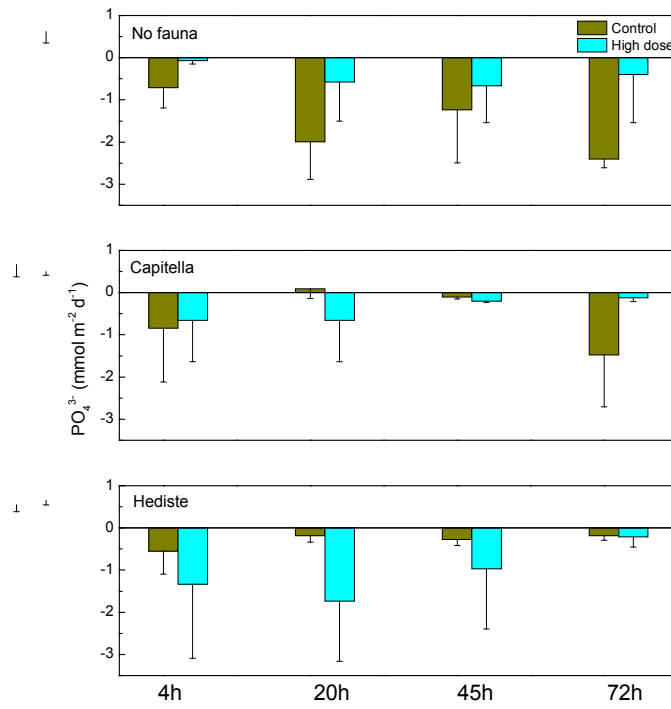


Fig. 7 Temporal changes in phosphate flux in treatments with high dose of mussel fecal pellets compared to no addition in sediment cores without polychaetes (Control) and with *Capitella* or *Hediste* added. (n=3±STDEV)

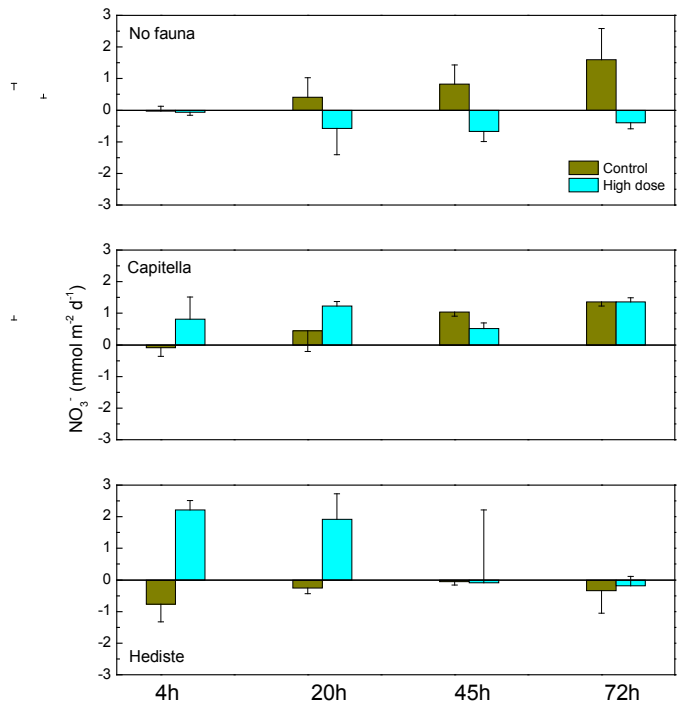


Fig. 8 Temporal changes in nitrate flux in treatments with high dose of mussel fecal pellets compared to no addition in sediment cores without polychaetes (Control) and with *Capitella* or *Hediste* added. ($n=3 \pm \text{STDEV}$)

Table 4. Total sediment oxygen consumption, *H. diversicolor* respiration (estimated from Banta et al. 1999) and microbial metabolism at the field site and in the laboratory experiment. Microbial metabolism was estimated as whole system respiration – macrofaunal respiration. (n=3±STDEV). For the laboratory experiment, the mean SOC (4-72 h) values were used. Fauna-induced stimulation represents the contribution of macrofaunal and enhanced microbial respiration, respectively, relative to benthic metabolism in the No fauna cores. n.a.= not available

| Station | Treatment | Metabolism (mmol m ⁻² d ⁻¹) | | | Fauna induced stimulation (%) | |
|------------------------|-----------|---|------------|-----------|----------------------------------|-----------|
| | | SOC | Macrofauna | Microbial | Macrofauna | Microbial |
| Mussel farm | No fauna | 42.9±24.7 | 0 | 42.9 | - | - |
| | H25 | 75.7±9.6 | 2.2 | 73.5 | +5 | +71 |
| | H100 | 93.4±49.3 | 8.7 | 84.7 | +20 | +97 |
| | H200 | 182.6±61.8 | 17.4 | 165.2 | +41 | +285 |
| Reference | No fauna | 52.8±n.a. | 0 | 52.8 | - | - |
| | H25 | 38.0±13.9 | 1.8 | 36.2 | +3 | -31 |
| | H100 | 39.7±20.8 | 7.3 | 32.4 | +14 | -39 |
| | H200 | 44.1±24.6 | 14.6 | 29.5 | +27 | -44 |
| Laboratory | | | | | | |
| No fauna | Control | 56.8 | - | 56.8 | - | - |
| | High dose | 56.3 | - | 56.3 | - | - |
| <i>H. diversicolor</i> | Control | 64.6 | 8.8 | 55.8 | +15 | -2 |
| | High dose | 84.9 | 10.5 | 74.5 | +19 | +32 |

Discussion

In the field, the farm sediments were affected by the organic enrichment and had higher oxygen consumption and larger efflux of ammonium compared to the reference sediments. The addition of the polychaetes increased the oxygen consumption, which could either be due to respiration by the polychaetes or from stimulation of the microbial processes (Banta et al. 1999, Heilskov and Holmer 2003). Polychaete respiration was not measured in this study, but by using literature values (Banta et al. 1999), this suggests that the increase in sediment oxygen consumption was to some extent (5-41%) due to polychaete respiration, whereas the microbial respiration was stimulated up with up to 285% compared to control sediments. This is a much higher microbial stimulation compared to what is observed in sediments enriched with organic matter in the form of dried yeast (labile organic matter), where the microbial respiration was only enhanced up to 89% (Banta et al. 1999). This increase is also much higher compared to fish farm sediments, where the microbial rates were already at maximum (Heilskov and Holmer 2003), suggesting that polychaetes has larger potential for positive effects in mussel farm sediments compared to other types of organic enrichments. *H. diversicolor* is an omnivore species and may consume the fecal pellets directly, removing organic matter from the sediment surface and thereby reducing the risk of oxygen depletion on the sediment surface. Their bioturbation effects may stimulate the bacteria in the deeper parts of the sediment by introducing oxidized compounds and organic matter from the sediment surface into the deep sediment (Kristensen et al. 2012). This mixing of sediments increase the overall decomposition of organic matter, although sulphate reduction may be an important microbial decomposition pathway (Heilskov et al. 2006). The bioirrigation of the sediments may be sufficiently strong to oxidize the sulfide pools maintaining a suitable environment for the polychaetes. The addition of *Capitella* also increased the sediment oxygen uptake in similar order of magnitude as the low densities of *H. diversicolor*. The mechanisms may be the same as for *H. diversicolor*, but there are no data available for *Capitella* respiration at the applied density and it is therefore not possible to estimate the microbial stimulation by *Capitella* addition. There was large variability in the sediment fluxes both at the farm and the reference site, and the potential effects by *Capitella* may be less due to the smaller size of the worms and less bioirrigation activity, as observed in fish farm sediments (Heilskov et al. 2006).

The lack of effects of polychaetes addition on the nutrient fluxes is in contrast to other studies, where for instance a direct relationship between bioirrigation activity and efflux of ammonium has been found (Heilskov et al. 2006). The bioirrigation activity was less than expected in this experiment (data not shown), suggesting limited impact of the polychaetes on the advection of dissolved nutrients at this

time of the year and under the *in situ* conditions. This could be attributed to the high efflux of ammonium during summer months in Limfjorden as a result of high pore water pools of ammonium (Carlsson et al. 2009), creating a steep concentration gradient between pore water and overlying water column and driving the efflux to the water column independent of the fauna. In contrast to the ammonium fluxes, the phosphate fluxes were low and directed into the sediments and there were no clear effects of fecal pellets or polychaetes on the fluxes. Previous studies have shown high efflux of phosphate during summer months in Limfjorden due to release of phosphate from the iron-bound pools (Holmer et al. 2003, Carlsson et al. 2009). The limited flux of phosphate into the sediments in the present study could reflect an oxidation of the sediment surface and binding of phosphate to iron independent of the fauna activity. The nitrate fluxes were in similar order of magnitude as found previously in Limfjorden (Carlsson et al. 2009), and the addition of *Capitella* and low numbers of *H. diversicolor* resulted in enhanced uptake of nitrate, which may be consumed in denitrification (for *Hediste*) or used in dissimilatory nitrate reduction (for *Capitella*) as has been found in mussel farm sediments previously (Carlsson et al. 2012). In the other treatments, nitrate fluxes showed no clear patterns, and were generally released from the sediments to the overlying water column possibly as a result of nitrification in the oxidized surface layers. The effects of polychaetes on nutrient fluxes is complex and has to be viewed in context of the biogeochemistry of the sediments and the seasonal dynamics in the physical, chemical and biological conditions.

Similar to the sediment oxygen consumption, the sediment sulphide pools were affected by *H. diversicolor*, which significantly decreased the AVS pools and increased the CRS pools in the sediments. *Capitella*, on the other hand, had no effect or caused only a slightly increase in the AVS pools. This is consistent with other findings, where *Capitella* maintain the sediments in a reduced stage, whereas *H. diversicolor* increase the oxidation of the sediments through their bioturbation activity and thereby decrease the AVS pools (Banta et al. 1999, Heilskov et al. 2006). The AVS pools in the Mussel farm sediments were, however, still higher compared to the Reference sediments after the 5 weeks of polychaete presence, and longer time is probably needed to reduce the AVS pools to reference levels. The AVS pools have been accumulating for several years, probably since mussel production was initiated at the farm. The CRS pools were lower in the Mussel farm sediment compared to Reference, indicating that mussel fecal pellet deposition affects the balance between AVS and CRS with a shift towards AVS. The AVS pools include the pore water sulphides and high AVS pools are consistent with the findings of higher pools of pore water sulphides in mussel farm sediments (Carlsson et al. 2009). Overall, the results suggest that addition of *H. diversicolor* to Mussel farm sediments improve the sediment conditions making it suitable for colonization of a diverse fauna.

In the laboratory experiments, the addition of fecal pellets to sediments generally affected the sediment oxygen consumption and nutrient fluxes similar to the field observations in the Mussel farm sediment. When comparing the short term effects of fecal addition on the sediment oxygen consumption, there was only a limited effect of the polychaetes and of fecal dose, with a decline in the presence of *H. diversicolor*. In contrast, the N fluxes were enhanced upon addition of *H. diversicolor* and *Capitella*, whereas there was only a limited effect on P fluxes. Short term (hours to days) temporal changes in fluxes were found reflecting a rapid response to biodeposits in sediments underlying mussel farms (Carlsson et al. 2010). Generally nitrogen fluxes were enhanced when *H. diversicolor* was present showing higher efflux of ammonium and nitrate both in sediments with and without organic matter addition. Addition of fecal pellets to *H. diversicolor* sediments enhanced the initial nitrate efflux, and fluxes nitrate and ammonium decreased with time, suggesting that *H. diversicolor* has immediate effect on the release of nitrogen from the fecal pellets either by physical disturbance of the pellets or through ingestion followed by excretion. *H. diversicolor* also stimulated the uptake of phosphate into the sediments in the organic matter added cores, probably due to oxidation of the surface layers and binding of P to oxidized iron, as observed at the field site (although not enhanced by fauna in the field). The oxygen consumption showed a maximum after 20 hours, which is consistent with findings of fecal pellet degradation in the water column and on the sediment surface, where bacterial colonization enhances the oxygen consumption in fecal pellets within the first day of release from the mussels (Carlsson et al. 2010). The presence of *H. diversicolor* enhanced the sediment oxygen uptake, although to a smaller extent (14-50%) compared to field (76-323%). This was primarily caused by lower microbial stimulation, probably reflecting the difference in time scales between lab and field experiments (days versus weeks).

The effects of *Capitella* on sediment fluxes were lower compared to *H. diversicolor* and more similar to the no-fauna sediments. The largest effects were found immediately after the addition of organic matter, where nitrogen efflux was enhanced, whereas there were no effects on sediment oxygen uptake suggesting limited effect on organic matter decomposition by the presence of *Capitella*. This is different from the observations in the field and compared to other studies with *Capitella*. The results may be caused by lower microbial stimulation, as discussed above for *H. diversicolor* or due to the low density of worms used here. Other studies were conducted with much higher density (typically $> 10.000 \text{ m}^{-2}$) and thus generating higher bioturbation activity (Heilskov et al. 2006).

In conclusion, both the field study and the laboratory experiment show that *H. diversicolor* enhance the decomposition of freshly-produced mussel fecal pellets, either by direct consumption of the pellets or by their bioturbation activity, stimulating the microbial activity in the sediments. The effect occurs right after the pellets reach the sediments (within the first day), and the stimulated decomposition

metabolism is maintained under field conditions. The nutrient fluxes are also affected by the presence of *H. diversicolor*. Nitrogen is released from the sediments right after deposition in a short pulse, whereas phosphate under the present conditions is bound in the sediments. It can thus be expected that the organic matter content of the sediments with polychaetes added will be lower compared sediments where no worms are added, although this was not possible to detect in this relatively short field experiment. Increasing C:N ratio in the sediments suggest a transformation of the organic matter to less labile composition. Combined with the observations in the field of improved sediment conditions due to lower sulphide pools, it is concluded that sediments with *H. diversicolor* have higher assimilative capacity of organic matter compared to sediments dominated by *Capitella* or devoid of benthic infauna. The stimulation of organic matter decomposition increased with increasing density of *H. diversicolor*, and the density can probably be increased to the double or even more, corresponding to natural densities in estuarine sediments. The effects of *Capitella* were less pronounced and no increase in organic matter decomposition or decrease in sediment sulphides could be detected suggesting limited effect on assimilative capacity of amendments with *Capitella* at this relatively low density. Further studies are needed to examine the population dynamics of *H. diversicolor* in mussel farm sediments, including their growth and survival under field conditions.

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Suppl. Table1. Supplementary results of 2-way ANOVA on surface pools of AVS and CRS (0-2 cm). Levels not connected by same letter are significantly different.

| AVS (0-2 cm) | | |
|----------------------|-------------------|----|
| Worms*station | Least Square mean | |
| Level | | |
| C40,MF | 22.97 | A |
| H200,MF | 22.22 | A |
| H100,MF | 21.61 | A |
| No,MF | 17.43 | AB |
| H25,MF | 7.08 | BC |
| H200,REF | 4.01 | C |
| H25,REF | 3.34 | C |
| C40,REF | 2.96 | C |
| H100,REF | 2.94 | C |
| No,REF | 1.26 | C |

| CRS (0-2 cm) | | |
|--------------|-------------------|----|
| Worms | Least Square mean | |
| Level | | |
| H100 | 82.80 | A |
| H25 | 73.71 | AB |
| C40 | 68.44 | AB |
| H200 | 60.76 | BC |
| No | 48.23 | C |

CRS (0-2 cm)

| Worms*station | Least Square mean | |
|----------------------|-------------------|----|
| Level | | |
| H100,REF | 127.54 | A |
| C40,REF | 97.10 | AB |
| H25,REF | 94.07 | B |
| H200,REF | 69.35 | BC |
| No,REF | 68.11 | BC |
| H25,MF | 53.35 | CD |
| H200,MF | 52.16 | CD |
| C40,MF | 39.80 | CD |
| H100,MF | 38.05 | CD |
| No,MF | 28.36 | D |

Suppl. Table2. Supplementary results of 3-way ANOVA on time-series of sediment fluxes

| Sediment oxygen fluxes | | | | |
|-------------------------------|----|-----------|---------|--------------------|
| Source | df | SS | MS | F ratio |
| Model | 23 | 89807963 | 3904694 | 6.1416 |
| Error | 48 | 30517259 | 635776 | Prob > F |
| C. Total | 71 | 120325222 | | <.0001* |

| Effects time | Nparm | df | SS | F ratio | Prob>F |
|---------------------|-------|----|----------|---------|---------|
| Time | 3 | 3 | 13651806 | 7.16 | 0.0005* |
| Dose | 1 | 1 | 2400021 | 3.77 | 0.0579 |
| Worm | 2 | 2 | 5298135 | 4.17 | 0.0214* |
| Time*Worm | 6 | 6 | 22417261 | 5.88 | 0.0001* |
| Time*Dose | 3 | 3 | 30016294 | 15.74 | <.0001* |
| Dose*Worm | 2 | 2 | 1257412 | 0.99 | 0.3794 |
| Dose*Worm*Time | 6 | 6 | 14767034 | 3.87 | 0.0031* |

| Ammonium fluxes | | | | |
|------------------------|----|---------|--------|--------------------|
| Source | df | SS | MS | F ratio |
| Model | 23 | 2466601 | 107244 | 1.7502 |
| Error | 48 | 2941278 | 61277 | Prob > F |
| C. Total | 71 | 5407879 | | 0.0511 |

| Effects time | Nparm | df | SS | F ratio | Prob>F |
|---------------------|-------|----|--------|---------|---------|
| Time | 3 | 3 | 468212 | 0.25 | 0.8576 |
| Dose | 1 | 1 | 307574 | 5.01 | 0.0297* |
| Worm | 2 | 2 | 814603 | 6.65 | 0.0028* |
| Time*Worm | 6 | 6 | 119767 | 0.33 | 0.9202 |
| Time*Dose | 3 | 3 | 158013 | 0.86 | 0.4685 |
| Dose*Worm | 2 | 2 | 635530 | 5.19 | 0.0091* |
| Dose*Worm*Time | 6 | 6 | 384293 | 1.05 | 0.4083 |

Phosphate fluxes**Source**

| | df | SS | MS | F ratio |
|----------|----|--------|------|--------------------|
| Model | 23 | 56786 | 2469 | 2.0045 |
| Error | 48 | 59121 | 1232 | Prob > F |
| C. Total | 71 | 115907 | | 0.0212* |

| Effects time | Nparm | df | SS | F ratio | Prob>F |
|---------------------|-------|----|-------|---------|---------|
| Time | 3 | 3 | 4475 | 1.21 | 0.3158 |
| Dose | 1 | 1 | 747 | 0.61 | 0.4397 |
| Worm | 2 | 2 | 5412 | 2.20 | 0.1222 |
| Time*Worm | 6 | 6 | 10725 | 1.45 | 0.2151 |
| Time*Dose | 3 | 3 | 5775 | 1.56 | 0.2106 |
| Dose*Worm | 2 | 2 | 21611 | 8.77 | 0.0006* |
| Dose*Worm*Time | 6 | 6 | 8039 | 1.09 | 0.3832 |

Nitrate fluxes**Source**

| | df | SS | MS | F ratio |
|----------|----|--------|------|--------------------|
| Model | 23 | 60047 | 2611 | 2.7702 |
| Error | 48 | 45238 | 942 | Prob > F |
| C. Total | 71 | 105285 | | 0.0015* |

| Effects time | Nparm | df | SS | F ratio | Prob>F |
|---------------------|-------|----|-------|---------|---------|
| Time | 3 | 3 | 4450 | 1.57 | 0.2079 |
| Dose | 1 | 1 | 9178 | 9.74 | 0.0031* |
| Worm | 2 | 2 | 6598 | 3.50 | 0.0381* |
| Time*Worm | 6 | 6 | 13973 | 2.47 | 0.0366* |
| Time*Dose | 3 | 3 | 7067 | 2.50 | 0.0707 |
| Dose*Worm | 2 | 2 | 8635 | 4.58 | 0.0151* |
| Dose*Worm*Time | 6 | 6 | 10148 | 1.79 | 0.1202 |



Havmeter Land

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Climate, water and spatial planning in collaboration

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Our results are useful for decision makers, planners, researchers and managers of natural resources.

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Digging worms for remediation of sediments impacted by mussel farms

Mussel farms affect the biogeochemical conditions in the sediments underneath the farms due to organic enrichment by mussel biodeposits and by mussels falling of the long-lines.

In this study, we conducted a number of field and laboratory studies using the organic tolerant polychaete, *Capitella* sp., and the less tolerant but larger polychaete *Hediste diversicolor*.

The results showed that *H. diversicolor* may be a strong candidate for remediation of mussel farm sediments.



Hav möter Land

The project Hav möter Land brings together 26 municipalities, regions, universities and governmental agencies from Sweden, Norway and Denmark. We work together on climate, water and spatial planning for the Kattegat and the Skagerrak. Our results are useful for decision makers, planners, researchers and managers of natural resources. Climate change alters the conditions for our life and livelihood here. We obtain collective knowledge for collective preparedness. The European Union is helping to finance the project.

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