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In situ characterization of benthic fluxes and denitrification efficiency in a newly re-established mussel farm



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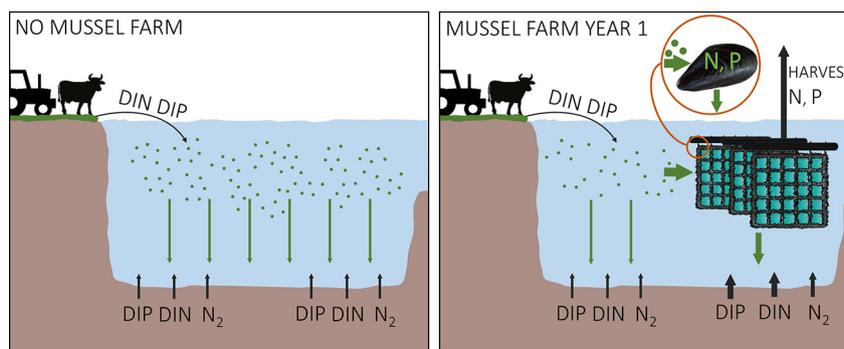
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HIGHLIGHTS

- Sediment impact from mussel farm were measurable during first year of establishment.
- Small rise in sediment DIN and DIP releases can approach removal at mussel harvest.
- Sedimentary denitrification efficiency was 49% lower compared to reference station.
- Benthic microalgae can be impacted under mussel farms.
- Initial changes are important when assessing environmental impact of mussel farms.

GRAPHICAL ABSTRACT



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ABSTRACT

Mussel farming has been proposed as a mechanism to mitigate eutrophication in coastal waters. However, localizing the intensive filtration of organic matter by mussels can cause a concomitant enrichment of organic matter in sediments below farms, which may influence biogeochemical processes and fates of nutrients in the system. In the context of mitigating eutrophication, it is important to quantify sedimentary changes induced at early life stages of mussel farms. Accordingly, this study investigated how a newly re-established mussel farm affected sedimentation rates, sediment characteristics, sediment-water solute fluxes and nitrate (NO_3^-) reduction rates (measured in situ) during the first year of production. Sedimentation rates were enhanced at the farm relative to a reference station, and both organic and inorganic carbon accumulated in the sediment with time. Increased organic matter input likely drove the slightly elevated sedimentary effluxes of ammonium (NH_4^+) and dissolved inorganic phosphorus (DIP) in the farm. Denitrification was the main NO_3^- reduction process, however, there was a relative increase in the remobilization of bioavailable nitrogen underneath the farm as dissimilatory nitrate reduction to ammonium (DNRA) rates were enhanced by >200% and the denitrification efficiency was 49% lower compared to the reference station. The sedimentary methane (CH_4) release tended to be higher at the farm, but fluxes were not significantly different from reference conditions. Low sedimentary pigment concentrations indicated a reduced presence of benthic microalgae at the farm, which likely influenced sediment-water solute fluxes. Over the production cycle, the release of dissolved inorganic nitrogen (DIN) and DIP underneath the farm were respectively 426% and 510% relative to reference conditions. Impacts of the mussel farm were thus

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measurable already during the first year of establishment. These immediate changes to the sediment biogeochemistry, as well as long-term effects, should be considered when estimating the environmental impact of mussel aquaculture.

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1. Introduction

High-density mussel cultivation is finding favor in nutrient management plans due to its potential to mitigate eutrophication, one of the largest threats to coastal environments (Diaz and Rosenberg, 2008; Fennel and Testa, 2019). Aquaculture is an important food source and was the fastest growing livestock sector in the world between 1990 and 2009 (Little et al., 2016), with mussel farming alone increasing about four times in volume over the last 40 years (Suplicy, 2018). Mussel farming is considered more sustainable than traditional fish farming as mussels feed on the algae and particles suspended in the water without the need of external food inputs (Suplicy, 2018).

By providing suspended hard substrate for mussel spat attachment and growth, mussel farms function as loci of particle filtration. A proportion of this organic material, with constituent nutrients, is assimilated in mussel somatic tissues. Harvest of mussels is thereby utilized as a means to extract nutrients from the marine environment and may prevent eutrophic ecological conditions (Kotta et al., 2020; Petersen et al., 2019). Mussel farming is used to reduce nutrient loads from internal sources in many coastal water bodies and estuaries, as well as from marine point sources (i.e. finfish culture). Furthermore, local particle depletion by mussel farming can reduce chlorophyll *a* concentrations, increase water transparency, and reduce basin-scale organic sedimentation (Timmermann et al., 2019).

It has been proposed, however, that the local impact of mussel farms on nutrient cycling might offset the benefits (Nizzoli et al., 2011; Stadmark and Conley, 2011). Sedimentation rates below farms can be up to a factor two to three times higher compared to reference stations in the same area (Carlsson et al., 2009; Hartstein and Stevens, 2005; Holmer et al., 2015). Like in other settings, the increased deposition of organic matter can lead to an increased release of dissolved inorganic carbon (DIC) and elevated oxygen (O_2) consumption rates in the sediment, followed by decreased sedimentary O_2 penetration depth (Brigolin et al., 2018; Christensen et al., 2003; Mirto et al., 2000) or even anoxia. As O_2 is depleted, anaerobic respiration processes take over, exemplified by increased sulfate reduction rates under many mussel farms (Carlsson et al., 2012; Dahlbäck and Gunnarsson, 1981; Holmer et al., 2015). Furthermore, the production of methane (CH_4) can increase in sediments with a high loading of organic matter (Egger et al., 2016). Yet there are few studies of CH_4 release from bivalve farms in general (Ray et al., 2019) and, to the best of our knowledge, no studies quantifying CH_4 release from sediments underneath mussel farms.

Changes in O_2 concentrations and redox conditions strongly impact the sedimentary nutrient cycling. Although denitrification may increase under mussel farms (Carlsson et al., 2012), nitrate (NO_3^-) reduction can also be diverted from denitrification to dissimilatory NO_3^- reduction to ammonium (DNRA, Christensen et al., 2003; Nizzoli et al., 2006). The result is a shift from removal (nitrous oxide (N_2O) and nitrogen gas (N_2) production) to recycling (ammonium (NH_4^+) production) of the bioavailable nitrogen within the system. This shift is believed to be a result of more reducing conditions underneath mussel farms, higher concentrations of organic matter and the presence of hydrogen sulfide; factors that favor DNRA over denitrification (Giblin et al., 2013; Hardison et al., 2015; Kraft et al., 2014). There are also observations of increased release of dissolved inorganic phosphorus (DIP, mainly phosphate) from sediments below mussel farms (Holmer et al., 2015; Nizzoli et al., 2011). Sedimentary iron(III)-oxides that adsorb DIP under oxic conditions are reductively dissolved in anoxic conditions, which enhances the DIP

release to the water column (Mort et al., 2010; Mortimer, 1941). Mussel farms can thus locally create sedimentary conditions that enhance nutrient recycling, and thereby hamper attempts to remove excess nutrients from coastal systems.

In shallow coastal systems, sediment-water nutrient fluxes can also be strongly regulated by benthic microalgae. The algae act as a filter by taking up nutrients produced in the sediment and thereby decrease the sedimentary nutrient release (Seitzinger et al., 2001; Sundbäck and Granéli, 1988). During photosynthesis, they also increase the O_2 penetration depth and thereby stimulate removal or retention of bioavailable nitrogen and phosphorus (Fenchel and Glud, 2001; Sundbäck and Granéli, 1988). Yet it is not clear how mussel farming affects benthic microalgae abundance and activity. Some studies have observed decreased abundance in benthic microalgae below farms (Christensen et al., 2003), while others report increases (Franzo et al., 2014; Mirto et al., 2000). Due to their important role as ecosystem engineers, changes in benthic microalgae abundance and activity could have large implications for nutrient cycling below mussel farms.

As mussel aquaculture is expanding, it is important to understand under what conditions, and to what extent, mussel farms influence the benthic environment. The effect of mussel farms on local sediment nutrient cycling can vary, seemingly dependent on factors such as hydrodynamics (Hartstein and Stevens, 2005) and general eutrophication status (i.e. ambient sedimentation rates) in the area (Carlsson et al., 2009). Another important aspect is the age of the farm (Carlsson et al., 2012; Franzo et al., 2014). Previous studies of sediment biogeochemistry underneath mussel farms have mainly focused on well-established farms, where years of impact have caused large changes in the sedimentary processes (e.g. Alonso-Pérez et al., 2010; Christensen et al., 2003; Nizzoli et al., 2006; although see exception in Holmer et al., 2015). Still, an understanding of how fast the sedimentary environment changes after establishment of a farm is required to accurately assess the environmental impact of mussel farms.

The goal of this study was to evaluate how a newly re-established mussel farm in As Vig, Denmark, affected the sediment-water nutrient exchange. To this end, we conducted in situ measurements of sediment-water fluxes and NO_3^- reduction rates below a mussel farm and at a reference station over a whole growing season, representative of the production cycle. This is to our knowledge the first study to characterize these processes in situ by measurements with a benthic chamber lander. We further measured sedimentation rates and examined sediment characteristics, to follow changes in the benthic environment over the growing season.

2. Materials and methods

2.1. Study area

As Vig is an exposed cove in eastern Jutland between the mouths of Horsens Fjord and Vejle Fjord (Fig. 1a). Sediments in the cove are mainly sand, muddy sand, or diamicton; the immediate study area is classified as sandy sediments (GEUS, 2015). Pertinent to nutrient compensation services, this region is host to many finfish aquaculture operations (*Oncorhynchus mykiss*).

An existing permitted mussel farming area was used as the experimental setup for this study. The farm was established in 2012 but was disused by 2014, before the beginning of this study in 2017. No cultivation units were present during the time the farm was disused. The 11.25 ha farm consisted of 40 tube-net mussel cultivation units

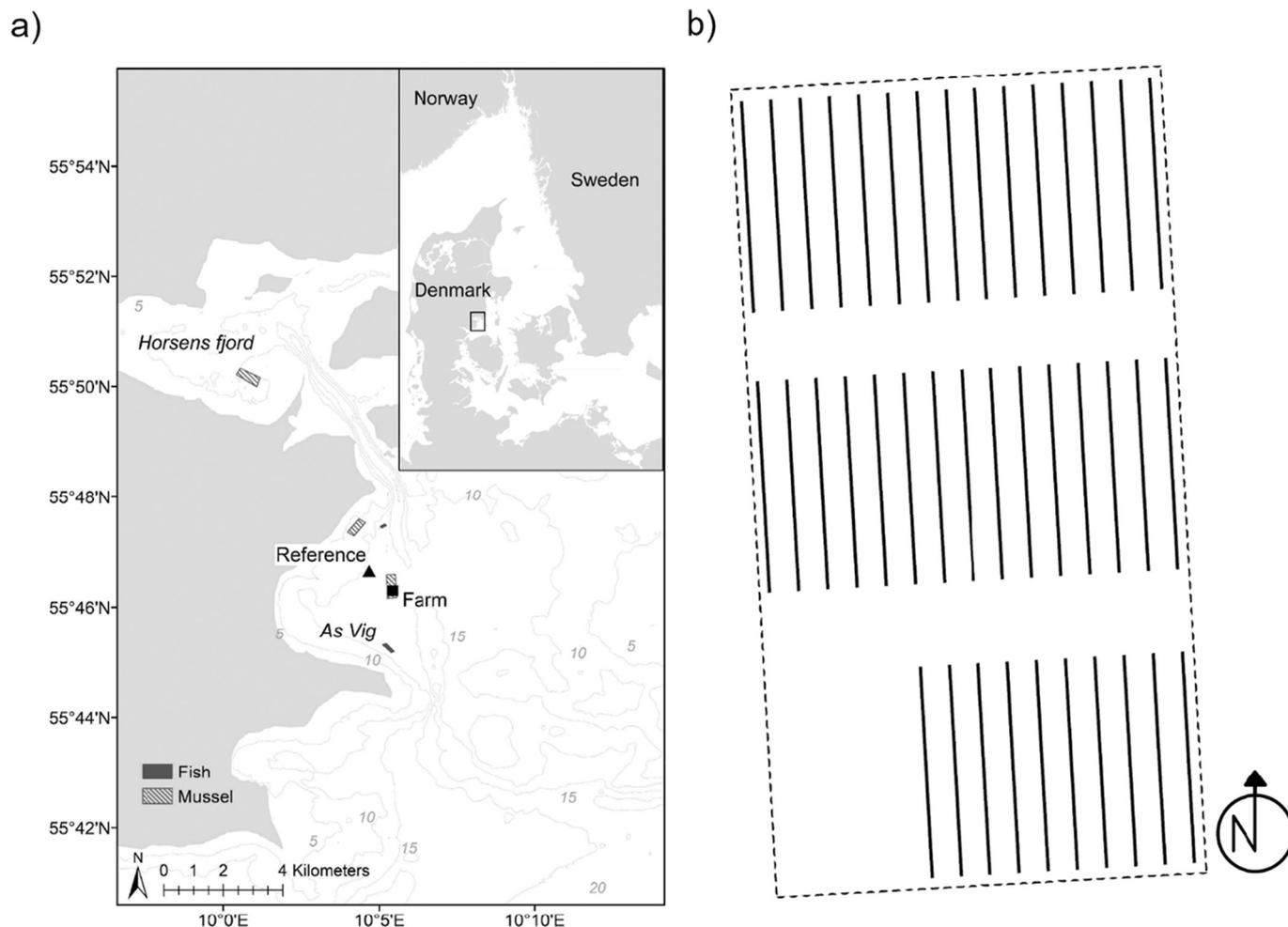


Fig. 1. Description of the study site. a) Location of stations and their relative placement in As Vig, Denmark. Other aquaculture sites in the area marked with solid (fish farms) or dashed (mussel farms) lines. b) Structure of the mussel farm. Solid lines represent cultivation units.

(SmartUnit, www.smartfarm.no), with 15 units each in the northern two sections and 10 units in the southern section (eastern half); each unit moored 10–20 m from each other, and each section was separated with a 30 m gap (Fig. 1b). These units serve the purpose of both wild mussel spat collection and on growth of attached mussels. Cultivation units were installed in the first week of June 2017, while the major settlement events occurred in October–November of 2017 and May 2018. Mussels were harvested at the beginning of November 2018 when the total harvestable mussel biomass was 729 ± 24.7 t, equivalent to 1406 mussels per m^2 (6.5 kg biomass m^{-2}). The total harvestable concentrations of nitrogen and phosphorus were 10,050 kg and 529 kg, respectively, calculated following methods described in Taylor et al. (2019) by assuming mussel nitrogen and phosphorus contents of 1.44 and 0.07% mussel wet weight, respectively.

The reference station was selected as it shared similar sediment characteristics, water depths, and current regimes as the farm (Table S1, Figs. S1–S3). It was further positioned contrary to predominant current directions, i.e. upstream from the mussel farm. The water depths were 9.5–10.5 m at the reference station and 10–12 m at the farm, varying by tides (generally <0.5 m), bathymetry, and barometric or wind-driven sea level in the Kattegat (Gustafsson, 1997). In order for the sites to reflect similar conditions with regards to depth, samplings in the farm were focused in the northern and mid parts, since the southeastern part was 1–1.5 m deeper. Four intensive sampling campaigns were carried out over the course of the study period (Table S1). A pre-settlement reference study was conducted on 26–30 June 2017; a post-settlement, high-growth period on 9–12 July 2018;

a pre-harvest biomass maximum period on 22–27 October; and a final post-harvest period on 18–26 February 2019 when the nets had been removed.

2.2. Water column conditions and sedimentation rates

Prior to any other work, CTD casts (EIVA Arop) were performed at 0.1 m depth intervals to profile the whole water column for salinity (conductivity), temperature, and chlorophyll *a* fluorescence. Illuminance (lux) was measured with pairs of sensors (HOBO Pendant Temperature/Light Data Logger 64 K) placed 0.5 m from the seafloor in both the farm and reference stations, logging every 15 min over the field campaigns. In July and October 2018 and February 2019, currents were measured with a 600 kHz Teledyne Marine Workhorse Sentinel acoustic Doppler current profiler (ADCP). Ensembles were collected every 30 s in 50 cm bins. At the farm, the ADCP was placed in the center of the farm, in between two nets.

Sedimentation rates were measured using twin tube (\varnothing 8.0 cm) sediment traps, equipped with a steering fin. Within the farm, two sets of traps were placed in between units of tube-nets (labelled 'Mid-farm'), and two sets were placed in close proximity to a net (labelled 'Net'). Two trap sets were simultaneously positioned at the reference station. Tubes were filled with filtered ($0.45 \mu m$) seawater on site from depth, and positioned 1.5 m above the seabed to avoid potential interference from resuspension of soft sediments. Traps were uncapped at depth by SCUBA diver and replaced with pre-filled capped tubes after 24–48-hour durations. Total particulate matter (TPM) was determined

by vacuum-assisted filtration of water samples through pre-dried Whatman GF/C glass microfiber filters (47 mm), which were dried at 80 °C for >48 h and weighed to the nearest 0.1 mg. The filters were then combusted in a muffle furnace at 500 °C for 4 h and weighed for determination of particulate organic matter (POM).

2.3. Sediment characterization and benthic microalgae

Sediment cores (\varnothing 9.5 cm) from the reference station and within the farm were captured by SCUBA diver or sub-sampled from a box corer. Triplicate cores were sectioned in intervals of 0.5 cm at 0–2 cm, 1 cm at 2–6 cm, and 2 cm at 6–10 cm depth. Porosity and dry sediment density were determined for each interval from 2 mL subsamples that were weighed, freeze dried and then weighed again for the difference in water content (corrected for salinity). The dried sediment was ground into a homogeneous powder and analyzed for total carbon (C_{tot}) and total nitrogen (N), as well as organic carbon (C_{org}) after treatment with HCl (37%) fumes, by elemental analysis isotope ratio mass spectrometry (EA-IRMS, Sercon). Inorganic carbon (C_{inorg}) was calculated as the difference between C_{tot} and C_{org} .

In 2018 and 2019, triplicate sediment cores were collected as described above for pigment analysis. Samples of 1 mL were collected from the top 0–0.5 and 0.5–1 cm sediment layers and were frozen immediately in liquid nitrogen. Pigments were extracted in 10 mL acetone:methanol (80:20 v:v) at –20 °C for 24 h. The samples were sonicated for 60 s and filtered through 0.45 μm nylon filters prior to separation and identification by high performance liquid chromatography (HPLC, Shimadzu LCsolutions System). Chlorophyll *a*, fucoxanthin, pheophorbide *a* and pheophytin *a* were quantified using pigment standards (DHI LAB). While chlorophyll *a* often is used as a proxy for photoautotrophic biomass, fucoxanthin is characteristic for diatoms. Pheophorbide *a* and pheophytin *a* are degradation products of chlorophyll *a*; in the rest of the text, their collective concentrations will be discussed using the term pheopigment.

2.4. Benthic in situ flux measurements

Over the course of the four field campaigns, a total of 18 benthic lander deployments were conducted to characterize fluxes of dissolved inorganic carbon (DIC), ammonium (NH_4^+), NO_3^- , nitrite (NO_2^-), O_2 , DIP and methane (CH_4). The Gothenburg big and small landers were deployed so that six chamber measurements were carried out at each station during all sampling occasions.

The big and small landers were equipped with four and two open-bottomed chambers, respectively, each covering 400 cm^2 of sediment surface and overlying water (Kononets et al., 2021). The average chamber volumes were 10.5 L at the reference station and 7.7 L in the mussel farm. The chambers were equipped with turbidity sensors, O_2 optodes and conductivity and temperature sensors (Aanderaa Data Instruments, Norway), operating at a 1-min sampling interval. To prevent concentration gradients, a paddle was carefully stirring the water in each chamber. Incubations were preceded by a 2-h ventilation period. Chambers were then sealed and injected with 60 mL of Milli-Q water for chamber volume determination (0.5–1% chamber volume). Nine sampling syringes per chamber collected 60 mL samples of chamber water at pre-set times, starting 10 min after the Milli-Q injection and every 150 min thereafter for a 21-h incubation period.

Water samples from the syringes were collected immediately after lander recovery. Nutrient samples were filtered through pre-rinsed cellulose acetate filters (0.45 μm , Sartorius) and frozen at –20 °C until segmented flow analysis (QuAAtro, XY-3 Sampler, Seal Analytical 2015) of NH_4^+ , NO_3^- , NO_2^- and DIP. The detection limits and precisions were 0.2 μM and 7% for NH_4^+ , 0.05 μM and 7% for NO_3^- , 0.02 μM and 7% for NO_2^- , and 0.02 μM and 4% for DIP. Samples for CH_4 were transferred to Exetainer vials (12 mL, Labco, UK) and biological activity was immediately terminated by adding 100 μL of a 7 M zinc chloride

(ZnCl_2) solution. Vials were preserved upside down at 4 °C until analysis, which was performed within 1 month from sampling. Concentrations of CH_4 were determined by headspace analysis on a gas chromatograph (GC 8A, Shimadzu) equipped with a PorapakTM N column (80–100 mesh) and a flame ionization detector (FID), and using dinitrogen (N_2) as carrier gas. The CH_4 concentrations in the water were calculated based on the distribution coefficient dependent on temperature and salinity (Yamamoto et al., 1976). The detection limit was 0.2 ppm. Analysis of DIC concentrations was performed immediately after sampling using an automated system based on infrared detection of CO_2 (Li-Cor 6262) (Nilsson et al., 2019). Dissolved carbonate species were converted into CO_2 by acidification with 8.5% phosphoric acid followed by N_2 stripping. Measurements of certified reference material (Dickson Laboratory, Scripps Inst. of Oceanography, San Diego) were conducted in parallel to acquire a two-point calibration with Milli-Q water as a zero point, as well as to correct for possible instrumentation drift. The analytical precision was typically better than 0.3% (relative standard deviation).

Benthic fluxes were calculated as the change in concentration per unit area and time in each chamber. The O_2 fluxes were calculated from linear fits of the chamber O_2 time series. To determine what range of the O_2 data to use in the calculations, we used the number of data points during a minimum of 30 min in the initial portion of an incubation that gave the highest r^2 value (typically better than 0.99).

Calculations of concentration changes measured in discrete samples were first corrected for the small dilution by ambient water that occurs each time water inside the chamber is withdrawn by a syringe. Subsequently, a simple or quadratic linear least square regression was performed to find the slope of concentrations in the water samples versus time. Cook's distance values and studentized deleted residual index were calculated for each data point to identify unusual observations such as leverage points and outliers, and diagnostic graphs of the data were used to ensure that assumptions of linear regression were fulfilled. The fluxes were then calculated by multiplying the chamber height with the slope of the regression line at the first time point. About 15% of the flux measurements resulted in slopes for which the *p* value was higher than 0.05 and are therefore generally seen as not being statistically different from zero. These non-significant fluxes could result either from genuinely low fluxes or from disturbances during the incubation (e.g. animal activity) or sample analysis. Since treating non-significant fluxes as zero may underestimate the flux in the second case, we used the calculated fluxes (as they are the most likely values even if $p > 0.05$) in further analyses. Including non-significant fluxes did not substantially change the results, so we believe that this approach is valid.

2.5. In situ measured nitrate reduction rates

Nitrate reduction processes were measured in situ in three out of the six chambers of the benthic landers, following the procedure described in De Brabandere et al. (2015) and Bonaglia et al. (2017b). A first sample was taken 10 min after the chamber was sealed, followed by the injection of a 9 mM $^{15}\text{NO}_3^-$ solution (in place of the Milli-Q water) 10 min later, giving final NO_3^- concentrations of about 70–100 μM depending on chamber volumes and initial NO_3^- concentrations. The remaining eight samples were collected 10 min later and then every 150 min for a 21-h period. Upon recovery, samples for measurement of the isotopic composition of N_2 and N_2O were transferred to 12 mL Exetainers (Labco) and immediately capped. To stop microbial activity, 100 μL of ZnCl_2 (7 M) was added to each sample before storage in cold and dark conditions until analysis. Samples for analysis of $^{15}\text{NH}_4^+$ were filtered through pre-rinsed cellulose acetate filters (0.45 μm , Sartorius) and frozen at –20 °C until analysis.

The isotopic compositions of N_2 and N_2O were analyzed by coupled gas chromatography–isotope ratio mass spectrometry (GC-IRMS). A 2 mL helium headspace was created in the Exetainers, which were then left upside down for 1 day in order for the gases to equilibrate.

Subsamples of the headspace were injected to a custom-made GC extraction line coupled to an IRMS (Thermo Delta V Plus). Denitrification rates were calculated using the isotope pairing technique (IPT; Nielsen, 1992). The $^{15}\text{NH}_4^+$ samples were treated with alkaline hypobromite to oxidize NH_4^+ to N_2 (Warembourg, 1993) prior to analysis as described above. Rates of DNRA were calculated according to Christensen et al. (2000). Since the lander only captures solutes that diffuse to the water column, total denitrification and DNRA rates were calculated by using a correction factor obtained from sediment core incubations (see below). Finally, the fractions of denitrification and DNRA driven by NO_3^- from the water column (D_w and DNRA_w) or sedimentary nitrification (D_n and DNRA_n) were calculated based on total rates and the water column concentrations of NO_3^- according to Risgaard-Petersen and Risgaard (1995). Fluxes of NO_3^- and NO_2^- were not measured in chambers where $^{15}\text{NO}_3^-$ was injected.

2.6. Sediment core and slurry incubations

In October and February, sediment core incubations with $^{15}\text{NO}_3^-$ were used to obtain a correction factor to account for any N_2 , N_2O and NH_4^+ that did not diffuse to the water in the benthic chambers. Sediment cores (\varnothing 4.6 cm, length 30 cm, $n = 3-4$), filled with half sediment and half water, were captured by SCUBA diver or sub-sampled from a box corer. Three replicate bottom water samples were also collected in situ and were divided into three subsamples. One aliquot for analysis of the isotopic composition of N_2 and N_2O was transferred to a 12 mL Exetainer to which 100 μL of 7 M ZnCl_2 was added. The other two aliquots were filtered through pre-rinsed cellulose acetate filters (0.45 μm , Sartorius) and frozen at -20°C until analysis of NO_3^- , NH_4^+ and $^{15}\text{NH}_4^+$.

Upon return to the laboratory, externally driven magnetic stirrers were positioned halfway down the water column of each core, gently stirring the water. One mL of a 9 mM $^{15}\text{NO}_3^-$ solution was carefully mixed into the water before a nutrient sample was collected, filtered through pre-rinsed cellulose acetate filters (0.45 μm , Sartorius) and frozen at -20°C until analysis. The cores were then capped with rubber stoppers, avoiding bubbles, and incubated at collection site in situ temperature for 4–6 h. The O_2 concentration was measured (OxyGuard Handy Polaris 2) immediately before capping and after opening of the cores to ensure that the concentration did not decrease below 30% of the original value. At the end of the incubation, water samples for measurement of nutrients and the isotopic compositions of N_2 , N_2O and NH_4^+ were collected and preserved as described above. The remaining water was then mixed with the top 7 cm of sediment. From the slurry, 12 mL of sample were transferred to an Exetainer to which 200 μL of 7 M ZnCl_2 was added, while pore water was extracted using Rhizon samplers (Rhizosphere research products) from two other samples which were then frozen at -20°C until analysis of NO_3^- , NH_4^+ and $^{15}\text{NH}_4^+$. The denitrification and DNRA rates in the water column (R_{WC}) and slurry (R_S) phases of the cores were used to calculate the total NO_3^- reduction rate in the lander:

$$\text{total rate} = R_{WC-L} \times (R_S/R_{WC})$$

where R_{WC-L} is the rate measured in the water column of the lander chambers. The October lander samples for N_2 and N_2O from the reference site were unfortunately damaged during storage, so denitrification rates from the core incubations are presented in the results instead. Since the total NO_3^- reduction rates (denitrification + DNRA) and contribution from denitrification to the total NO_3^- reduction rates could not be calculated for the lander chambers at the reference station in October, average values based on the core incubations (denitrification) and lander incubation (DNRA) are presented for that sampling.

During the same sampling campaigns, slurry incubations were used to estimate the relative contribution of anammox to the N_2 production. At each site, two sediment cores (\varnothing 9.5 cm) were captured by SCUBA

diver or by box corer. The top 2 cm of each core were homogenized and 100 mL of sediment were mixed with 900 mL of filtered (0.45 μm cellulose acetate filters, Sartorius) anoxic bottom water in a glass bottle. The slurry was bubbled with helium for 10 min to remove any O_2 entering during sampling. Using Viton™ tubing, the slurry was transferred to 12 mL Exetainers ($n = 35$), each containing a 4-mm glass bead. The samples were left to pre-incubate for about 10 h to remove any residual O_2 and NO_3^- . The slurries were then treated either with 150 μL of 9 mM $^{15}\text{NO}_3^-$ ($n = 15$) or $^{15}\text{NH}_4^+ + ^{14}\text{NO}_3^-$ ($n = 15$), injected through the vial septa (target $^{15}\text{NO}_3^-$ or $^{15}\text{NH}_4^+ + ^{14}\text{NO}_3^-$ concentration 113 μM each), or were left without additions to serve as controls ($n = 5$), before incubation for 8 h. Both the pre-incubation and the incubation were conducted at in situ temperature and the samples were shaken every 15 min to keep the slurries in suspension. Directly after addition of the tracers and every 2 h during the incubation, 200 μL of 7 M ZnCl_2 were injected to triplets of Exetainers from each treatment and one control ($n = 7$) to stop the incubation. The samples were stored dark and cold until analysis of the isotopic composition of N_2 as described above.

2.7. Statistical analyses

All analyses were conducted using a purpose-built script applied in the statistical software R (R Core Team, 2019) and the RStudio desktop interface (R Studio Team, 2019). Fluxes across the sediment-water interface were fitted with linear mixed effects models using the 'lmerTest' package (Kuznetsova et al., 2017). All tests were two-sided, using a significance level of 0.05. Data was checked visually for normality and homogeneity of variance before analysis and data were $\log(+1\text{-minimum value})$ transformed if deemed necessary. The functions *anova()* and *ranova()* were used to conduct a type III ANOVA with Satterthwaite's method and obtain *p* values for fixed and random factors respectively, as implemented in the 'lmerTest' R package. The model involved two fixed factors (station and time, where time represents sampling occasion) and one random factor, deployment (nested within station and time). Sedimentation and pigments in surface sediment content were evaluated using analysis of variance with station and time as factors. For sedimentation rates factors, sampling(time) and site(sampling, time, station) were also included in the analysis. Two-tailed *F*-ratios were constricted by dividing the largest variance by the smallest and tested at $\alpha = 0.025$. Light data (lux) were transformed from time of day into directional time (0–360), and were then analyzed in a Gaussian generalized additive model with an identity link function in the 'mgcv' R package (Wood, 2017). The correlation between chlorophyll *a* and fucoxanthin concentrations was tested using Pearson's correlation coefficient. Full results from the statistical analyses are presented in Tables S2–S6.

3. Results

3.1. Water column profiles and sedimentation rates

The water column profiles and bottom water conditions were generally similar at the two sites (Fig. 2). On all occasions apart from the October samplings, a pycnocline was present around 2–3 m above the sea floor, with the strongest stratification observed in June and July. The chlorophyll *a* concentration did not differ between sites. Bottom water O_2 concentrations measured with the lander were within 197–251 μM at all samplings (Table S1). Current roses are presented in Figs. S1–S3.

The sedimentation rates changed with the season at both sites ($F_{3,54} = 26.4$, $p = 0.002$, Fig. 3), with the highest rates measured in October. Over the course of the entire study, there was no statistically significant difference in sedimentation rates between sites, but there was a trend of higher rates in the farm and net relative to the reference station. In October, the sedimentation rate of TPM was notably high at both sites and the POM fractions were low compared to the other sampling occasions (~20% vs 30–58%). This event coincided with persistent high

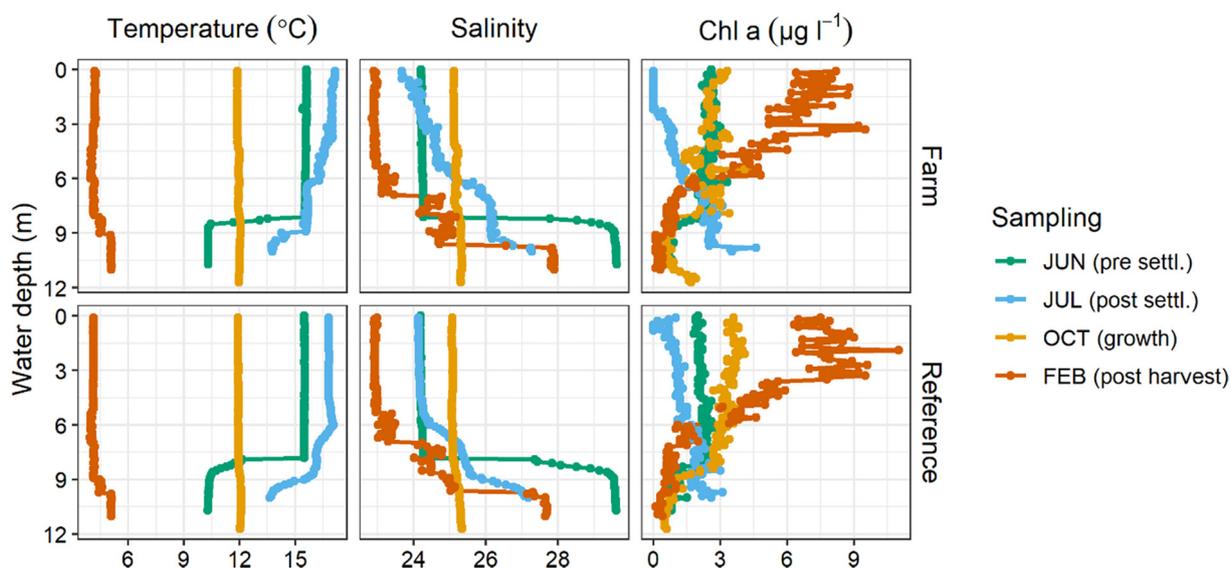


Fig. 2. Water column profiles of temperature (°C), salinity and chlorophyll a (Chl a; $\mu\text{g l}^{-1}$) in the mussel farm (top) and reference station (bottom).

winds, likely leading to transport of inorganic sedimentary materials from shore.

There were statistically significant differences between the net traps and reference site for TPM in June ($p = 0.003$), July ($p = 0.029$) and October ($p = 0.001$) as well as for POM in October for both the reference ($p < 0.001$) and mid-farm ($p = 0.004$); while no other pairwise differences were found. Sampling site (trap location), nested within sampling, time, and station, was a significant effect for both TPM ($p = 0.002$) and POM ($p < 0.001$) rates, indicating considerable spatial variability in sedimentation within the farm. A negative correlation between current velocities (mm s^{-1} , Fig. S1–3) and both TPM ($r = 0.79$, $p < 0.001$, slope = -0.084) and POM ($r = 0.37$, $p < 0.001$, slope = -0.008) sedimentation rates was observed at both stations in July and October, where the highest TPM sedimentation rates occurred over days with the lowest current velocities. No correlation was found in February, when sedimentation rates were very low. Comparison of hydrodynamics between the farm and reference is limited as only one ADCP was used over the campaign.

Lux levels (Fig. 4) differed significantly ($p < 0.001$) between the farm and reference station, with lower light at the farm. The difference was most notable in July when total light levels were highest. Interestingly, one sensor in the farm in February (Farm 2 in Fig. 4) registered substantially lower illuminance levels than at the reference station. That sensor was placed 0.9 m deeper than the other sensors due to a depth gradient, which is likely the cause of the lower light levels measured during the campaign as the farm superstructure had been removed.

3.2. Sediment characteristics and benthic microalgae

Porosity profiles at the farm site were homogeneous with depth, displaying values between 0.6 and 0.8 (Fig. S4). The C_{org} content at the farm site was 3–4% (Fig. 5), with slightly lower values at the surface than at depth in July and October and a homogeneous profile in February. The C_{inorg} content was around 0.5% throughout the sediment in July. In October, the surface C_{inorg} content had increased to over 2%,

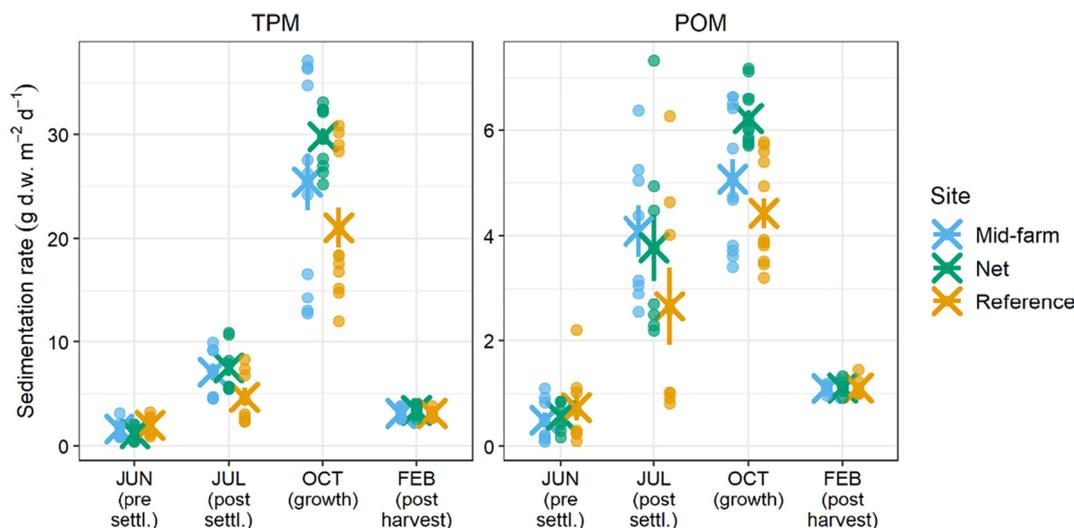


Fig. 3. Sedimentation rates of total particulate matter (TPM) and particulate organic matter (POM) at the reference station and farm (traps in between sets of tube-nets = ‘Mid-farm’, close proximity to a net = ‘Net’). Crosses mark average values, small circles are values from replicate traps and error bars show the standard errors.

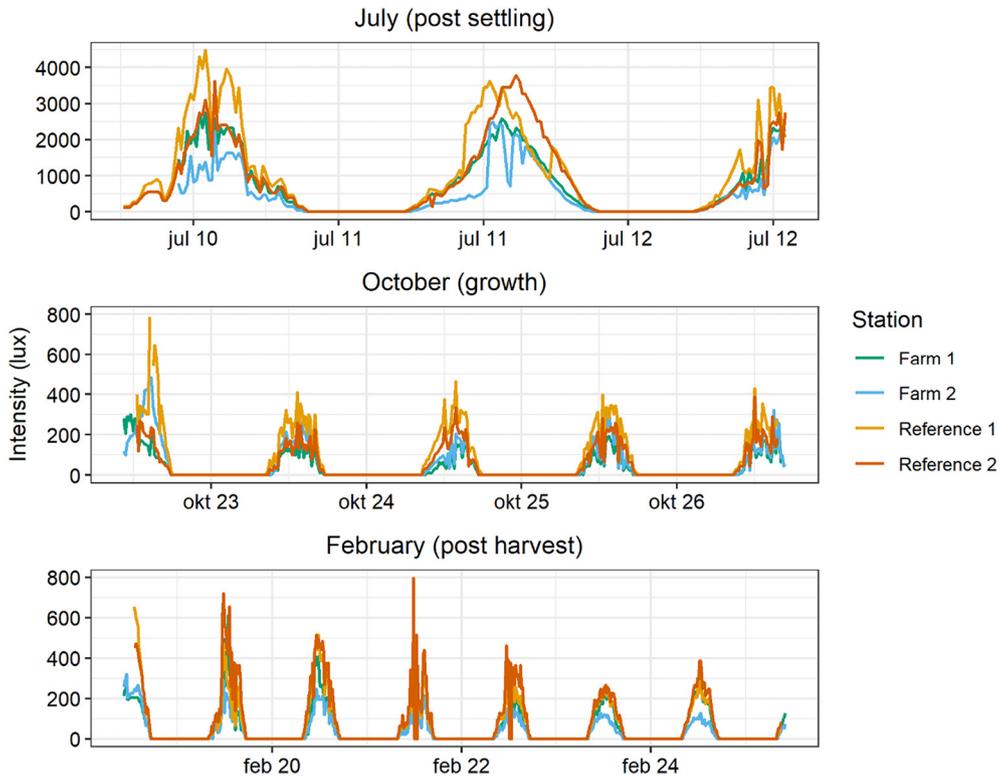


Fig. 4. Light intensity (lux) measured at the farm and reference station in July (top), October (middle) and February (bottom).

followed by a slight decrease to about 1.5% in February. The N content was stable around 0.45% throughout the sediment and over all seasons, apart from a slight increase at the surface in February. The $C_{org}:N$ ratio was 8–10 throughout the sediment and during all samplings, with slightly lower values at the surface.

At the reference station, the porosity values decreased with sediment depth. In July and February the profiles decreased from around 0.75 at the surface to 0.5–0.6 at depth, whereas the porosity was consistently lower in October with values of 0.4 at the surface and 0.3 at depth. At the reference station, the C_{org} content in the surface sediment was

always slightly higher than at depth. However, the C_{org} content varied substantially between samplings, from around 1% in July to 3.5% in October. The C_{inorg} and N profiles also decreased slightly with depth, with values of 0–1.5% and 0–0.5%, respectively. The $C_{org}:N$ ratios showed the same pattern as at the farm site, with ratios around 8–10 and slightly lower values at the surface.

Benthic microalgae were visibly present on the sediment surface at both the reference station and underneath the farm. The chlorophyll *a* and fucoxanthin concentrations in the sediment correlated strongly ($r = 0.94, p < 0.001$). The concentrations of both these pigments were

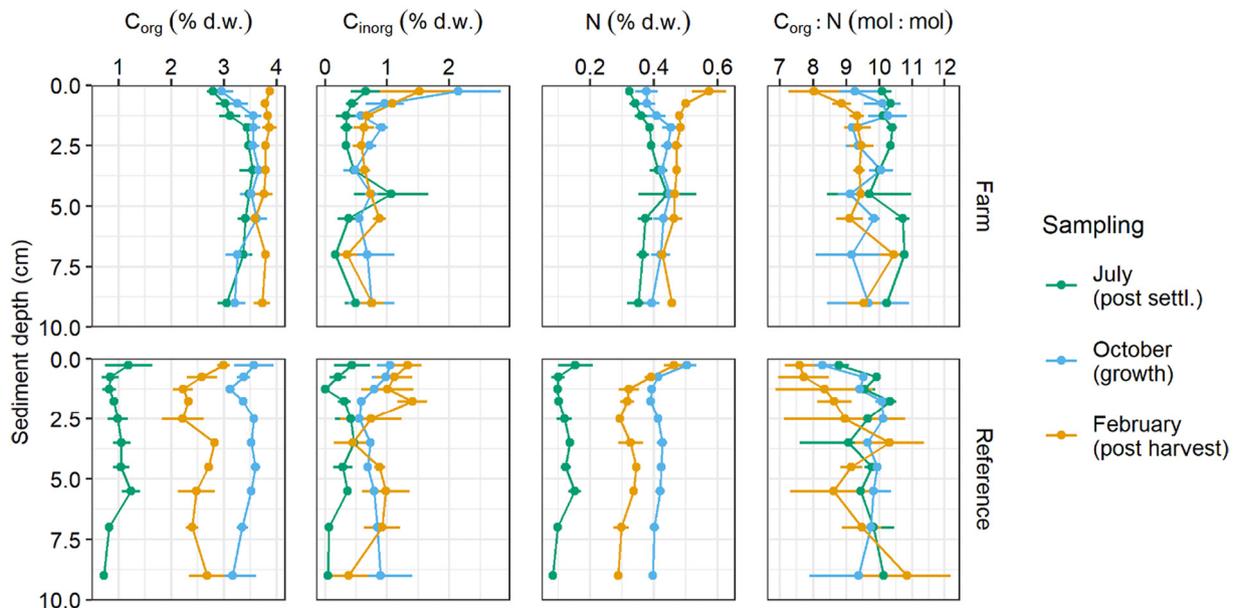


Fig. 5. Sediment solid phase profiles of organic carbon (C_{org}), inorganic carbon (C_{inorg}), nitrogen (N) and $C_{org}:N$ ratio. Error bars show standard errors.

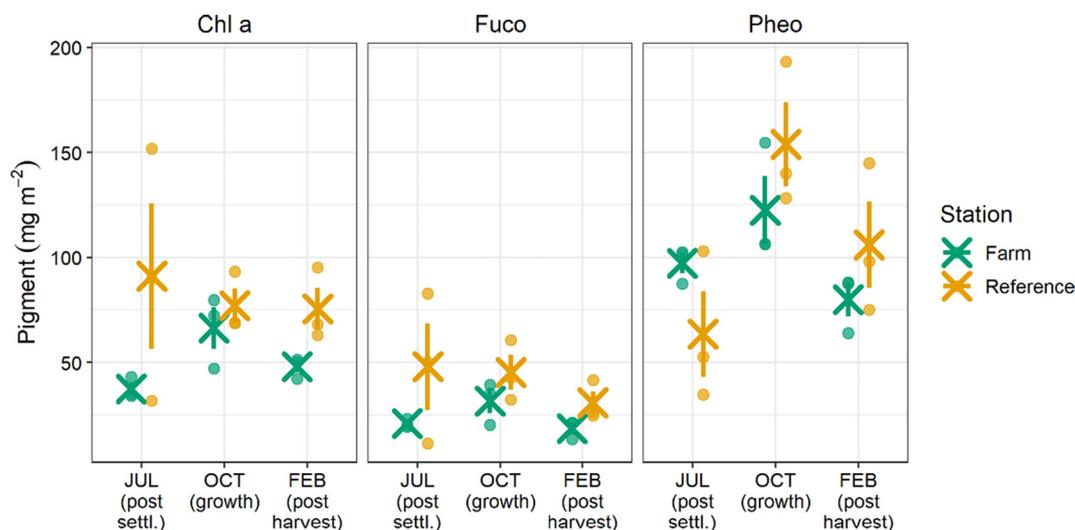


Fig. 6. Inventories of chlorophyll *a* (Chl *a*), fucoxanthin (Fuco) and pheopigments (Pheo) in the top cm of sediment in July, October and February. Crosses mark average values, small circles are values from replicate cores and error bars show standard errors.

generally higher at the reference site (Fig. 6), and there were statistically significant differences between sampling sites (chlorophyll *a*: $p = 0.034$, fucoxanthin: $p = 0.046$). On the other hand, a statistically significant seasonal variability drove differences in pheopigment inventories ($p = 0.046$). The highest concentrations were measured in October and thus coincided with the peak in sedimentation rates.

3.3. Benthic fluxes

In July 2017, one non-significant DIC flux at the reference station was strongly negative ($-107 \text{ mmol m}^{-2} \text{ d}^{-1}$). The data of concentration versus time showed substantial scatter and we see no reasonable explanation for this flux value. Similarly, one O_2 flux at the farm site in October 2018 was inexplicably large compared to any of other measurement ($-407 \text{ mmol m}^{-2} \text{ d}^{-1}$). In neither of these cases did the fluxes of other compounds in the same chambers exhibit unusual behavior. We therefore believe that the odd flux values were caused by issues with measurements of these specific compounds at these specific occasions, and excluded them from further analysis.

The sediment-water fluxes (Fig. 7) of O_2 and NH_4^+ followed statistically significant seasonal patterns ($p < 0.001$ and $p = 0.007$, respectively) while the DIC fluxes showed a strong tendency to vary with season ($p = 0.082$). The O_2 fluxes were always directed into the sediment and were highest in July. The O_2 fluxes furthermore showed significant differences between lander deployments ($p = 0.028$), indicating that the deployment positions differed in metabolic activity even within stations. The differences between deployment could be attributed to the patchy deposition of organic matter reflected in the sedimentation rates (Fig. 3). During sampling in the farm in July and October 2018, some chambers became hypoxic toward the end of the incubations, which could affect the fluxes of several compounds. However, since the flux was calculated from the initial slope in cases when the concentration change was not constant with time, we are confident that the presented fluxes represent initial incubation conditions.

The fluxes of DIC and NH_4^+ were elevated in June and October, with the highest fluxes measured in the farm in October. These elevated fluxes coincided with the peak in sedimentation rates, indicating that October was the sampling campaign with the highest sedimentary organic matter mineralization rates. The NH_4^+ and DIP fluxes were generally higher in the farm than at the reference station, and were statistically different between sites ($p < 0.001$). The CH_4 fluxes appeared to vary between stations; however, this trend was not significant ($p = 0.397$), probably due to small sample sizes (up to 3 chambers per site

and sampling occasion). The CH_4 fluxes at the farm followed the same trend as the DIC and NH_4^+ fluxes and peaked in October, suggesting that they were strongly related to organic matter mineralization. At the reference site, the CH_4 fluxes were near zero at all occasions.

The NO_3^- fluxes tended to follow seasonal patterns ($p = 0.054$). The sediment was generally a source of NO_3^- , except for the reference station in June when an uptake was observed. High effluxes of NO_3^- were measured at both sites in February. The NO_2^- fluxes followed statistically significant seasonal patterns ($p = 0.009$) and differed between sites ($p = 0.012$). The NO_2^- fluxes in the farm peaked in October 2018.

3.4. Nitrate reduction rates

Complete denitrification (NO_3^- to N_2) and DNRA (NO_3^- to NH_4^+) were measured at both stations on all occasions (Fig. 8), while anammox or production of N_2O by incomplete denitrification were not detected during any of the sampling campaigns (data not shown). Denitrification was the dominant NO_3^- reduction process at all sampling occasions and there were no trends in the ratio between denitrification and DNRA. The highest denitrification rates were measured in the farm in July, while DNRA rates peaked in the farm in October. The DNRA rates were significantly different between stations ($p < 0.001$) and seasons ($p < 0.011$). There were similar trends in the denitrification and total NO_3^- reduction rates, but these were not statistically significant.

In June, the fractions of denitrification and DNRA driven by nitrification were relatively low (around 25 and 50%, respectively). The low D_n and DNRA_n fractions in June coincided with uptake of NO_3^- at the reference station and a low NO_3^- efflux in the farm. During the following samplings, D_n and DNRA_n increased to almost 100% of the total NO_3^- reduction. The D_n fraction decreased in February, but was still $>75\%$.

4. Discussion

4.1. Benthic fluxes and sediment metabolism

While we did observe increases in solute fluxes (i.e. NH_4^+ , NO_2^- and DIP) at the farm, these changes were smaller and affected fewer solutes than seen at other bivalve farms. In October, fluxes of NH_4^+ , NO_2^- and DIP were 5.2, 5 and 8 times higher at the farm site compared to the reference station, whereas previous studies have observed 2.5–7 times higher O_2 fluxes, 6–17 times higher NH_4^+ fluxes and DIP fluxes ranging from 4 times higher to infinitely higher (no release from control station; Alonso-Pérez et al., 2010; Carlsson et al., 2009, peak growth;

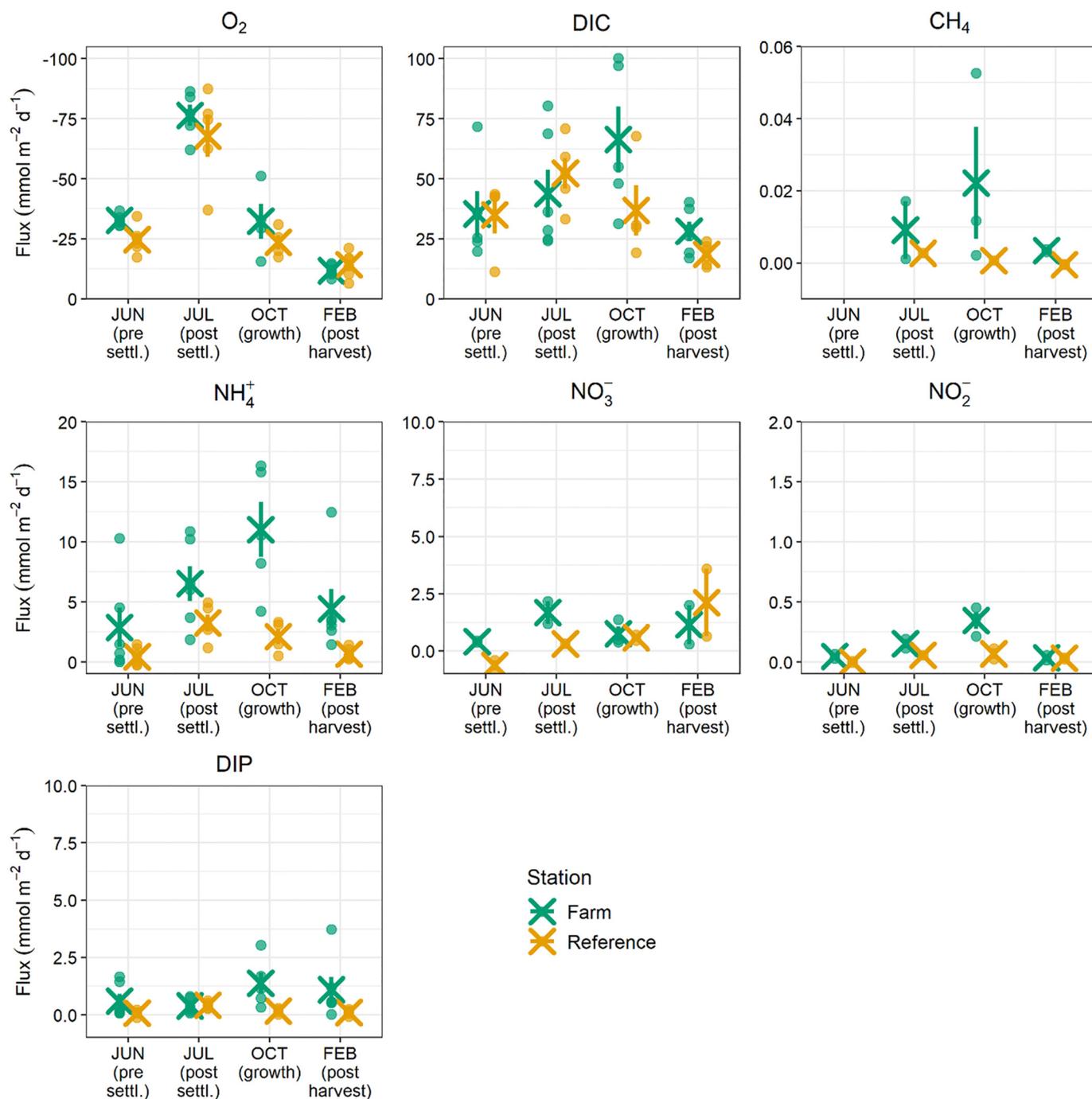


Fig. 7. Sediment–water fluxes measured with the benthic lander. Positive fluxes are directed out of the sediment, negative fluxes are sedimentary uptake. Crosses mark average values, small circles are values from individual chambers and error bars show standard errors. No CH₄ samples were collected in June.

Christensen et al., 2003; Nizzoli et al., 2011, peak growth). The higher degree of sedimentary impact in those farms may reflect their longer operational lifetime, however, whereas the farm in this study was newly re-established. Our results thus agree with those from Holmer et al. (2015), showing that effects of mussel farming on the sediment are relatively small during the first year of production.

Both stations showed clear seasonal patterns in the benthic fluxes. Concomitant with increased sedimentation of POM in July, there was an increase in the sedimentary uptake of O₂ and release of DIC and NH₄⁺ (Fig. 7), likely as a result of intensified organic matter mineralization. The DIC fluxes did not differ significantly between stations and the ratio between the fluxes of DIC and NH₄⁺ were close to the Redfield ratio (6.6), thus we conclude that the elevated input of C_{inorg} to the sediment

underneath the farm did not affect the DIC fluxes during this first year of establishment. In July, the sedimentary O₂ uptake was higher than the DIC release at both stations, likely due to oxidation of reduced species that had accumulated in the sediment during remineralization of algae blooms in spring and early summer (Glud et al., 2003). While the O₂ uptake and DIC efflux were in the same range again at the reference station in October, the O₂ uptake at the farm station was surprisingly low compared to the releases of DIC and NH₄⁺, especially considering the high bottom water O₂ concentration (218 μM, Supplementary Table 1). Higher POM sedimentation rates at the farm compared to the reference station in October may have shifted more of the organic matter remineralization to anaerobic processes. This could have caused a temporary accumulation of reduced compounds below

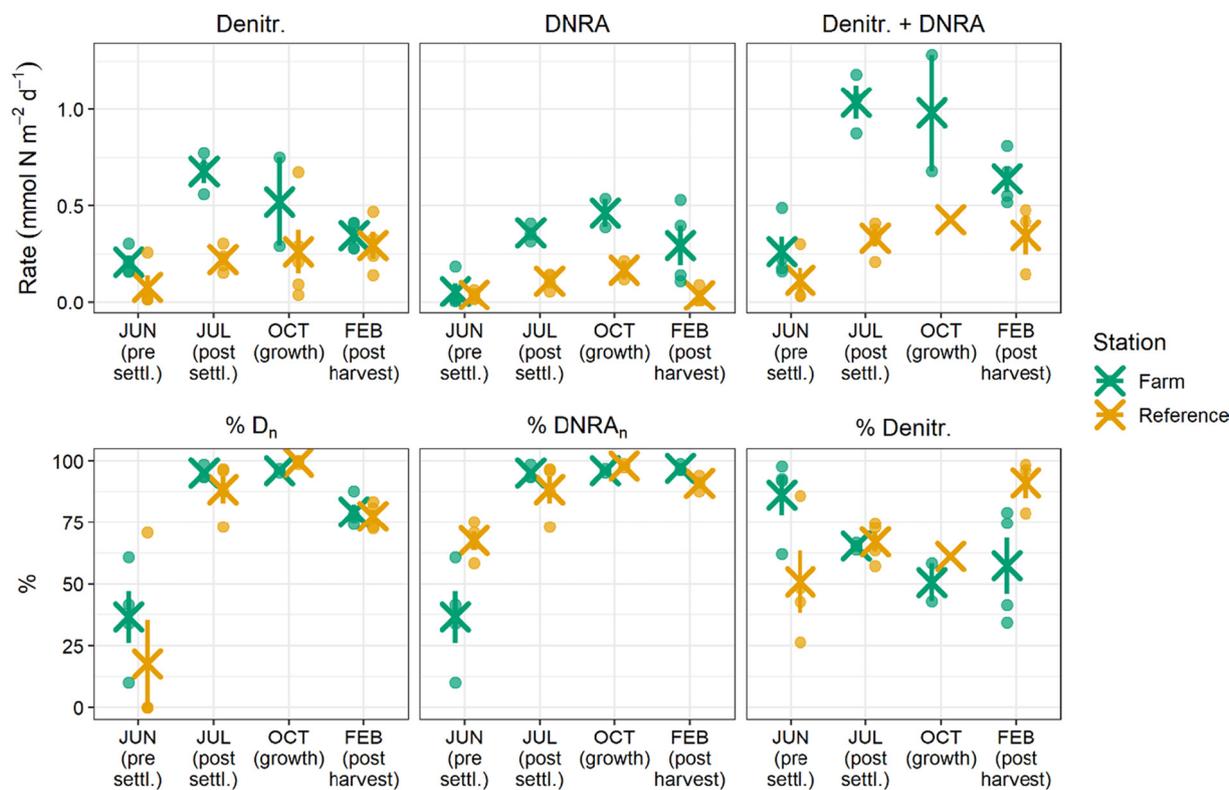


Fig. 8. Top panel: denitrification rates, dissimilatory nitrate reduction to ammonium (DNRA) rates and total nitrate reduction (denitrification + DNRA) rates. Bottom panel: fraction of denitrification (D_n) and DNRA ($DNRA_n$) driven by nitrification, contribution of denitrification to the total nitrate reduction (denitrification + DNRA). Crosses mark average values, small circles are values from replicate cores and error bars show standard errors.

the O_2 penetration depth. Shifts toward a higher proportion of anaerobic respiration and more reduced conditions in sediments below bivalve farms have indeed been reported previously (e.g. Carlsson et al., 2009; Gilbert et al., 1997; Hargrave et al., 2008).

The difference in POM sedimentation rates between the sites in October was relatively small, however, indicating that differences in the organic matter composition could have contributed to the flux discrepancy in the farm. A substantial fraction of the sedimenting organic matter at the farm was likely fecal matter from the mussels. Nutrients, DIC and CH_4 contained in the fecal particles, produced during organic matter remineralization in the mussels' digestive systems, may have been released once the particles were deposited on the seafloor, thereby disconnecting the release of these compounds from the sedimentary O_2 uptake.

Release of CH_4 from the farm sediment followed the same seasonal trend as DIC and NH_4^+ . The trend of higher release of CH_4 at the farm suggested that methanogenesis was more active and/or methane oxidation was less efficient than at the reference site, where CH_4 fluxes were negligible. Sedimentary release of CH_4 can be connected to high organic matter loading (Egger et al., 2016), but production of CH_4 has also been linked to methanogens in the digestive systems of bivalves in coastal environments (Bonaglia et al., 2017a). Both enhanced organic matter deposition and mussel excretion of methanogens or fecal particles containing CH_4 could thus have contributed to the CH_4 efflux observed at the farm. Due to the low CH_4 efflux and oxic water column, however, the released CH_4 was likely oxidized before reaching the atmosphere. Reduced sediments can also enhance the production of the greenhouse gas N_2O . Low O_2 concentrations and presence of H_2S can limit or inhibit nitrification and denitrification (Henriksen and Kemp, 1988; Senga et al., 2006; Sørensen et al., 1980), thereby causing the release of N_2O . In this study, however, no N_2O production through incomplete denitrification was detected using ^{15}N isotope methods. Net N_2O fluxes were not measured, so we cannot exclude N_2O consumption or

production through nitrification. However, previous studies from oyster farms have shown unchanged sedimentary N_2O release compared to control sites (Ray et al., 2019), or even an N_2O uptake (Erler et al., 2017). Our results are thus in line with previous studies, suggesting that bivalve farms have a minor effect on sedimentary greenhouse gas production (Ray et al., 2019).

A small increase in organic matter deposition is expected to provide NH_4^+ for nitrification and thereby stimulate nitrification-driven NO_3^- reduction (Newell et al., 2002). Conversely, high loads of organic matter can lead to O_2 depletion and inhibition of nitrification in the sediment, which may decrease NO_3^- reduction rates unless there is a sufficient supply of NO_3^- from the water column. Apart from the June (pre-settlement) sampling, the contribution of D_n and $DNRA_n$ to the total NO_3^- reduction approached 100% both under the farm and at the reference station. It thus seems clear that nitrification was active, an observation supported by a sedimentary efflux of NO_3^- at all but one occasion. Despite the increase in nitrification- NO_3^- reduction rates, elevated NH_4^+ fluxes at the farm show that the nitrifying capacity of the microbial community did not increase at the same rate as the production of NH_4^+ from organic matter. Higher mineralization rates at the farm may have led to shallower sedimentary O_2 penetration or accumulation of hydrogen sulfide, which could have limited or inhibited nitrification (Henriksen and Kemp, 1988; Joye and Hollibaugh, 1995). The higher NO_2^- fluxes at the farm could also be a result of low O_2 availability, since the first step of nitrification (NH_4^+ oxidation) is less sensitive to O_2 deficiency than the second step (NO_2^- oxidation) (Henriksen and Kemp, 1988). It should be noted, however, that the IPT can underestimate denitrification rates in bioturbated sediments (Ferguson and Eyre, 2007). In a comparison with net N_2 flux measurements using the $N_2:Ar$ method (Kana et al., 1994), lower denitrification rates were obtained with the IPT method, likely due to non-homogeneous mixing of the labelled and ambient NO_3^- in burrow structures (Ferguson and Eyre, 2007). Furthermore, the release of NO_3^- from the sediment

suggests that this electron acceptor was not limiting the NO_3^- reduction processes, whereas one of the assumptions behind the IPT is that the system is NO_3^- limited (Robertson et al., 2019). These factors suggest that the NO_3^- reduction rates presented in this study could be underestimated and should be seen as a lower limit. However, relative patterns between stations are likely not affected. The potential underestimation of denitrification rates is also unlikely to fully explain the apparent lack of increase in nitrification capacity. Inhibition of heterotrophic denitrification by hydrogen sulfide (Sorensen et al., 1980) or lack of electron donors for chemolithotrophic denitrification and DNRA (e.g. Caffrey et al., 2019; Roberts et al., 2014) could be other possible explanations for the apparent mismatch between nitrification and NO_3^- reduction.

The load of organic matter affects the rate of NO_3^- reduction as well as the partitioning between NO_3^- reduction processes. Studies from other marine environments have shown that organic-rich and reduced sediment conditions generally favor DNRA over denitrification (Christensen et al., 2000; Giblin et al., 2013; Hardison et al., 2015; Kraft et al., 2014). Since denitrification constitutes a sink for bioavailable nitrogen while DNRA retains nitrogen in a bioavailable form, the rates of and ratios between these processes strongly impact the fate of nitrogen in the marine environment (Kuyppers et al., 2018; Thamdrup, 2012). Several studies have investigated the effect of increased organic matter loading on NO_3^- reduction processes underneath bivalve farms. Reported results include increased denitrification rates (Carlsson et al., 2012), a dominance of DNRA but increases in both denitrification and DNRA rates (Erler et al., 2017; Gilbert et al., 1997; Lunstrum et al., 2018; Nizzoli et al., 2006), as well as decreased NO_3^- reduction rates (Carlsson et al., 2012; Christensen et al., 2003; Nizzoli et al., 2006). Although bivalve density or sedimentation rates were not always reported, the general trend points toward increased denitrification and DNRA rates at moderately impacted farm locations, while increases in DNRA rates are higher at strongly impacted farms or after pulses of organic matter input (e.g. phytoplankton blooms), thus agreeing with findings from other marine systems.

In accordance with previous studies, we observed elevated DNRA rates in the farm, although the contribution from DNRA to the NH_4^+ flux was low (1–10%). Still, denitrification dominated at both stations, although the differences in denitrification and total NO_3^- reduction rates between stations were not statistically significant. The seasonal trends in denitrification and DNRA rates suggest that input of organic matter to the sediment was favoring these processes, either directly by the supply of an electron donor (organic matter) or indirectly by providing NH_4^+ for nitrification.

4.2. Benthic microalgae as a nutrient filter

In this study, we measured higher concentrations of chlorophyll *a* and fucoxanthin at the reference station. The ratios between fucoxanthin and chlorophyll *a* of 0.47–0.49 agree with those found in diatoms (Sundbäck et al., 2004). Although pigment concentrations are not always easily converted to biomass or abundance (e.g. de Jonge, 1980), chlorophyll *a* concentrations are routinely used as a proxy for algal biomass (De Jonge et al., 2019; McGlathery et al., 2012; Sundbäck et al., 2004). Our observations thus indicate that the benthic algae biomass was higher at the reference station. Light can be one factor affecting the abundance, since we did observe lower illuminance levels at the farm, likely due to shading from the superstructure or potentially reduced backscatter from the sea floor as a result of relatively increased organic enrichment (Fogarty et al., 2018). Furthermore, the benthic solute fluxes suggested that the sediment environment at the farm site was more reduced and thus could have been harmful to benthic microalgae.

It is unclear under what circumstances mussel farms affect benthic microalgae abundance, as previous studies have shown varying results. Christensen et al. (2003) found that the abundance of benthic microalgae was substantially lower under a mussel farm compared to

a reference station. It has been suggested by these and other authors that shading cultivation units may decrease the amount of light reaching the seafloor under mussel farms, which would negatively impact benthic microalgae (Barranguet et al., 1996; Christensen et al., 2003; Franzo et al., 2014). Simultaneously, water transparency within the farm can in fact be significantly higher than ambient conditions, due to filtration of particles in the water column (Cranford, 2019). The transformation of higher concentrations of small particles (phytoplankton) into lower concentrations of larger particles (feces) within the farm may counteract some of the shading effect of the farm structure. The configuration of cultivation units, density of mussels, ambient suspended particle concentrations, and currents are all factors that can affect shading and fecal particle dispersion (Weise et al., 2009) and thereby have an effect on the light field conditions underneath mussel farms. In addition to decreased light, high sulfide concentrations in sediments under mussel farms have also been proposed to negatively impact benthic microalgae (Christensen et al., 2003). Despite many unfavorable environmental factors, Franzo et al. (2014) measured higher diatom abundance underneath an old mussel farm compared to a reference station. The relative abundance of certain diatom species nevertheless differed between stations, as a species that prefer organic matter dominated under the farm while another species with higher light requirements was more common at the reference station.

Differences in benthic microalgae abundance may have impacted solute fluxes at the two stations. The algae can affect sediment-water nutrient exchange directly by uptake of solutes, and indirectly through photosynthetic production of O_2 which in turn regulates the sediment geochemistry. The lander incubations were effectively dark incubations and did not include the effect of primary production (i.e. uptake of DIC and production of O_2). The DIC and O_2 fluxes thus represent community respiration rates, which seem to have been equal at the two stations. Incubations with benthic chamber landers are generally believed to disturb the sediment less than core incubations, and each chamber incubate a larger sediment area than a sediment core (Hammond et al., 2004; Kononets et al., 2021). One resulting advantage with lander incubations is that they are thought to better reflect the biogeochemical effects of animal behavior, since larger animals generally are excluded from cores and animals have been shown to become less active in core incubations (Glud et al., 2003; Hammond et al., 2004). This shows the importance of combining methods (e.g. benthic lander incubations and core incubations in light) when investigating complex ecosystems. It is worth noting that if the benthic microalgae biomass was indeed lower in the farm, the presented sediment-water fluxes could underestimate the effect of mussel farming on sediment-water fluxes. In light conditions, the uptake of nutrients and production of O_2 would be expected to be higher at the reference station. The total nutrient release integrated over a daily cycle would then be even higher at the farm station.

Although light incubations were not conducted in this study, benthic microalgae could have affected the dark incubations as well. Benthic microalgae are able to continue the uptake of phosphorus and nitrogen in the dark (Clark et al., 2002; Cochlan et al., 1991; Cornwell et al., 2014), so the higher release of DIP and NH_4^+ at the farm could have reflected a lower algae abundance at that location. Furthermore, production of O_2 during light hours can contribute to the formation of iron(III)-oxides, which continue to act as a DIP trap during dark conditions (Cornwell et al., 2014). However, the effect of benthic microalgae on the fate of bioavailable nitrogen is complicated. Firstly, benthic N_2 fixation can occur in shallow systems, even under high bottom water NO_3^- and NH_4^+ concentrations (Knapp, 2012; Newell et al., 2016). In systems where N_2 fixing organisms constitute a substantial fraction of the benthic primary producers, N_2 fixation could potentially decrease as a result of mussel farming. Secondly, benthic microalgae can affect the removal of bioavailable nitrogen. Production of O_2 can increase nitrification, potentially benefiting NO_3^- reducing organisms (Risgaard-Petersen, 2003; Risgaard-Petersen et al., 1994). At the same time, benthic microalgae

compete with nitrifying and NO_3^- reducing organisms for substrate in nitrogen-limited systems (Risgaard-Petersen, 2003; Risgaard-Petersen et al., 1994; Sundbäck et al., 2004). Previous studies show that coupled nitrification-denitrification is generally suppressed in settings with benthic microalgae, thereby lowering the removal of nitrogen through denitrification (Risgaard-Petersen, 2003). In this study, the elevated NO_3^- reduction rates at the farm station were driven by increases in D_n and DNRA_n . It is thus possible that a lower benthic microalgae abundance under the farm decreased the competition for substrate, and favored NO_3^- reduction. However, NO_3^- assimilation by benthic microalgae has been shown to be a more efficient nitrogen sink than sedimentary denitrification, even at deeper sites than those in this study (Risgaard-Petersen, 2003; Sundbäck et al., 2004). This balance between benthic microalgae and denitrification thereby needs to be taken into account when evaluating changes to the sediment biogeochemistry. Our results are thus in line with those from Christensen et al. (2003), suggesting that loss of the benthic microalgae filter function could increase the sedimentary nutrient release under bivalve farms.

4.3. Sedimentation rates and sediment characteristics

As observed, sedimentation rates were elevated inside the farm, and particularly underneath nets, relative to the reference station. Significantly heightened rates of sedimentation in the farm directly underneath nets relative to between nets indicates that patterns of sedimentation were very local. Since the tube-net units were staggered throughout the farm and could drift with currents within a ~20 m space, the patterns of sedimentation might have shifted and led to a patchy distribution of organic matter. Yet the extent of dispersion will depend on sinking advection, fecal composition (density), resuspension, and rates of decay (Weise et al., 2009). Local current conditions strongly impact how much of the organic matter that reaches the sediment, and consequently is available for remineralization, underneath and around shellfish farms (Testa et al., 2015). Prior study of fecal particle dispersion has demonstrated little or no impact beyond 50 m from the canopy edge in similar depths and higher current velocities (Hartstein and Stevens, 2005). The presence of mussels thus led to an increase in sedimentation rates, but likely very locally.

Although sedimentation rates were elevated directly underneath the nets, spatial and temporal variability were the most important drivers of differences in rates. While sedimentation rates generally followed total mussel biomass growth in time, wind-driven transport of inorganic material from shore likely contributed to the very high ambient TPM sedimentation rates observed in October, as POM rates were less elevated. The rates of TPM sedimentations were in the range of what has been measured previously in mussel farms and reference stations in another Danish estuary (Skive Fjord, Holmer et al., 2015) and on the Swedish west coast (Carlsson et al., 2012). The POM sedimentation rates were similar to previously reported values in this area for June and higher for the productive periods in July and October (Christiansen et al., 1997).

The cultivation units were deployed already in June 2017, but without successful recruitment of mussels. Since suspended canopies can influence current flows (Tseung et al., 2016), it is possible that the sedimentation rates were affected by their presence alone. Yet the sedimentation rates in June (when the nets were empty) were similar to those in February (when the nets had been removed), and did not differ between the farm and the reference station. Nets were oriented longitudinal to predominant current directions (Fig. S1-S3) and were spaced at 10–20 m intervals, further reducing potential influence on ambient hydrodynamics and sedimentation rates (Stevens and Petersen, 2011; Tseung et al., 2016). We therefore consider the June sampling to represent true pre-settlement conditions and suggest that nets alone did not measurably affect particle sedimentation.

Whereas the changes in sedimentary C_{org} content followed the sedimentation rates at the reference station, the same pattern was not as

clear at the farm. Increasing C_{org} content with sediment depth in June 2018 indicates that the deposition of organic matter previously had been higher at the farm. This observation is in agreement with a mussel farm being situated at the location between 2012 and 2014. After one season with an active mussel farm and seemingly elevated POM deposition on the site, the C_{org} distribution was uniform throughout the sediment. The C_{inorg} content also increased in the sediment underneath the farm over the course of the sampling campaign, likely due to deposition of mussel shells.

Although the deposition of organic matter seems to have increased underneath the farm, the sediment composition did not change. Unlike many older mussel farms (e.g. Carlsson et al., 2012; Christensen et al., 2003; Hartstein and Stevens, 2005), the sedimentary $C_{\text{org}}:\text{N}$ ratio was not elevated at the farm compared to the reference site. High $C_{\text{org}}:\text{N}$ ratios underneath mussel farms have been suggested to result from efficient uptake of N by mussels, since mussel tissue generally has a lower $C_{\text{org}}:\text{N}$ ratio than the phytoplankton they feed on (Jansen et al., 2012). In accordance with this study, however, no changes in the $C_{\text{org}}:\text{N}$ ratio were observed underneath another newly established mussel farm (Carlsson et al., 2012). Furthermore, elevated concentrations of pheopigments are often observed underneath bivalve farms due to biodeposition of degraded phytoplankton (Christensen et al., 2003; Dahlbäck and Gunnarsson, 1981; Mirto et al., 2000). Yet changes in pheopigment concentrations at our sites were driven by season rather than station. It thus seems like these changes to sediment composition generally take place at longer time scales than during the first year of establishment.

4.4. Ecosystem implications

The total releases of DIP and dissolved inorganic nitrogen ($\text{DIN}; \text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$) were 5.1 and 4.3 times higher at the farm than at the reference station, respectively, when integrated over the production period (231 days) and the area affected by mussel biodeposition (19.25 ha, assuming deposition up to 50 m from the canopy edge (Hartstein and Stevens, 2005); Supplementary material, Table S7). The increased release of DIP and DIN immediately underneath the farm amounted to 1313 and 3716 kg, respectively, equaling 248% of the phosphorus and 35% of the nitrogen removed by mussel harvest. Bioavailable nitrogen was removed less efficiently at the farm, where the denitrification efficiency ($\text{N}_2\text{-N}/(\text{N}_2\text{-N} + \text{DIN}) \times 100\%$, sensu Eyre and Ferguson (2002)) was diminished by half relative to the reference site (6.14 and 12.43%, respectively). Regression estimates for the integrated flux models exhibited generally weak correlations and high variability, however, particularly in farm data points (Supplementary material). The integrated flux estimates should therefore be interpreted as general trends in the degree of difference between the farm and reference site. Still, these results evoke questions about the effect of mussel farming on an ecosystem scale.

In eutrophic basins, it is a fundamental goal to reduce the total nutrient loads on the system, whether they are regularly introduced from the watershed, atmosphere, or internal loads from legacy pools. As mussels feed on suspended organic matter present within the ecosystem, their active feeding and growth reduces the total load of organic matter from the system (Timmermann et al., 2019). Nutrients transported by suspended organic matter are transformed by mussels into somatic tissues, metabolic byproducts (i.e. NH_4^+ , DIP), and solid/semi-solid fecal waste. One fraction of this nutrient pool is removed when mussels are harvested, while other fractions are buried or transferred to further ecological processes, namely the regeneration of organic matter or removal by denitrification (Petersen et al., 2019). At the ecosystem or basin-scale, bivalves function both as sinks (harvest and denitrification) and sources (enhanced organic matter degradation and DNRA) of nutrients within the system (Jansen et al., 2019; Newell et al., 2002).

Although the increased benthic recycling of nutrients underneath the mussel farm could counteract attempts to mitigate eutrophication,

several factors will affect the total ecosystem impact of the farm. First, the fates of the recycled nutrients are unclear. Release of DIN and DIP from mussel farms into the water column can stimulate phytoplankton growth further afield (Hulot et al., 2018). This has yet to be demonstrated in situ, however, as water column chlorophyll *a* concentrations around mussel farms typically are reduced relative to reference conditions (Cranford, 2019). One possible fate of the released DIN is removal within the suspended canopy, as the high surface area in 3D and availability of interstitial organic matter can promote denitrification (Kaspar et al., 1985; Nizzoli et al., 2006). This relocation of denitrification could potentially balance the decrease in sedimentary denitrification efficiency observed here and in other bivalve farms (Carlsson et al., 2012; Lunstrum et al., 2018). Second, decreased sedimentation rates in a larger part of the basin can compensate for increased deposition of organic matter underneath a mussel farm (Timmermann et al., 2019). Increased sedimentation rates can in turn lead to a higher burial efficiency (fraction of organic matter input that is buried; Blair and Aller, 2012) and an increased sink of bioavailable nutrients. Organic matter burial efficiencies underneath mussel farms could thus substantially influence the net removal of bioavailable nutrients; yet, to the best of our knowledge, the topic has not been studied sufficiently and should be a focus of future investigations.

Bivalve farms have been discussed in the context of nutrient cycling for a long time, however attention has only recently been given to their possible roles as sinks or sources of greenhouse gases (e.g. Filgueira et al., 2015; Ray et al., 2019; Suplicy, 2018). In this study, the CH₄ fluxes were not significantly different between the farm and the reference station. However, the CH₄ release was generally higher in the farm and the lack of a significant difference between sites was likely due to a low number of samples. If the flux trend is assumed to be correct, upscaling calculations similar to those for DIP and DIN suggest that the integrated release of CH₄ was 17.6 times higher at the farm than at the reference station, corresponding to a total increased release of 11.29 kg CH₄ at the farm. Assuming a protein to nitrogen ratio of 5.6 (Mariotti et al., 2008) and a CH₄ global warming potential of 28 (Myhre et al., 2013), the CH₄ release corresponded to 5.62 g CO₂ equivalents per kg mussel protein. Although this number does not include potential CH₄ production within the canopy, it is three or five orders of magnitude lower than the corresponding values for poultry and beef protein, respectively (Ray et al., 2019). Similarly to the CH₄ fluxes, the DIC fluxes tended to be higher at the farm although the difference between stations was not significant. Elevated sedimentary DIC release underneath farms due to focusing of organic matter can generally not be assumed to increase the carbon availability in the system, since the material would have likely been degraded elsewhere in a scenario without mussels (Filgueira et al., 2019). As discussed above, however, if the burial efficiency increases underneath farms due to elevated particle deposition, the mussel farms could be regarded as enhancing the sedimentary carbon sink.

During the first year of production in the studied farm, we observed elevated sedimentary releases of NH₄⁺, NO₂⁻ and DIP, and a tendency toward higher release of CH₄. Removal of nutrients at mussel harvest compensated for the increased sedimentary release of DIN, but not DIP. Relative to nitrogen, a larger proportion of the ingested phosphorus is excreted by the mussels (Jansen et al., 2012), which will lead to enrichment and possibly enhanced recycling of phosphorus underneath mussel farms. The enhanced recycling of phosphorus suggests that mussel farms may not be efficient tools to mitigate eutrophication in phosphorus limited systems, where other mitigation mechanisms may be more suitable. Alternatively, if mussel farms are established, extensive monitoring should be undertaken to quantify and avoid adverse environmental effects.

4.5. Conclusions and future outlook

In this study, we investigated biogeochemical changes and solute exchange in sediment below a mussel farm during the first year of

establishment. Although impacts at this site were generally low, sedimentation rates were elevated directly underneath the farm and DNRA rates and fluxes of NH₄⁺, NO₂⁻ and DIP were higher at the farm site compared to a nearby reference site. The sedimentary release of CH₄ tended to be higher in the farm, but fluxes were not significantly different from reference conditions. Furthermore, sediment pigment data suggested that the benthic microalgae biomass was lower underneath the farm. Our study points to the importance of benthic microalgae as ecosystem engineers in these settings. Long-term effects of bivalve farming on the role of benthic microalgae as a nutrient filter are currently unknown and require investigation.

While local conditions and cultivation practice will determine the degree and extent of benthic impacts from mussel farming, sedimentary nutrient cycles can be negatively affected already during the first year of production. The results presented here suggest that over the whole production cycle, relatively small increases in the sedimentary releases of DIN and DIP under mussel farms can decrease or offset the removal of bioavailable nitrogen and phosphorus through harvest. Although other potential changes to the system have to be taken into account, these results highlight the need to classify the sensitivity of benthic habitats in the local system before establishment of a farm. In some environments, it can be expected that consecutive years of production will increase the sedimentary release of bioavailable nutrients (Alonso-Pérez et al., 2010; Carlsson et al., 2009; Christensen et al., 2003; Nizzoli et al., 2011). An understanding of how sedimentary processes underneath mussel farms change over time should be used in conjunction with monitoring to accurately assess environmental impacts. This would allow informed management decisions and efficient use of mussel farms as tools to mitigate coastal eutrophication.

Data availability

All data included in this manuscript can be downloaded from the Swedish National Data Service (doi:<https://doi.org/10.5878/as06-g207>).

CRediT authorship contribution statement

Astrid Hylén: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Daniel Taylor:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration. **Mikhail Kononets:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Mats Lindegarh:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Anna Stedt:** Formal analysis, Investigation, Writing – review & editing. **Stefano Bonaglia:** Formal analysis, Investigation, Writing – review & editing. **Per Bergström:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.146853>.

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