



## Mixed-habitat assimilation of organic waste in coastal environments – It's all about synergy!



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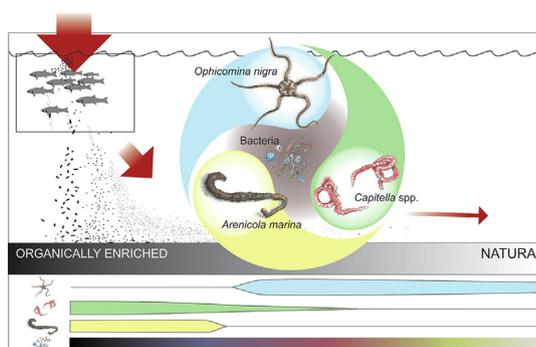
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### HIGHLIGHTS

- Benthic assessments of enrichment often exclude functionally important fauna.
- *Arenicola marina* occupies subtidal opportunistic niche in response to organic waste.
- *Ophiocomina nigra* exhibits tolerance threshold and positive response in near-field.
- Synergy between 3 contrasting fauna, microbes responsible for high assimilation capacity
- Sediment microbe composition suggests inoculation from/to overlying farm fish.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Fish farms are increasingly situated in strong current sites above or near to mixed-bottom habitats that include organisms not normally considered in the context of organic enrichment. This study takes a holistic view of the benthic enrichment process by combining different survey techniques on complimentary spatial scales: conventional macrofaunal cores, larger-scale visual quantification of epibiota and environmental-DNA metabarcoding of microbial communities. A large tube forming polychaete (*Arenicola marina*), normally found intertidally and living too deep for conventional sampling, was observed occupying an opportunistic niche in areas of high deposition and in very close association with Capitellid worm complexes. The surface-dwelling brittlestar, *Ophiocomina nigra*, was abundant at distances of 250–1000 m from Farm-B, suggesting a positive response to enrichment, but was displaced where sedimentation exceed  $5 \text{ g m}^{-2} \text{ d}^{-1}$ . A corresponding gradient was evident within the sediment microbial communities, supporting established theories about ecosystem engineering and multi-species synergies for organic waste assimilation. Many of the bacteria present in the near-farm sediments were linked to the farmed fish and fish health issues suggesting one or two-way inoculation pressures. These functionally different benthic organisms are intrinsically linked and the resulting synergy has

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the potential to assimilate significant quantities of anthropogenically produced organic waste contributing to environmental sustainability.

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## 1. Introduction

Assessments of organic enrichment of benthic marine habitats are invariably based on the pronounced changes that occur in the diversity of infauna, which is exemplified by extreme proliferation of a few, globally ubiquitous opportunistic polychaetes (Ritz et al., 1989; Tomassetti and Porrello, 2005; Villnas et al., 2011). Such ecological changes are usually measured in conjunction with similarly responsive geochemical indicators of hypoxic state (Cranford et al., 2017; Hargrave et al., 2008) and used in various ways and combinations to assess enrichment levels around fish farms for management purposes (Hansen et al., 2001; MPI, 2015; Wilson et al., 2009). The small and hardy opportunistic worms such as *Capitella* sp. that proliferate in response to organic enrichment are very effective metabolizers of organic waste (Chareonpanich et al., 1994; Kunihiro et al., 2008), and therefore represent an important component in the assimilative capacity of the receiving environment (Keeley et al., 2019). One such genus (*Vigtoriniella*) has evolved in naturally occurring, nutrient-rich ephemeral habitats such as whale carcasses (Dahlgren et al., 2004; Wiklund et al., 2009). There are however other benthic organisms that may play equally important roles in the breakdown and assimilation of organic waste that remain poorly understood.

By convention, assessments of macrofauna communities are usually constrained to fauna that are retained on a 1 mm mesh after sieving sediment from a grab (0.1 m<sup>2</sup>) or a core (0.01 m<sup>2</sup>) that penetrates to a depth of ca. 10 cm (ASC, 2012; Wilson et al., 2009). The sampled fauna are used to calculate different biotic indices, ranging from simple statistics (e.g. number of species, abundance and diversity) to more complex metrics based on contrasting ecological functions of the different species and empiric relationships such as the AZTI Marine biotic Index (AMBI, Borja et al., 2000) and the Norwegian Sensitivity Index (NSI, Rygg and Norling, 2013). The effectiveness of the method is contingent upon the target organisms (macrofauna) being relatively abundant and homogeneously distributed (to ensure reliable capture from a practically small sample area), ubiquitous (with regard to geographic region and depth), and predictable in their responses. Therefore, by convention, soft-sediment grab samples comprise mostly organisms in the 1 mm to 10 mm size range, and larger more sparsely distributed infauna (a few individuals per m<sup>2</sup>), such as some errant polychaetes (e.g. *Nereis* spp.) or large bivalves (e.g. *Arctica islandica*) are poorly quantified. Notwithstanding that, larger-bodied organisms may comprise a significant part of the benthic biomass and/or have a disproportionately large influence on sediment composition, and therefore benthic functioning.

Many surface-dwelling fauna (epifauna), such as echinoderms (predominantly starfish, sea urchins and sea cucumbers), crustaceans and surface-dwelling bivalves (e.g. scallops), are also poorly enumerated by traditional benthic sampling techniques and rarely featured in benthic enrichment assessments. Some of which also have the potential to significantly influence the physical and biological properties of the sediment and furthermore, represent important foodweb links between the benthic and pelagic environments. For example, asteroids (starfish) may predate directly on other benthic fauna, while urchins break down and metabolize depositing organic matter including drift macroalgae, and both invertebrate groups represent prey for fish (Aronson, 1989; Bonaviri et al., 2009). Likewise, some crustaceans (e.g. *Cancer*

*pagurus*), can be responsible for large-scale sediment disturbances, affect benthic community structure (Hall et al., 1991; Hall et al., 1993) and predate on large benthic bivalves (Mascaró and Seed, 2001).

Multiple video surveys around coastal salmon farms in mid-Norway revealed another seemingly enrichment tolerant species that has been overlooked in terms of its role in the assimilation of fish farm wastes. The large (ca. 10 cm) tube dwelling polychaete *Arenicola marina* was observed in high densities alongside fish farms in 30 to 50 m water depth. This was peculiar, as *A. marina* is a species that is normally associated with intertidal sand-flats (e.g., Grossmann and Reichardt, 1991; Philippart, 1994). *Arenicola marina* is noted for having high bioturbation (Kristensen et al., 2012) rates and ability to metabolize organic carbon and re oxygenate sediments (Reichardt, 1988; Volkenborn et al., 2007; Wendelboe et al., 2013). It's ability to alter the physical structure and geochemical state of the sediment is such that it is considered to be 'an ecosystem engineer' (Riisgård and Banta, 1998). Interestingly, it is also capable of altering the bacterial communities in the sediment (Grossmann and Reichardt, 1991), possibly to its own advantage (i.e., a 'microbial gardener', Hylleberg, 1975). Interestingly, the widely recognized, highly numerous, but much smaller opportunistic polychaete, *Capitella* spp. is also thought to stimulate the microbial activity in surface sediments and benefit from the presence of certain bacterial species (Kunihiro et al., 2008; Wada et al., 2008), albeit a functionally different worm.

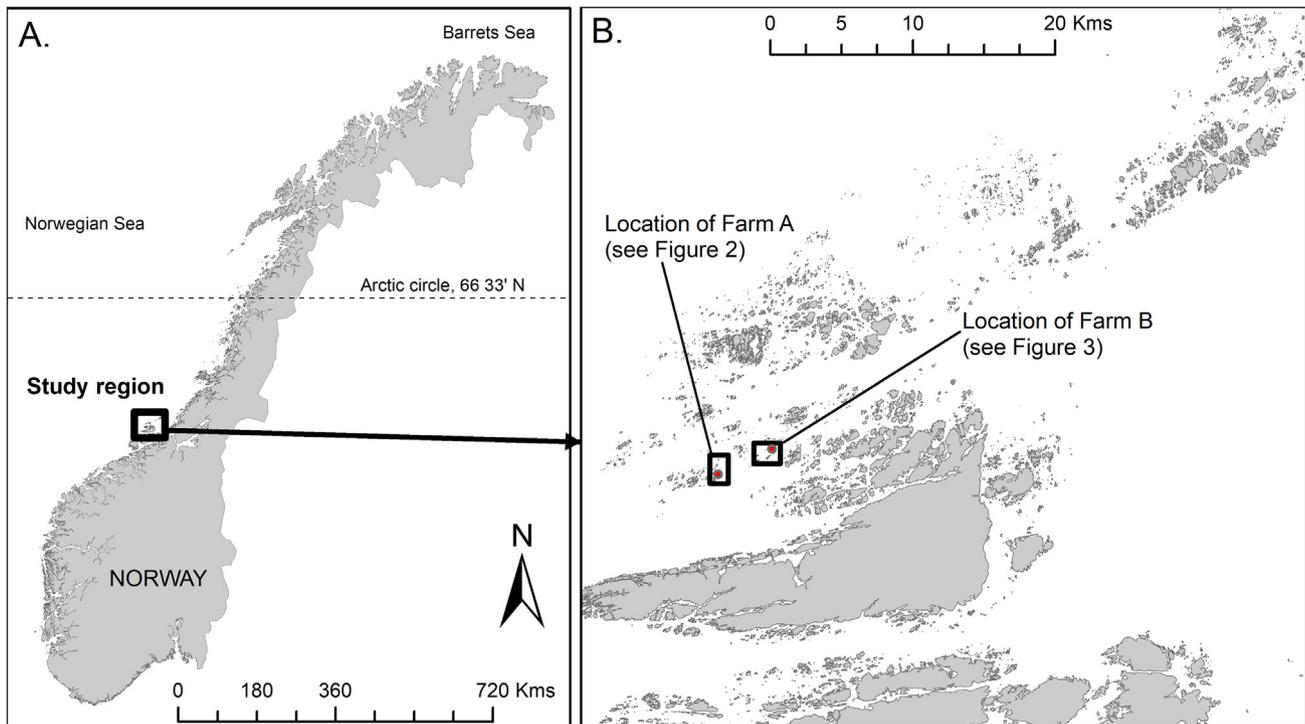
The ecological functioning of these two polychaeta species both therefore implicate the microbial community, which represents the microscopic end of the size spectrum, but is still a very important component of benthic functioning. Microbial communities are implicit in many benthic processes, including photosynthesis, nitrogen fixation, denitrification, sulfate reduction, methanogenesis and iron and metal reduction reactions, and are closely linked to the oxic status of the seabed (Paerl and Pinckney, 1996). The compositional change that occurs with bacteria in a response to enrichment is such that it can be utilized to elucidate benthic enrichment gradients around salmon farms with the use of modern metabarcoding tools (Dowle et al., 2015; Keeley et al., 2018; Stoeck et al., 2018). Hence, there is presumably a high level of coincidence and interactions between macrofauna, epifauna and bacteria with respect to organic enrichment and benthic function.

In this study we combine complimentary datasets to examine how the different biological components of the benthic environment – bacteria and benthic invertebrates – respond and contribute to waste metabolism in dynamic coastal ecosystems affected by aquaculture.

## 2. Methods

### 2.1. Survey design

Density estimates of dominant epibiota made from video quadrats were combined with measurements of depositional flux (waste inputs) and relatively spatially sparse, but intensive conventional assessments of sediment infauna and sediment grain size to elucidate the ecological responses and functional roles related to organic enrichment. The data were complimented by analysis of environmental DNA (eDNA) via metabarcoding of the microbial communities in the sediment at one of the two study farms.



**Fig. 1.** Location of study region A. and farms B., Frøya, mid-Norway coastline. For a close-up of the farm and sampling stations arrangement see Figs. 2 and 3.

### 2.1.1. Study sites

The study was conducted at two comparable, large scale Atlantic salmon (*Salmo salar*) farms (between 8000 and 13,000 T fish/farm/18 mo. production cycle) situated in dynamic, coastal waters of 28–50 m depth in mid-Norway (Lat: 63.7° N, Fig. 1). Both **Farm-A** and **Farm-B** utilized circular 60 m diameter net pens. The seabed in the wider area comprised mixed substrates, with a complex bathymetry consisting of many large rock/reef structures separated by soft sediments (Figs. 2A and 3A). Substrate information presented was obtained from multibeam and backscatter surveys conducted by Norges geologiske undersøkelse and is modified from Plassen et al. (2014).

Study transects were orientated as much as possible in the down-current directions from the two farms, over areas of relatively flat, homogenous, unconsolidated sediments (Figs. 2A and 3A). At Farm-B the transects were orientated in a secondary (less dominant) down-current direction as the primary direction to the north did not offer suitably consistent bathymetry of substrate. The sediments along the study transect at Farm-A comprised a mixture of shell-sand (average grain size 300 to 600  $\mu\text{m}$ ), gravel and cobble interspersed with patches of bedrock, while the substrate at Farm-B comprised predominantly coarse sand (average grain size 700 to 1200  $\mu\text{m}$ ). Average near-bottom (4 m above sea level) current speeds were influenced by tide and seasonal conditions (wind and swell), with peak velocities (during flooding tide) ranging between 18 and 32  $\text{cm s}^{-1}$  at Farm-A and 4 and 12  $\text{cm s}^{-1}$  at Farm-B (from AANDERAA SeaGaurd® multi-frequency Doppler Current Sensor collected during the study period).

### 2.2. Video surveys of epifauna

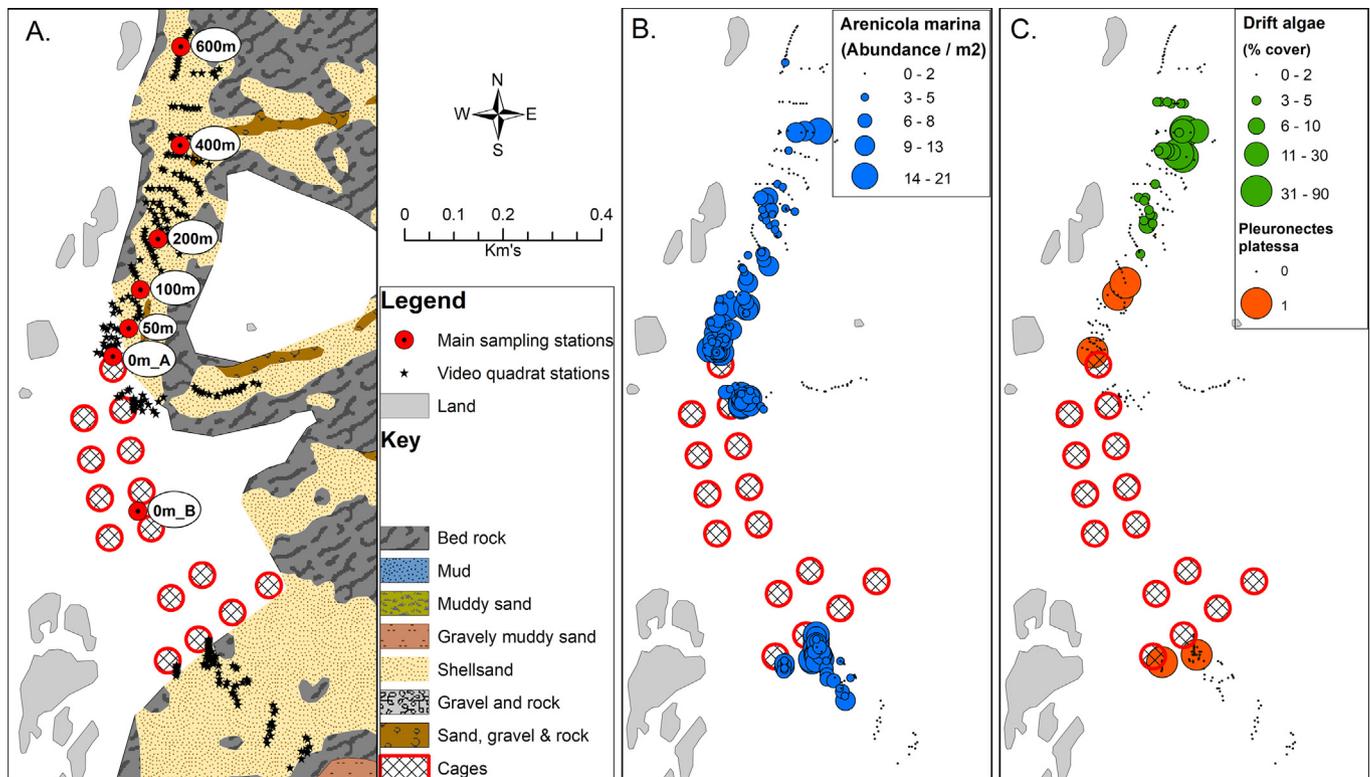
In June 2016 (during peak fish production), a 1  $\text{m}^2$  video quadrat was lowered repeatedly at haphazard intervals as the boat was moved slowly around in the desired transect area, usually following multiple approximate transects either toward or away from the cages in accordance with wind or currents. The sampling effort was

focused in pre-defined areas of consistent bathymetry and substrate type until relatively consistent coverage and density was achieved with respect to the substrate area and distance from the farm. At Farm-A TA-1 ( $n = 162$ ) and TA-2 ( $n = 82$ ) were orientated at opposing angles (to the N-NE and S-SW, respectively) in the predominant (flood and ebb) directions of flow, while TA-3 ( $n = 49$ ) was orientated in an East direction perpendicular to the main currents, but along a channel (between reefs) where currents also flowed to and from the farm (Fig. 2A). During the same survey, 248 quadrat drops were obtained along the main transect at Farm-B (TB-1) in a NE-SW direction off from the SW-most cage out to approximately 1200 m away (Fig. 3A). Field observations and preliminary analysis indicated possible ecological changes approximately 200 m from the cage. In September 2016, an additional replicate transect (TB-2) was surveyed (total of 98 additional quadrats) out to approximately 400 m away from the neighboring cages.

### 2.2.1. Image analysis and density estimates

Quantitative estimates of benthic epifauna densities were obtained from intensive spatial surveys with a 1  $\text{m}^2$  video quadrat. GPS, time and depth information (from an on-board Garmin depth plotter) was overlaid on to high definition (Seaviewer 6000 HD Sea-Drop) camera images in real-time using a Proteus II overlay box to capture spatial (x,y,z) and temporal information. The camera was attached to a custom-made quadrat frame along with a Keldan 9000 lm (5600 K) CRI82 video light which was lowered to the seabed and left motionless for 2–5 s on each drop to capture a stationary, clear video clip. The use of video clips instead of still images aided in the identification of fauna as their movement could be detected and the image could be advanced or reversed to allow silt plumes to dissipate to reveal all areas of the quadrat.

Each video quadrat was analyzed retrospectively (on high resolution monitors) for all conspicuous, identifiable fauna, including, but not limited to: brittle stars (*Ophiocoma nigra*), lugworms (*Arenicola marina*), scallops (*Pecten maximus*), urchins (mostly



**Fig. 2.** A. Site and substrate map of Farm-A (substrate information modified from Plassen et al., 2014). Red circles indicate fish cages, red dots indicated positions of main benthic sampling stations (macrofauna and sedimentation), and black stars indicate video quadrat stations. B. Blue graduated circles indicating relative abundance and distribution of *Arenicola marina*, C. green graduated circles indicating percent cover and distribution of drift macroalgae. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*Echinus esculentus* and *E. acutus*), tubeworms (cf. Ampharetidae), flatfish (mostly European plaice: *Pleuronectes platessa*, Witch flounder: *Glyptocephalus cynoglossus* and Kveite: *Hippoglossus hippoglossus*) and several species of starfish.

Lugworm densities were estimated by counting fecal casts as per Riisgård and Banta (1998), which are clearly apparent on the surface of the seabed. Because *A. marina* is typically described from the intertidal regions, the taxonomy of the lugworm found in this study was confirmed by DNA sequencing. Species determination was carried out with the universal metazoan barcode marker COI (Hebert et al., 2003) using standard PCR and sanger sequencing protocols. The resulting sequences were compared with publicly available barcode sequences on GenBank using BLAST (Altschul et al., 1990). Biological information for each quadrat was combined with depth, GPS coordinates, and percent cover estimates of primary, secondary and tertiary substrates (categories: mud, sandy mud, medium sand, coarse sand, shell sand, broken shell & cobble, gravel/cobble, broken rock, patchy bed rock, bed rock). Any organisms that were only partly visible were counted if more than half of the body was inside the quadrat frame. For highly numerous species (e.g. brittlestars) the screen was divided into quadrants, which were each enumerated in a random order and the average quadrat count was extrapolated up to the whole area once the variance in the mean had stabilized with each new quadrant count. A distance to farm (meters to nearest salmon cage) variable was added retrospectively by importing the x,y,z data to ArcGIS 10.3.1 and using the 'Near' function in the 'Proximity' toolbox.

### 2.3. Sampling of complimentary variables

Sedimentation rates and the biological and physical properties of the sediment were assessed at seven main benthic sampling sta-

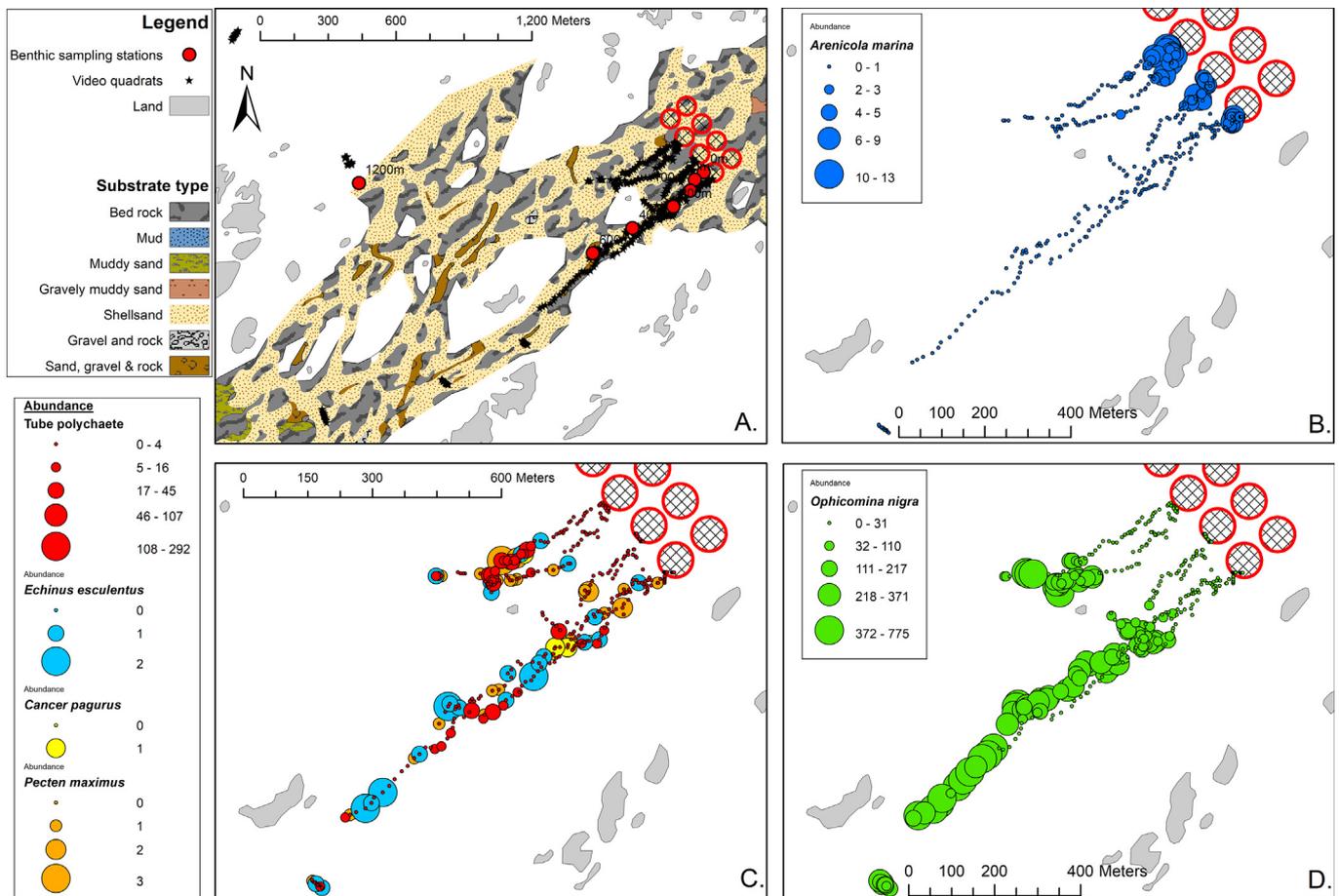
tions at each farm ('0 m', '50 m', '100 m', '200 m', '400 m', '600 m' and '1200 m' from the cages; Figs. 2A and 3A). Triplicate sediment samples were collected using a heavily weighted 0.1 m<sup>2</sup> van-Veen grab and each successful grab was subsampled for macrofauna, sediment grain size and microbial eDNA (Farm-B only) as follows:

#### 2.3.1. Benthic macrofauna

Sediment samples were obtained using a boat operated Van Veen grab and sediment dwelling infauna were subsampled using a 10 cm diameter, 10–15 cm deep core. The contents were sieved, and the material retained on 1 mm mesh was preserved with buffered 4% formalin. Full taxonomy was conducted by the Cawthron taxonomy lab using standard protocol, whereby all fauna were picked out, counted and identified to the lowest practically possible taxonomic level.

#### 2.3.2. Sediment grain-size

Approximately 10 g of sediment was also collected and frozen for subsequent analysis. Any gravel or stones larger than approximately 0.5 mm diameter, or large organic debris (e.g. shells) were excluded. Samples were prepared for analysis on a Beckman Coulter Laser Diffraction Particle Size Analyzer (LS 13 320) by subsampling approximately 3 g of sediment into 30 mL beakers. Samples were digested with approximately 10 mL of 70% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) at 60 °C to remove all organic material. Prior to analysis sediment was suspended in clean water (i.e. filter at 1 μm) and sonicated for 3 min with a sonic probe (Misonix, Sonicator 3000) to disaggregate the particles. Sediment samples were then rinsed with clean water into the sample chamber of the LS13 320 through a 2000 μm sieve to remove larger particles and shell fragments if they existed. The Coulter Laser analyzes particles from 0.375 μm to 2000 μm in size. Samples with particles larger than 2000 μm



**Fig. 3.** A. Site and substrate map of Farm-B (substrate information modified from Plassen et al., 2014). Red circles indicate fish cages, red dots indicated positions of benthic sampling stations and black crosses indicate photo quadrat stations. B. Blue graduated circles indicating relative abundance and distribution of *Arenicola marina*, C. Red, light blue, yellow and orange graduated circles indicate relative abundance and distribution of a Tube-forming polychaete, *Echinus esculentus*, *Cancer pagurus* and *Pecten maximus*, respectively, and D. green graduated circles indicate relative abundance and distribution of *Ophicomina nigra*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were additionally processed through a series of sieves to determine grain-sizes >2000  $\mu\text{m}$ . Sieve sizes of 2000  $\mu\text{m}$ , 2360  $\mu\text{m}$ , 2800  $\mu\text{m}$ , 3350  $\mu\text{m}$  and 4000  $\mu\text{m}$  were used to complete the distribution. Coulter Laser and sieve data were then merged and normalized to 100% for the complete grain size distribution and are expressed as log of diameter ( $\mu\text{m}$ ) vs. weight %.

### 2.3.3. Microbial composition of sediments (eDNA)

The microbial composition of the sediments at the main benthic sampling stations of Farm-B were assessed from DNA samples using metabarcoding following the methods outlined in Keeley et al. (2018) and Pawlowski et al. (2014). In brief, surface sediment (top 1–2 cm) was collected from across the surface of the Van Veen grab using a small sterile scoop into 3 mL Eppendorf tubes, which were then placed on dry ice before transportation to a  $-80$  freezer. Environmental DNA was extracted from 2 g of surface sediment using the DNeasy PowerSoil™ DNA extraction kit (Qiagen), including DNA extraction blanks (i.e. extraction controls), followed by DNA quality and purity verification using a spectrophotometer (Eppendorf, Leipzig, Germany). A two-step tailed PCR amplicon procedure (Kozich et al., 2013) was applied to all eDNA samples, targeting an ca. 400 base-pair fragment of the nuclear 16S rRNA bacterial gene (V3–4 region), using the primers, reagents and PCR thermocycling conditions described in Keeley et al. (2018). PCR products were purified with AMPure® XP PCR Purification beads (Agencourt®, MA, USA), quantified using a Qubit® Fluorometer (Life

Technologies, Carlsbad, CA, USA), and diluted to 3 ng/ $\mu\text{L}$  with ddH<sub>2</sub>O. Purified amplicon libraries were individually indexed using the Nextera™ DNA library Prep Kit (Illumina, California, USA) through an eight cycle PCR, and sequenced on a MiSeq Illumina™ sequencer at the Genomics Facility, University of Auckland, New Zealand. Sequences have been deposited in the NCBI's Sequence Read Archive under BioProject ID PRJNA548397 (SRR9277578-SRR9277598).

Bioinformatics analyses followed the same procedure as in Dowle et al. (2015). The sequencing run was demultiplexed using MiSeq Reporter v2.0. All further analyses were performed using MOTHUR (Schloss et al., 2009). Sequences corresponding to the forward and reverse primers were trimmed and merged into single contigs. Contigs were aligned to the SILVA bacteria reference alignment (Pruesse et al., 2007) and chimera removal performed using the UCHIME algorithm (Edgar et al., 2011). Sequences were clustered into Operational Taxonomic Units (OTUs). Sequences were first grouped at the class level before OTUs were created independently in each of the corresponding bins using a 0.02 pairwise sequence distance cut-off value. The dataset was rarefied to 20,000 sequences per sample using QIIME (Caporaso et al., 2010) and OTUs represented by <2 reads across all samples were removed and the remainder classified to identify taxonomic annotation using the Ribosomal Database Project (RDP) taxonomic database Version 9 (Cole et al., 2013). Unknown archaeal or eukaryotic sequences were filtered and removed.

The microbial eDNA dataset contained several examples where multiple OTUs were highly correlated and belonged to the same taxa. These OTUs were up-grouped at the lowest available taxonomic level, producing 648 unique taxa for the purposes of multivariate community composition analysis. Bray-Curtis similarities between samples were calculated (with dummy variable = 1) and the distance among centroids function was used to visualize differences between stations based on the resemblance matrix and then displayed in 2-dimensional space using Principle Co-ordinates analysis (PCO1 and PCO2, Anderson et al., 2008). The dominant bacterial OTUs that were responsible for the differences between stations were identified by placing the stations into three broad groups based on their placement on the two primary PCO axes, corresponding reasonably well to distance from farm; namely: '0–100 m', '200–600 m' and 'Reference'. SIMPER analysis was conducted on these groups and the OTUs that contributed to  $\geq 0.45\%$  of the dissimilarity between groups are provided in Supplementary information Tables S1–S3.

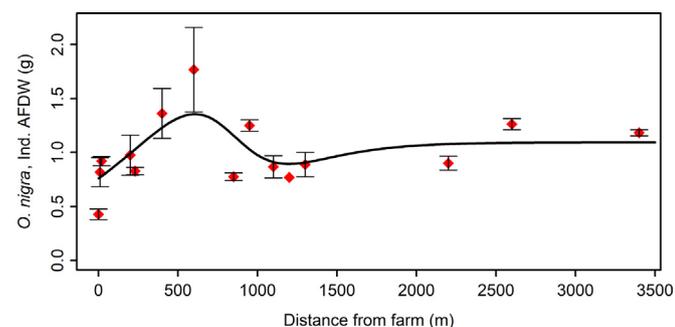
### 2.3.4. Sedimentation rates

Estimates of the flux of suspended particulate matter were measured during the period of peak production by deploying sediment traps for 10 days at 2.5 m above the seabed at the main benthic stations and at two more distant reference locations. Depositional fluxes of total and organic particulate matter ( $\text{g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) were analyzed following the methods of Keeley et al. (2019).

### 2.4. Fauna biomass

Fauna density and mass information was converted to equivalent biomass per unit area for comparative purposes and to estimate potential metabolic capacity and carbon utilization and storage in response to organic enrichment and distance from the farms. *Ophiocomina nigra* biomass was determined from replicate specimens ( $n = 2\text{--}6$  per station) obtained in the farm area (from 0 m to 3.5 km away). Average, distance-specific, individual biomass was determined from a non-linear, parametric SponnegRichards curve ( $A = 3.05$ ,  $k = 0.003$ ,  $i = 654$ ,  $m = 836$ ,  $\text{df} = 15$ ,  $\text{RSSE} = 0.241$ ) fitted using the FlexParamCurve function in R (Oswald et al., 2012), which automatically selects and parameterizes the best fitting curve (Fig. 4).

*Arenicola marina* biomass was determined non-destructively by using the fecal cast diameter to individual biomass (g, wet weight) relationships from Valdemarsen et al. (2011). During video analysis, each fecal cast pile was classified into two size categories: small (2–4 mm diameter, estimated average diameter of 3 mm with an associated individual mass of 4.6 g WW) and large (>4 mm, estimated average size of 5 mm diameter with an associated individual mass of 14.1 g WW). On-screen dimensions were



**Fig. 4.** Average individual (Ind.) biomass (AFDW, g) of *Ophiocomina nigra* with distance from Farm-B (error bars  $\pm$ se). Line represents best-fit non-linear, parametric SponnegRichards curve ( $A = 3.05$ ,  $k = 0.003$ ,  $i = 654$ ,  $m = 836$ , Oswald et al., 2012) used to determine individual, distance-specific brittle star mass.

scaled to actual dimensions by relating the on-screen size of the quadrat frame to the actual size. Total biomass was calculated for each of size categories (abundance  $\times$  estimated individual biomass), and then summed to give estimated total biomass per  $\text{m}^2$  which was then converted to ash-free dry weight (AFDW)- $\text{m}^2$  assuming an AFDW - wet weight ratio for polychaetes of 0.16 (Ricciardi and Bourget, 1998).

Total capitellid biomass was determined by multiplying abundance by the average individual biomass (0.000761 gAFDW) that was determined from a composite subsample of 260 individuals collected from the fauna samples during processing, as described in Keeley et al. (2019).

### 2.5. Estimating carbon processing capacities

Potential organic carbon processing rates ( $\text{gC}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) were estimated for *A. marina* and *Capitella* sp. based on biomass information and existing literature. For *A. marina*, the gross sediment reworking rate ( $\text{cm}^3$  sediment- $\text{m}^{-2}\cdot\text{d}^{-1}$ ) was calculated from the estimated total biomass  $\text{m}^2$  using the relationship derived by Valdemarsen et al. (2011):

$$\text{Sedimentreworkingactivity} \left( \text{cm}^3 \cdot \text{m}^{-2} \cdot \text{d}^{-1} \right) = 2.2 \text{ Biomass DW}^{1.21}.$$

Sediment organic carbon content was calculated from site-specific measurements of average sediment mass (1.59  $\text{g}/\text{cm}^3$  wet weight), water content (37.2%), percent organic matter (5.65%; average for near-farm sediments) and an assumed carbon content of the organic matter (43%, Luczak et al., 1997). When combined with *A. marina* sediment, reworking rates ( $\text{cm}^3\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ , Valdemarsen et al., 2011) gave an estimate of the rate at which sediment carbon passed through the gut of ( $\text{gC}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ). The proportion of the organic material that gets assimilated seems to remain undefined, therefore for the purpose of the exercise it was conservatively assumed to be 0.75.

Organic matter decomposition potential for *Capitella* sp. was calculated from the biomass (dry weight, DW) estimates and the organic carbon decomposition potential derived by Chareonpanich et al. (1994): carbon decomposition ( $\text{gC}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) =  $0.15 \times \text{DW} + 0.23$  ( $r^2 = 0.86$ ).

## 3. Results

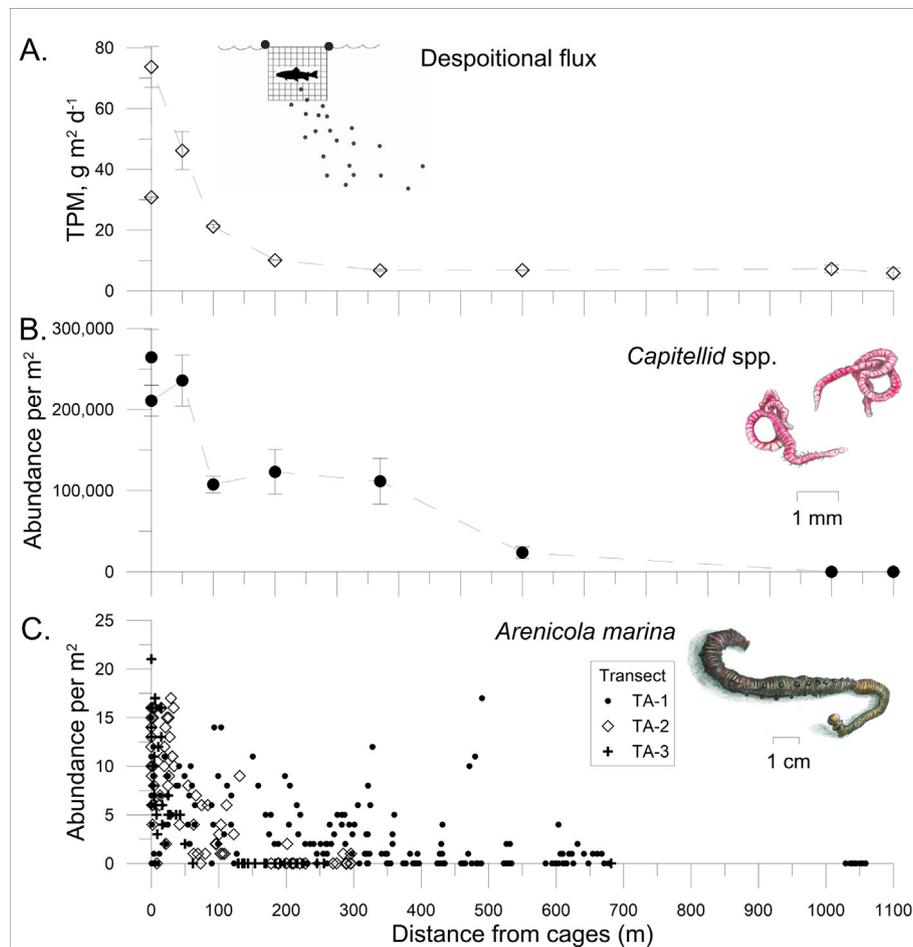
### 3.1. Benthic substrates

The sediment grain size beneath the cages ('0 m-A', '0 m-B'), at '50 m' and '600 m' at 'Farm-A' was dominated by 200 to 400  $\mu\text{m}$  particles with very few particles larger than 1 mm. At 100 m from the cages, a peak was evident at the 200  $\mu\text{m}$  grainsize range as well as in the larger 1–3 mm size range (Fig. S-1). The '200 m' and '400 m' stations had a mixture of grainsizes >200  $\mu\text{m}$  and a peak in the coarse grainsize fraction (>3 mm).

The grainsize at Farm-B was dominated by particles in the 1 mm size range. Similar distributions were evident at '50 m' and '100 m' stations only there was a greater proportion of smaller particles between 300  $\mu\text{m}$  and 1 mm. The more distant ('200 m', '400 m' and '600 m') stations were noticeably different from the closer stations, being strongly dominated by coarser sediments peaks at 2–3 mm grainsize and relatively few particles in the smaller 300–1000  $\mu\text{m}$  size range.

### 3.2. Conspicuous epibiota

The majority of the video quadrats at Farm-A were situated on flat homogenous sandy sediments between rock outcrops radiating



**Fig. 5.** Farm-A: A. Depositional flux ( $\text{gTPM}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) at benthic sampling stations with distance along main transect, B. abundance of *Capitella capitata* along main transect, C. abundance of *Arenicola marina* along transects TA-1 (black dots), TA-2 (open diamond symbols) and TA-3 (black crosses).

from the farm, to the north, south and east (Fig. 2A). Diversity of conspicuous biota was consistently low and evidenced by *A. marina* fecal cast mounds (in some areas), polychaete tubes, with the very occasional scallop (*Pecten maximus*), urchin (*Echinus esculentus*), starfish (*Asterias rubens*), brown crab (*Cancer pagurus*) and hermit crab. A large, discrete area of decomposing macroalgae was observed 350–450 m away from the farm along the north transect (Fig. 2C). Drift algae was also common, but at a low percentage cover (3–10%) 200–300 m away. *A. marina* densities were consistently elevated (5–25  $\text{m}^{-2}$ , Figs. 2B & 5C) within ca. 100 m of the farm on all three transects. Densities generally decreased with distance but remained elevated out to approximately 200 m to the north and south (Fig. 2B). On the north transect, densities were also elevated in patches 200–300 m and at ca. 420 m from the farm, both of which corresponded to the patches of decomposing drift macroalgae, where *A. marina* mounds were observed mostly around the perimeter of the kelp mass.

Video quadrats at Farm-B were also situated on sand flats radiating away from the farm (Fig. 3A), but the substrate was more complex than at Farm-A, with a mixture of coarse sand, gravel and stones. Conspicuous fauna was generally more prolific, with more frequent occurrences of tube forming polychaetes, *P. maximus* and *E. esculentus* (Fig. 3C). The epifaunal brittle star *Ophiocornia nigra* did not occur close to the farm but was prolific along and reached densities between ca. 100 and 500 per  $\text{m}^{-2}$  at >200 m distance from the farm (Figs. 3D and 6D). A converse relationship existed with respect to *A. marina*, which was prolific close to and directly beneath the cages, achieving densities of between 3

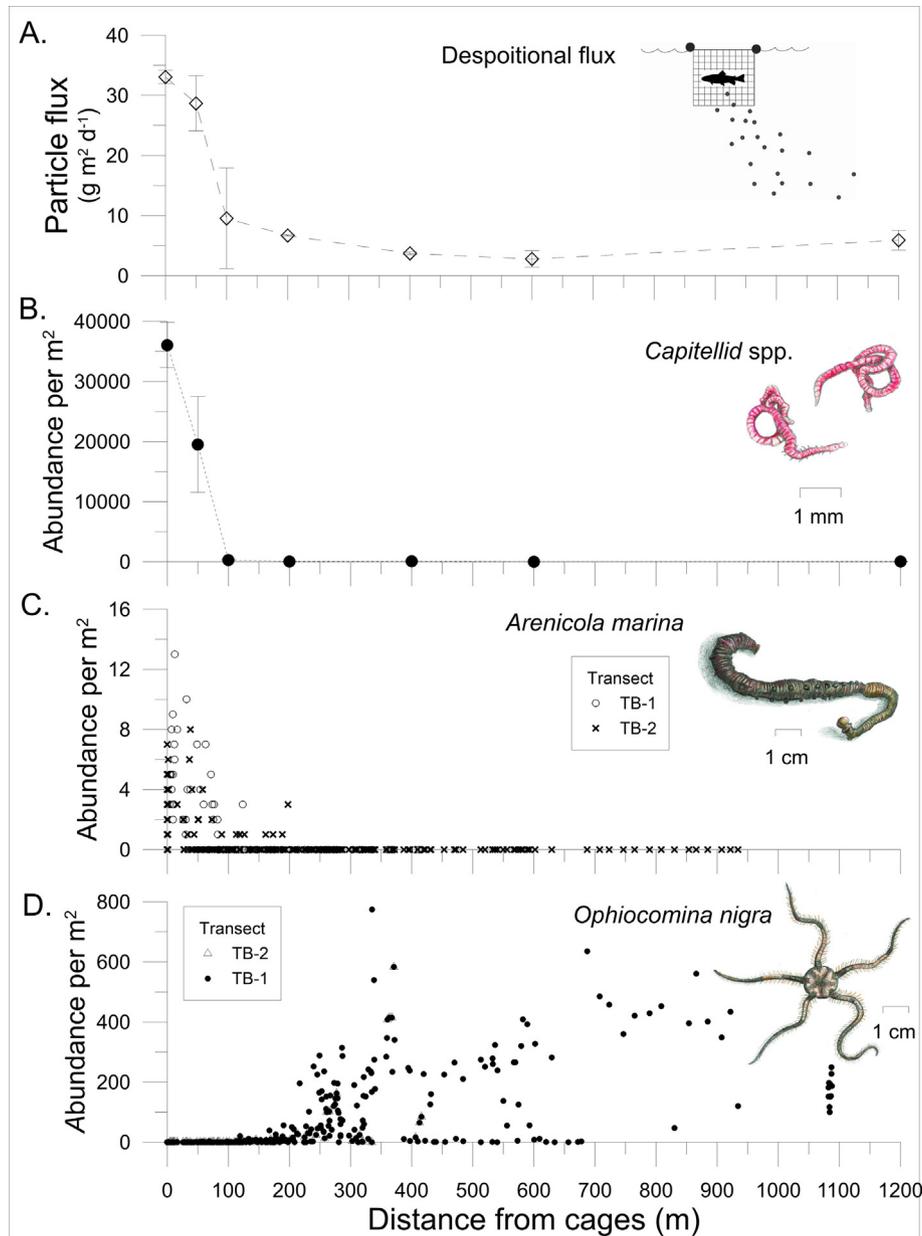
and 13 per  $\text{m}^{-2}$ . Low densities of *A. marina* were observed between 100 m and 200 m away, beyond which it was absent (Fig. 3C).

### 3.3. Depositional flux

The flux of organic waste deposition 2.5 m above the seabed at Farm-A was highest next to the cages (0 m, average  $73 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  TPM, Fig. 5A) and decreased rapidly with distance. At 200 m from the cages the flux was still slightly elevated ( $10 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) above the three reference stations ( $2.7\text{--}5.9 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ). Similar patterns were evident at Farm-B, with strongly elevated flux at the cages (0 m,  $33 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  TPM) and 50 m away ( $28 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ), reducing significantly, but still elevated above reference stations at 200 m and 400 m from the cages along the main transect (Fig. 6A).

### 3.4. Dominant opportunistic infauna (*Capitella* spp.)

The numerically dominant species in the macrofaunal datasets from both Farm-A and Farm-B was the opportunistic polychaete genus *Capitella*. At Farm-A, extremely high average densities were recorded beside the cages and 50 m away (210,848 to 264,494 per  $\text{m}^{-2}$ , Fig. 5B). Densities were also strongly elevated 100 m and 400 m from the cages (107,546 to 123,165 per  $\text{m}^{-2}$ ) and moderately elevated (23,725 per  $\text{m}^{-2}$ ) 600 m away. Densities at the reference stations were negligible, ranging between 0 and 42 per  $\text{m}^{-2}$ . Farm-B had generally lower macrofauna densities beside the cages



**Fig. 6.** Farm-B: A. Depositional flux (gTPM·m<sup>2</sup>·d<sup>-1</sup>) at benthic sampling stations with distance along main transect, B. abundance of *Capitella capitata* along main transect, C. abundance of *Arenicola marina* along transects TB-1 (open circles) and TB-2 (black crosses), and D. abundance of *Ophiocomina nigra* along transects TB-1 (open triangles) and TB-2 (black dots).

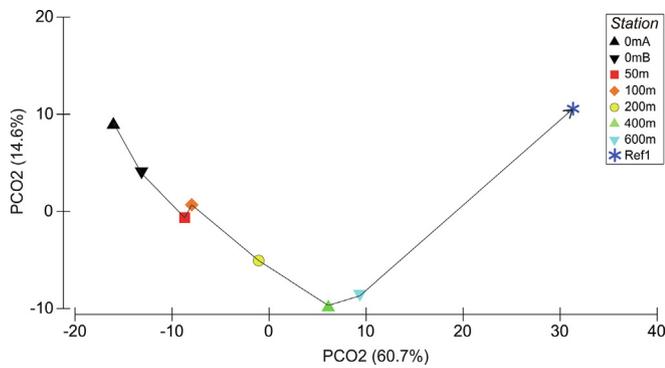
(36,065 per m<sup>2</sup>) and at '50 m' (19,523 per m<sup>2</sup>), and beyond 100 m, densities were comparable to or only slightly elevated above the reference (42 to 297 per m<sup>2</sup>, Fig. 6B).

### 3.5. Microbial communities

The raw sequencing data produced 2,996,133 reads, representing 115,235 ( $\pm 70,914$  standard deviation) sequences per sample ( $N = 24$ ). Following the filtering (trimming, merging, and removal of chimeras), rarefaction and removal of <2 singletons steps, the total number of sequences per sample used for downstream statistical analyses was 427,100 reads, or 17,795 reads per sample ( $\pm 3521$  standard deviation), grouped into 3361 OTUs. Globally, seven dominant (>1%) bacterial phyla were recorded across the entire dataset: Proteobacteria (56%), Bacteroidetes (21%), Verrucomicrobia (6%), Acidobacteria (5%), Actinobacteria (3%), Planctomycetia (3%), and Firmicutes (2%).

Metabarcoding derived OTU read-abundance data describing bacterial communities in sediments from Farm-A clearly distinguished the sampling stations in a manner consistent with proximity to farm (Fig. 7). The two primary principle coordinates axes (PCO1 and PCO2) accounted for 75.3% of the variation between sampling stations. The most distant (1200 m 'reference') station was distinct from the near-farm stations (0–600 m), and most similar to the most distant of those stations (600 m). Differences between the near-farm stations were discerned on both PCO1 and PCO2 axes, with the most distant station (600 m) on bottom right, progressing to the nearest-farm (0 m, and most impacted) stations, top left (See Fig. 7).

The average dissimilarity (29.6%, one-way SIMPER analysis) between the three main groups of stations (i.e. 0–100 m, 200–600 m, Reference) was discerned by small differences (<0.6 contributing % per individual OTU) in a diverse range of bacteria. The main taxa that distinguished the highly enriched (0–50 m) stations



**Fig. 7.** Two-Dimensional Principal Coordinates (PCO) representation of distance among centroids for sampling stations based on Bray Curtis Similarities using log-transformed metabarcoding derived Operational Taxonomic Units (OTUs) read-abundance data for sediment bacteria. Bacteria OTUs most responsible for the differences between groups of similar stations provided in Supplementary information Tables S1–S3.

from the mildly enriched (200–600 m) stations were *Psychromonas* sp. (Psychromonadaceae, 0.55 Contrib.%), *Arcobacter* sp. (Campylobacteraceae, 0.53 Contrib.%) and *Aliivibrio* sp. (Vibrionaceae, 0.46 Contrib.%), which had increased prevalence of near to the cages. Whereas, *Eudoraea* sp. (Flavobacteriaceae, 0.58 Contrib.%), *Nakamurella* sp. (Nakamurellaceae, 0.57 Contrib.%), *Syntrophorhabdus* sp. (0.56 Contrib.%), *Nitratireductor* sp. (Phyllobacteriaceae, 0.56 Contrib.%), were more prevalent 200 m to 600 m away from the cages (Table S1, Supplementary information).

Bacterial taxa that were more prevalent in mildly enriched sediments (200–600 m away) than at the distant reference location and contributed most to the difference included: *Cocleimonas* (0.62 Contrib.%), *Pelagicola* sp. (Rhodobacteraceae, 0.59 Contrib. %), *Polaribacter* sp. (Flavobacteriaceae, 0.55 Contrib.%), *Sulfurimonas* sp. (Helicobacteraceae, 0.53 Contrib.%), *Acetivibrio* sp. (Ruminococcaceae, 0.49 Contrib.%), *Sulfurovum* sp. (Helicobacteraceae, 0.48 Contrib.%) and *Psychromonas* sp. (Psychromonadaceae, 0.48 Contrib.%) (Table S2, Supplementary information). *Psychromonas* sp. (Psychromonadaceae, 0.65 Contrib.%), *Polaribacter* sp. (Flavobacteriaceae, 0.52 Contrib.%), and *Sulfurovum* sp. (Helicobacteraceae, 0.46 Contrib.%) had notably higher prevalence underneath the cages (0–50 m) than at the reference location (Table S3, Supplementary information).

### 3.6. Relative biomasses and waste decomposition potentials

The average individual biomass of *O. nigra* at Farm-B increased in a near-linear manner from ca. 1 g to 2.5 g with increasing distance out to 600 m. Estimated individual biomass of *O. nigra* obtained beyond 1000 m away ranged between approximately 0.8 and 1.3 g (Fig. 4). The area-standardized biomass ( $\text{gAFDW}\cdot\text{m}^{-2}$ ) of *O. nigra* at Farm-B was negligible between 0 m and 200 m from the farm, but increased abruptly between 200 and 300 m, and continued to increase out to 600–700 m away, where peak biomass of approximately  $550 \text{ gAFDW}\cdot\text{m}^{-2}$  was achieved (Fig. 8). By comparison, the *Capitella* spp. and *Arenicola marina* biomass at Farm-B were low (peaking at 27 and  $14 \text{ gAFDW}\cdot\text{m}^{-2}$ , respectively) and only elevated within 100 m of the farm. At Farm-A, *Capitella* spp. biomass peaked at approximately  $200 \text{ gAFDW}\cdot\text{m}^{-2}$  (one third of the peak biomass observed for *O. nigra* at Farm-B) alongside the cages and decreased gradually, but remained elevated, out to 600 m. *Arenicola marina* followed a very similar spatial pattern at Farm-A, however the relative biomass was approximately one order of magnitude lower, with a maximum average biomass (near

to the cages) of approximately  $25 \text{ gAFDW}\cdot\text{m}^{-2}$ . Biomass of *O. nigra* at Farm-A was negligible.

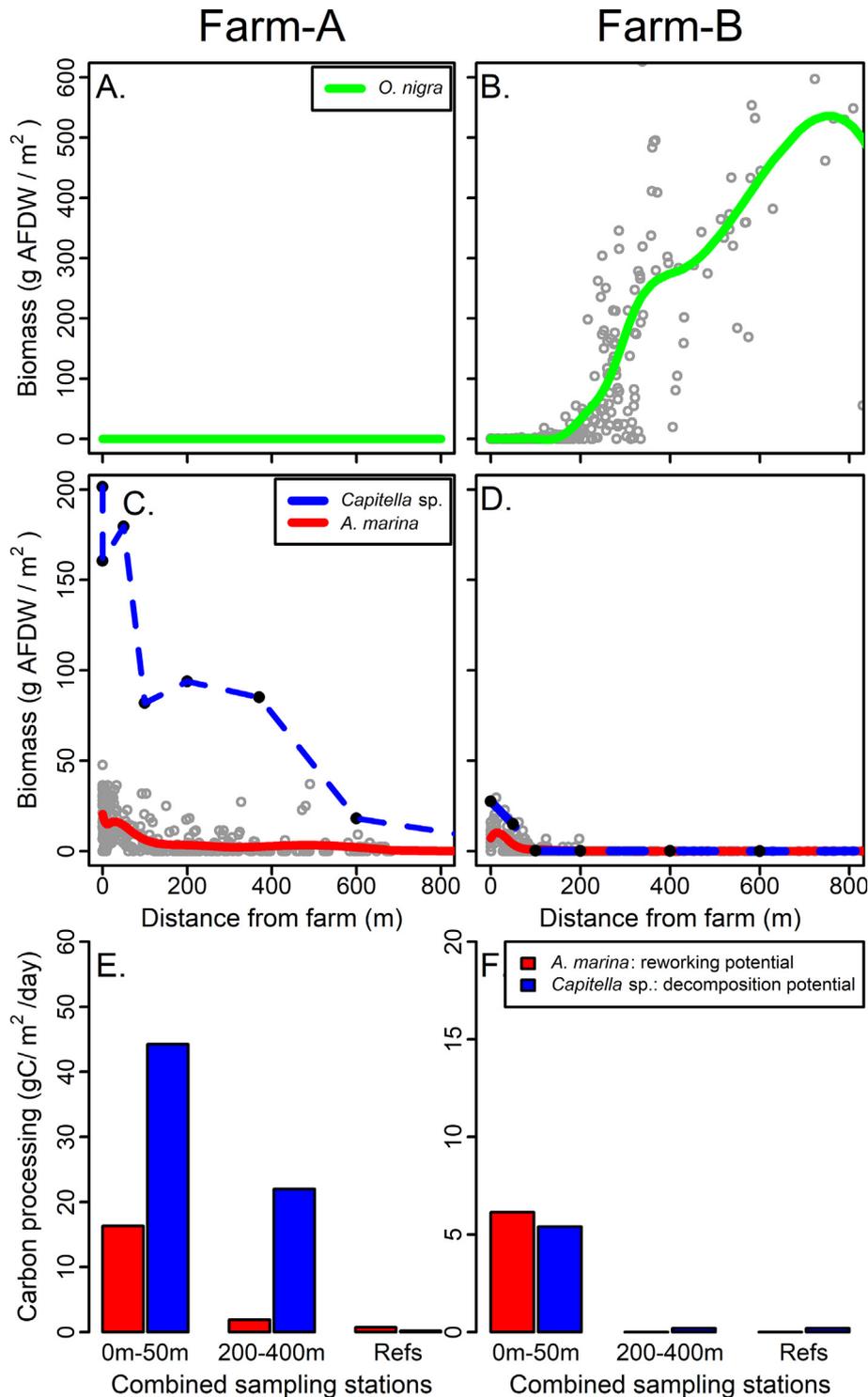
The amount of carbon processed through the gut passage of *A. marina* near to the cages at Farm-B was estimated to be  $6.1 \text{ gC}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  (Fig. 8). A slightly lower, but comparable amount of carbon decomposition was estimated for *Capitella* spp. ( $5.4 \text{ gC}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) at the same location. By comparison, in accordance with the increased biomass, estimated carbon processing by *A. marina* close to the cages at Farm-A was  $16.4 \text{ gC}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ , which was double that of Farm-B. At  $44.2 \text{ gC}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ , the decomposition capacity of *Capitella* spp. was an order of magnitude higher than estimated for Farm-B, and approximately three times as high as the carbon processing rate for *A. marina* in the same near-cage sediments.

## 4. Discussion

### 4.1. The ecological role of *Arenicola marina* beneath marine farms

The literature related to *Arenicola marina* principally describes observations from intertidal sandflats, where *A. marina* can be found in high abundances and readily studied. However, it is hereby documented that the species also occurs subtidally where it has equally important ecological functions. *Arenicola marina* occurs in coastal zones in mid-Norway at depths of 30–50 m in relatively high current, organically enriched areas associated with both anthropogenic and natural sources. Notably, *A. marina* was observed directly beneath and near to salmon farms in close association with *Capitella* sp., which is a well-known opportunistic polychaete assemblage and widely utilized indicator taxon (Blake et al., 2009; Chareonpanich et al., 1994). *Arenicola marina* appears to prefer similar environmental conditions and may be considered an opportunistic species (i.e., Eco-Group V; Borja et al., 2000). Extensive benthic videos in the wider region also revealed isolated aggregations of *A. marina* around accumulated mounds of decomposing macroalgae, suggesting it plays an important ecological role subtidally, breaking down natural organic debris. Such an association was evident along the north transect from Farm-A, which interrupted the gradient in response to farm inputs. These naturally occurring decomposition zones may well act as source populations for inoculating farms where *A. marina* subsequently assumes a similar role processing the significant, extraneous (unnatural) waste inputs. Yet, *A. marina* rarely features in subtidal benthic ecology assessments, presumably because conventional benthic monitoring equipment ( $0.03\text{--}0.1 \text{ m}^2$  and 10–20 cm depth) does not penetrate deep enough (*A. marina* burrows are up to 40 cm deep, Kristensen, 2001) and/or the sample area is inappropriate for the relatively sparse distribution ( $1\text{--}15 \text{ m}^2$ ).

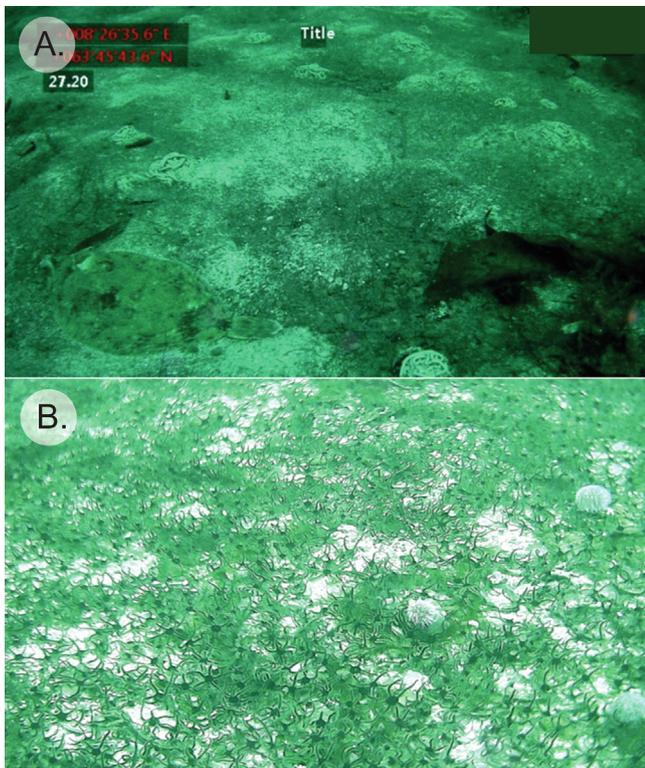
The occurrence of *A. marina* in an opportunistic role beneath salmon farms is functionally significant as it is a known ecosystem engineer with the ability to alter the chemical and physical structure of the sediments as well as the geochemical processes (Riisgård and Banta, 1998). *Arenicola marina* lives in J-shaped burrows where it ingests subsurface sediment, which is defecated at the surface (Kristensen, 2001). This sediment reworking process creates ‘feeding funnels’ where flocculent organic material (such as salmon faeces, Fig. 9) naturally accumulates and subsequently sinks downward and is ingested along with the sediment at rates as high as  $40 \text{ cm}^3 \text{ sediment}\cdot\text{d}^{-1}$  (based on a 9 g individual, Valdemarsen et al., 2011). Sediments beneath sea-based salmon farms are invariably subjected to excessive organic enrichment, with profound geochemical and ecological effects exemplified by anaerobic conditions with high sulfate reduction and the production of toxic free sulfides (Hargrave et al., 2008). The presence of *A. marina* can lower sediment organic content by 50% (Papaspyrou



**Fig. 8.** Between-farm (Farm-A and Farm-B) comparison of biomass (gAFDW·m<sup>-2</sup>) of: A. and B. *Ophicomina nigra* (green lines (fitted smooth.splines)), C. and D. *Capitella* sp. (blue dashed lines), and *Arenicola marina* (red lines (fitted smooth.splines)). Barplots E. and F. present estimated organic carbon reworking potential by *A. marina* and carbon decomposition potential by *Capitella* sp. (gC·m<sup>-2</sup>·d<sup>-1</sup>). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2007), irrigate sediments and strongly stimulate benthic metabolism by promoting anaerobic processes and reducing sulfate reduction rates (Banta et al., 1999; Wendelboe et al., 2013). In particular, the increased availability of dissolved reactive nutrients and solute transport can fuel *Capitella* spp. production and microbial decay, thereby facilitating degradation of organic matter (Papaspyrou et al., 2007). Also significant from a marine farming

waste mitigation perspective, sediment egested by *A. marina* tends to be enriched with fine particles which are more readily eroded (Wendelboe et al., 2013), thereby promoting dispersion and dilution of enriched farm sediments. Furthermore, the pits and mounds (fecal casts) created by the feeding process will likely alter the critical erosion threshold, and further promote erosion and dispersion of wastes (Wendelboe et al., 2013; Widdows and Brinsley, 2002).



**Fig. 9.** Photograph of: A. seabed beneath 0 m-A station, Farm-A, showing *Arenicola marina* fecal cast piles and depressions where farm biodeposits have accumulated, some of which are presumed 'feeding pits'; B. a carpet of *Ophiocomina nigra* overlying coarse sediments near to Farm-B.

The concept of 'microbial gardening' (Hylleberg, 1975) and bio-irrigation, may logically be extended to commensalism or mutualism with other co-inhabitants and more importantly generally enhanced ecosystem function, and there is evidence in the current study to support this. The concept of microbial gardening stems from observations of selective feeding and the stimulation of growth of favorable microorganisms (Grossmann and Reichardt, 1991; Hylleberg, 1975). Subsequent studies have also demonstrated how *A. marina* affects solute transport and has the potential to redistribute and facilitate the removal of particulate organic matter via enhanced microbial degradation (Papaspyrou et al., 2007). The resulting sediments are well flushed and oxidized despite high rates of benthic metabolism (Banta et al., 1999). The sediments beneath Farm-A in this study, where *A. marina* was most prolific, also contained extreme abundances of opportunistic polychaetes (ca. 250,000·m<sup>-2</sup>) and during peak production had very high levels of respiratory activity (Keeley et al., 2019). Yet, the sediments remained visually relatively unimpacted (Author, Pers. Obs.), the *Capitella* assemblage showed no signs of collapse despite very high depositional flux rates, and the benthic state recovered rapidly during a subsequent following period (Keeley et al., 2019). Thus, the ability of *A. marina* to influence microbial communities, irrigate and oxidize the sediments may have facilitated the proliferation of *Capitella* sp. and buffered against excessive enrichment and anoxia.

#### 4.2. Comparison of carbon processing capacity between *A. marina* and *Capitella* spp.

In terms of the relative abilities of *A. marina* and *Capitella* spp. to process organic waste, the estimates suggest comparable capacities. While the simple biomass calculations indicated *Capitella*

spp. had a considerably higher biomass per unit area, the decomposition potential of *A. marina* was comparable, especially beneath the cages at Farm-B where abundances of *Capitella* spp. were less extreme. Although the estimated carbon processing capacity of *A. marina* at Farm-A was approximately one third that of *Capitella* spp., the total for *A. marina* at Farm-B was still three times higher than for either species. *Capitella* spp. was clearly the dominant processor of carbon at Farm-A due to the very high biomass (ca. 500–1200 gWW·m<sup>-2</sup>) and therefore decomposition rate (49 gC·m<sup>-2</sup>·d), which was more than an order of magnitude higher than other published values (Tsutsumi et al., 2002). However, direct comparison between the species is made difficult due to the differing forms of feeding and niche occupation (deep sediment versus near surface). The estimates derived here for the more selective deposit feeding *Capitella* spp. are for carbon decomposition rates, whereas the estimate for *A. marina* is the carbon processed, but not necessarily ingested based on the sediment reworking potential. *Arenicola marina* is considered to be a head-down, non-selective deposit feeder sustained by ingesting large amounts of sediment with low nutritional value (Riisgård and Banta, 1998) with a seemingly unknown absorption efficiency. But even with the conservative assumed assimilation efficiency of ca. 30%, the rate of carbon processing by *A. marina* would still have been significant in the context of the macrofauna assemblage present. Hence, both *Capitella* spp. and *A. marina* appeared to have comparative and significant roles in terms of waste assimilation in dispersive, sandy subtidal environments.

#### 4.3. Ecological role of *Ophiocomina nigra* beneath fish farms

The spatial distribution of the brittle star, *O. nigra*, was inversely related to that of the two opportunistic polychaete species, indicating contrasting tolerances to organic waste. Densities of the opportunistic polychaetes increased abruptly when depositional fluxes exceeded ca. 5–10 gTPM·m<sup>-2</sup>·d<sup>-1</sup> (responding positively to the waste), and almost exclusively occurred in close association with the fish cages. Whereas, *O. nigra* demonstrated a negative response to the waste with an apparent prohibitive threshold approximately 200 m from the farm, inside of which abundances diminished to near-zero. The 200 m proximity corresponded to a depositional flux threshold of approximately 5 gTPM·m<sup>-2</sup>·d<sup>-1</sup>. While the positive association between *Capitella* spp. and farm waste discharges is well established (e.g., Kutti et al., 2007; Wada et al., 2008), a negative association between *O. nigra* and organic waste is seemingly undocumented.

However, the relationship with *O. nigra* appears more complex than just suppression in close proximity to the enrichment source. The results of this study indicate that both the abundance and individual biomass was elevated between 300 m and 1000 m away and a recent study in the same area demonstrated that *O. nigra* utilizes fish feed-derived organic matter out to comparable distances (Woodcock et al., 2018), which together suggest a peripheral zone where growth and potentially reproduction are stimulated (i.e. a low-level 'fertilization' effect). In the absence of controlled experiments, it is difficult to specify the cause of the very-near-farm inhibition. However, likely mechanisms for displacement include: i) suffocation or smothering from periods of extremely elevated deposition, ii) adverse biogeochemical conditions in the sediments due to organic enrichment (localised near-bottom anoxia and elevated total free sulfides, which are toxic to most fauna), iii) changes to the physical substrate and iv) a combination thereof. Sediment grainsize analysis and visual observations suggested that sediments close to the farm tended to comprise finer particles, which may be less conducive to colonization by *O. nigra* as it lacks bed-roughness and therefore attachment points. All three processes are linked to (if not caused by) elevated biodeposition and as such

the relative abundances of *A. marina*, occupying an opportunistic ecological group (i.e., EcoGroup V, Borja et al., 2000) and *O. nigra*, occupying a semi-tolerant ecological group (e.g. EcoGroup III) may serve as useful visual indicators of an approximate effects boundary for environmental management purposes.

#### 4.4. Changes in physical substrates and interactions with ecology

The relatively minor changes in the physical composition of the sediments with proximity to the farm likely had implications for the benthic geochemical state and for general habitat suitability for fauna. Differences in sediment grain size can have a profound influence on solute distribution and transport within the sediments and the geochemical reaction zones that facilitates the decomposition of organic matter (Huettel et al., 2014; Martinez-Garcia et al., 2015). Sediment grain size is also linked to the microbial composition of the sediments (Miller, 1989). Near-farm changes in sediment composition was most apparent at Farm-B where the dominance of coarser particles (2–3 mm) further than 100 m away was replaced by dominance of 0.3 mm to 1 mm grain-sizes close to the cages. Although these changes could simply be a product of spatial heterogeneity in substrates, the same changes were evident in video surveys from other sides of the farm and at other farms (data not shown) and the overall perception was that it was at least partly due to farm derived biodeposits. The increased prevalence of finer grained soft-sediments would have favoured some soft-sediment invertebrates over others, and facilitate colonization by larger, deeper-living species such as *A. marina*. Once established, *A. marina* would, in turn, mobilize, irrigate and sort the sediment, and further influence the microbial community, thus maintaining a particular ecotype that favors itself and other like species.

It is also possible that the feeding behavior and niche occupied by *O. nigra* may have influenced the biological and potentially chemical state of the underlying sediments. At distances of between 200 and 400 m from Farm-B, *O. nigra* was highly abundant yet the opportunistic polychaeta densities (especially *Capitella* spp.) were substantially lower than for comparable distances at Farm-A, where *O. nigra* was absent. In many cases, the densities of *O. nigra* effectively formed a carpet over the sediment (Fig. 9B) with feeding appendages protruding upward intercepting particles and in effect creating a greater seabed roughness, which influences turbulence and near-bottom boundary layers and therefore particle settlement (Trowbridge and Lentz, 2018). One possible explanation for the reduced underlying polychaete abundances is that the Farm-B transect was not situated in the predominant down-current direction where the majority of waste was transported, which would explain why the peak flux of particulate matter was approximately half that for Farm-A. In which case, the scope for enrichment and proliferation of opportunistic polychaetes on the study transect at Farm-B was proportionally less. However, it is unlikely that this accounts for the order of magnitude lower infaunal abundances at Farm-B, especially given that the waste influence was extensive, with *O. nigra* known to ingest the waste out to 1000 m away (Woodcock et al., 2018). Regardless, the biomass and niche that was occupied by opportunistic polychaetes >50 m from Farm-A was effectively replaced by brittle stars at Farm-B. Once established, the epibenthic positioning of this active suspension feeder (Fontaine, 2009; Warner and Woodley, 2009), *O. nigra* would provide it with first opportunity to intercept the waste thereby reducing the amount reaching the underlying sediments where it would otherwise induce typical soft-sediment enrichment effects.

The assessment of relative equivalent biomass and waste metabolizing capacity supports this niche displacement/replace theory. The highest average area standardized biomass of

*O. nigra* at Farm-B was approximately 20 times that of *Capitella* spp. and 40 times that of *A. marina*, whereas at Farm-A where *O. nigra* was absent, the combined biomass of *A. marina* and *Capitella* spp. was comparable to (approximately half that of) *O. nigra* at Farm-B. The greater relative biomass does not necessarily imply greater functional role, because although *O. nigra* actively intercepts particles, the opportunistic polychaetes are also capable of modifying their surroundings providing active positive feedback mechanisms and facilitating geochemical changes. It was not possible to make a direct comparison between the functionality of the polychaete assemblage and the brittle star beds due to a paucity of information pertaining to feeding behavior of *O. nigra*. However, when in coexistence it is suggested that the three species with their differing feeding strategies were providing an effective natural biological mitigation mechanism against organic enrichment that very likely has many synergistic effects.

There are also other higher trophic species that will be playing a role that are still being largely ignored. For example, the video surveys suggested that red-spotted flounder ('Plaice', *Pleuronectes platessa*) was at least indifferent to the waste discharges and may in fact be attracted to the farmed areas. This is plausible given that it feeds on polychaetes and other small invertebrates (including ophiuroids) and maybe benefit from increased prevalence of highly productive, smaller shorter-lived species (Rijnsdorp and Vingerhoed, 2001). The commercially valuable large Brown crab *Cancer pagurus* was also recorded in the vicinity, which interacts strongly with the benthic environment through bioturbation and feeding on benthic invertebrates (Hall et al., 1993; Mascaró and Seed, 2001).

#### 4.5. Synergistic effects and the importance of bacteria

Many of the positive and negative interactions between species as well as the potential 'synergistic effects' may be explained through changes to the diverse and ubiquitous microbial communities and the role of these bacteria in ecosystem functioning. Analysis of the microbial communities using metabarcoding showed very clear changes in composition with proximity to Farm-A. This finding was consistent with previous studies that also describe pronounced microbial changes along aquaculture waste gradients (Dowle et al., 2015; Hamoutene, 2014; Keeley et al., 2018), and in naturally organic rich environments such as wood and whale falls (Palacios et al., 2006; Treude et al., 2009). The roles of bacteria in the environment and the drivers of their composition are diverse and complex, however, the gross changes observed here can be attributed to some general farm-related causes. Many bacteria are closely associated with either anaerobic or aerobic states and/or implicit in the nitrification/denitrification process that is central to the cycling of nitrogen in the environment and alleviates the effects of eutrophication (Blackburn and Blackburn, 1992). *Sulfurimonas* sp. was an example of species that was more prevalent close to the farm relative to reference locations, that oxidizes sulfide (produced under organically enriched anoxic conditions), to sulfate using nitrate as electron acceptor (Zhang et al., 2009). The same species is known for its ability to survive in extreme conditions. Similarly, *Psychromonas* sp. is an example of a facultative anaerobe that was also strongly associated with the enriched cage sediments which uses carbohydrates, that are typically produced under anoxic conditions, for growth (Miyazaki and Nogi, 2014). Therefore, much of the observed gradient is assumed to be a natural response to the artificially elevated organic load stimulating various decomposition processes (Bissett et al., 2006; Bissett et al., 2007; Kunihiro et al., 2008).

There was however also some evidence to suggest that the observed microbial gradient may be directly related to the composition of the fish diet and the fish microbiomes, inoculating the

sediments with an unnatural microflora. For example, a *Flavobacterium* species was significantly elevated in the near-farm sediments, and it may be significant that a species within this group (e.g. *Flavobacterium columnare*) is responsible for large mortalities events in wild and farmed fish (Madetoja et al., 2002). Additionally, near-farm sediments were distinguished by elevated prevalence of bacteria (*Aliivibrio* sp.) from the group Vibrionaceae, which includes *Vibrio anguillarum*; a species known to cause septicemia in Atlantic salmon (Thompson et al., 2006). *Psychromonas* sp., that was also more prevalent close the cages, has also been found in association with rapeseed meal feeds (Long et al., 2017), which can be a component of modern salmon feeds (Shepherd et al., 2017). Although the inference here is that the fish feed and fish themselves were inoculating the sediments, it is also conceivable that the sediments were also a source of bacteria which may in-turn influence the overlying waters. As such, the bacterial composition of sediments is a subject that should be investigated from a fish health and farm sustainability perspective.

Some of the observed differences in the microbial communities may also be a product of the interactions with key infaunal inhabitants such as *A. marina* and *Capitella* spp. In addition to influencing the structure and chemical nature of the seabed, *A. marina* is suspected of microbial 'gardening', which in this context refers to an ability to cultivate or enrich certain bacteria during the gut passage (in the oesophagus), thereby also enriching the sediment in the immediate vicinity upon excretion (Grossmann and Reichardt, 1991; Hylleberg, 1975). Most related studies talk in general terms of enhanced microbial biomass, and details with respect to changes in actual species composition are sparse. However, *Flavobacterium* spp. is among the few taxa that have been implicated (Grossmann and Reichardt, 1991). It may therefore be significant that two *Flavobacterium* species (*Polaribacter* sp. and *Maribacter* sp.) were noticeably more prolific in the near-farm sediments where *A. marina* was also most abundant. In the case of *Capitella* spp., previous studies have found that it strongly promotes the growth of, and has synergistic effects with (in terms of organic matter decomposition) certain bacterial species (Kunihiro et al., 2008; Wada et al., 2008). The species that have been implicated to date are from the  $\alpha$ -subclass of Proteobacteria, including two *Vibrio* spp. and a *Roseobacter* sp. Therefore, it is probably no coincidence that a Vibrionaceae species was prevalent in, and characteristic of, the sediment beneath the cages at Farm-A and that a *Roseobacter* sp. was also characteristic of sediments between 400 m and 600 m in coincidence with abundances of *Capitella* spp. Whether these altered microbial communities are just a symptomatic response to organic enrichment or the product of faunal interactions and microbial gardening cannot be determined from this study, however there is sufficient anecdotal evidence to suggest that the overall assemblage was a significant factor in the apparent resilience of the seabed at this location (Keeley et al., 2019).

## 5. Conclusion

*Capitella* spp., *Arenicola marina*, and *Ophiocomina nigra* have differing but similarly effective strategies for assimilating waste that contributed to a high total benthic assimilation capacity. Living within the sediment, both polychaete species have significant sediment reworking and carbon decomposition capacity, actively modifying the sediments in addition to directly ingesting organic matter. Whereas *O. nigra* represents a much larger biomass and has the ability to filter and intercept the organic particulates before it reaches the seabed, thereby moderating sediments enrichment. *Arenicola marina* has the capacity not just to assimilate organic waste, but to modify the physical and chemical composition of the sediments in a way that is conducive to the breakdown, physical resuspension and dispersion of organic matter; very likely to

the benefit and proliferation of non-specific opportunistic fauna. In addition to being an 'ecosystem engineer' (Riisgård and Banta, 1998), when occurring subtidally, *A. marina* appears to occupy the enrichment tolerant opportunistic niche analogous to that of *Capitella* spp. Sources for the introduction of *A. marina* to anthropogenic sources of organic waste is likely to be provided by natural depositional areas, e.g. where kelp debris accumulate. These results provide a strong argument for incorporating biological components in depositional models that aim to predict dispersion and fate of organic waste and impact to sediments.

We hypothesize that the total benthic assimilation rate was greater than the sum of the individual contributions of the dominant species, and that this may be explained by dynamics within the microbial community, much of which remains poorly understood. Organic enrichment processes and changes in oxic state are synonymous with changes in microbial communities, while capitellids occur in association with certain bacteria and *A. marina* can manipulate the composition of microbes in nearby sediments. It is therefore no surprise that clear gradients in microbial composition are readily observed with respect to distance from farm. However, the dominance of certain species also suggested inoculation pathways between the sediment and overlying farm stock, with potential implications for fish health. The roles played by larger epifauna species (not assessed in conventional biomonitoring) in combination with the synergistic effects help explain differences that have been observed between the rates of organic matter deposition and benthic assimilation (Keeley et al., 2019). More broadly, the results highlight potential for higher-order food-web effects as well as for enhanced bioremediation of the assimilative capacity of the seabed through the introduction or enhancement of certain species.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.134281>.

## Declaration of Competing Interest

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

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