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Understanding the effects of mussel aquaculture on denitrification

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Prepared by	Emily Douglas, Jenny Hillman, Drew Lohrer (NIWA)
For	Waikato Regional Council Private Bag 3038 Waikato Mail Centre HAMILTON 3240
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March 2024

Prepared by:
Emily Douglas
Jenny Hillman
Drew Lohrer

For any information regarding this report please contact:

Emily Douglas
Marine Ecologist
Marine Ecology
+64 7 856 1728
emily.douglas@niwa.co.nz

National Institute of Water & Atmospheric Research Ltd
PO Box 11115
Hamilton 3251

Phone +64 7 856 7026

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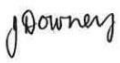	Formatting checked by:	Jo Downey
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Executive summary

- Waikato Regional Council engaged NIWA to complete a survey and comparison of nitrogen removal rates beneath and outside mussel farms in Manaia Harbour in the Firth of Thames in late summer and spring 2023. The purpose of this work was to investigate the influence of mussel aquaculture on benthic processes to help inform spatial management.A key aim was to investigate the potential influence of mussels on the seabed that have fallen from the lines above, on ecosystem functions and services.
- In collaboration with colleagues at the University of Auckland, we conducted field surveys and benthic chamber incubations beneath and outside mussel farms at two sites (inner and outer harbour) in March and November 2023.
- This report presents the results of this work, including discussion and comparison with nitrogen removal rates and environmental drivers measured elsewhere.
- During each sampling event, we measured nitrogen removal rates (N_2 flux), fluxes of dissolved inorganic nutrients (PO_4^{3-}, NH_4, NO_3^-, NO_2^-) and dissolved oxygen between the seabed and the water column. Environmental variables were quantified including sediment and environmental variables, metrics of benthic mussels, benthic macrofaunal communities, and water column dissolved inorganic nutrient concentrations.
- The seabed at mussel farm sampling locations consisted of layers of shell hash and clumps of live mussels. Sampling locations 'outside' mussel farms were ~200m from farm locations and seabed conditions consisted of undulating fine sediments with burrows and no live mussels or shell hash.
- Water column dissolved inorganic nutrient concentrations were consistently higher within the farm rather than outside sampling locations, and higher in November compared with March.
- Dissolved oxygen fluxes were significantly different between sites (inner/outer harbour), locations (farm/outside), and seasons (March/November). The influence of the mussel farm on oxygen flux was dependent on the site; for the inner harbour there was higher sediment oxygen demand inside the farm, whereas for the outer harbour the outside site had higher sediment oxygen demand.
- Nitrogen removal rates (N_2 flux) were not significantly different between farm and outside sampling locations but were greater overall at the inner harbour site compared with the outer harbour. Benthic mussels did not significantly influence nitrogen removal rates, rather the environmental drivers of N_2 flux were water column nutrient concentrations, microphytobenthic biomass and sediment organic matter content.
- Nitrogen removal rates (N_2 flux) and drivers of nitrogen removal (sediment organic matter content, Chl *a*, water column nitrate concentration) were similar to those measured in restored mussel beds in other studies.
- The influence of mussel farms on benthic nitrogen cycling and removal in the Firth of Thames is context dependent and seasonally variable. Similar studies of farms in different locations (e.g., offshore) may produce different results.

- Further research such as investigating gradients in key environmental drivers of nitrogen removal (e.g., sediment organic matter content, Chl *a*, water column nitrate concentration) relative to mussel farm location will help to assess the influence of mussel aquaculture on nitrogen cycling in sheltered waters such as Manaia Harbour.

1 Purpose/Scope

Waikato Regional Council contracted NIWA in partnership with University of Auckland researchers to undertake field surveys to investigate the possible effects of mussel aquaculture on denitrification and other benthic processes. Of particular interest is the influence of mussels that have fallen from culture lines and continue to live on the seafloor. Understanding the possible effects of mussel farming and associated benthic mussels on ecosystems services such as denitrification will help Waikato Regional Council manage activities in the coastal marine area and generate knowledge pertinent to the dynamics of nutrients. To this end, the analyses and discussion presented in this report focuses on the comparison of nitrogen flux measurements beneath and outside mussel farms.

2 Background

Mussel aquaculture has become a well-recognised and large-scale activity in the Firth of Thames and is expected to influence the nitrogen budget of this eutrophication-sensitive system. However, few studies have investigated the effects of mussel aquaculture on benthic nitrogen transformation processes, knowledge that is required to make informed decisions around nitrogen budgets and aquaculture management areas.

Denitrification is a key ecosystem service in coastal marine ecosystems, reportedly responsible for removing up to 50% of the bioavailable nitrogen in estuaries (Seitzinger 1988). Denitrification is a biological process that occurs within marine sediments that converts bio-available nitrogen (i.e., nitrate) to nitrogen gas, removing it from the ecosystem. Modelling studies have concluded that denitrification plays a major role in the nitrogen budget of the Firth of Thames, since concentrations of land- and oceanic nitrogen inputs are unbalanced (Green and Zeldis 2015). As yet, modelled estimates of nitrogen removal rates have not been verified by empirical measurements due to the difficultly of measuring denitrification at appropriate scales *in situ* (Groffman et al. 2006). It has been suggested that denitrification rates may be higher under mussel farm structures based on differences in the balance of nitrogen fluxes between summer and winter (Giles et al. 2006) which would be a beneficial ecosystem service (Stenton-Dozey and Broekhuizen 2019) .

Denitrification and other benthic nitrogen transformation processes are highly temporally and spatially variable (Crawshaw et al. 2018; Douglas et al. 2022) and are dependent on local factors including sediment composition (Douglas et al. 2018), concentration of nutrients in the water column and sediment pore water (Magalhães et al. 2005; Kessler et al. 2018), availability of sediment organic matter (Eyre and Ferguson 2009; Eyre et al. 2013), as well as factors that control sediment oxygen concentrations, especially benthic macrofaunal communities (Kristensen et al. 1991; Cornwell et al. 1999; Douglas et al. 2017).

Beneath mussel farms the sedimentary environment of the seabed can be substantially altered through the build-up of shell material from mussels falling off the lines above, and the biodeposition of faeces and pseudofaeces produced by the living mussels. Biodeposition from mussel aquaculture causes increased sedimentation and flux of organic matter to the seabed (Giles and Pilditch 2006). Over time, dependent on local flushing and hydrodynamics, the seabed below mussel farms can become dominated by shell material (whole shells and fragments) and fine organic rich sediments (Giles et al. 2006). These alterations in sedimentary environment and flux of material from mussel lines results in significant changes in benthic-pelagic coupling and benthic metabolism (Christensen et al. 2003). International literature suggests that the benthos beneath mussel farms can have significantly increased rates of sedimentation, benthic nitrogen flux, and oxygen consumption within as little as 1.5 years after establishment (Carlsson et al. 2012). In addition to alterations in the physical and chemical characteristics of the seabed, mussel aquaculture can also have a significant influence on benthic macrofaunal communities (see McKindsey et al. 2011 for review) which in turn can influence local biogeochemistry.

The Firth of Thames previously had extensive subtidal reefs (estimated up to 1300 km^2) of green-lipped mussels (*Perna canaliculus*) which were decimated by dredge fishing (Martin et al. 2021). Since the collapse of the fishery in the 1960s, populations have not recovered (Reid 1969; Paul 2012). Benthic sediment conditions in the Firth of Thames have also changed significantly due to land clearance and changes in catchment land use (Green and Zeldis 2015). Sediments have become muddier, and sedimentation rates and turbidity are high (Green and Zeldis 2015); conditions that are

thought to have contributed to low recruitment, low survival and therefore lack of recovery of benthic mussels (McLeod et al. 2012). Additionally, dredging activities removed settlement surfaces (i.e., live mussel clumps and shell material) and habitat required for recruitment (Greenway 1969). These historic mussel beds likely performed a range of ecosystem services and would have played a role in benthic nitrogen cycling and denitrification in the Firth of Thames. It has been estimated that in their original abundances, soft sediment mussel beds would have filtered the entire volume of the Firth of Thames every day (McLeod 2009).

Restoration of soft sediment mussel beds have been carried out in the Hauraki Gulf in recent years and studies have shown these beds significantly alter carbon and nitrogen fluxes including denitrification (Hillman et al. 2021; Sea et al. 2021; Sea et al. 2022b). Soft sediment mussel reefs are an important habitat for both epifauna and infauna (McLeod et al. 2014). Live mussels are often present on the seafloor below mussel aquaculture lines, presumably from individuals that have dropped off the lines above. Drop-off rates or survivorship of green-lipped mussels below farms in New Zealand have not been quantified to our knowledge, however, mussel long lines have been installed to successfully establish mussel reefs in soft sediment environments in other parts of the world (Goedefroo et al. 2022) and in Ohiwa Harbour, New Zealand (Paul-Burke et al. 2018). These benthic mussels are expected to influence ecosystem functioning of the seabed, including benthic metabolism and nitrogen cycling. Addition of mussels to the seabed from aquaculture may have positive ecological effects including provision of heterogeneous habitat, sediment stabilisation, and increased biodiversity (Martin et al. 2021).

Relative to the other areas of the Firth of Thames, Manaia Harbour hosts a few small mussel farms in shallow water (~8-15 m). Sites within Manaia Harbour with suitable depth were selected to represent different mussel farm situations; the outer and innermost farms in the harbour which were presumed to have differing hydrodynamics and seafloor conditions including sediment grain size, organic matter content and light availability. Encompassing a gradient in environmental conditions allowed analyses of the interactions of different environmental conditions on benthic ecosystem functions.

3 Methods

Data collection for this work was carried out in two sampling trips: 14 - 15 March 2023 (early autumn) and 7 - 8 November 2023 (late spring). Two sites were selected within Manaia Harbour and sampled on both occasions. Site 1 was located at the outermost mussel farm in Manaia Harbour where the water depth was 10 m and Site 2 was located at the innermost mussel farm in Manaia Harbour and had a water depth of 7 m (Figure 3-1:). The tidal range for the area is 2.9m.The two sites were selected to encompass the greatest possible gradient in exposure and nutrient enrichment based on the premise that inner estuarine sites are usually less flushed and tend to have higher proportions of mud and organic content in the sediments, promoting a higher degree of eutrophication and higher denitrification activity (Douglas et al. 2018). Seafloor locations in the interior of the farm blocks at Sites 1 and 2 ('farm' locations), and adjacent locations outside of those farm blocks ('outside' locations) were sampled on each date.

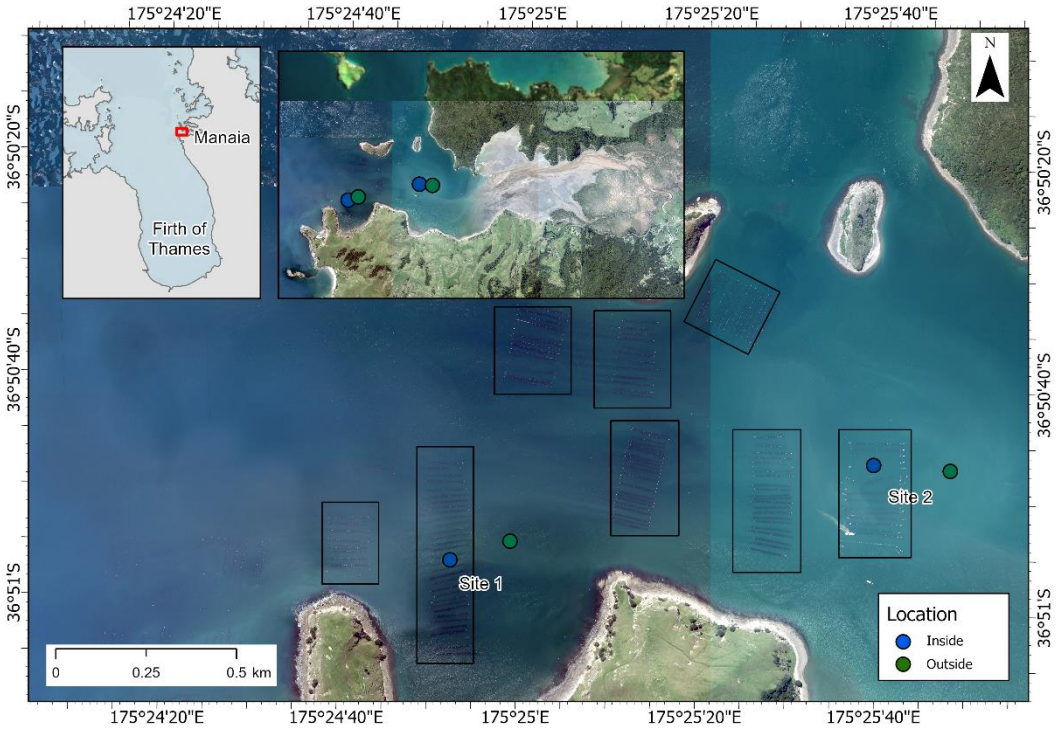


Figure 3-1: Location of sampling sites in Manaia Harbour, Firth of Thames. Black boxes outline mussel farms in Manaia Harbour and dots indicate farm (blue) and outside (green) sampling sites.

Mussel farm sites were selected on the March sampling trip by identifying mussel farm lines with medium to fully grown mussels on the culture lines to ensure the presence of the maximum number of drop-off mussels on the sea floor. For the November sampling trip, sampling locations were selected by finding full mussel lines as close as possible (<20 m) to the March sampling locations.

Outside locations were approximately 200 m east of each farm location (Figure 3-1:), with the intention that they would be away from the influence of the mussel farms (especially biodeposition) but have similar sedimentary and hydrodynamic conditions. However, we observed biodeposits in the water in similar quantities at all sampling locations, suggesting that the mussel farm influence

may be greater than the distance from our outside locations. To quantify potential differences in biodeposition between the mussel farm and outside farm locations, sediment traps were deployed at all sites during the November sampling (see methods section 2.6). The outside sampling locations were the same locations in March and November.

3.1 Site characteristics

Seafloor video was collected along a 25 m transect at each site with a hand-held downward-facing video camera (GoPro 9 camera mounted between two BigBlue® video lights) held ~50 cm above the transect tape. Divers captured footage of benthic mussel clumps (mussel farm locations only), sediment characteristics, and chamber setup. Footage was used to generate still images that could be used to characterize drop-off mussel populations, presence of epifauna, and environmental features.

Seawater temperatures and ambient oxygen concentrations were measured every minute at the seafloor using D-Opto® loggers (Zebra-Tech Ltd) placed outside chambers for the duration of incubations for the November sampling event. For the March sampling event, ambient oxygen seafloor oxygen concentrations and temperatures were determined using D-Opto® logger measurements from inside chambers immediately before lids were installed (see Table 4-1).

Ambient incident sunlight radiation (Photosynthetically Active Radiation) was measured above the water surface (mounted on top of the boat) and on the seafloor using Odyssey PAR loggers (Dataflow systems Pty Ltd). One Odyssey logger was installed at each location (farm and outside at each site) for the duration of the chamber incubations. All PAR loggers recorded at a one-minute interval. Light data were presented as average PAR recorded during the corresponding incubation periods for each site.

3.2 Chamber incubations and flux sampling

Benthic chambers (0.25 x 0.25 m, 41 L volume) were deployed by divers to quantify fluxes of nitrogen gas (N_2), oxygen (O_2), and dissolved inorganic nutrients (Dissolved Reactive Phosphorous (DRP, PO_4^{3-}), ammoniacal nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), and nitrite-nitrogen (NO_2^--N)) following well-established protocols (Lohrer et al. 2004; Hillman et al. 2021). At each site, five chambers were deployed beneath the mussel farm and five chambers outside the mussel farm (approximately 200 m away). Chamber placement beneath the farms targeted clumps of live drop-off mussels. Each chamber contained a submersible pump for intermittent non-directional water stirring to prevent the formation of solute gradients within chambers that can influence flux estimates. To correct for water column processes, an opaque 1L bottle was filled with ambient seawater and deployed at the seabed at each location (i.e., farm and outside) at each site for the duration of the incubations.

One D-Opto® logger was positioned inside every chamber. The D-Opto® loggers were recording data before the chamber lids were clamped in place providing ambient bottom water DO concentrations and throughout the incubation. All incubations were made in the dark (i.e., chambers had opaque lids) to exclude any photosynthetic activity. Unlike light chambers, conditions in dark chambers are standardised across sites of differing depth and across sunny versus cloudy weather. Moreover, light levels were expected to be generally low at both sites due to high turbidity (later confirmed, see Results section 4.1). Therefore, we maximised replication of dark treatments instead of attempting to assess light-dark differences in solute fluxes. Nitrogen removal rates have been shown to be no different in light and dark treatments in at least one prior New Zealand study (Petersen et al. 2022). Two water samples were collected from each chamber using 60 ml syringes fitted with luer lock taps

at the beginning and end of incubations (approximately 4 h) for analysis of fluxes of oxygen, nutrients (PO_4^{3-}, NH_4^+, NO_3^-, NO_2^-) (syringe 1), and nitrogen gas (N_2) (syringe 2). Extreme care was taken to avoid contamination by air bubbles during the sampling.

For N_2 flux samples (syringe 2), triplicate 15 ml glass exetainers were filled to overflowing (to avoid introducing air bubbles), preserved with zinc chloride, capped, and stored 2-3 degrees below the ambient seawater temperature from which they were collected. Samples were transported to the Institute of Marine Science, University of Auckland, where they were analysed on a quadrupole membrane inlet mass spectrometer (with Pfeiffer Vacuum Prisma Plus QMG220 M1 QMS, Bay Instruments) (MIMS) (Kana et al. 1994). MIMS is a high precision method (<0.05%) that uses the ratio of N_2 and Argon to determine N_2 flux; a positive number indicating net nitrogen removal (usually dominated by denitrification) and a negative number indicating net nitrogen fixation.

Oxygen measurements were recorded manually from each sample (syringe 1) using a Firesting oxygen probe (FSGO2). Samples were then filtered (Whatman GF/C filter, 1.2 µm pore size), transported on ice and then frozen (-20°C) until nutrient analysis. In the laboratory, analysis of nutrient concentrations were performed using standard methods for seawater on an Astoria-Pacific 300 series segmented flow auto-analyser. Detection limits were 1 mgm^{-3} for DRP, NO_3^--N, NO_2^--N, and 2 mgm^{-3} for NH_4^+-N.

3.2.1 Data processing

Oxygen flux for each chamber was calculated from the slope of the linear change in DO concentration within that chamber over the course of the incubation period (based on the D-Opto® logger data and expressed as µmol O_2 $m^{-2}h^{-1}$). Time-concentration relationships were visually assessed to ensure linearity in benthic oxygen flux, and where this did not occur, the initial linear phase of oxygen drawdown was used as the flux measurement (see Results). Water column processes were found to make a negligible contribution to chamber fluxes.

Nutrient and N_2 fluxes (expressed as µmol m^{-2} h^{-1}) were calculated using the difference between the final and initial concentrations and dividing by the incubation time, while accounting for the chamber volume and area (O'Meara et al. 2020). A few nutrient concentration values were below detection limit (NH_4^+-N values recorded at Outside Site 2 in November) and these were assigned zero values prior to flux calculations.

Denitrification efficiency (DE) is the proportion of the total benthic nitrogen flux (dissolved inorganic nitrogen (DIN) and N_2 gas) that is N_2 gas. DIN is the sum of NH_4^+, NO_3^- and NO_2^-. This was calculated using the equation:

$$DE = \frac{N_2\ flux}{N_2\ flux\ +\ DIN\ flux}$$

Denitrification efficiency was only calculated for chambers where both N_2 flux and DIN flux were positive since we use DE as a metric of nitrogen removal (Eyre and Ferguson 2009).

3.3 Sediment sampling

Sediment cores (2x 26 mm dia., 2 cm depth) were taken from the inside of each chamber for analysis of grain size, sediment organic matter content, and microphytobenthic biomass (chlorophyll-*a* (Chl-*a*) and phaeopigments). Chl-*a* was extracted from freeze dried sediments by boiling in 90% ethanol. The

extract was measured spectrophotometrically, and an acidification step was included to separate degradation products (phaeophytin) from Chl-*a* (Sartory 1982).

For analysis of organic matter content, samples were dried to a constant weight at 60 °C for 48 h then combusted at 400 °C for 5.5 h. Organic matter was expressed as percentage dry weight lost on ignition. Sediment grain size samples were homogenised then digested in ~9% hydrogen peroxide to remove organic material before wet sieving through 2000 µm, 500 µm, 250 µm, 125 µm, and 63 µm mesh size. Pipette analysis was used to separate the <63 µm fraction into >3.9 µm and ≤3.9 µm. Fractions were dried to a constant weight (60 °C for 48 h) to quantify the percentage weight of gravel/shell hash (>2000 µm), coarse sand (500–2000 µm), medium sand (250–500 µm), fine sand (125–250 µm), very fine sand (62.5–125 µm), silt (3.9–62.5 µm) and clay (≤3.9 µm).

3.4 Macrofauna and mussel sampling

To characterize the benthic macrofaunal community, one core (13 cm dia., 15 cm depth) was taken from inside each chamber at the end of incubations. Cores were sieved (500 µm) on board the boat and preserved in 70% isopropyl alcohol. In the laboratory samples were stained, sorted and macrofauna were identified to the lowest possible taxonomic level (usually species) and counted. It was not possible to collect macrofaunal cores from beneath the mussel farm at Site 1 during either sampling event due to a deep layer of compacted shell hash (i.e., divers could not get cores into the substrate to get samples of a standard area/volume).

All mussels and shell hash from within each chamber were collected by hand to a depth of 15 cm and placed in onion bags. At the surface, shell debris and live mussels were separated, photographed, and weighed. Live mussels were counted and measured (length). Shell debris and mussel clumps were only present under mussel farm sites.

3.5 Other environmental measurements

Five sediment traps were deployed alongside chambers beneath the mussel farm and five outside the mussel farm at each site during the November sampling. This was following the observation of heavy biodeposition on the chambers during the March sampling beneath and outside the mussel farms at both sites. Sediment traps consisted of modified centrifuge tubes (30 mm dia, 213 mm length, aspect ratio: 7.1) on stakes pushed into sediment so that openings were 5 cm above the sediment surface. Caps were removed at the beginning of incubations and replaced at the end. Samples were stored on ice, transported to the laboratory and frozen. Samples were thawed and analysed for Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) by vacuum filtration (Whatman GF/C glass fibre filter) using pre-weighed and dried filter papers (APHA 2017). Following filtration, each sample was dried at 105°C until constant weight, weighed, then combusted at 400°C for 6 h, then weighed to determine TSS and VSS, respectively. Sedimentation rates were expressed in g m^{-2} h^{-1}.

Apparent Redox Potential Discontinuity (aRPD) (Gerwing et al. 2013) measurements were planned, however, sediment conditions did not permit comprehensible results.

3.6 Data analysis

To analyse differences in measured variables and ecosystem functions between sites, under and outside of mussel farms, and between seasons, Permutational Analysis of Variance (PERMANOVA, Anderson et al. (2008)) were conducted with three fixed factors (Site, Location, Season) each with two levels (Site 1/Site 2, Farm/Outside, March/November). Initial models were created with

permutation of residuals under a reduced model with 9999 permutations. Where significant differences or interactions occurred, post-hoc pair-wise tests were performed.

Multiple regression analyses were used to explore the interaction and contribution of environmental factors that drive nutrient fluxes and nitrogen removal (DistLM (Anderson et al. 2008)). Environmental predictor variables were standardised to balance the relative weighting of each variable. A backwards selection procedure was used with the AICc criterion. When predictor variables were highly correlated ($R>0.8$) the variable accounting for the least amount of variability was excluded from the final model.

4 Results

4.1 Sampling sites

Beneath the farm at Site 1 the seafloor consisted of a dense layer (>30 cm) of mussel shell hash, with silty deposits and 5-10 live mussels per m^2. The seafloor outside the mussel farm at Site 1 consisted of very soft sediment with undulating hummocks and a silt layer >10 cm. Large burrows (2-3 cm dia.) were present at densities of 4-5 per m^2 (Figure 4-1), likely attributed to the Stalk Eyed Mud Crab (*Hemiplax hirtipes*; Table B-2).

Beneath the farm at Site 2 the seafloor consisted of a 20 cm deep shell hash layer (much less than that at Site 1), with muddy sediment. The benthos was characterized by the presence of burrows (1-2 cm dia.) in densities of about 3 per m^2, lots of small *Costinasterias* sea stars and a few cushion stars (*Patiriella regularis*) (Figure 4-1). The seafloor outside the mussel farm at Site 2 was similar to that of Site 1 with undulating soft sediment with burrows (2-3 cm dia.,) at densities of 4-5 per m^2.

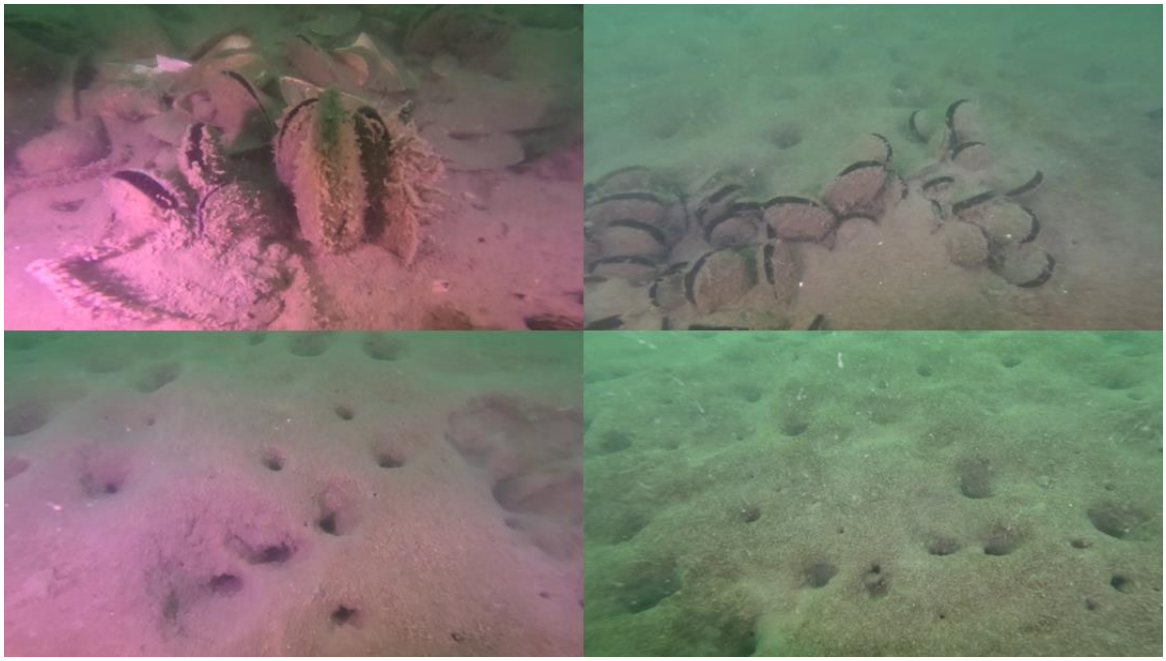


Figure 4-1: Seafloor conditions at sampling locations in March. Site 1 (left) and Site 2 (right), and farm (top) and outside (bottom) of the mussel farm blocks.

Observations of seafloor conditions at all sampling locations were similar in March and November except for dense films of microalgae at all sampling locations in November (Figure 4-2), and small differences in mussel size and density (see Section 4.3). Similar to Site 1, sediments at the outside location were characterised by crustacea burrows likely from *H. hirtipes* (Table B-2). After incubations, divers observed that the sediments inside chambers at Site 2 'outside' had turned a dark rusty colour.

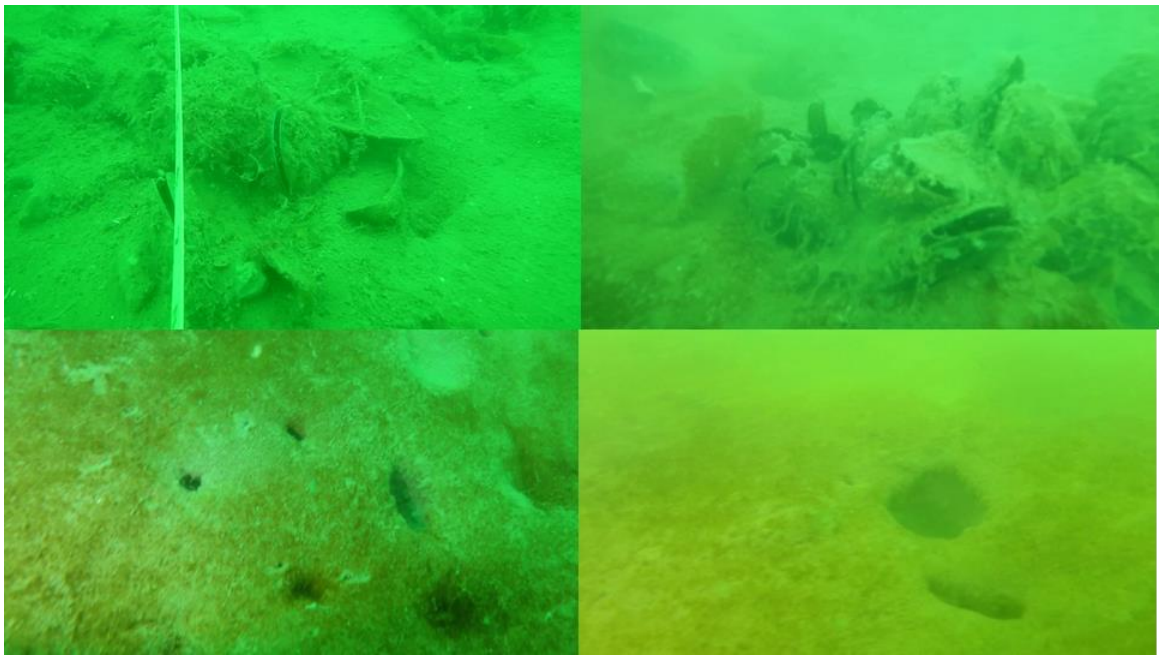


Figure 4-2: Seafloor conditions at sampling locations in November. Site 1 (left) and Site 2 (right), and mussel farm (top) and outside (bottom).

During the March sampling, bottom water temperatures were 20.8 ^{o}C which was consistent across sites (Table 4-1). In November bottom water temperatures were around 16 ^{o}C with the inner harbour (Site 2) being ~0.5 ^{o}C warmer than the outer harbour (Site 1, Table 4-1). Ambient bottom water oxygen concentrations were lower beneath mussel farms compared with outside at both sites in March and November (Table 4-1).

Table 4-1: Ambient seafloor water temperature and dissolved oxygen concentrations. November values were averages from loggers placed outside chambers during the incubation period. March values were obtained from the average of logger values for each site 5 minutes prior to chamber lids being installed.

		Temperature (ºC)		DO (%)		DO (mg L^{-1})	
		March	November	March	November	March	November
Site 1	Farm	20.8	15.9	68.2	87.1	6.1	8.6
	Outside	20.8	15.9	73.5	96.2	6.6	9.5
Site 2	Farm	20.8	16.4	77.9	83.2	7.0	8.1
	Outside	20.8	16.5	83.0	102.2	7.4	10.0

Ambient incident sunlight irradiance values above the surface of the water were similar in March and November, with slightly higher surface PAR recorded on the sampling days for Site 1 compared with Site 2 on both sampling occasions (Table 4-2). Seafloor light conditions at each site reflected site depth; where Site 1 (12m) received less light than Site 2 (8m). At all seafloor locations, PAR was less than 3% of ambient surface levels. Divers observed high turbidity (low water clarity), and there was a thick layer of bio-deposits covering the chambers at the end of the incubations, so high suspended sediment concentrations likely contributed to low seabed light levels. Light levels beneath mussel farms were expected to be lower than outside due to shading from the mussel lines, however, this was only the case in November but not March.

Table 4-2: Photosynthetically active sunlight irradiance. Photosynthetically active radiation (PAR) measured on the seafloor and above the water surface. Seafloor values are averages (±SE) during corresponding incubation periods, and surface values are averages (±SE) of above-water values from farm and outside locations during the incubation periods at each site.

		March	Proportion of surface PAR	November	Proportion of surface PAR
		PAR	%	PAR	%
Site 1	Farm	25.6 (0.6)	1.03	47.2 (1.0)	1.77
	Outside	15.2 (0.3)	0.61	60.1 (0.5)	2.25
	Surface	2497 (24.3)		2675 (32.4)	
Site 2	Farm	61.2 (1.0)	2.66	62.8 (1.6)	2.72
	Outside	39.8 (0.8)	1.73	101 (2.5)	4.39
	Surface	2302 (21.8)		2311 (35.9)	

4.2 Sediment characteristics

Sediment mud content was higher overall at outside locations than at locations inside the mussel farm blocks (overall effect of 'farm'), but there was no overall effect of site or season. There were significant Farm*Site and Site*Season interactions (Figure 4-3, Table A-1). For sediment organic matter content, there was no main effect of farm, but overall organic matter content was greater at Site 2 than Site 1 (Table A-2). Sediment organic matter content was overall higher in November than it was in March.

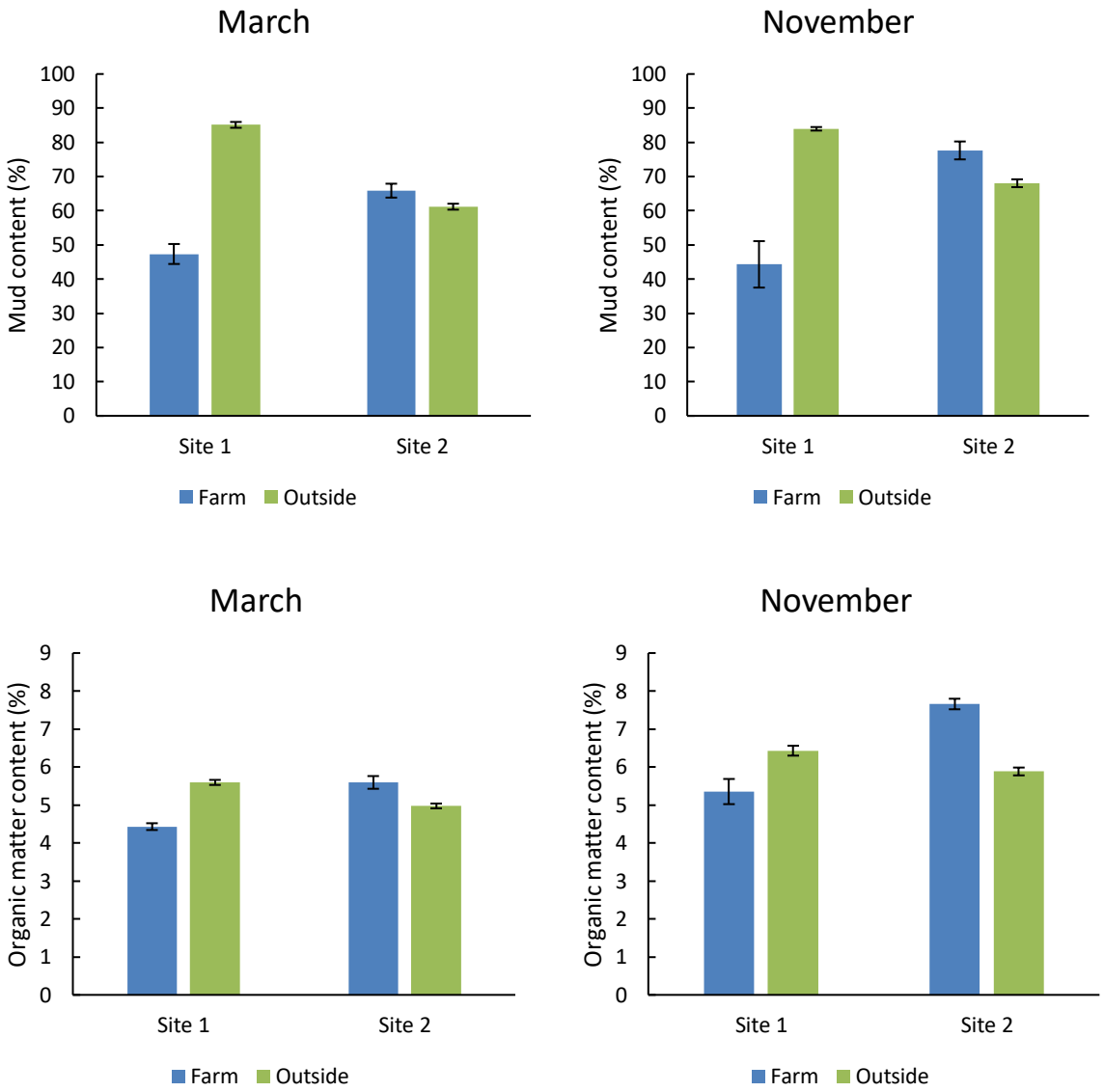


Figure 4-3: Sediment mud and organic matter content. Values are means (+/-SE).

The was no significant effect of mussel farms on sediment chlorophyll *a* concentration but there were significant effects of Site and Season (Figure 4-4, Table A-3). Overall, Chl-*a* levels were higher at Site 2 than Site 1, and higher in November than March (Figure 4-4,Table A-3). There was also a significant Farm*Site interaction and Farm*Site*Season interaction. Sediment phaeophytin content was overall higher beneath the mussel farms than outside and was greater in November than March (Figure 4-4).

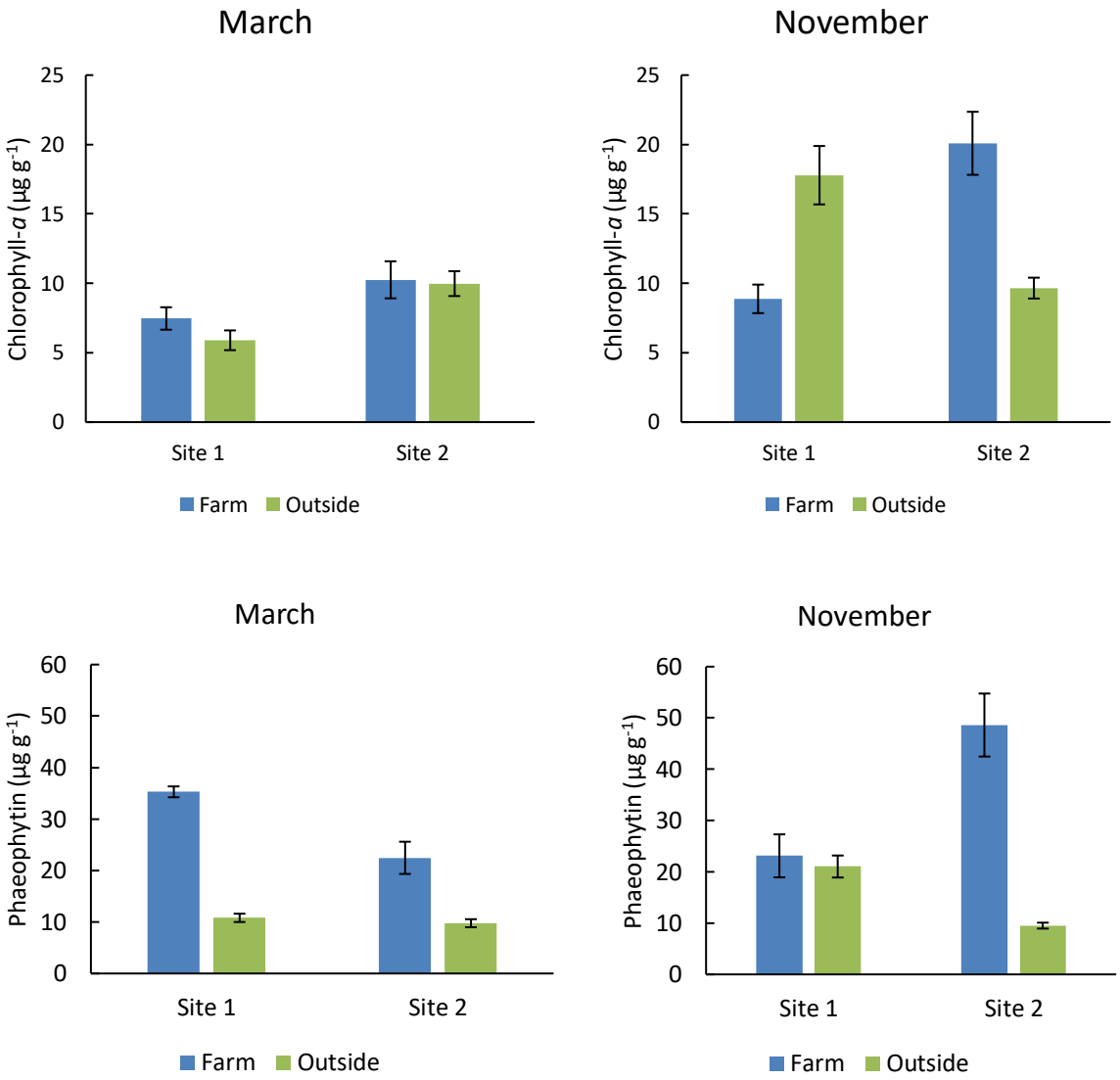


Figure 4-4: Sediment chlorophyll-*a* and phaeophytin concentrations. Values are means +/- SE

4.3 Mussel clumps

Mussels collected from the chambers deployed within the two mussel farm sites were distinct from one another. In March, the mussel lines above Site 1 contained mature mussels and in November these lines had been harvested and had not yet been reseeded. Beneath this farm, the live mussels present were large and existed in small clumps or individually on the seafloor (Figure 4-5, Figure 4-6). The average length of mussels at Site 1 was 106 mm in March and 101 mm in November (Figure 4-6).

Figure 4-5: Live mussels sampled from chambers. Photos show mussels from beneath the farm at Site 1 (left) and 2 (right) in March (top) and November (bottom).

The mussel lines above Site 2, in contrast, contained medium sized mussels in both March and November. Beneath this farm, the live mussels present were medium sized and mostly existed in large clumps on the seafloor (Figure 4-5, Figure 4-6). These clumps often appeared to have fallen directly from farms as they had mussock/seeding material entangled in them. The average length of mussels at Site 2 was 77 mm in March and 66 mm in November (Figure 4-6).

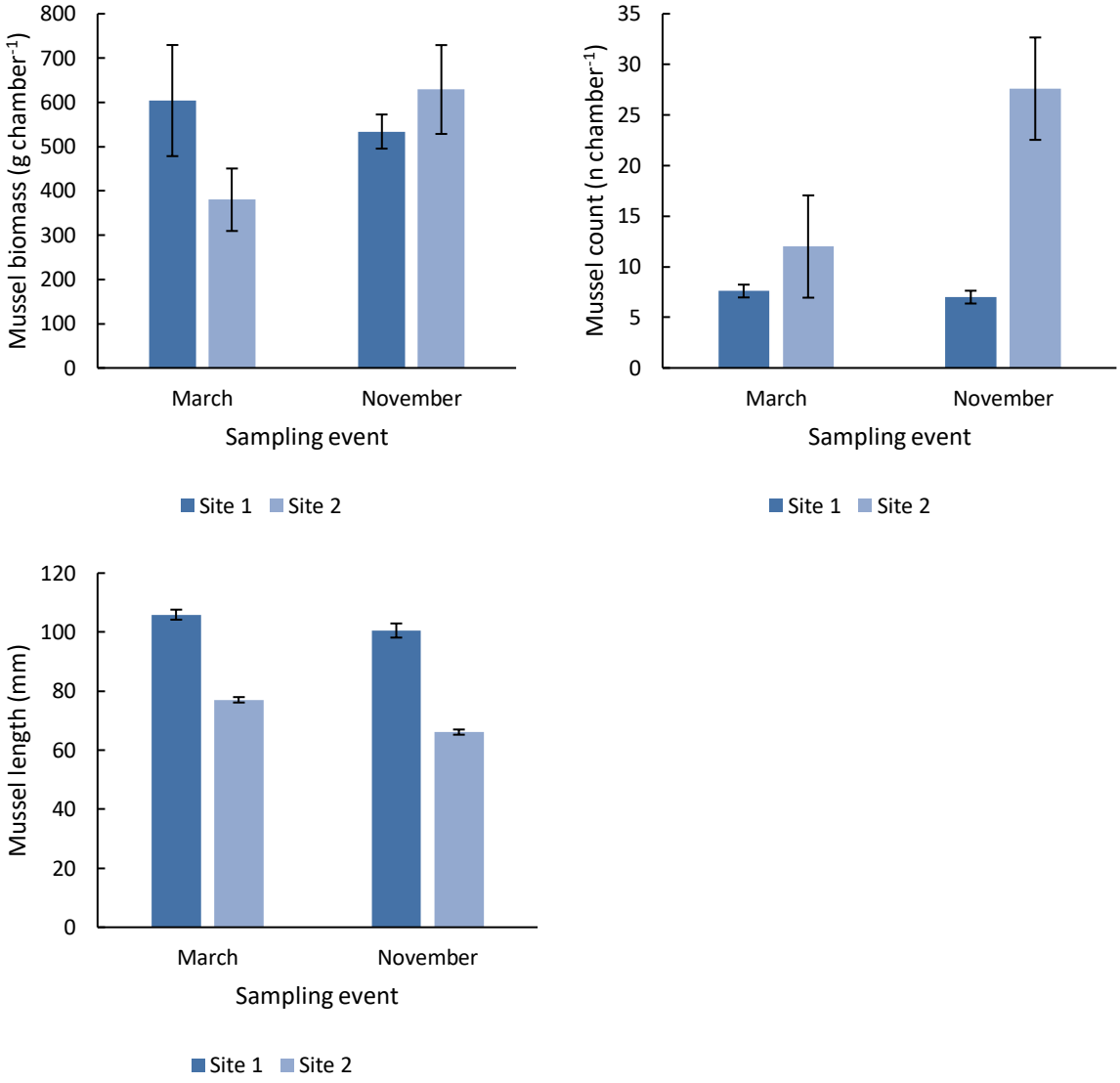


Figure 4-6: Biomass, abundance and average length of mussels within chambers. Values are Mean ± standard error.

4.4 Water column conditions

Water column dissolved inorganic nitrogen (NH_4^+, NO_2^-, NO_3^-) and dissolved reactive phosphorus (DRP) concentrations were elevated in November compared with March, and farm locations had higher concentrations than outside locations in both seasons (Figure 4-7, Table A-5).

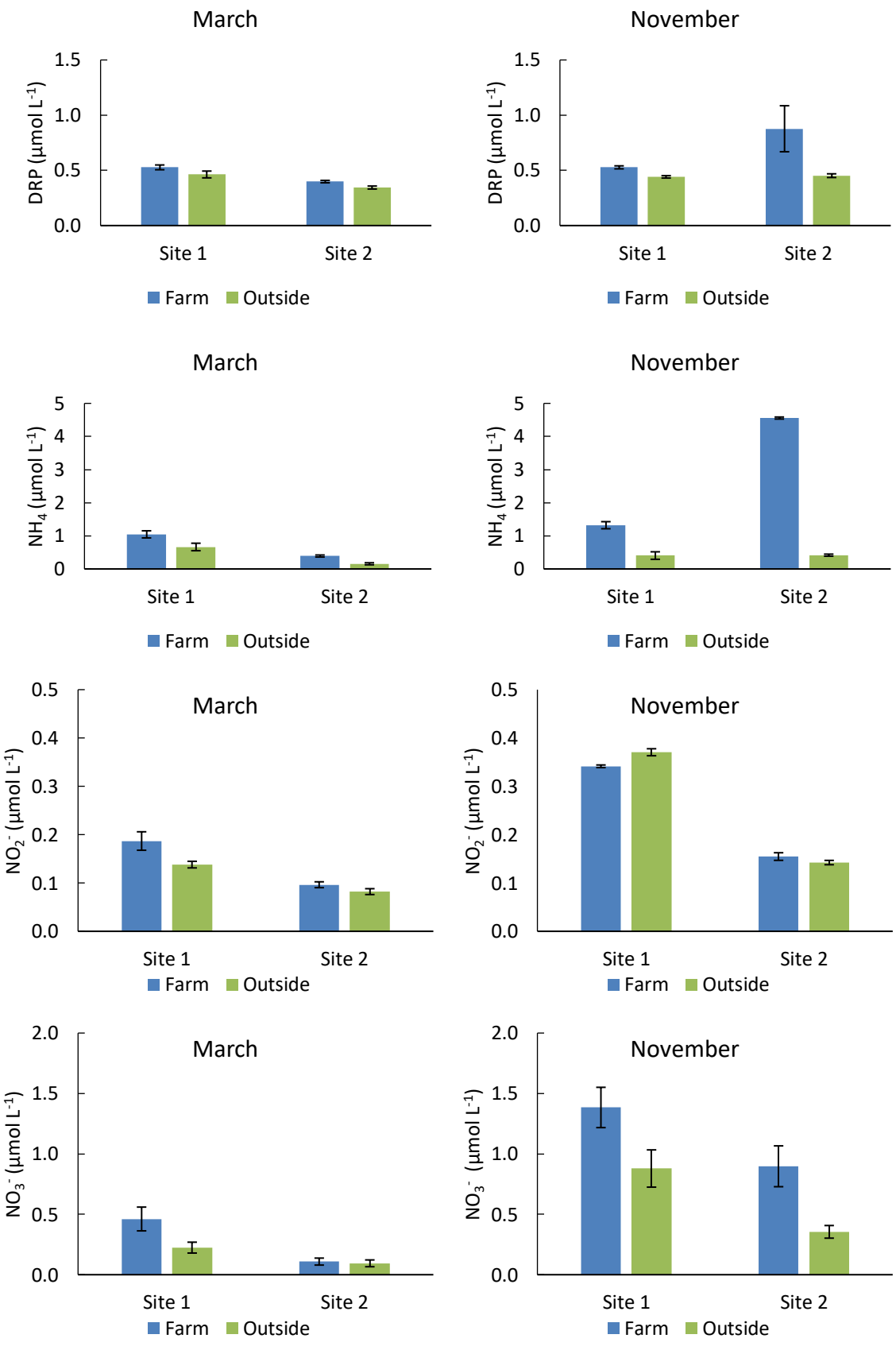


Figure 4-7: Water column nutrient concentrations within and outside mussel farms. Values are means (+/- SE). Note difference in y-axis scales.

The fall of suspended material (total and volatile suspended solids) from the water column to the benthos in November was higher inside than outside mussel farms (Figure 4-8, Table A-6). For total suspended solids rates were greater at Site 1 than Site 2, and there was a significant Site*Farm interaction effect.

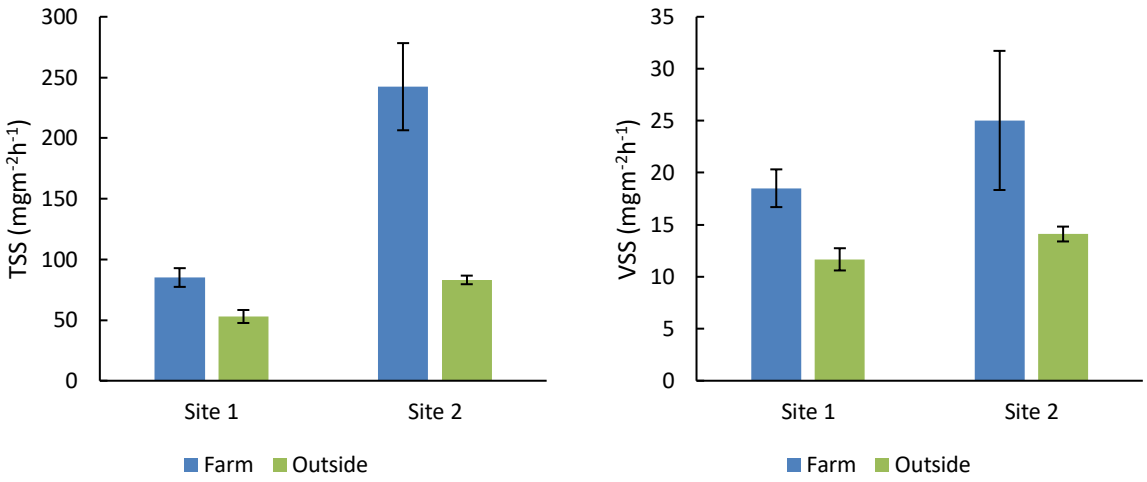


Figure 4-8: Total and volatile suspended solids collected in benthic sediment traps. Measured beneath and outside mussel farms in November 2023. Values are means +/- SE.

4.5 Oxygen flux

There were significant effects of Site, Location and Season on benthic oxygen fluxes, and there was also a significant interaction between Location and Site (Figure 4-9, Table A-7). Despite the large differences in flux values between seasons, the patterns seen among sites and locations were the same, with highest oxygen demand measured at Site 2 inside the farm, and at Site 1 outside the farm (Figure 4-9).

Benthic oxygen fluxes varied between farm and outside locations, between sites, and between seasons (Figure 4-9, Table A-7). Benthic oxygen demand was much higher at the Site 2 farm location relative to all other locations during both sampling events. In November, two of the five chambers at the Site 2 farm showed rapid oxygen drawdown within ~30 mins of the lids being clamped into place. Upon collection, some mussels at this site were found to be dead, although it could not be determined whether or not the mortality was a result of the incubations. The chambers where mortalities were noted were not those with the highest mussel densities or biomass. Microbial decomposition of dead mussel tissue may have contributed to elevated oxygen consumption. However, without definitive information, values from these chambers were not considered outliers or removed from analysis. The median O_2 flux rate for the farm location at Site 2 in November was -9419 µmol m^{-2} h^{-1}, and the mean excluding these two chambers was -7773 ± 2165 µmol m^{-2} h^{-1}.

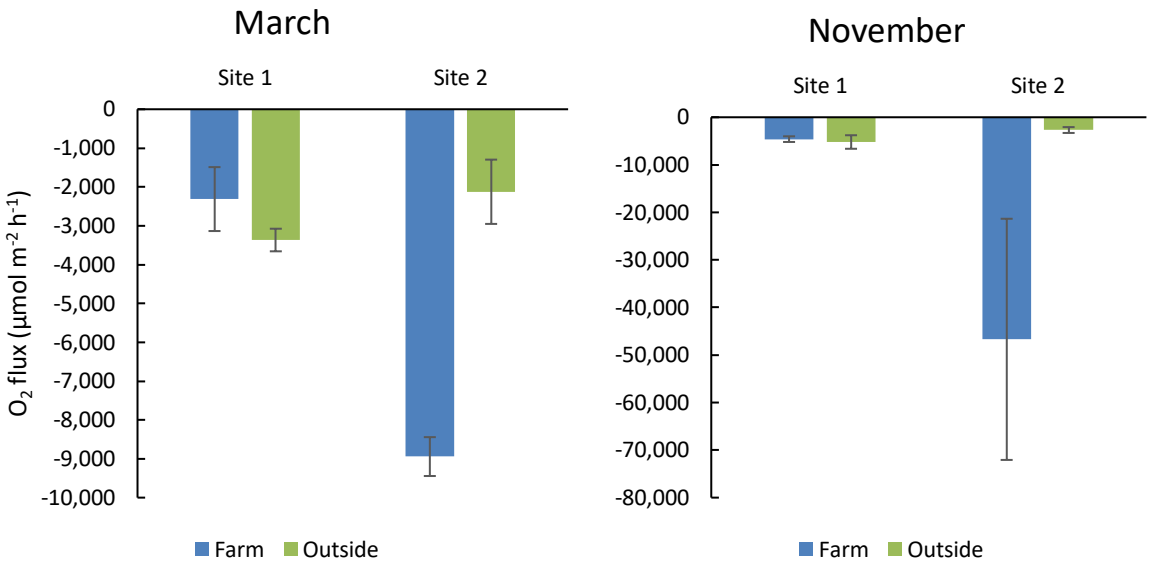


Figure 4-9: Oxygen fluxes beneath and outside mussel farms. Values are mean (+/- SE). Note difference in y-axis scales.

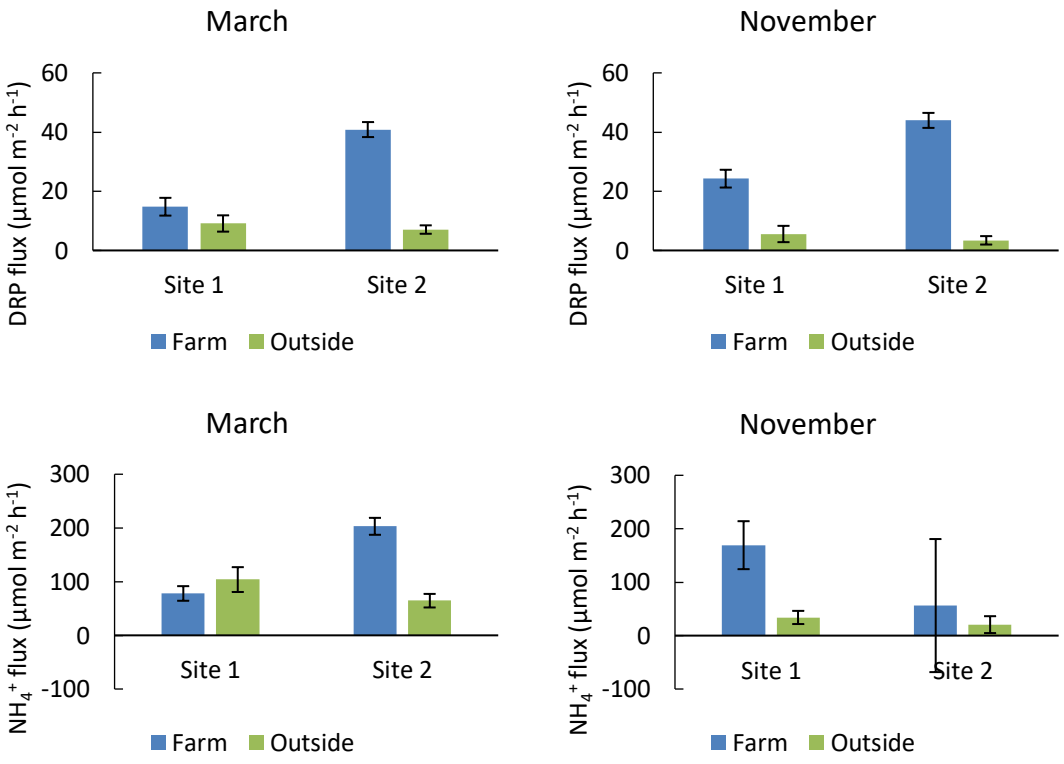
A large proportion of the variability in dissolved oxygen flux could be explained by the variables measured (R^2 = 0.86), with water column DRP and the number of mussels the most influential in the final model (Table 4-3). Higher mussel count resulted in a greater drawdown of oxygen (i.e., a negative correlation). Excluding the November Site 2 farm location chambers that had the high O_2 drawdown, results showed a full model that explains much less variability (R^2 = 0.17) but with similar predictor variables (water column DRP and mussel count). These chambers were not excluded from further analyses as outliers, because they were considered a fair representation (i.e., 40% of the data) of the benthic conditions at that site and location.

Table 4-3: Environmental drivers of dissolved oxygen flux. Results of DistLM analysis. P indicates probability significance of each variable explaining model variance. Prop. shows proportion of variability accounted for by each variable included in the final models. Dir. indicates direction of relationship between response and predictor variables. WC = water column.

DO flux				
Variable	**Pseudo-F**	**P**	**Prop.**	**Dir**
WC DRP	140.8	0.0003	0.79	-
Mussel Count	18.7	0.004	0.33	-
Chl-*a*	15.4	0.002	0.29	-
WC NO_3^--N	5.2	0.04	0.12	-
WC NO_2^--N	0.04	0.90	0.001	+
Final model	AICc	740.1		
	R^2	0.86		

4.6 Nutrient flux

In March (late summer) at the Site 2 mussel farm, nutrient release rates to the water column were greater than outside the mussel farm, but at site 1, farm and outside nutrient flux rates were similar (Figure 4-10, Table A-8, Table A-9, Table A-10, Table A-11). In November (spring), mussel farm sites released more nitrogen and phosphorus to the water column than sites outside mussel farms. The high variability in NH_4^+ flux at the Site 2 farm location in November was partly attributed to the two chambers that had high O_2 drawdown (Figure 4-9). One of these chambers had very high (533 µmol m^{-2} h^{-1}) and the other very low NH_4^+ flux (-190 µmol m^{-2} h^{-1}) compared to the other three chamber replicates. The mean NH_4^+ flux for Site 2 farm in November without these chambers was -20.5 µmol m^{-2} h^{-1}.



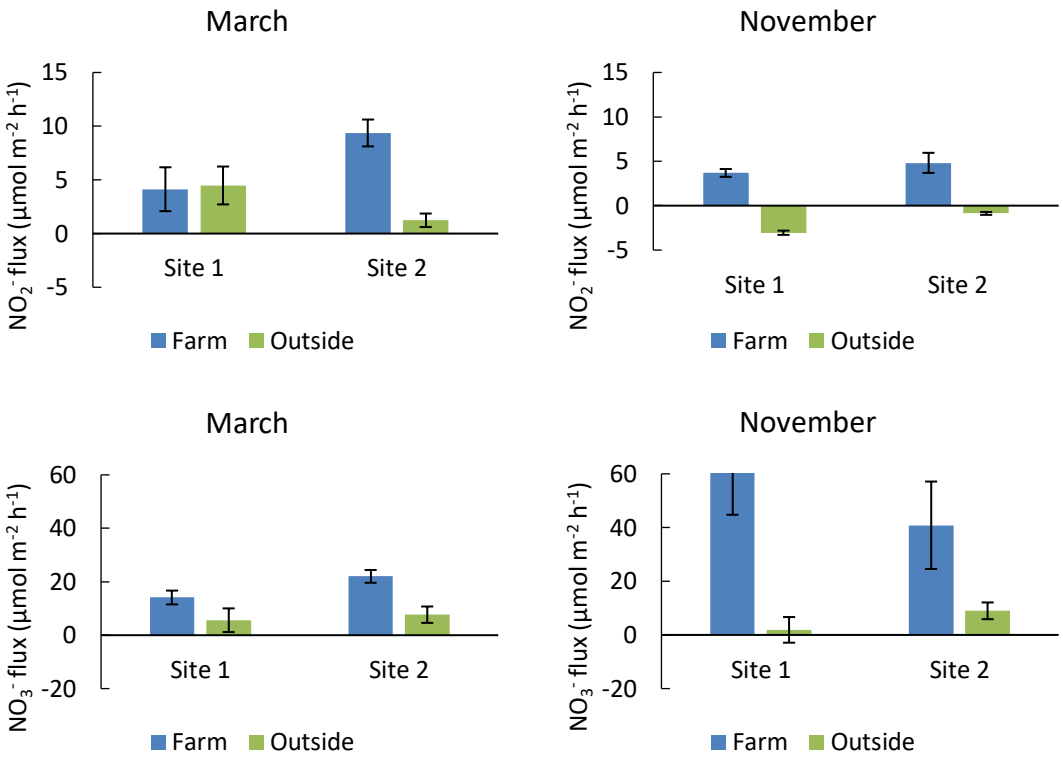


Figure 4-10: Nutrient fluxes beneath and outside mussel farms. Values are means (+/-SE).

Mussel density had a positive effect on DRP flux and accounted for most of the explained variability (Table 4-4). Other variables accounting for variability in DRP flux were water column DRP concentration, and sediment variables (shell, mud, Chl-*a* and organic content).

Table 4-4: Environmental drivers of DRP flux. Results of DistLM analysis. P indicates probability significance of each variable explaining model variance. Prop. shows proportion of variability accounted for by each variable included in final models. Dir. indicates direction of relationship between response and predictor variables. WC = water column.

Variable	Pseudo-F	P	Prop.	Dir.
Mussel Count	42.73	0.00	0.529	+
Org	6.06	0.02	0.138	+
Chl-*a*	2.34	0.14	0.058	+
WC DRP	1.30	0.27	0.033	+
Shell hash	1.36	0.26	0.035	+
Mud	0.30	0.60	0.008	-
Final model	AICc	181.20		
	R^2	0.78		

Environmental factors driving the variability in benthic NH_4^+ fluxes were water column concentrations of phosphorus (WC DRP) and ammonium (WC NH_4^+), mussel biomass and sediment Chl-*a* and organic matter content (Org) (Table 4-5). The relationship between water column NH_4^+ concentration and NH_4^+ fluxes was negative, but areas with higher WC DRP and mussel biomass had higher NH_4^+ flux from the sediment to the water column.

Table 4-5: Environmental drivers of NH_4^+ flux. Results of DistLM analysis. P indicates probability significance of each variable explaining model variance. Prop. shows proportion of variability accounted for by each variable included in final models. Dir. indicates direction of relationship between response and predictor variables. WC = water column.

Variable	Pseudo-F	P	Prop.	Dir.
WC DRP	8.03	0.03	0.170	+
WC NH_4^+-N	3.69	0.07	0.089	-
Mussel biomass	1.43	0.24	0.036	+
Chl-*a*	1.02	0.31	0.026	-
Org	0.028	0.87	0.001	-
Final model	AICc	366.5		
	R^2	0.70		

Mussel biomass, water column nitrite concentrations and sediment shell content accounted for most of the variability in NO_2^- flux, these variables all positively influencing flux rates (Table 4-6). Sediment Chl-*a* content was also important, having a negative influence on flux rates.

Table 4-6: Environmental drivers of NO_2^- flux. Results of DistLM analysis. P indicates probability significance of each variable explaining model variance. Prop. shows proportion of variability accounted for by each variable included in final models. Dir. indicates direction of relationship between response and predictor variables. WC = water column.

Variable	Pseudo-F	P	Prop.	Dir.
WC NO_2^--N	8.05	0.008	0.17	+
Mussel Count	7.53	0.008	0.17	+
Shell hash	7.19	0.01	0.16	+
Chl-*a*	2.31	0.14	0.06	-
Final model	AICc	100.02		
	R^2	0.50		

Mussel density had a positive influence on NO_3^- flux and accounted for the largest amount of explained variability (Table 4-7). Water column concentrations of NO_2^- and NO_3^- were also included in the final model and had a positive influence on NO_3^- flux, whereas sediment Chl-*a* had a minor negative influence.

Table 4-7: Environmental drivers of NO_3^- flux. Results of DistLM analysis. P indicates probability significance of each variable explaining model variance. Prop. shows proportion of variability accounted for by each variable included in final models. Dir. indicates direction of relationship between response and predictor variables. WC = water column.

Variable	Pseudo-F	P	Prop.	Dir.
Mussel Count	7.81	0.02	0.171	+
WC NO_3^--N	4.14	0.05	0.098	+
WC NO_2^--N	1.91	0.17	0.048	+
Chl-*a*	0.07	0.79	0.002	-
Final model	AICc	242.800		
	R^2	0.65		

4.7 Nitrogen removal

Benthic nitrogen removal rates were variable, and the only significant factor was site, where rates were overall higher at Site 2 than Site 1 (Figure 4-11, Table A-12). There was no main effect of season and there were no patterns in N_2 fluxes when comparing March to November. There was a significant three-way interaction between Farm, Site and Season. At Site 1 in November there was a net uptake of N_2 beneath the mussel farm which was not evident outside the farm, or beneath the farm in the previous sampling in March.

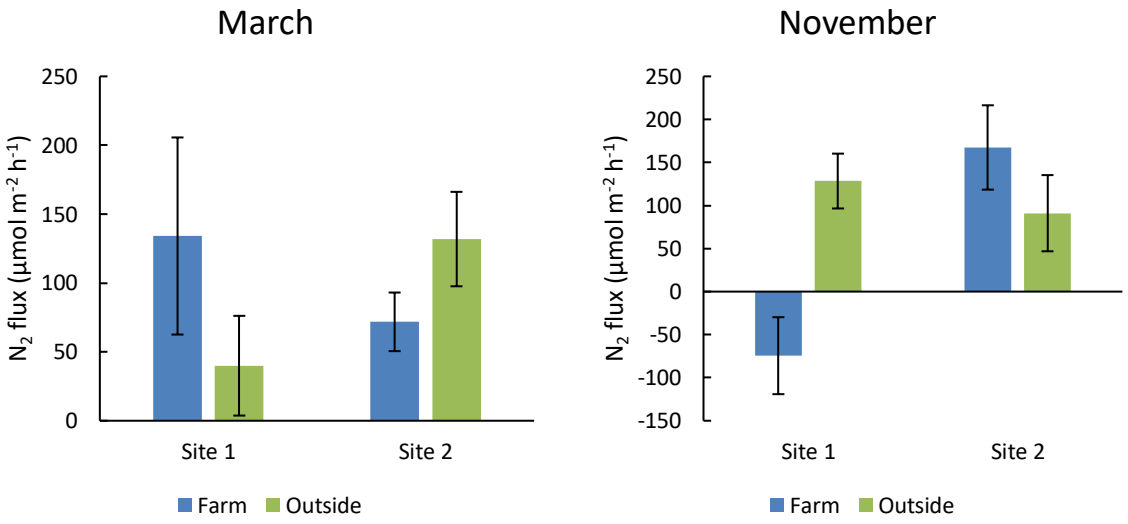


Figure 4-11: Nitrogen flux rates beneath and outside mussel farms. Values are mean (+/- SE).

The environmental variables measured could account for 42% of the variability in N_2 flux rates but final models did not include metrics of benthic mussel clumps (Table 4-8). Sediment Chl-*a* content was the most influential predictor variable and was positively related to N_2 flux. Other variables included in the final model were water column nitrate concentration, which had a negative influence on N_2 flux rates, and sediment organic matter content and water column DRP which were negatively related to N_2 flux rates. Excluding data from the November Site 2 farm location chambers that had the high O_2 drawdown, does not have a significant influence on the N_2 flux results.

Table 4-8: Environmental drivers of N_2 flux. Results of DistLM analysis. P indicates probability significance of each variable explaining model variance. Prop. shows proportion of variability accounted for by each variable included in final models. Dir. indicates direction of relationship between response and predictor variables. WC = water column.

Variable	Pseudo-F	P	Prop.	Dir
Chl-*a*	6.38	0.02	0.14	+
WC NO_3^--N	3.09	0.08	0.08	-
Org	1.51	0.22	0.04	+
WC DRP	1.14	0.27	0.03	+
Final model	AICc	368.5		
	R^2	0.42		

Overall DE was greater outside mussel farms, and there was a seasonal effect with DE greater in spring (November) (Figure 4-12, Table A-13). There was a significant Farm*Site*Season interaction where the farm and outside locations at Site 2 in March were significantly different, and where there was a significant difference in DE between the Site 1 and Site 2 mussel farms in March.

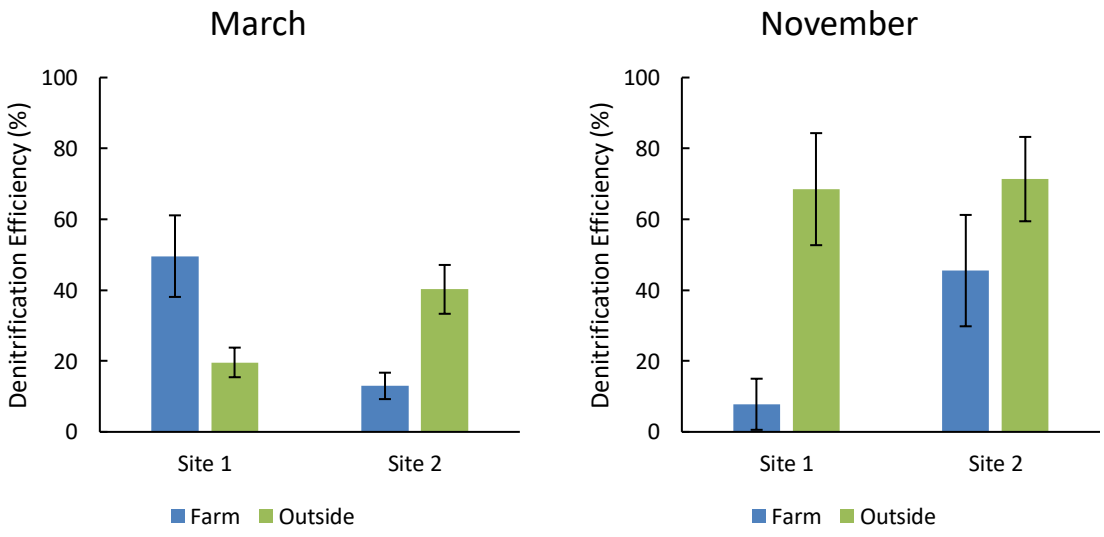


Figure 4-12: Denitrification efficiency of sediments beneath and outside mussel farms. Values are mean (+/-SE).

Denitrification efficiency was poorly explained by the environmental variables measured (Table 4-9). Together, mussel count and Chl-*a* content were the only variables included in the final model where DE was negatively related to the number of mussels and positively related to Chl-*a* content.

Table 4-9: Environmental drivers of Denitrification Efficiency. Results of DistLM analysis. P indicates probability significance of each variable explaining model variance. Prop. shows proportion of variability accounted for by each variable included in final models. Dir. indicates direction of relationship between response and predictor variables.

Variable	Pseudo-F	P	Prop.	Dir.
Mussel count	2.1595	0.14	0.067	-
Chl-*a*	4.1088	0.049	0.12	+
Final model	AICc	212.4		
	R^2	0.31		

4.8 Macrofaunal community

The benthic macrofaunal community varied among Sites, Location and Season (Figure 4-13, Table A-14, Table B-2). Cores could not be sampled from beneath the mussel farm at Site 1 in March or November so a fully orthogonal comparison could not be made. SIMPER analysis showed that *Theora lubrica* was the dominant taxa at both outside and farm locations, contributing to 59% and 39% of community dissimilarity, respectively. This was followed by Phoxocephalidae (Farm, 11%) and *Heteromastis filiformis* (Outside, 11%).

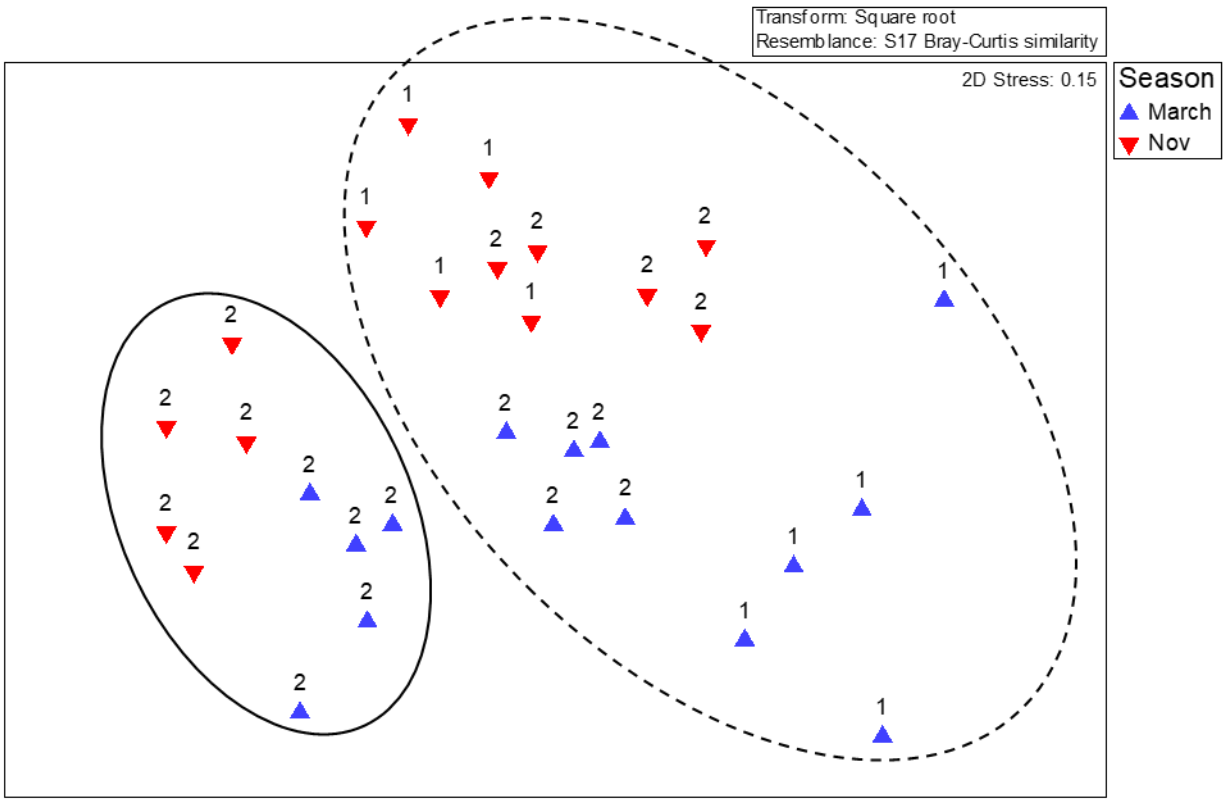


Figure 4-13: Macrofaunal community structure ordination. Plot is a non-Metric Multidimensional Scaling (nMDS) ordination from all replicates collected beneath and outside mussel farms in March and November. Mussel farm samples are within the solid ellipse and outside samples in the dashed ellipse. Numbers indicate samples from Site 1 and 2.

5 Discussion

The main aim of the study was to investigate the influence of mussel aquaculture on the nitrogen cycling functions of benthic habitats in the Firth of Thames, specifically their role in nitrogen removal. Understanding nitrogen removal rates is important for several reasons, some of which are discussed below.

The Firth of Thames receives high levels of inorganic nitrogen and organic matter from rivers draining through agricultural land in the Waikato Region. For example, the Piako, Waihou Rivers and other smaller rivers together deliver at least 3730 tonnes of N to the southern Firth of Thames each year (Vant 2016). Other work indicates that loading is highly variable and may be much higher, between 4600 and 7000 t N yr^{-1} (Zeldis, J 2008; Zeldis, J. R. et al. 2010), highlighting the uncertainty in Firth of Thames nutrient loading. As a result, the Firth of Thames is showing signs of eutrophication and degradation and has recently been classified as a degraded water body (Waikato Regional Council 2023). Biologically mediated nitrogen removal (e.g., via denitrification) represents a key ecosystem service. Being able to attribute this valuable ecosystem service to particular habitats or areas of seafloor would help Waikato Regional Council justify the protection of key habitats and better understand the influence of their consenting decisions.

To date, our understanding of natural nitrogen removal processes in the Firth of Thames is limited to the 'missing' quantity estimated from box models (Green and Zeldis 2015). Empirical measurements are needed to validate the broader scale estimates and understand how nitrogen removal rates vary by habitat type and location, and to identify the habitat variables that drive it. Without understanding spatial variation in nitrogen removal rates and an ability to identify 'hot spots' (Lohrer et al. 2020; Douglas et al. 2022), the nitrogen removal ecosystem service cannot be adequately weighed or utilised in marine spatial management in the Firth of Thames.

Mussel aquaculture is a relatively widespread practice in the Firth of Thames, an activity that is known to enrich seafloor sediments with organic matter. Organic enrichment of sediments can elevate sediment oxygen demand and increase the potential for bottom water hypoxia. The potential for bottom water hypoxia will likely increase with climate-related ocean warming, because oxygen solubility decreases with increasing temperature. However, aquaculture-related sediment organic matter enrichment may also increase the capacity of the sediment to remove nitrogen, given that sediment-associated microbially-mediated processes such as denitrification occur in the absence of oxygen and require organic carbon. Mussel aquaculture is also known to increase the supply of shell material and live mussels to the seafloor immediately under farms. Seafloor mussel beds have been shown to be hotspots of ecosystem service delivery, with the shelly habitats harbouring a greater density and diversity of invertebrates and fish, and supporting higher rates of inorganic nitrogen removal (Hillman et al. 2021; Sea et al. 2021; Benjamin et al. 2022; Sea et al. 2022a). Therefore, these 'positive' contributions of mussel farms to ecosystem health—if shown to be consistently quantifiable—could perhaps be considered alongside the negative aspects of farm establishment in resource consent decision making.

5.1 Nitrogen removal rates

Nitrogen removal (N_2 flux) rates measured beneath and outside mussel farms in this study (-185 – 355 μmol N_2 m^{-2} h^{-1}) are consistent with rates measured elsewhere in New Zealand: in intertidal and subtidal sites from estuaries across the country (0 – 300 μmol N_2 m^{-2} h^{-1} (Douglas et al. 2022)), in a eutrophic South Island lagoon estuary (0 – ~350 μmol N_2 m^{-2} h^{-1} (Crawshaw et al. 2018)), and in

subtidal restored mussel beds in the Hauraki Gulf (~-1500 μmol N_2 m^{-2} h^{-1} (i.e., net fixation) to ~900 μmol N_2 m^{-2} h^{-1} (Hillman et al. 2021; Sea et al. 2021)). In the present study, sediments beneath and outside mussel farms exhibited net nitrogen removal except for beneath the mussel farm at Site 1 in November (spring) which showed net N_2 fixation. This differs from previous measures of N_2 flux in the Firth of Thames which showed dominance of net N_2 fixation at most sites in late summer and dominance of net N_2 removal in spring (Drylie and Vopel 2019).

Net uptake of N_2 by sediment does not necessarily indicate low rates of denitrification, but rather that nitrogen fixation is exceeding it, and this needs to be considered when comparing N_2 flux rates across sites or seasons (Drylie and Vopel 2019). Denitrification is highly variable in space and time, and the measurements made in this study may have missed particular 'hot moments' where the right conditions for denitrification occur simultaneously (Douglas et al. 2022). Nitrogen flux is typically dominated by denitrification, however, other processes including anaerobic ammonium oxidation (annamox) can also produce nitrogen gas but the contribution of this is likely to be insignificant in coastal sediments (Thamdrup and Dalsgaard 2002). In places where salinity is high and nutrient enrichment low, annamox may contribute between 1–14% of the overall gaseous nitrogen flux (Nicholls and Trimmer 2009; Hou et al. 2015). Furthermore, reports of N_2 fixation could be spurious since fixing nitrogen is energetically unfavourable especially in an environment with plentiful inorganic nitrogen in both the porewater and the overlying water column (Vieillard et al. 2020).

Our results are also consistent with previous N_2 flux measurements below South Island mussel farms in Beatrix Bay which were between -6 – 11 μmol N_2 m^{-2} h^{-1} (Christensen et al. 2003), and in Kenepuru Sound which were between 13.8 – 126 μmol N_2 m^{-2} h^{-1} (Kaspar et al. 1985). However, these studies were conducted 20 and 40 years ago, respectively, and used different methods of measuring denitrification. These studies compared rates below mussel farms with those of nearby unfarmed seabed finding conflicting results; rates below farms were significantly elevated (Kaspar et al. 1985) and slightly reduced (Christensen et al. 2003) compared with reference sites (see Stenton-Dozey and Broekhuizen 2019 for discussion). Mussel farms in our study did not increase or decrease nitrogen removal rates overall, although an interaction effect suggests a site-specific influence of the mussel farm in springtime, where N_2 flux was higher outside the farm at Site 1.

Rates of denitrification associated with cultured benthic oysters have been shown to be comparable to those of restored oyster (*Crassostrea virginica*) beds (Humphries et al. 2016). For New Zealand green-lipped mussel aquaculture, it is desirable to know whether drop-off mussels below farms provide similar ecosystem services to restored mussel beds. However, to understand the true effect of drop-off mussels on benthic processes relative to the effect of mussel farms, a comparison of fluxes under mussel farms with and without mussel clumps may be warranted. However, denitrification rates measured within a restored mussel bed were similar for chambers with and without mussels concluding that mussels influence denitrification at the whole-bed scale (Sea et al. 2021).

In addition to benthic nitrogen removal, denitrification also occurs on suspended mussel farm lines with up to twice the rates of the seabed below, and ten times the denitrification rates of reference sites (Kaspar et al. 1985; Stenton-Dozey and Broekhuizen 2019). Rates of mussel line denitrification are difficult to quantify and compare with benthic rates but are likely to contribute to the overall nitrogen budget of the Firth of Thames.

5.2 Environmental drivers of N_2 removal

The main environmental drivers of N_2 flux rates (Chl-*a*, water column NO_3^-, sediment organic matter, water column DRP) were similar to those in other New Zealand studies (organic content, water column NOx, Chl-*a*, mud content) (Crawshaw et al. 2018; Sea et al. 2021; Douglas et al. 2022; Cheung et al. 2024), but differed to those found in another Firth of Thames study (coarse sand, phaephytin) (Drylie and Vopel 2019) Sediment organic matter content provides a carbon source for heterotrophic denitrifying bacteria as well as a source of ammonium for the nitrification process which provides nitrate for denitrification, thus it is expected to be a key driver of denitrification. This is consistent with other denitrification studies in oligotrophic waters (low water column nitrate concentration) where organic matter mineralisation is the main nitrogen source for denitrification (Cheung et al. 2024). Nitrogen removal rates were higher in the inner harbour (Site 2) where sediment organic content was higher.

Sediment organic matter was the most important predictor of N_2 flux rates in restored mussel beds where higher rates were associated with moderate sediment organic matter loading (from biodeposits) (Sea et al. 2021). However, the range in sediment organic matter values in the restored mussel beds in the Sea et al. (2021) study were much lower (2.7–3.4%) than those inside and outside farms in our study (4.2–8.1%). In another study of restored mussel beds with sediment organic matter content similar to our study (1.97–4.93%), nitrogen removal rates from within mussel beds (-16 – 330 μmol m^{-2} h^{-1}) were similar to those presented here (Hillman et al. 2021).

The dense layer of shell hash below the farm at Site 1 may reflect the age of the farm, and farm age is expected to influence other sediment properties such as organic matter accumulation and therefore denitrification rates (Onorevole et al. 2018). Organic content accumulation over time can also lead to buildup of hydrogen sulphide which can inhibit coupled nitrification denitrification (Joye and Hollibaugh 1995). Organic loading increases result in increased decomposition and subsequent increased benthic oxygen demand. In such scenarios, more nitrogen is recycled within the ecosystem as ammonium than is removed as N_2 gas (Kemp et al. 1990). Increased organic loading beneath Marlborough Sounds mussel farms was shown to decrease denitrification, where it removed only ~2% of the mussel farm derived nitrogen (Christensen et al. 2003). There may be a threshold in sediment organic matter where further increases no longer contribute to higher denitrification rates. Furthermore, the relationship between sediment composition and denitrification may be context-dependent; for example the presence of bioturbating macrofauna may increase the threshold due to enhancing the coupling of nitrification-denitrification within the sediment profile (Douglas et al. 2019). Changes in denitrification over time below mussel farms and within restored mussel beds should be anticipated as sediment properties change. Shell hash can mitigate the negative impact of organic enrichment in marine sediments (e.g., through changes in biogeochemistry) however this effect may be dependent on the presence of bioturbating macrofauna (Casado-Coy et al. 2017; Bergström et al. 2020).

Phosphorus is not an environmental variable commonly associated with denitrification (Wallenstein et al. 2006). In this study water column DRP concentration accounted for a small proportion of N_2 flux variability (0.03), which could be attributed to the ability of phosphorus to enhance the performance and diversity of denitrifying bacteria (Fan et al. 2018). Water column DRP was also an influential driver of dissolved oxygen flux (0.79) and NH_4^+ flux (0.17). These relationships may be from bacterial organic matter mineralisation of mussel faeces and pseudo-faeces accumulations, resulting in sediment oxygen consumption, and nitrogen and phosphorus release. Water column DRP and

sediment organic content were correlated (0.54) indicating that DRP may be acting as a proxy for organic matter mineralisation or availability.

The importance of microphytobenthos (Chl-*a*) in governing N_2 flux rates is consistent with other studies (Sea et al. 2021; Douglas et al. 2022). Microphytobenthos may influence denitrification by competing for nitrate, by reducing fluxes of other solutes through binding and stabilising sediments, or through the effect of photosynthesis-produced oxygen which can accelerate coupled nitrification-denitrification (Harris et al. 2015; Serpetti et al. 2016; Bartoli et al. 2021). Chamber incubations in this study were conducted in the dark removing the influence of photosynthesis, however microphytes continue to assimilate nitrogen in the dark for at least the duration of the incubation period used in this study (Cochlan et al. 1991; Rysgaard et al. 1993).

Water column nitrate concentration was the second most important factor controlling N_2 flux in this study (although only marginally significant, p=0.08) despite consistently low concentrations (oligotrophic conditions), however higher N_2 flux rates were associated with lower concentrations (i.e., negatively correlated). At all sites and sampling events, nitrite and nitrate are being released from the seafloor to the water column (with the exception of the outside locations in November which showed net nitrite uptake), suggesting that direct water column denitrification is insignificant and most denitrification is coupled to nitrification in the sediments.

Denitrification and other benthic nitrogen transformations are known to be influenced by benthic macrofauna, particularly large burrowing animals (Rysgaard et al. 1995; Gilbert et al. 2003; Michaud et al. 2006). Some of the variability in N_2 flux may have been attributed to difference in macrofaunal communities among sampling locations and seasons. For example, burrowing crabs (*Hemiplax hirtipes*) were likely responsible for the surface features seen at the outside locations at both sites in November and March. This species was present in similar abundances at farm and outside locations (Table B-2), although bioturbator influence on fluxes can differ with sedimentary environment (Needham et al. 2011). However, with the data obtained during this study, we were unable to fully analyse macrofauna -N_2 flux relationships.

5.3 Denitrification efficiency

Denitrification efficiency (DE) is the proportion of benthic nitrogen flux that is N_2 gas and can be used as a measure of nutrient enrichment in coastal ecosystems (Eyre and Ferguson 2009). In this study DE was lower beneath mussel farms than outside and was greater in spring. Denitrification efficiency values beneath farms were on average less than those reported for farmed clams (~67%, *Ruditapes philippinarum*) and greater than those within (-15-10%) and outside (-5-1%) restored green-lipped mussel beds (Hillman et al. 2021). Mussel count was included in the final model for DE although they were negatively correlated indicating that benthic mussels reduce DE. Other work has suggested that high organic loading in the Firth of Thames decreases DE (Green and Zeldis 2015), however this study shows a positive relationship between DE and sediment organic matter content (Appendix B). Both the quantity and quality (availability to denitrifiers) is important for denitrification (Eyre et al. 2013) and optimum DE may occur at a certain carbon loading (Eyre and Ferguson 2009). Further analysis of organic carbon associated with Firth of Thames mussel farms is required to fully understand the influence on N_2 removal and DE in these systems.

5.4 The influence of mussel farms and benthic mussels on benthic ecosystem function

Biodeposits from mussel farms can have a significant influence on local sediment conditions and biogeochemistry (McKindsey et al. 2011). The amount of biodeposition is dependent on local hydrodynamics and in some places has little influence on the seabed more than 50 m away from farms (Hartstein and Stevens 2005). Higher fluxes of suspended solids from the water column to the seabed were measured beneath the mussel farms compared with outside sites in Manaia Harbour. Outside sites were approximately 200 m from farms and appeared to be subject to biodeposition from mussel farms. This is unsurprising given the extent of aquaculture in the harbour (Figure 3-1) and underscores the difficulty in conducting a true comparative study, i.e., a site away from the sphere of influence of the farms while maintaining similar environmental conditions does not exist. Unfortunately, sediment traps were only deployed during the spring sampling so could not be used in analyses of environmental drivers of N_2 flux. The fall of suspended material showed a site*farm interaction indicating context dependency of organic matter flux from farm to seabed which could have implications for benthic nitrogen cycling in farms in different places.

Mussel farms in this study had a significant influence on nutrient fluxes which were consistently higher beneath farms when compared with outside mussel farms, and at least half of the variability in these fluxes were explained by water column nutrient concentrations and metrics of benthic mussels. Benthic mussels below farms had a positive influence on the release of DRP, NH_4^+ NO_3^- and NO_2^- from the sediments which will indirectly influence nitrogen removal rates. Greater release of phosphorous, ammonium and nitrates from the seabed to the water column below mussel farms results from the breakdown of organic biodeposits.

Mussel farms and subsequent organic matter deposition are commonly associated with increased benthic oxygen demand (Matisson and Lindén 1983; Christensen et al. 2003). Changes in benthic organic and oxygen conditions lead to a shift in biological communities, often lower taxonomic richness and higher overall abundance (Pearson and Rosenberg 1978). A balanced analysis of differences in benthic communities could not be done because cores from the Site 1 mussel farm could not be sampled. However, there were differences in the benthic communities between farm and outside locations, between sites, and across seasons. Benthic oxygen consumption rates beneath and outside mussel farms were similar or greater than those in restored mussel beds (Sea et al. 2022b) apart from the high rates beneath the farm at Site 2.

5.5 Conclusions

Overall, it appears that the benthos beneath mussel farms are areas of net nitrogen removal at times. However, whether these areas are ‘hotspots’ of net nitrogen removal (i.e., with higher nitrogen removal rates than areas outside the sphere of influence of mussel farms) remains inconclusive. The data suggests that the seafloor beneath mussel farms does not have higher nitrogen removal rates than the seafloor adjacent to mussel farms. Nitrogen removal rates were higher inside farms on some occasions/sites and higher outside on some occasions/sites. In other words, the influence of mussel aquaculture on sediment biogeochemistry and nitrogen removal is probably dependent on the farm location and local environmental conditions. A similar study in a location where the mussel farm effects are contained to areas beneath farms (e.g., an offshore site with higher flushing) may produce different results. To further assess the influence of mussel aquaculture on nitrogen cycling in sheltered waters such as Manaia Harbour, a similar study

encompassing gradients in the key variables driving denitrification (sediment organic content, Chl-*a*, water column nitrate concentration) and/or distance from farms may be beneficial.

Nitrogen removal rates may be indirectly influenced by seafloor mussels below farms due to the effect of mussels on nutrient fluxes and therefore the availability of nitrogen for denitrification. However, we did not find clear evidence to support the enhancement of the nitrogen removal ecosystem service by benthic mussels or mussel farms. Furthermore, benthic mussels had a negative influence on denitrification efficiency, which was lower on the seafloor inside mussel farms compared with outside.

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Appendix A Supplementary tables: PERMANOVA results

Table A-1: PERMANOVA results for sediment mud content. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
Mud content	Fa	1	2491.7	57.956	0.001	Farm < Outside
	Si	1	90.06	2.0948	0.152	
	Se	1	130.61	3.038	0.117	
	FaxSi	1	5256.5	122.26	0.001	Site 1: Farm < Outside, Site 2: Farm > Outside, Farm: Site 1 < Site 2, Outside: Site 1 > Site 2
	FaxSe	1	5.7456	0.13364	0.728	
	SixSe	1	325.13	7.5624	0.010	Nov: Site 1 < Site 2, Site 2: March < Nov
	FaxSixSe	1	28.832	0.67063	0.405	
	Res	32	42.993			
	Total	39				

Table A-2: PERMANOVA results for sediment organic matter content. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
Organic	Fa	1	0.019492	0.15509	0.671	
	Si	1	3.3761	26.861	0.001	Site 2 > Site 1
	Se	1	14.115	112.3	0.001	March < November
	FaxSi	1	13.302	105.83	0.001	Site 1: Farm < outside, Site 2: Farm > outside
	FaxSe	1	0.93667	7.4524	0.014	March: Farm < outside, Farm: March < Nov, Outside: March < Nov
	SixSe	1	0.88899	7.0731	0.014	Site 1: March < Nov, Site 2: March < Nov, March: Site 1 < Site 2, November: Site 1 < Site 2
	FaxSixSe	1	0.74234	5.9063	0.024	
	Res	32	0.12569			
	Total	39				

FaxSixSe			
Site 1 March:	Farm	<	Outside
Site 1 Nov:	Farm	<	Outside
Site 2 March:	Farm	>	Outside
Site 2 Nov:	Farm	>	Outside
Farm March:	Site 1	<	Site 2
Farm Nov:	Site 1	<	Site 2
Outside March:	Site 1	>	Site 2
Outside Nov:	Site 1	>	Site 2
Site 1 Farm:	March	<	November
Site 2 Farm:	March	<	November
Site 1 Outside:	March	<	November
Site 2 Outside:	March	<	November

Table A-3: PERMANOVA results for sediment chlorophyll a. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	source	df	MS	Pseudo-F	p(perm)	post-hoc pair wise tests
Chl-*a*	Fa	1	6.97	0.75	0.38	
	Si	1	61.8	6.62	0.02	Site 1 < Site 2
	Se	1	326.6	35.0	0.001	March < November
	FaxSi	1	203.0	21.8	0.001	Site 1: Farm < Outside, Site 2: Farm > Outside, Farm: Site 1 < Site 2
	FaxSe	1	0.07	0.01	0.93	
	SixSe	1	8.74	0.94	0.33	
	FaxSixSe	1	264.7	28.4	0.001	
	Res	32	9.33			

FaxSixSe			
Site 1 Nov:	Farm	<	Outside
Site 2 Nov:	Farm	>	Outside
Farm Nov:	Site 1	<	Site 2
Outside March:	Site 1	<	Site 2
Outside Nov:	Site 1	>	Site 2
Site 2 Farm:	March	<	November
Site 1 Outside:	March	<	November

Table A-4: PERMANOVA results for sediment phaeophytin content. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
Phaeo	Fa	1	3837.7	84.735	0.0001	Farm > Outside
	Si	1	0.004	8.83E-05	0.9917	
	Se	1	357.6	7.8958	0.0075	March < November
	FaxSi	1	399.42	8.8192	0.0049	Site 1: Farm > Outside, Site 2: Farm > Outside, Farm: Site 1 < Site 2, Outside: Site 1 > Site 2
	FaxSe	1	10	0.2208	0.6434	
	SixSe	1	483.03	10.665	0.0021	March: Site 1 > Site 2, Site 2: March < Nov
	FaxSixSe	1	1490.8	32.917	0.0001	
	Res	32	45.29			
	Total	39				

FaxSixSe			
Site 1 March:	Farm	>	Outside
Site 2 March:	Farm	>	Outside
Site 2 Nov:	Farm	>	Outside
Farm March:	Site 1	>	Site 2
Farm Nov:	Site 1	<	Site 2
Outside Nov:	Site 1	>	Site 2
Site 1 Farm:	March	>	November
Site 2 Farm:	March	<	November
Site 1 Outside:	March	<	November

Table A-5: PERMANOVA results for water column nutrient (DRP, NH_4^+, NO_x^-) concentrations. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated. WC = water column.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
WC NH_4^+	Fa	1	24.461	35.618	0.0001	Farm > Outside
	Si	1	4.7748	6.9528	0.0036	Site 1 < Site 2
	Se	1	16.564	24.119	0.0001	March < November
	FaxSi	1	8.8925	12.949	0.0001	Farm: Site 1 < Site 2, Outside: Site 1 > Site 2, Site 1: Farm > Outside, Site 2: Farm > Outside
	FaxSe	1	16.667	24.269	0.0001	March: Farm > Outside, November: Farm> Outside, Farm: March < November
	SixSe	1	17.109	24.912	0.0001	March: Site 1 > Site 2, November: Site 1 < Site 2, Site 2: March < November
	FaxSixSe	1	9.4478	13.757	0.0001	
	Res	32	0.68675			
	Total	39				

FaxSixSe			
Site 1 Nov:	Farm	>	Outside
Site 2 March:	Farm	>	Outside
Site 2 Nov:	Farm	>	Outside
Farm March:	Site 1	>	Site 2
Farm Nov:	Site 1	<	Site 2
Outside March:	Site 1	>	Site 2
Site 2 Farm:	March	<	November
Site 1 Outside:	March	>	November
Site 2 Outside:	March	<	November

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
WC NO_x	Fa	1	1.156	19.806	0.0002	Farm > Outside
	Si	1	2.6317	45.088	0.0001	Site 1 > Site 2
	Se	1	6.084	104.24	0.0001	March < November
	FaxSi	1	0.02401	0.41136	0.527	
	FaxSe	1	0.33124	5.6751	0.0217	March: Farm > Outside, November: Farm > Outside, Farm: March < November, Outside: March < November
	SixSe	1	0.37249	6.3818	0.0147	March: Site 1 > Site 2, November: Site 1 > Site 2, Site 1: March < November, Site 2: March < November
	FaxSixSe	1	0.07225	1.2378	0.2694	
	Res	32	0.058368			
	Total	39				

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
WC DRP	Fa	1	0.31152	8.9016	0.0001	Farm > Outside
	Si	1	0.019803	0.56585	0.6094	
	Se	1	0.23256	6.6454	0.0004	March < November
	FaxSi	1	0.095062	2.7164	0.0589	
	FaxSe	1	0.1575	4.5006	0.005	March: Farm > Outside, November: Farm > Outside, Farm: March < November
	SixSe	1	0.31506	9.0028	0.0001	March: Site 1 > Site 2, November: Site 1 < Site 2, Site 2: March < November
	FaxSixSe	1	0.1092	3.1204	0.0368	
	Res	32	0.034996			
	Total	39				

FaxSixSe			
Site 1 Nov:	Farm	>	Outside
Site 2 Nov:	Farm	>	Outside
Farm March:	Site 1	>	Site 2
Farm Nov:	Site 1	<	Site 2
Outside March:	Site 1	>	Site 2
Site 2 Farm:	March	<	November
Site 2 Outside:	March	<	November

Table A-6: PERMANOVA results for total suspended solids (TSS) and volatile suspended solids (VSS). Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
TSS	Fa	1	45793	26.337	0.0001	Farm > Outside
	Si	1	43920	25.259	0.0001	Site 1 > Site 2
	FaxSi	1	20213	11.625	0.0013	Site 1: Farm > Outside, Site 2: Farm > Outside, Farm: Site 1 < Site 2, Outside: Site 1 < Site 2
	Res	16	1738.8			
	Total	19				

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
VSS	Fa	1	394.45	6.3512	0.025	Farm > Outside
	Si	1	100.35	1.6158	0.224	
	FaxSi	1	20.849	0.33569	0.581	
	Res	16	62.107			
	Total	19				

Table A-7: PERMANOVA results for dissolved oxygen (DO) flux. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	p(perm)	post-hoc pair wise tests
DO flux	Fa	1	1.51E+09	3.7358	0.0006	Outside > Farm
	Si	1	1.26E+09	3.1234	0.007	Site 1 > Site 2
	Se	1	1.13E+09	2.7838	0.0211	March > November
	FaxSi	1	1.72E+09	4.2562	0.0001	Site 1: Farm > Outside, Outside: Site 1 < Site 2
	FaxSe	1	8.86E+08	2.1897	0.1259	
	SixSe	1	7.31E+08	1.806	0.2949	
	FaxSixSe	1	8.44E+08	2.0862	0.1696	
	Res	32	4.05E+08			

Table A-8: PERMANOVA results for ammonium (NH_4^+) flux. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
NH_4^+ flux	Fa	1	50410	4.2343	0.0414	Farm > Outside
	Si	1	1020.1	0.085685	0.776	
	Se	1	17893	1.5029	0.2406	
	FaxSi	1	2689.6	0.22592	0.647	
	FaxSe	1	2131.6	0.17905	0.6866	
	SixSe	1	28196	2.3684	0.141	
	FaxSixSe	1	43560	3.6589	0.0593	
	Res	32	11905			
	Total	39				

FaxSixSe			
Site 1 Nov:	Farm	>	Outside
Site 2 March:	Farm	>	Outside
Farm March:	Site 1	<	Site 2
Site 1 Farm:	March	<	November
Site 1 Outside:	March	>	November

Table A-9: PERMANOVA results for dissolved reactive phosphorus (DRP) flux. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
DRP flux	Fa	1	6088.6	100.87	0.0001	Farm > Outside
	Si	1	1076.4	17.833	0.0003	Site 1 < Site 2
	Se	1	17.822	0.29527	0.5886	
	FaxSi	1	1558.8	25.825	0.0001	Site 1: Farm > Outside, Site 2: Farm > Outside, Farm: Site 1 < Site 2
	FaxSe	1	244.53	4.0513	0.0537	
	SixSe	1	25.44	0.42148	0.5256	
	FaxSixSe	1	25.122	0.41622	0.5186	
	Res	32	60.359			
	Total	39				

Table A-10: PERMANOVA results for nitrite (NO_2^-) flux. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
NO_2^- flux	Fa	1	255.32	37.799	0.0001	Farm > Outside
	Si	1	17.624	2.6092	0.1126	
	Se	1	133.79	19.808	0.0004	March > November
	FaxSi	1	34.505	5.1084	0.0311	Site 1: Farm > Outside, Site 2: Farm > Outside, Farm: Site 1 < Site 2
	FaxSe	1	13.501	1.9987	0.167	
	SixSe	1	1.0876	0.16102	0.6898	
	FaxSixSe	1	56.764	8.4039	0.0064	
	Res	32	6.7546			
	Total	39				

FaxSixSe			
Site 1 Nov:	Farm	>	Outside
Site 2 March:	Farm	>	Outside
Site 2 Nov:	Farm	>	Outside
Outside Nov:	Site 1	<	Site 2
Site 2 Farm:	March	>	November
Site 1 Outside:	March	>	November
Site 2 Outside:	March	>	November

Table A-11: PERMANOVA results for nitrate (NO_3^-) flux. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
NO_3^- flux	Fa	1	8439.7	19.68	0.0001	Farm > Outside
	Si	1	18.566	0.043292	0.8423	
	Se	1	2696.6	6.2879	0.0125	March < November
	FaxSi	1	353.88	0.82518	0.3812	
	FaxSe	1	3107.4	7.2458	0.0074	March: Farm > Outside, November: Farm > Outside, Farm: March < November
	SixSe	1	403.71	0.94136	0.3536	
	FaxSixSe	1	785.37	1.8313	0.1926	
	Res	32	428.86			
	Total	39				

Table A-12: PERMANOVA results for nitrogen gas (N_2) flux. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	p(perm)	post-hoc pair wise tests
N_2 flux	Fa	1	5341	0.56	0.47	
	Si	1	34269	3.58	0.07	Site 2 > Site1
	Se	1	2663	0.28	0.61	
	FaxSi	1	9753	1.02	0.32	
	FaxSe	1	16120	1.69	0.21	
	SixSe	1	19114	2.00	0.16	
	FaxSixSe	1	117440	12.28	0.001	Site 1 Nov: Farm < Out, Site 1 Farm: March > Nov, Farm Nov: 1 < 2
	Res	32	9565			
	Total	39				

FaxSixSe			
Site 1 Nov:	Farm	<	Outside
Site 1 Farm:	March	>	November

Table A-13: PERMANOVA results for denitrification efficiency (DE). Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant (p<0.05), post-hoc pair-wise test results are indicated.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
DE	Fa	1	3213	6.40	0.018	Farm < Outside
	Si	1	277	0.55	0.50	
	Se	1	2294	4.57	0.04	March < November
	FaxSi	1	229	0.46	0.48	
	FaxSe	1	3649	7.26	0.014	November: Farm < Outside, Outside: March < November
	SixSe	1	1466	2.92	0.09	
	FaxSixSe	1	3888	7.74	0.018	
	Res	24	502			
	Total	31				

FaxSixSe			
Site 2 March:	Farm	<	Outside
Farm March:	Site 1	>	Site 2

Table A-14: PERMANOVA results for the benthic macrofaunal community. Permutational Analysis of Variance (PERMANOVA) with three Factors: Farm (Fa) – Farm/Outside, Site (Si) – Site 1/Site 2, Season (Se) – March/November. Shading indicates significant effect and where significant ($p<0.05$), post-hoc pair-wise test results are indicated. Separate tests for the whole community, number of taxa and number of individuals are shown.

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
Whole community	Fa	1	1.12E+04	10.6	0.0001	Farm ≠ Outside
	Si	1	2463	2.33	0.019	Site 1 ≠ Site 2
	Se	1	7787	7.38	0.0001	March ≠ November
	FaxSi**	0		No test		
	FaxSe	1	2922	2.77	0.007	March: Farm ≠ Outside, Nov: Farm ≠ Outside
	SixSe	1	4439	4.21	0.0004	Site 1: March ≠ November, Site 2: March ≠ November
	FaxSixSe**	0		No test		
	Res	24	1055.1			
	Total	29				

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
Taxa	Fa	1	1423	31.06	0.0001	Farm > Outside
	Si	1	22.1	0.48	0.52	
	Se	1	152	3.31	0.08	
	FaxSi**	0		No test		
	FaxSe	1	111	2.43	0.13	
	SixSe	1	6.86	0.15	0.77	
	FaxSixSe**	0		No test		
	Res	24	45.8			
	Total	29				

Variable	Source	df	MS	Pseudo-F	P(perm)	post-hoc pair wise tests
Individuals	Fa	1	2662	54.3	0.0001	Farm > Outside
	Si	1	364	7.43	0.008	Site 1 < Site 2
	Se	1	2260	46.1	0.0001	March < November
	FaxSi**	0		No test		
	FaxSe	1	6.12	0.12	0.80	
	SixSe	1	2766	56.4	0.0001	March: Site 1 < Site 2, November: Site 1 > Site 2, Site 1: March < November
	FaxSixSe**	0		No test		
	Res	24	49.0			
	Total	29				

Table A-15: Pearson's correlation coefficient matrix. Relationships between environmental variables and fluxes. Includes data from all sites and seasons.

	WC DRP	WC NH_4^+-N	WC NO_2-N	WC NO_3-N	WC DIN	Mussel biomass	Mussel count	Shell	Mud	Org	Chl *a*	Phaeo	N_2 flux	NH_4^+ flux	DRP flux	DIN flux	O_2 flux	DE	NO_2^- flux
WC DRP																			
WC NH_4^+-N	0.53																		
WC NO_2-N	0.08	0.00																	
WC NO_3-N	0.42	0.40	0.75																
WC DIN	0.56	0.96	0.23	0.63															
Mussel biomass	0.44	0.55	0.15	0.39	0.58														
Mussel count	0.64	0.72	-0.10	0.27	0.69	0.79													
Shell	0.00	-0.06	0.02	0.00	-0.05	0.53	0.14												
Mud	0.14	0.14	-0.08	-0.17	0.08	-0.39	0.05	-0.64											
Org	0.54	0.62	0.12	0.28	0.61	0.08	0.53	-0.55	0.64										
Chl*a*	0.44	0.56	0.26	0.39	0.59	0.20	0.50	-0.32	0.45	0.75									
Phaeo	0.62	0.76	0.15	0.50	0.79	0.75	0.77	0.37	-0.05	0.40	0.55								
N_2 flux	0.17	0.11	-0.18	-0.27	0.03	-0.03	0.13	-0.06	0.27	0.20	0.38	0.13							
NH_4^+ flux	0.42	-0.30	0.00	0.03	-0.25	0.19	0.18	0.16	-0.11	-0.03	-0.16	-0.02	-0.10						
DRP flux	0.18	0.60	-0.16	0.09	0.54	0.62	0.73	0.19	-0.09	0.37	0.24	0.53	-0.03	0.12					
DIN flux	0.43	-0.22	0.04	0.09	-0.17	0.30	0.27	0.18	-0.19	0.00	-0.17	0.03	-0.16	0.97	0.24				
O_2 flux	-0.88	-0.50	0.02	-0.35	-0.52	-0.32	-0.55	0.10	-0.23	-0.54	-0.53	-0.59	-0.11	-0.37	-0.15	-0.34			
DE	-0.10	0.02	0.26	0.11	0.08	-0.28	-0.26	-0.18	0.08	0.12	0.35	-0.06	0.41	-0.67	-0.41	-0.67	0.18		
NO_2^- flux	0.10	0.14	-0.42	-0.20	0.06	0.41	0.41	0.40	-0.22	-0.11	-0.24	0.20	-0.20	0.43	0.67	0.51	-0.06	-0.61	
NO_3^- flux	0.20	0.24	0.22	0.31	0.29	0.49	0.41	0.09	-0.40	0.13	-0.04	0.20	-0.29	0.18	0.49	0.40	0.03	-0.15	0.38

Appendix B Macrofaunal community

Table B-1: Full taxa list of macrofaunal species collected in March and November 2023.

Phylum	Class	Order/Subclass	Family	Taxa
Annelida	Polychaeta	Phyllodocida	Nephtyidae	*Aglaophamus verrilli*
Arthropoda	Malacostraca	Decapoda	Alpheidae	*Alpheus spp*
Annelida	Polychaeta	Terebellida	Ampharetidae	*Ampharetidae*
Echinodermata	Ophiuroidea	Ophiurida	Amphiuridae	*Amphiura sp*
Mollusca	Bivalvia	Pectinoida	Anomiidae	*Anomiidae*
Arthropoda	Malacostraca	Isopoda	Anthuridae	*Anthuridae*
Arthropoda	Malacostraca	Amphipoda	Aoridae	*Aoridae*
Mollusca	Bivalvia	Mytilida	Mytilidae	*Arcuatula senhousia*
Annelida	Polychaeta	Scolecida (infra class)	Opheliidae	*Armandia maculata*
Annelida	Polychaeta	Scolecida (infra class)	Maldanidae	*Asychis theodori*
Annelida	Polychaeta	Sedentaria	Capitellidae	*Barantolla lepte*
Arthropoda	Malacostraca	Amphipoda	Oedicerotidae	*Bathymedon sp*
Annelida	Polychaeta	Scolecida (infra class)	Capitellidae	*Capitella spp*
Annelida	Polychaeta	Spionoda	Spionidae	*Carazziella sp*
Chaetognatha				*Chaetognatha*
Arthropoda	Malacostraca	Amphipoda	Cheirocratidae	*Cheirocratus sp*
Annelida	Polychaeta	Terebellida	Cirratulidae	*Cirratulidae*
Mollusca	Gastropoda	Neogastropoda	Cominellidae	*Cominella sp*
Arthropoda	Copepoda			*Copepoda*
Annelida	Polychaeta	Scolecida (infra class)	Cossuridae	*Cossura consimilis*
Arthropoda	Malacostraca	Cumacea	Bodotriidae	*Cyclaspis elegans*
Mollusca	Bivalvia	Venerida	Mactridae	*Cyclomactra ovata*
Arthropoda	Malacostraca	Cumacea	Diastylidae	*Diastylis insularum*
Annelida	Polychaeta	Eunicida	Dorvilleidae	*Dorvilleidae*
Echinodermata	Echinoidea	Spatangoida	Loveniidae	*Echinocardium cordatum*
Annelida	Polychaeta	Terebellida	Flabelligeridae	*Flabelligeridae*
Annelida	Polychaeta	Phyllodocida	Glyceridae	*Glycera ovigera*
Arthropoda	Malacostraca	Decapoda	Hymenosomatidae	*Halicarcinus varius*
Annelida	Polychaeta	Phyllodocida	Polynoidae	*Harmothoe sp*
Arthropoda	Malacostraca	Decapoda	Macrophthalmidae	*Hemiplax hirtipes*
Annelida	Polychaeta	Scolecida (infra class)	Capitellidae	*Heteromastus filiformis*
Mollusca	Bivalvia	Adapedonta	Hiatellidae	*Hiatella sp*
Cnidaria	Hydrozoa			*Hydrozoa*
Arthropoda	Malacostraca	Amphipoda	Ischyroceridae	*Ischyroceridae*
Annelida	Polychaeta	Phyllodocida	Sigalionidae	*Labiosthenolepis sp*
Annelida	Polychaeta	Terebellida	Pectinariidae	*Lagis australis*
Annelida	Polychaeta	Phyllodocida	Nereididae	*Leonnates stephensoni*
Annelida	Polychaeta	Phyllodocida	Polynoidae	*Lepidastheniella comma*
Arthropoda	Malacostraca	Cumacea	Leuconidae	*Leucon sp*

Phylum	Class	Order/Subclass	Family	Taxa
Arthropoda	Malacostraca	Amphipoda	Liljeborgiidae	*Liljeborgia sp*
Mollusca	Bivalvia	Limida	Limidae	*Limaria orientalis*
Annelida	Polychaeta	Eunicida	Lumbrineridae	*Lumbrineridae*
Arthropoda	Malacostraca	Amphipoda	Lysianassidae	*Lysianassidae*
Annelida	Polychaeta	Spionida	Magelonidae	*Magelona dakini*
Mollusca	Bivalvia	Venerida	Mactridae	*Maorimactra ordinaria*
Arthropoda	Malacostraca	Mysida		*Mysida*
Arthropoda	Malacostraca	Nebaliacea		*Nebaliacea*
Nematoda				*Nematoda*
Nemertea				*Nemertea*
Arthropoda	Malacostraca	Decapoda	Pinnotheridae	*Nepinnotheres novaezelandiae*
Mollusca	Bivalvia	Nuculida	Nuculidae	*Nucula sp1*
Annelida	Clitellata	Oligochaeta (subclass)		*Oligochaeta*
Annelida	Polychaeta	Eunicida	Onuphinae	*Onuphis aucklandensis*
Arthropoda	Ostracoda			*Ostracoda*
Annelida	Polychaeta	Phyllodocida	Hesionidae	*Oxydromus angustifrons*
Arthropoda	Malacostraca	Amphipoda	Paracalliopiidae	*Paracalliope novizealandiae*
Annelida	Polychaeta	Scolecida (infra class)	Paraonidae	*Paradoneis lyra*
Arthropoda	Malacostraca	Cumacea	Leuconidae	*Paraleucon sp*
Annelida	Polychaeta	Spionoda	Spionidae	*Paraprionospio sp*
Mollusca	Gastropoda	Cephalaspidea	Philinidae	*Philine sp*
Phoronida				*Phoronida*
Arthropoda	Malacostraca	Amphipoda	Phoxocephalidae	*Phoxocephalidae (other)*
Annelida	Polychaeta	Scolecida (infra class)	Orbiniidae	*Phylo sp*
Annelida	Polychaeta	Phyllodocida	Nereididae	*Platynereis australis*
Annelida	Polychaeta	Spionida	Spionidae	*Prionospio ehlersi*
Annelida	Polychaeta	Spionoda	Spionidae	*Prionospio spp*
Annelida	Polychaeta	Spionida	Spionidae	*Prionospio yuriel*
Annelida	Polychaeta	Spionida	Spionidae	*Pseudopolydora paucibranchiata*
Mollusca	Polyplacophora	Chitonida	Chitonidae	*Rhyssoplax stangeri*
Mollusca	Scaphopoda			*Scaphopoda*
Annelida	Polychaeta	Sabellida	Serpulidae	*Serpulidae*
Annelida	Sipuncula			*Sipuncula*
Annelida	Polychaeta	Phyllodocida	Syllidae	*Sphaerosyllis semiverrucosa*
Annelida	Polychaeta	Phyllodocida	Syllidae	*Syllinae*
Mollusca	Bivalvia	Venerida	Veneridae	*Tawera spissa*
Annelida	Polychaeta	Terebellida	Terebellidae	*Terebellidae*
Mollusca	Bivalvia	Venerida	Semelidae	*Theora lubrica*
Arthropoda	Malacostraca	Amphipoda	Phoxocephalidae	*Torridoharpinia hurleyi*
Mollusca	Gastropoda	Neogastropoda	Nassariidae	*Tritia burchardi*

Table B-2: Average abundance of macrofaunal species observed at sampling locations in March and November 2023. Note: Ostracods, considered meiofauna, were not included in analyses of community composition.

	March Site 1 Outside	March Site 2 Farm	March Site 2 Outside	November Site 1 Outside	November Site 2 Farm	November Site 2 Outside
Aglaophamus verrilli	0.2	0	0.2	0.6	0	0.8
Alpheus spp	0	0.8	0	0	0	0
Ampharetidae	0	0	0	0.8	0.6	0.8
Amphiura sp	0.2	0.2	0	0	0.2	0
Anomiidae	0	1.2	0	0	0	0
Anthuridae	0	0.6	0	0	0	0
Aoridae	0	0	0	0	0.6	0.4
Arcuatula senhousia	0	0	0	0	0.4	0
Armandia maculata	0	0.2	0	0	2.6	0
Asychis theodori	0.2	0	0	0	0	0
Barantolla lepte	0	0	0	0	0.2	0
Bathymedon sp	0	0	0	0	1	0
Capitella spp	0	0.6	0	0	0	0
Carazziella sp	0	0	0	0	0	0.8
Chaetognatha	0	0	0.4	0.4	0	0
Cheirocratus sp	0	1	0.2	0	0	0
Cirratulidae	0.2	0.2	0.2	0	0	0.4
Cominella sp	0	0	0	0.2	0	0
Copepoda	0	0	0	0	0	0.2
Cossura consimilis	1.6	0.8	1	0.6	0.2	1.2
Cyclaspis elegans	0	0	0	0.4	0	0
Cyclomactra ovata	0	0	0	0	0.4	0
Diastylis insularum	0	0	0	3.8	0.2	0.2
Dorvilleidae	0	1	0	0	0.8	0
Echinocardium cordatum	0	0	0	0.8	0	0
Flabelligeridae	0	0	0	0	0	0.2
Glycera ovigera	0.2	0.2	0	0	0	0
Halicarcinus varius	0	0.2	0	0	0.2	0
Harmothoe sp	0	0.2	0	0	0	0
Hemiplax hirtipes	0.4	0	0.2	0.4	0.2	0.2
Heteromastus filiformis	1.6	3.2	2.8	0.6	0.4	1.6
Hiatella sp	0	0.2	0	0	0	0.2
Hydrozoa	0.2	0.4	0	0	0	0
Ischyroceridae	0	0	0	1	0	0
Labiosthenolepis sp	0.2	0	0.2	1.6	0.4	1
Lagis australis	0	0.2	0.2	0	0.6	0
Leonnates stephensoni	0.2	1	1	0	0	0.2
Lepidastheniella comma	0	0.2	0	0	0	0
Leucon sp	0	0.6	0	0	5.6	0

	March Site 1 Outside	March Site 2 Farm	March Site 2 Outside	November Site 1 Outside	November Site 2 Farm	November Site 2 Outside
Liljeborgia sp	0	0	0.2	0	0	0
Limaria orientalis	0	0.4	0	0	0	0
Lumbrineridae	0.4	0.2	0.2	0.8	0.4	0.2
Lysianassidae	0	0.4	0	0	0	0
Magelona dakini	0	0	0	0	0.2	0
Maorimactra ordinaria	0	0	0	0.2	0	0.2
Mysida	0	0.2	0.2	0.6	0	0.4
Nebaliacea	0	0.2	0	0	0.2	0
Nematoda	0.2	0.8	0	0	0	0
Nemertea	0.4	0.4	0	0	0	0
Nepinnotheres novaezelandiae	0	0	0	0.4	0	0
Nucula sp1	0	0	0	0	0.2	0
Oligochaeta	0.2	2.8	0	0.2	2.6	0.2
Onuphis aucklandensis	0.2	0	0	0	0	0
Ostracoda	0.8	4.6	11	15.8	6.8	15.8
Oxydromus angustifrons	0.8	0	0.2	0	0.2	0
Paracalliope novizealandiae	0	0	0	0	0.2	0
Paradoneis lyra	0	6.2	0.2	0.2	2.6	0
Paraleucon sp	0	0	0	1.4	0	0
Paraprionospio sp	0	0	0	0	0.6	0.2
Philine sp	0	0	0	0	0.4	0.2
Phoronida	0	0	0	0	0.2	0
Phoxocephalidae (other)	0	11	0.6	1	9	0
Phylo sp	0	0	0	0	0	0.2
Platynereis australis	0	0.2	0	0	0	0
Prionospio ehlersi	0	0.8	0	0	10.2	0
Prionospio spp	0	0	0	0	0.2	0
Prionospio yuriel	0.8	3.2	2	0.2	9.4	0.2
Pseudopolydora paucibranchiata	0	0.2	0	0	1	0
Rhyssoplax stangeri	0	0.4	0	0	0	0
Scaphopoda	0	0	0	3.2	0	3.2
Serpulidae	0	0	0	0	0.6	0
Sipuncula	0	0	0	0	0.2	0
Sphaerosyllis semiverrucosa	0	0	0	0.2	0	0
Syllinae	0	0.2	0	0	0	0
Tawera spissa	0	0	0	0	0.4	0
Terebellidae	0.4	1	0	0.4	0	0
Theora lubrica	5	75.8	37	70	47.8	30.4
Torridoharpinia hurleyi	0	0.8	1	1	10	0.6
Tritia burchardi	0	0	0	0.2	0.4	0.2