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Effects of Long-line Mussel Farming on Sediment Nitrogen Mineralisation; Reaction Rates and Pathways

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Abstract

Benthic nitrogen mineralisation underneath long-line mussel farms was studied at three locations around Mollösund on the Swedish West Coast. Organic matter mineralisation rates and nitrogen mineralisation pathways were compared for farm-affected sediment and unaffected reference sediment (references situated 100m away from the farm).

Organic matter mineralisation rates, measured as ammonium pore-water production in anoxic incubations were significantly higher in farm-affected sediment as compared to reference.

Sediment oxygen consumption and ammonium fluxes in farm-affected sediments were shown to be between 3-7 and 17-200 times higher than at the corresponding reference site respectively.

The sediment at the site that seemed to be most affected by farm derived organic matter was totally reduced and patches of the sulphur oxidising bacteria *Beggiatoa spp.* were present on the sediment surface. Denitrification was largely suppressed, the trend being that the favoured pathway for nitrate reduction was dissimilatory nitrate reduction to ammonium.

At the two other farm-affected sites mineralisation pathways resembled those found at reference sites. Mineralisation rates were elevated but denitrifying bacteria were still actively producing N₂, although denitrification rates seemed to be somewhat suppressed.

Impacts from organic matter enrichment induced by mussel farms in the underlying sediment differed substantially between the investigated areas, despite similar conditions concerning age and stock size. Findings presented here indicate that nutrient removal through extensive mussel farming could be achieved without changing nitrogen mineralisation pathways, but there will be an impact on the benthic environment. The depth beneath the farm and water column current velocities are proposed as factors controlling sedimentation rates, and thereby the impact on benthic nitrogen mineralisation below farms.

Introduction

Background

The marine nitrogen cycle is a complex yet interesting and important field to study. Since nitrogen generally limits the growth of photosynthetic organisms in marine and estuarine environments, primary production is in part controlled by the nutritional status in the system (e.g. Herbert, 1999; Cloern, 2001). The size and quality of the primary production will have an impact on the whole ecosystem since it is the base of the food web, i.e. directly and indirectly feeding all heterotrophic organisms. Large anthropogenic inputs of nutrients causing excess phytoplankton production (eutrophication) change the abundance as well as the species composition of primary producers (Herbert, 1999; Cloern, 2001). The effects of anthro-

pogenically induced eutrophication of estuaries, fjords and coastal waters has received public attention only the last decades, even though the process has been going on since the beginning of the twentieth century (Cloern 2001). A large amount of bioavailable nitrogen is introduced to these systems through e.g. runoff from agricultural activities, fossil fuel combustion and outlets from municipal wastewater treatment plants. Large-scale mussel farming has been suggested as one way to reduce plankton biomass and thereby reduce the amount of bioavailable nutrients in coastal areas.

Eutrophication

The effects of increased nutrient supply in coastal waters are many and diverse, depending on physical, chemical and biological factors within the system. Generally; eutro-

phication means a larger phototrophic biomass, which feeds heterotrophic organisms, resulting in a larger biomass as a whole (Cloern, 2001). A larger biomass alters the prerequisites for life within the system. These changed conditions can lead to more or less extreme environmental effects. E.g. as phytoplankton stocks increase, water transparency decreases. Less penetration of light to benthic plants shrinks their habitat and less light demanding species could take their place. The growth of phytoplankton and fast growing pelagic macro algae is promoted while rooted plants and benthic algae are suppressed, changing not only species composition, but also the oxygen production distribution pattern from benthic to pelagic (Herbert, 1999; Cloern, 2001). A change in primary producer abundance and diversity directly and indirectly affects several marine animals by e.g. disappearance of natural habitats and/or food sources. This may have an impact on species composition among animals as well as plants, and possibly alter the whole ecosystem (Pihl et al., 1999; Cloern, 2001). Increased primary production increases sedimentation, causing larger inputs of organic matter to the sediment. The amount and quality of deposited material affects the microbial community and solute distribution patterns in the sediment. Thus, eutrophication may not only change sediment biology, but also have an impact on sediment biogeochemistry.

Benthic decomposition of organic material

Shallow coastal sediments are important sites for the mineralisation of organic material. Microbial communities in marine sediments are essential to the biogeochemical cycling of nutrients. The microbes energy-gaining oxidation of organic compounds decompose sediment debris, and release nutrients to the surrounding environment e.g. the water column. In this sense they perform the process responsible for sustaining primary production (Herbert, 1999). Benthic fauna (where present) also plays an important role in diagenetic processes. Bioturbation and digestion of organic matter enhances transport of solutes produced and

utilised in microbial metabolism (Brune et al., 2000).

Microbial respiration of organic material consumes oxidants (e.g. oxygen and nitrate) and regenerates nutrients. Since oxygen is produced through photosynthesis, the sediment will only be oxygenated at the surface (if enough light for photosynthetic benthic plants or algae reaches the sea floor). Sediments without photosynthetic organisms are oxygenated through a downward transport of oxygen (diffusion and bioturbation). Since organic material is continuously deposited, oxygen has to be continuously supplied or the sediment will become anoxic.

Organic matter mineralisation proceeds even during anoxic conditions, but with other electron acceptors than O_2 in respiration. Aerobic respiration is just one of several possible metabolic pathways utilised by microorganisms. In fact alternative respiratory processes proceed parallel to the aerobic. Different microbial species use different oxidants, though some are able to use several electron acceptors. The most common oxidants utilised include: O_2 , NO_3^- , $Mn(IV)$, $Fe(III)$ and SO_4^{2-} . Conceptual models of mineralisation suggest that the oxidant supplying the highest free energy yield will be consumed first. Oxygen is the most favourable e^- acceptor, while sulphate will give the lowest yield of free energy in the order listed above. When sulphate is respired hydrogen sulphide is the major by-product (Aller, 1982; Sherr and Sherr, 2000). If the sinking organic material is easily degraded (high reactivity) and the accumulation rates are high, the bottom water could become anoxic or hypoxic accompanied by sulphide release from the sediment to overlying water. This sort of event, known as dystrophic crisis, suffocates benthic fauna. Since sulphide and anoxia is lethal to all multi-cellular fauna, decomposition is left to anaerobic microbes (Herbert, 1999; Cloern, 2001).

Microbial nitrogen mineralisation

Benthic nitrogen mineralisation is a complex matrix of microbial processes performed by a range of different organisms under completely different environmental

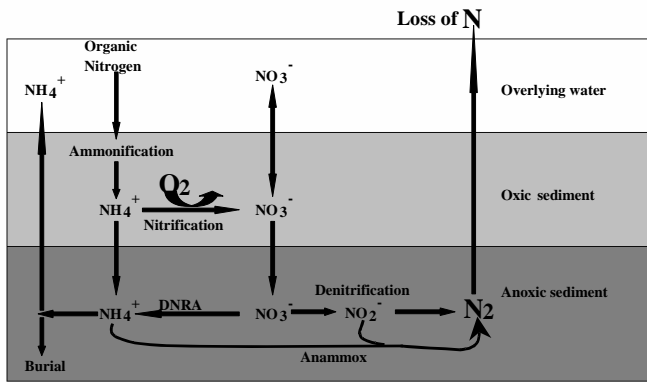


Fig. 1 a) Nitrogen mineralisation pathways in a well oxygenated sediment: Organic nitrogen deposited onto the sediment is mineralised through several different steps. The nitrification process in the oxic zone supplies anaerobic denitrifiers with substrate. The N_2 -producing pathways denitrification and anammox removes bioavailable nitrogen.

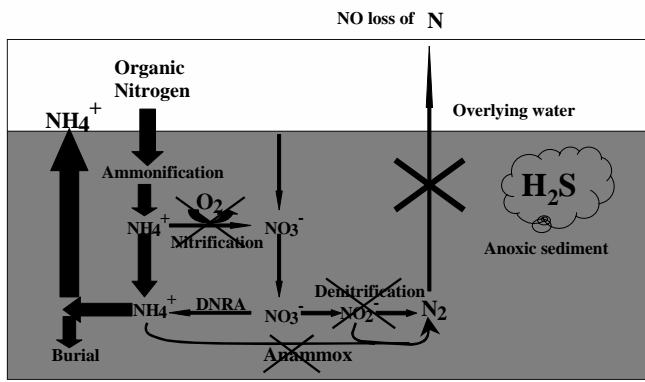


Fig. 1 b) Nitrogen mineralisation pathways in an organically enriched anoxic sediment: When the organic loading is high and the oxygen supply rate is lower than the consumption rate, the sediment becomes anoxic. Nitrification is shut off by oxygen deficiency and since the only nitrate source is diffusion from overlying water, denitrification rates are low. In case of hydrogen sulphide production the N_2 -producing pathways denitrification and anammox are inhibited and the ammonium production (DNRA and ammonification) is increased, stimulating primary production.

conditions (fig. 1 a) and b)). The major sources of dissolved inorganic nitrogen (DIN) in shallow (5-50m) marine ecosystems are fluvial inputs and solutes originating from the sediment water interface. It is possible to estimate mineralisation rates of nitrogenous compounds by measuring benthic fluxes of DIN. DIN fluxes are dependent on deposition rate, bioturbation and the reactivity of the accumulating material (Herbert 1999). Many aerobic bacteria are facultative nitrate respirers; i.e. they use nitrate as terminal electron acceptor when oxygen is depleted. There are several possible end products, depending on which species or process being responsible for the nitrate respiration. Dissimilatory nitrate reduction could produce dinitrogen gas, nitrous oxide, ammonia or nitrite. When nitrate is dissimilatively reduced according to: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$, the process is called denitrification (fig. 1 a)). Denitrification is a very important microbial process since it turns bioavailable nitrogen (NO_3^-) into, almost, biologically inert molecules (NO , N_2O and N_2) (Sherr and Sherr, 2000). Nitrogen removal from the marine ecosystem is to a large extent dependent on denitrification.

Since denitrification is an anaerobic process one would expect anoxic sediments to be an optimal state. However, as mentioned

above, anoxic conditions are known to stimulate sulphate-reducing bacteria. The produced sulphide is suggested to inhibit denitrifying bacteria, although these complex processes are not yet fully described (Joye and Hollibaugh, 1995). Denitrifying bacteria needs nitrate supplied by nitrification, an aerobic process, and therefore depend on sediment oxygen penetration depth and the increased solute transport induced by benthic fauna that determines the size of the nitrifying community as well as the amount of nitrate they produce (Brune et al., 2000). Since nitrification demands ammonium, nitrifiers need to live close to ammonifying organisms (Ward, 2000).

Ammonification occurs throughout the pelagic and benthic environments, but ammonium rarely accumulates in oxidised environments since it is nitrified and also the preferred nitrogen source for phytoplankton as well as bacteria (Newell, 2004; Hulth et al., 2005). In the anoxic part of the sediment, there is an accumulation of NH_4^+ since nitrification is absent and extensive organic matter mineralisation sustains a high ammonium production. The optimal zone for nitrifiers is therefore in the oxic-anoxic interface where oxygen is present and NH_4^+ diffuses from the anoxic part of the sediment (Ward, 2000). Bioturbation by macro fauna increase

the area of oxic-anoxic interfaces and can thereby stimulate denitrification in the sediment.

In recent years an alternative microbial pathway for N_2 -production has been described; anammox (anaerobic ammonium oxidation by nitrite). Anammox is an autotrophic strictly anaerobic process in which N_2 is produced without producing any of the greenhouse gases CO_2 or N_2O . Anammox has been shown to be a very important source of N_2 -formation in some environments, sometimes constituting as much as 67% of total N_2 -production. The anammox pathway appears to be less important in eutrophic systems, since it has been reported to be sensitive to organic enrichment (Thamdrup and Daalgaard, 2002; Engström et al. 2005).

Since denitrification is closely linked to nitrification, for both processes to occur the sediment needs to be oxic as well as anoxic (fig. 1 a)). Coupled nitrification-denitrification is only achievable in the oxic-anoxic interface and a very shallow or absent oxic layer shuts off nitrification, and denitrifiers has to rely on nitrate diffusing from the overlying water column, which is generally slower (Capone, 2000) (fig. 1 b)). In case of H_2S -production coupled nitrification-denitrification is inhibited (Joye and Hollibaugh, 1995) and the major nitrate-reducing pathway will be dissimilatory nitrate reduction to ammonium (DNRA) (fig. 1 b)). This implies that the amount of organic material deposited on the sediment surface could change the routes and rates of nitrogen mineralisation, and since nitrogen removal is the aim with nutrient-removing mussels their effect on benthic nitrogen fluxes is very important.

Mussels and mussel farming

It has been suggested that the culturing and harvest of the blue mussel (*Mytilus edulis*) can be a way to reduce the amount of bioavailable nutrients in coastal areas (Lindahl et al., 2005). Mussels are filter feeders, and as such they clear the water from phytoplankton and other organic particles, collecting the nutrients bound in the phyto-

plankton tissue and incorporating it into their own. In a sense they are concentrating nutrients and when harvested it is possible to withdraw nitrogen and phosphorus from the sea. Estimations made indicates that one ton harvested mussels will remove ~ 36 kg C, $\sim 8,3$ kg N and $\sim 0,5$ kg P from the system (Loo and Petersen, 2004).

When mussels feed they do not only concentrate nutrients into their tissue, they also produce faeces and pseudo-faeces and excrete ammonia nurturing their food-source phytoplankton. The faecal pellets and mussels falling off the lines are concentrated beneath the farm. This debris consumes oxygen when decomposed. This could change mineralisation rates and pathways as mentioned above. But, one of the great advantages with suspended mussel cultures is the possibility to geographically relocate a farm if there is an unwanted impact on the benthic environment. The amount of biodeposition beneath a mussel farm is usually several times higher than what an average sediment surface receives (Dahlbäck and Gunnarsson, 1981). Mussel faeces also contain more C, N and P than what the sediment usually is made up of (Loo and Petersen, 2004). This changes the chemical environment for microorganisms in the sediment, and it may have an impact on nitrogen mineralisation rates and pathways.

Objectives

This study was a part of a larger research project with the aim to investigate possibilities of mussel farms as a sink for anthropogenically released nutrients in the coastal waters of western Sweden. In this project 17 mussel farms will be set up outside Lysekil. When harvested the mussels will be used for human consumption and chicken feed or as an organic fertiliser, closing an agriculture-aquaculture-cycle. The efficiency of nutrient removal and associated environmental effects of mussel farming will be evaluated. Further development of nutrient-removing mussel farms could include creating a nutrient trade system (Lindahl et al., 2005). This could be environmentally as well as economically beneficial.

Since the main objective in the Lysekil project is to remove nutrients from the marine environment (nitrogen being the most important element), the effects of farming activities on benthic nitrogen mineralisation will be studied in a time series, from establishment and three years ahead.

This study is a part of the pilot study for the time series surveillance plan of the benthic nitrogen cycling beneath farm sites. As such it has been its main objective to clarify whether farm-induced particulate organic material (POM) loads affect benthic nitrogen mineralisation, and if so; in what way. This was achieved through experiments with sediment from beneath three sites commercially farmed for 10-15 years. A range of variables relevant to nitrogen mineralisation in the farm sediment was compared with sediment from reference sites. The hypothesis that nitrogen mineralisation routes and rates would differ between reference sites and farm-affected sites was tested and evaluated.

Materials & Methods

Investigated areas

In the current study (March 2005), sediment was sampled from three areas at six different sites around the island of Lyr at farms managed by the Scansford™ mussel farming company in Mollösund, Orust, on the Swedish West Coast (Fig. 2). Three long-line mussel farms (F1, F2 & F3) were studied where sediment was collected in the centre of the farm. For each mussel farm a reference site (R1, R2 & R3) was also studied, located ~100m outside the farm where no farm-effects are found (L-O Loo, pers. comm.). All three areas were situated in-shore and moderately exposed to waves and currents. The tidal amplitude in the area ranges from 0-20cm. *In situ* environmental conditions are displayed in Table 1.

Sampling

Sediment was collected with an Olausen box-corer. Plexiglas cores (Ø10cm) were

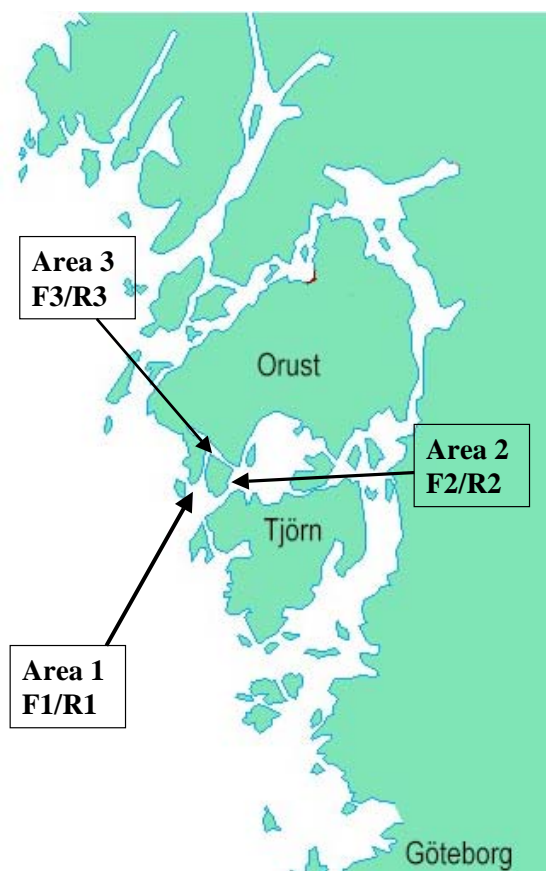


Fig. 2: Sampling sites, F=farm, R=reference

inserted and intact sediment cores could be collected from the box-corer, sealed with bottom plates and lids and brought to the laboratory. These cores were used for oxygen and nutrient (NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$, Si and PO_4^{3-}) flux-measurements.

From the box-corer, surface (~0-2cm) sediment was retrieved and sieved (1mm) to remove macro fauna. The sieved sediment was stored in 25 litre plastic containers with lids during transport to the laboratory. Samples were stored in 8°C until start of experiment.

Bottom water was sampled for *in situ* oxygen and nutrient concentration analyses. Samples for *in situ* nutrient analyses were filtered (0,45µm, cellulose acetate), and stored frozen (-20°C) immediately upon arrival at the laboratory. *In situ* oxygen samples were immediately precipitated and analysed within 24h.

Oxygen and nutrient flux measurements

The intact sediment cores were allowed to settle ~1h (after sampling and transport) in contact with the atmosphere and in constant temperature (8°C). Once settled a continuous flow of seawater (35m, 8°C, from the Gullmar Fjord) was applied to each tube securing oxic conditions similar to the natural ones.

Sealing the cores with lids started the incubations. During incubation, mixing of the overlying water was achieved by rotating (40rpm) Teflon-coated magnetic stirring-bars attached to the lids, receiving momentum from external motorised magnets.

Samples from the overlying water in the cores was taken out in a time series (six times, $t=0-t=5$), each time ~50ml was withdrawn (the exact volume recorded) and replaced by refill-water with known oxygen and nutrient concentrations. Refill-water consisting of filtered (0,3µm) sea water (35m, from the Gullmar Fjord).

Incubation times were determined from oxygen consumption in cores. The oxygen concentration was never allowed to fall by more than 20%.

The overlying water was analysed with respect to O₂ and nutrient concentrations. O₂ samples were taken in 12ml gas tight glass vials (Exetainer, Labco, High Wycombe, UK) immediately precipitated and analysed within 48h. Nutrient samples were stored in 9ml plastic vials, frozen (-20°C) until analysis.

Oxygen concentrations were determined by modified Winkler titration (Svensk Standard, 1975) and nutrient concentrations with an automatic analyser (TRAACS 800 Bran+Luebbe, Germany) using standard colorimetric methods (Strickland and Parsons, 1972).

Anoxic jar-incubations; mineralisation rate estimations

Sieved (1mm) surface (0-2cm) sediment was homogenised and distributed to 60ml polypropylene centrifuge tubes (jars). The jars were top-filled with sediment and sealed.

Jars were placed in plastic zip-lock bags, which were flushed with N₂, and sealed. All handling was performed in an N₂-atmosphere (glove-bag). Bags were placed in anoxic mud (8°C) to secure totally anoxic incubations according to procedure in Hansen et al., (2000) and Hulth et al., (1999). Anaerobic microbes decomposing the organic material in the jars changes the concentration of dissolved NH₄⁺ and PO₄³⁻. Thus measuring NH₄⁺ and PO₄³⁻ concentrations at several times produced a time series estimating mineralisation rates, i.e. potential microbial decomposing activity. Each time, triplicate jars were centrifuged (3000rpm in 25min). The supernatant was filtered (0,45µm, cellulose acetate) and stored frozen in 9ml plastic vials, later analysed for NH₄⁺ and PO₄³⁻ content (TRAACS). Reversible adsorption of ammonium to negatively charged mineral particles and/or POM was estimated. The fraction of adsorbed NH₄⁺ was desorbed by the addition of KCl. 20ml of sediment was shaken with 30ml 2M KCl in a centrifuge tube, then centrifuged and supernatant was treated as above. Ammonium production rates were corrected by multiplying with the factor (1+K), where K is the linear adsorption coefficient according to Mackin and Aller (1984).

Anoxic jar-incubations; measurements of denitrification- and anammox-potential with ¹⁵N-species

A set-up similar to the mineralisation rate estimations (above) was used, but ¹⁵N-species was added to the sediment. Three treatments were used in parallel, i) ¹⁵NH₄⁺, ii) ¹⁴NO₃⁻ + ¹⁵NH₄⁺ and iii) ¹⁵NO₃⁻, and the production of ²⁹⁻³⁰N₂ in pore water was detected with GC-MS (Thamdrup and Daalgaard, 2002). Nitrogen species utilised in the three treatments was mixed with sieved (1mm), homogenised surface (0-2cm) sediment to ~50µM final concentration respectively. Jars were then filled, sealed, packaged and incubated as above. Denitrification and anammox in the jars changes the concentration of N₂. Thus the measuring of ²⁹⁻³⁰N₂ concentrations at several times produced a time series. The i) and ii) treatments were only to serve

as control jars (analysed at $t=0$), thus rationalising experimental conditions to contain a complete time series only from the iii) treatment. Each time triplicate jars were centrifuged (3000rpm in 25min). 2ml of the supernatant was transferred with a gas-tight syringe to a 6ml Exetainer containing 0,1ml 50% $ZnCl_2$. The Exetainer was previously flushed with He (creating an anoxic atmosphere) and the $ZnCl_2$ was used to stop microbial processes in the sample. Samples were later analysed for $^{29-30}N_2$ with an isotope ratio GC-MS (Roboprep G⁺ on-line with a TracerMass, Europa Scientific, Crewe, UK).

Sediment characteristics

Sediment porosity was determined from the weight loss after drying 5ml wet sediment to constant weight at 60°C. Sediment used for analysis was from 0-2cm and sieved (1mm).

Carbon and nitrogen content was determined from the sediment dried for porosity measurements. The analysis was performed on a Fison Instruments NA 1500 NC, Elemental Analyzer.

Results & Discussion

Effects of biodeposition from mussel farms have been subject of several studies e.g. Dahlbäck and Gunnarsson (1981); Chamberlain et al. (2001). The more specific effects from organic enrichment from cultured mussels on nitrogen mineralisation have been studied as well (Christensen et al., 2003; Newell, 2004), often with different conclusions concerning ecosystem effects.

The effect on benthic nitrogen cycling imposed from organic matter derived from mussel farms is determined by a range of biogeochemical and physical variables, e.g.; water depth and current velocities. This implies that effects could differ substantially in different sites and seasons.

Bottom water chemistry

The *in situ* conditions at the studied areas reflect the differences and similarities between sites. The values of *in situ* bottom water variables measured here (tab. 1) represents normal average values at the area this time of the year (Bohuskustens Vattenvårdsförbund, web site).

As can be seen from table 1, bottom water oxygen concentrations do not seem to be affected by biodeposition from farms. There is, however, considerable differences in O_2 concentrations between the three areas; the oxygen concentrations at the second area (F2/R2) is $\sim 100\mu M$ lower than at the first (F1/R1) and third (F3/R3) area respectively. Salinity was not measured, but since oxygen solubility is related to both salinity and temperature, this difference is probably due to water column stratification, where the second (deeper) site probably is situated below the pycnocline and the two other above. This depth related effect can also be seen in nitrate + nitrite, silica and phosphate concentrations. The bottom water ammonium concentrations do, however, indicate effects from the farms. This could be due to ammonium release from anoxic sediments or excretion from feeding mussels (e.g. Newell et al., 2004). At the first area, the NH_4^+ concentration is elevated in bottom water from below the farm compared to reference site.

Table 1: *In Situ* environmental properties. (* = not detectable, $n=2$)

Station	Depth/Temp (m/°C)	[O ₂] (μM) \pm SE	[NH ₄ ⁺] (μM) \pm SE	[NO ₃ + NO ₂] (μM) \pm SE	[SiO ₃] (μM) \pm SE	[PO ₄ ³⁻] (μM) \pm SE
F1	10 / 4,2	355,8 \pm 0,5	2,00 \pm 0,51	1,53 \pm 1,08	0,68 \pm 0,08	*
R1	10 / 4,2	358,6 \pm 0,4	0,63 \pm 0,00	0,66 \pm 0,00	0,53 \pm 0,08	*
F2	15 / 4,7	271,3 \pm 1,5	0,98 \pm 0,12	5,50 \pm 0,02	2,46 \pm 0,11	0,13 \pm 0,02
R2	15 / 4,7	260,1 \pm 0,6	1,59 \pm 0,22	8,84 \pm 1,41	3,83 \pm 0,19	0,43 \pm 0,13
F3	9 / 3	362,9 \pm 0,9	0,78 \pm 0,08	0,49 \pm 0,17	0,42 \pm 0,11	*
R3	8 / 3	365,6 \pm 1,6	0,69 \pm 0,18	1,18 \pm 0,35	0,57 \pm 0,27	*

This difference is not statistically significant (students t-test; $p > 0,05$; $n=2$), neither are there any significant trends in data (concerning bottom water ammonium concentrations) from the other areas.

Sediment chemistry

When visually inspected, the sediment at station F1 appeared to be strongly reduced all the way to the surface and patches of sulphur oxidising *Beggiatoa spp.* was visible. At the other two farm-affected sites a thin oxic layer was observed. At the reference sites the sediment appeared to be well oxygenated. These observations imply a general effect on sediment reactivity from farm debris deposited.

Mussel faeces have a higher C:N ratio than their planktonic food source, mussel tissue generally have a lower C:N ratio than their food. Thus, mussels are literally concentrating nitrogen, and the sediment below farms would therefore become impoverished in nitrogen as compared to carbon content, i.e. show elevated C:N ratios (Kautsky and Evans, 1987; Christensen et al., 2003). The same result (elevated C:N ratios) is the effect from microbial degradation of POM (Libes, 1992), hence the C:N ratios are often used as an indication of POM reactivity, with lower ratios being more reactive and more easily biodegraded.

The C:N ratios for the first area are significantly higher (students t-test; $p=0,003$, $n=3$) in farm-affected sediment compared to reference (tab. 2). This is most likely the result of sinking mussel faeces since the mineralisation rate at F1 is slower than at the other farm sites (fig. 4). The other two areas do not show any significant differences in

C:N ratios. Christensen et al. (2003) demonstrated differences in C:N ratios between farm-affected and unaffected sediments, possibly due to higher sedimentation rates, weaker bottom currents or slower mineralisation.

F1 seems to be most affected by a high supply of organic matter with the highest total content of carbon and nitrogen in the sediment. Carbon and nitrogen content is significantly higher in all farm-affected sediments when compared to corresponding reference sites (students t-test; $p < 0,05$; $n=3$), except for nitrogen content in the first area where no significant trends were found. This indicates that the second and third areas are organically enriched, implying a general effect from farming activities.

Oxygen and nutrient fluxes

Fluxes of oxygen and nutrients over the sediment-water interface (figs. 3 and 4) further support the indications of increased organic matter mineralisation rates beneath farms. These data also points out the protrusion of values from the first area compared to the second and third. The oxygen consumption in farm-affected sediments was significantly higher when compared to its corresponding reference site for all three areas sampled (students t-test, $p < 0,01$; $n=5$).

Christensen et al. (2003) compared sediment from two bays in New Zealand, one with several long-line mussel farms and one (reference) with prospected farm sites. Christensen and co-workers (2003) found oxygen consumption rates in reference sites to be ~ 650 , and farm site consumptions were $\sim 1700 \mu\text{mol m}^{-2} \text{h}^{-1}$, corresponding to a ~ 3 times higher consumption at farm sites. Dahlbäck and Gunnarsson (1981) estimated the oxygen consumption rate in farm-affected sediments to be $\sim 2500 \mu\text{mol m}^{-2} \text{h}^{-1}$. These results are in reasonable agreement with findings in this study (fig. 3), where oxygen consumption in farm-affected sediments is between ~ 3 and 7 times higher than at the corresponding reference site.

Table 2: Sediment characteristics ($n=3$)

Station	C:N-ratio (mol:mol) \pm SE	N-content (mg $\text{g}_{\text{drysed}}^{-1}$) \pm SE	C-content (mg $\text{g}_{\text{drysed}}^{-1}$) \pm SE
F1	11,40 \pm 0,20	6,00 \pm 0,06	58,63 \pm 1,49
R1	9,85 \pm 0,15	6,07 \pm 0,09	51,20 \pm 0,26
F2	10,54 \pm 0,12	5,23 \pm 0,03	47,23 \pm 0,50
R2	10,64 \pm 0,17	4,87 \pm 0,09	44,33 \pm 0,20
F3	10,60 \pm 0,37	5,70 \pm 0,26	51,87 \pm 3,75
R3	10,73 \pm 0,17	3,77 \pm 0,18	34,67 \pm 2,09

Since nitrate and nitrite (fig. 4) are used as oxidants in bacterial respiration, these chemical species are primarily consumed in the sediment (i.e. negative fluxes since consumption exceeds production). There are no significant trends in nitrate data, but the pattern is still concise with higher consumption rates in farm-affected sediment. The apparent peak in nitrate consumption at F1 coincides with a large ammonium flux from the sediment, indicating that DNRA probably is the favoured nitrate consumption route.

Elevated ammonium fluxes from sediments beneath farms is of great concern. Since ammonium-N is in the preferred oxidation state (-III) for assimilation by primary producers, increased release would actually nurture primary production (Newell, 2004; Hulth et al., 2005). Results from this study (fig. 4) shows that significantly more ammonium is released from farm-affected sediments compared to corresponding reference

sites (students t-test, $p < 0,01$; $n=5$). Ammonium fluxes from farm-affected sediments are from ~ 17 up to 200 (average ~ 30) times higher than from unaffected reference sediments. In a similar study (Christensen et al., 2003) the corresponding result is also ~ 30 times, demonstrating reasonable agreement.

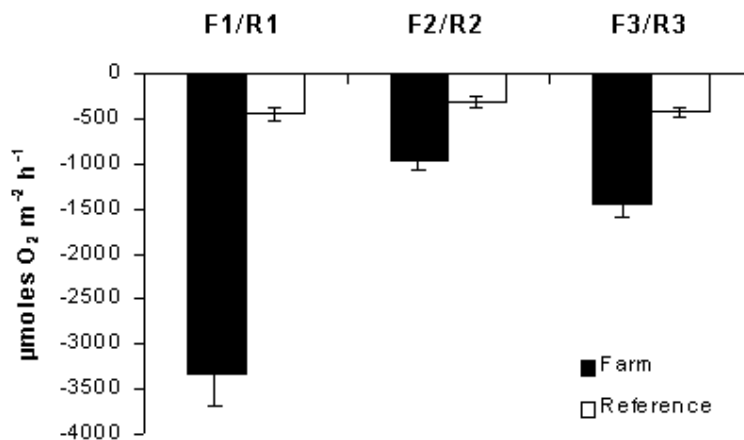


Fig. 3: Sediment-Water oxygen fluxes from whole-core sediment incubations. Negative values indicate oxygen fluxes into the sediment (Error bars indicate SE, $n=5$, F=farm; R=reference)

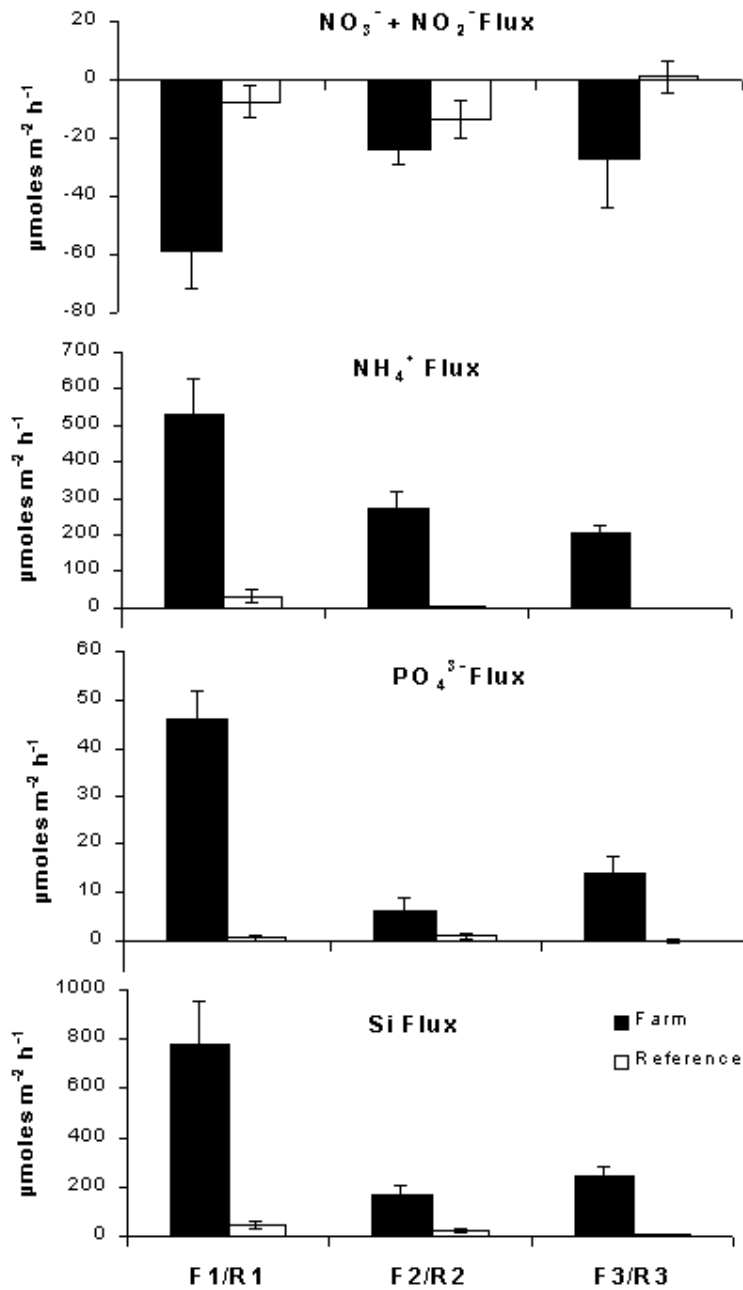


Fig 4: Sediment-water nutrient fluxes from whole-core sediment incubations. Negative fluxes are directed into the sediment, while positive are directed out from the sediment (Error bars indicate SE, $n=5$; F=farm; R=reference)

The phosphate and silica fluxes (fig. 4) are also significantly higher from farm sediments compared to reference, with one exception; the F2/R2 phosphate flux does not show any significant trend. Nitrogen mineralisation is not directly affected by the re-

lease of phosphate and silica, but the similarities between ammonium, phosphate and silica fluxes further supports the indices of increased nutrient release from farm-affected sediments, all in all stimulating primary production.

Organic matter mineralisation rates

The increased supply of POM below mussel farms generates increased ammonium fluxes from the sediment (Dahlbäck and Gunnarsson, 1981; Chamberlain et al., 2001). Ammonium production rate measurements provide rate estimates of organic matter mineralisation, quantifying stimulated microbial activity in sediments affected by farm debris (Christensen et al., 2003; Newell, 2004).

Mineralisation rates were estimated from ammonium production rates in sediment pore water during anoxic incubations (fig. 5). Ammonium production rates at farm-affected sites were found to be significantly higher when all farm sites were compared to all references (students t-test,

$p < 0,05$, $n=3$). This implies that farm induced POM has a general effect on sediment organic matter mineralisation rates. This result was largely expected since mussel farming has been shown to increase sedimentation approximately three times (Dahlbäck and Gunnarsson, 1981). When testing within each area ammonium production rates were shown to be significantly higher at farm-affected sites in all three areas (students t-test, $p < 0,05$, $n=3$). These results also further supports the indications of increased nutrient release from farm-affected sediments shown earlier (fig. 4).

While solute production indicates mineralisation rates, pore water NH_4^+ start concentrations reveals natural sediment conditions. A high NH_4^+ start concentration indicates that ammonium production rates ex-

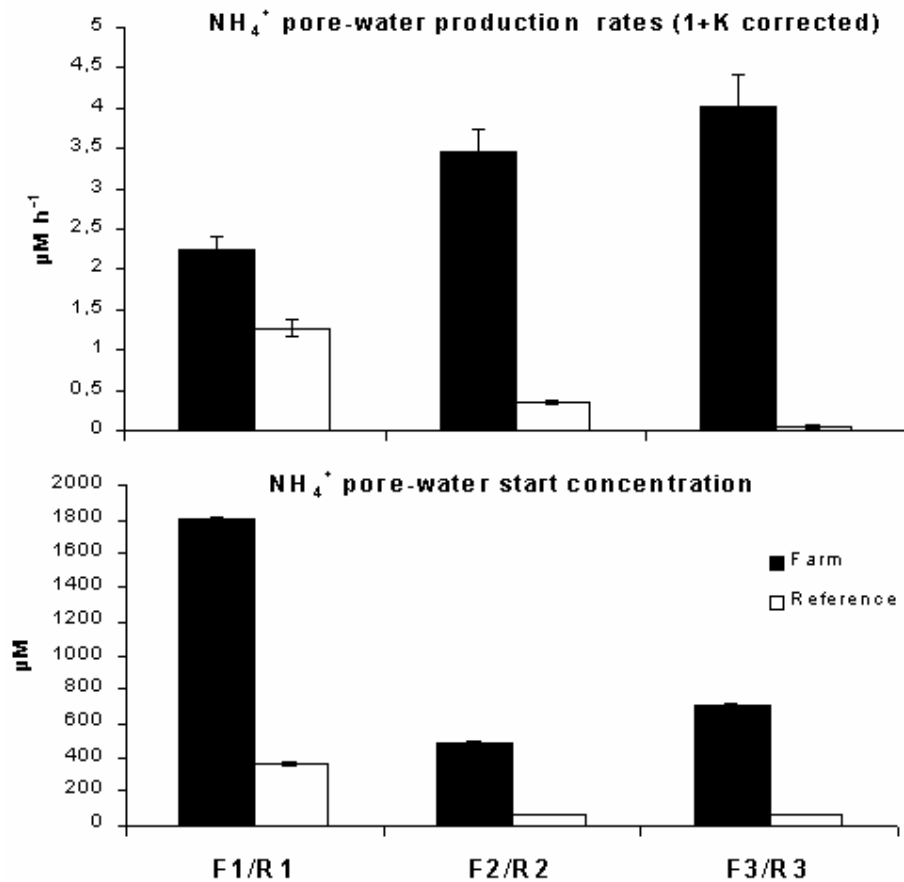


Fig 5: Ammonium production rates and start concentrations from anoxic jar incubations estimating mineralisation rates. Production rates are calculated from pore water concentration changes over time, $1+K$ is the term used to correct for reversible adsorption to particles. Pore water start concentration is the measured concentration at $t=0$ in the time series measuring production rate (Error bars indicating SE, $n=3$, F=farm; R=reference).

ceed consumption rates, i.e. the microbial degradation of organic matter is substantial.

Pore water start concentrations does not show any significant differences when all farm sites are compared to all references, but when concentrations in each farm sediment is compared to its corresponding reference there is a significant difference (students t-test, $p < 0,01$, $n=3$). This result indicates that there are large differences between areas, but increased biodeposition seems to affect ammonium pore water concentrations.

The slightly lower mineralisation rate at F1 could be due to the extreme concentration gradient against which ammonification at this site must occur ($\sim 1800 \mu\text{M}$ start concentration; fig. 5). Fig. 5 also shows higher pore water concentrations at the first reference site, in fact the $[\text{NH}_4^+]$ at R1 is almost on the same level as farm sites F2 and F3. The mineralisation rate at R1 appears to be slightly elevated as well which, again, implies that local natural differences in sedimentation rates and current velocities could, in part, explain differences in sediment organic matter mineralisation. This points out the importance of choosing the best location when a farm is to be established.

Denitrification- and anammox-potential

Figure 6 illustrates the potential denitrification rates in tested sediments. The controls for anammox activity did not indicate any activity (data not shown), thus the only

N_2 -producing pathway is denitrification. Furthermore, the results from figure 6 indicates lower denitrifying activities in farm-affected sediment, though there is no statistically significant difference when all farm sites are compared to all references (students t-test; $p > 0,05$, $n=3$).

The denitrifying community at the investigated sites does not seem to benefit from an increased POM load, even though denitrification is reported to correlate positively with the supply of reactive organic carbon to the sediment (Engström et al., 2005). The N_2 production at F1 is probably inhibited by sulphide (Joye and Hollibaugh, 1995), while the other farm sites seems to be less affected, still represented by denitrification rates similar to R1. Christensen et al. (2003), found denitrification rates in farm-affected sediments to be ~ 14 times lower than in reference sediments.

Results from other studies (Dahlbäck and Gunnarsson, 1981; Joye and Hollibaugh, 1995; Christensen et al., 2003) shows that organic enrichment in marine sediments increases oxygen consumption and stimulates sulphate-reducing bacteria producing sulphide. Sulphide appears to inhibit nitrification as well as denitrification, in particular uncoupling the two processes, thereby increasing the fraction of nitrate dissimilatively reduced to ammonium, increasing recirculation of bioavailable nitrogen. Increased POM loading in coastal sediments,

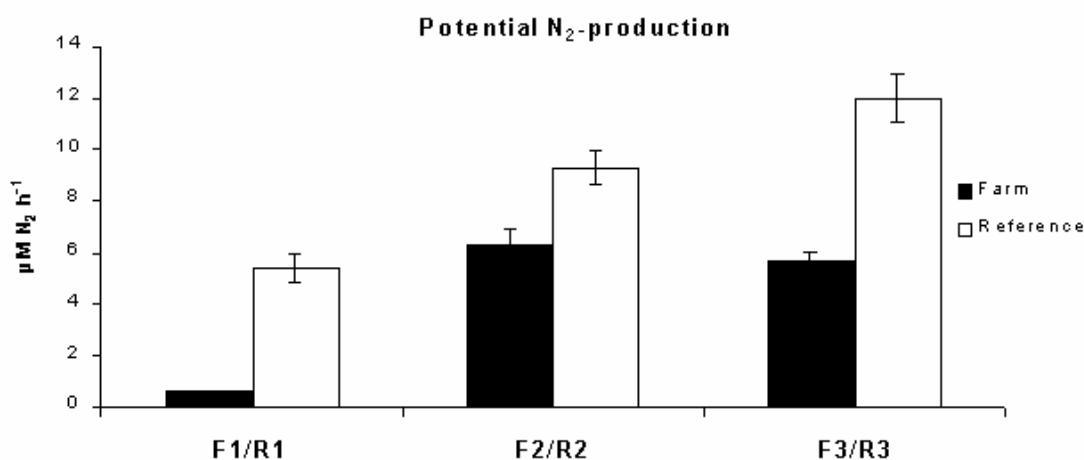


Fig 6: Potential N_2 -production from anoxic jar incubations with ^{15}N -species. N_2 -production is calculated from linear regression (Error bars indicate SE of linear fit, $n=3$; F=farm; R=reference).

changing routes of nitrogen mineralisation, regenerating nutrients utilised in primary production, could thus act as a positive feedback loop increasing eutrophication (Joye and Hollibaugh, 1995; Newell, 2004).

Implications on nutrient trade mussel farming

Effects on the benthic nitrogen cycle could substantially diminish nitrogen removal. It would be reasonable to compare sediment-water fluxes with respect to POM load per area unit (assuming linear mineralisation reactions). If, as according to Dahlbäck and Gunnarsson (1981), the sedimentation rate under the farms are ~ 3 times higher than at reference sites; then ammonium fluxes from these sediments should be ~ 3 times higher. Thus, comparing farm sites with references should include compensating for differences in sedimentation rates. In this study ammonium fluxes in average increased ~ 30 times, not ~ 3 , i.e. farm sites showing ~ 10 times higher ammonium fluxes when corrected for increased sedimentation!

Newell, (2004) states that increased nitrogen removal by denitrification will provide an additional ecosystem service beneath well managed mussel farms. The condition for this extra service is “fully oxygenated sediments”. There are no significant differences between farm and reference in fig. 6, but the absence of negative effects does not mean that there is a positive one. In order to claim that there is no effect the fraction of sedimenting POM denitrified at unaffected sites, should be similar to the one denitrified at farm-affected sites. Analogous to assumptions concerning ammonium fluxes, denitrification should have shown an approximate three-time increase. The lack of increase in N_2 production implies that denitrification rates in farm-affected sediments is at least three times lower than at reference sites when corrected for increased sedimentation. This doesn't necessarily mean that there is a large total effect on nitrogen mineralisation, since absolute denitrification rates are unknown in this study.

Furthermore, these calculations and assumptions are general in character since

actual sedimentation rates also are unknown, and some important variables (e.g. mussel faeces C:N ratio, effects on mineralisation routes and rates) are not accounted for. However, the results from this study do not indicate additional denitrification nitrogen removal from mussel farms, rather the contrary.

The farm where the least negative effects where shown, was F2. This was also the deepest site (15m). The depth below farms could be a key factor in organic matter distributions, since sinking distance probably is correlated to faecal dispersion (Chamberlain et al. 2001). Newell (2004) argues that deeper sites would show less nutrient recirculation and higher denitrification rates. Since deeper situated sediments will not be reached by enough photosynthetic active radiation to sustain algal growth, nitrifiers will not have to compete with benthic algae for NH_4^+ , thus a larger fraction of nitrogen would be permanently removed from the system as N_2 . I cannot present an explanation to the differences in impact found here, though faecal dispersion is one probable cause. However, lack of data concerning sedimentation rates and current velocities leaves this question unanswered.

Perhaps some of the more shallow sites would have shown less impacted if the study had been performed in summer, with light conditions allowing algal mat formations. Benthic algae have the capacity to sequester a large fraction of the released ammonia (Christensen et al. 2003; Newell, 2004). On the other hand sedimentation rates should be higher during the more productive season (Kautsky and Evans, 1987), possibly increasing impacts at all sites.

The observed differences between farm sites could perhaps be explained by differences in sedimentation rates (Chamberlain et al. 2001). There is probably some (site-specific) range, within which the sediment receives an acceptable load, exceeding this range could successively alter mineralisation routes. Outlining a “best practice” scheme for nutrient trade mussel farming involves finding the optimal range of organic enrichment, optimising nutrient removal. In order

to fully understand the cycling of nitrogenous compounds, a balanced budget needs to be established. Budget calculations would include the quantification of all nitrogen-transport across the sediment-water interface, including the fraction of particulate, dissolved and gaseous compounds. To achieve this further research is needed.

Finally, the possibility to geographically relocate a farm if there is a negative impact on the benthic environment should not be overlooked. This is one of the great advantages with suspended mussel cultures.

Conclusions

Results from this study shows that mussel farming has an effect on nitrogen mineralisation. It has been statistically inferred that mineralisation rates and thereby mobilisation of dissolved inorganic nutrients are increased from sediments underlying mussel farms. Furthermore, results indicate changes in mineralisation pathways, manifested by decreased denitrification rates accompanied by increased ammonium fluxes. It should, however, be noted that the magnitude of the impact is very different between sites, despite apparently similar conditions concerning age and stock size. This observation leads to the conclusion that it could be possible to optimise nitrogen removal, if the site-specific pre-requisites controlling POM dispersion and subsequent nitrogen mineralisation are known. In other words; a farm established at the right place and well managed might be less negative, or even positive, with respect to benthic nitrogen removal.

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