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Effects of bottom trawling and environmental factors on benthic bacteria, meiofauna and macrofauna communities and benthic ecosystem processes

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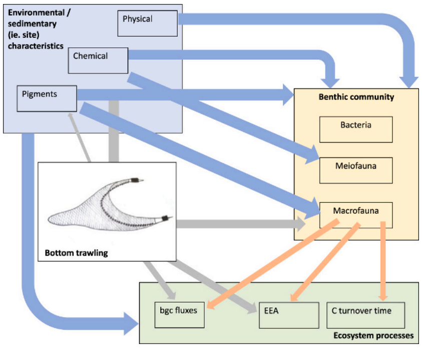
HIGHLIGHTS

- Bottom trawling affected macrofauna but not meiofauna or bacteria community structure.
- Trawling affected the sedimentary carbon pool and increased carbon degradation rates.
- Key bioturbating macrofauna were strongly linked to biogeochemical processes.
- Environmental variables affected fauna and ecosystem processes more than trawling did.
- Anthropogenic impacts must be considered in the context of environmental variability.

GRAPHICAL ABSTRACT



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ABSTRACT

Soft sediment marine benthic ecosystems comprise a diverse community of bacteria, meiofauna and macrofauna, which together support a range of ecosystem processes such as biogeochemical cycling. These ecosystems are also fishing grounds for demersal species that are often caught using bottom trawling. This fishing method can have deleterious effects on benthic communities by causing injury or mortality, and through alteration of sediment properties that in turn influence community structure. Although the impacts of bottom trawling on macrofauna are relatively well studied, less is known about the responses of meiofauna and bacteria to such disturbances, or how bottom trawling impacts benthic ecosystem processes. Quantifying trawling impacts against a background of natural environmental variability is also a challenge.

To address these questions, we examined effects of bottom trawling and a range of environmental variables (e.g. water chemistry and physical and biochemical surface sediment properties) on a) bacterial, meiofaunal and macrofaunal community structure and b) benthic ecosystem processes (nutrient fluxes, extracellular enzyme activities and carbon turnover and degradation rates). We also investigated the link between the benthic macrofauna community and the same ecosystem processes.

While there was a significant effect of bottom trawling intensity on macrofaunal community structure, the same was not seen for bacterial or meiofaunal community composition, which were more affected by

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environmental factors, such as surface sediment properties. The labile component of the surface sediment carbon pool was higher at highly trawled sites. Carbon degradation rates, extracellular enzyme activities, oxygen fluxes and some nutrient fluxes were significantly affected by trawling, but ecosystem processes were also strongly linked to the abundance of key bioturbators (*Macoma balthica*, *Halicryptus spinulosus*, *Scoloplos armiger* and *Pontoporeia femorata*). Although benthic ecosystems were affected by a combination of trawling and natural variability, disentangling these showed that the anthropogenic effects were clearest on the larger component of the community, i.e. macrofauna composition, and on ecosystem processes related to sedimentary carbon.

1. Introduction

Soft sediment marine benthic ecosystems harbour one of the largest pools of macro- and microorganisms in the oceans (Snelgrove, 1999) and are crucial to global biogeochemical cycles, serving as major nitrogen and carbon sinks through sedimentation processes (Snelgrove et al., 2014, 2018; Thrush et al., 2021a). Benthic community composition is strongly determined by environmental factors such as water depth, hydrodynamic energy level (which affects sediment grain size, carbon content and food availability), salinity, and nutrient and oxygen concentrations (Thrush et al., 2021b). The type and intensity of macrofaunal bioturbation activities also affect sediment oxygenation and thus conditions for meiofaunal and bacterial survival, community composition and biogeochemical processes (Mermillod-Blondin and Rosenberg, 2006; Braeckman et al., 2011; Mermillod-Blondin, 2011; Aller and Cochran, 2019). Like most ecosystems, the functioning and productivity of benthic soft bottoms rely on their biodiversity which provides ecological niches and functional resilience against environmental disturbances (Danovaro et al., 2008; Pusceddu et al., 2014; Snelgrove et al., 2014).

Marine benthic ecosystems also host productive grounds for invertebrates and fish that are important natural resources. These species are caught using a range of demersal fishing gears, all of which physically disturb the seabed to some degree, and the use of which has increased globally since the 1950s (Watson et al., 2006). The most common gear types are beam trawls and otter trawls, the former comprising a net held open at the leading edge by a solid beam and with a 'shoe' at each end that moves over the seafloor. There is often additional contact with the seabed through 'tickler chains' across the opening of the net (Depestele et al., 2016). Otter trawls have two heavy trawl doors that keep the net open and close to the seabed and which displace and suspend sediment, while the ground gear, comprising a row of rubber or plastic rollers across the leading edge of the net, also disturbs the seafloor but penetrates less deeply than the trawl doors (O'Neill and Ivanović, 2016). European waters are among the most heavily trawled areas globally (Amoroso et al., 2018). The trawling footprint, i.e. the seabed area affected by bottom trawling, between 0 and 200 m water depth in European regional seas ranges from 28 % to 84 %, with some hotspot areas being trawled >10 times a year (Eigaard et al., 2017).

These fishing methods impact macrofaunal community structure, both directly through displacement, damage or mortality of organisms, and indirectly by altering the natural sediment structure and abundance of habitat-forming species, or through changes in species interactions (competition, predation) in the food web (Groenewold and Fonds, 2000; Thrush and Dayton, 2002; Hiddink et al., 2017; van de Wolfshaar et al., 2020). The net response of the benthic community to demersal fishing disturbance depends on how sensitive species are to this disturbance and how quickly they can recover (Kaiser et al., 2006; Hiddink et al., 2017), as well as sediment type, gear type and fishing history of the site (Sciberras et al., 2018). Although impacts of demersal fishing on macrofaunal community structure may vary on different spatial and temporal scales, some trends are frequently seen; for example, large, long-lived, sessile species are commonly most affected, while small, short-lived mobile, opportunistic species are less impacted or even favoured (e.g. Kaiser et al., 2006; Tillin et al., 2006; Sciberras et al., 2018). Fewer studies have been done on the impact of demersal fishing on the smaller meiofauna, and detected effects are inconsistent across different spatial and temporal scales, water depth or across taxa (Schratzberger and Jennings, 2002; Lampadariou et al., 2005; Pusceddu et al., 2014, 2020; Good et al., 2022). Effects on microbial communities are even less understood, but the few existing studies suggest that biomass, community composition and activity may be affected, at least in the short term (Watling et al., 2001; Polymenakou et al., 2005; Ramalho et al., 2020). The small size, fast life cycles and high recolonization capacity of meio- and microfauna may make them more resilient to such physical disturbance (Leduc et al., 2016) and their community composition may be driven more by changes in macrofaunal species and/or benthic sediment composition (Ingels et al., 2014; Ramalho et al., 2020). However, to our knowledge there are no studies that simultaneously examine the effects of demersal fishing on macrofauna, meiofauna and bacterial communities.

Demersal fishing also has the potential to alter the physical and biogeochemical environment. For example, changes have been seen in sediment grain size distributions and in the quantity, biochemical composition and nutritional value of sedimentary organic matter (OM) in fished areas, with implications for ecosystem functions such as carbon and nutrient cycling (Duplisea et al., 2001; Falcão et al., 2003; Percival et al., 2005; Olsgard et al., 2008; van der Molen et al., 2013; Smith et al., 2016; Ferguson et al., 2020; Morys et al., 2021). These effects can be caused by direct physical disruption of biogeochemical processes and gradients at the sediment-water interface or indirectly, mediated by ecological changes (Thrush et al., 2016; Hale et al., 2017; Tiano et al., 2019). The magnitude and direction of these physical and biogeochemical changes is, however, variable, and apparently determined by the fishing intensity, environmental setting and temporal scale at which the studies are performed (Martín et al., 2014; Pusceddu et al., 2014; Sciberras et al., 2016; Paradis et al., 2021; Epstein et al., 2022).

In summary, there is a complex array of possible interactions between ecological, biogeochemical and environmental factors and demersal fishing disturbance and few studies have addressed all these factors together. Focussing on only one aspect of this complex picture may lead to an incomplete understanding of the complex biotic and abiotic interactions mediating the relative impacts of demersal fishing on benthic ecosystems. We therefore conducted a field study to quantify the role of commercial otter trawling and an array of environmental variables on benthic community structure and ecosystem functioning. To do this, we quantified bacteria, meio- and macrofauna community structure, surface sediment OM content, biochemical composition and degradation rates, extracellular enzymatic activities and nutrient fluxes at six sites characterized by differing bottom trawling intensities in the southern Baltic Sea. The aims were to determine the effect of bottom trawling on a) benthic community structure, b) surface sediment properties and c) benthic ecosystem processes, in the context of environmental variability.

2. Methods

2.1. Study area

The study area was in the Swedish waters of the southern Baltic Sea, west and northwest of the island of Bornholm (Fig. 1). This area lies between the Arkona and Bornholm Basins, where waters are strongly stratified, with a salinity of 7–8 in surface waters and c. 15 in bottom waters. The halocline occurs between 40 and 60 m depending on the area and season. Anoxic or hypoxic waters from the Bornholm Deep, as well as occasional inflows of deep saline water from the Kattegat, may also periodically influence this area (Carstensen et al., 2014). The seabed at 50–60 m water depth is dominated by silty substrates, with some areas of harder clay, and is strongly influenced by large annual inputs of organic carbon from spring and summer plankton blooms. This area has been fished for cod and flatfish, using mainly otter trawls, since the end of the 19th century (Eero et al., 2007). In 2019, the European Commission stopped commercial fishing of the Eastern Baltic cod, only allowing a bycatch rate in other fisheries from mid-2019 onwards (EU, 2019a, 2019b).

Six study sites were chosen, on seabeds of similar depths (50-60 m), and with similar sediments (upper 10 cm comprising mainly silt to fine sand), but characterized by different trawling intensities; three sites (A, C, E) were classified as highly trawled ("High") and the other three (B, D, F) as "Low" trawled areas with only occasional or no trawling (Fig. 1), based on fisheries intensity data (see Section 2.2).

2.2. *Fishing intensities*

Fishing intensity in the study area was determined from Swedish fishing vessels ≥12 m equipped with vessel monitoring systems (VMS) and logbooks. VMS information on vessels' speed and course was coupled to logbook information on vessel size and gear used and interpolated to 1-min temporal resolution (Hintzen et al., 2012). Swept area ratio (SAR) was calculated by combining the high-resolution vessel tracks point data with modelled bottom trawl gear width following the approach of Eigaard et al. (2016). SAR values were then calculated and used in two ways. Firstly, SAR was used for *a priori* selection of areas of 'High' and 'Low' fishing intensity; a regular spatial grid (250 × 250 m) was applied, and swept area was calculated per grid cell for the years 2012–2016 (Fig. 1A). These years were used since they are more representative of longer-term spatial patterns of fishing effort; after 2016 there was a decrease in fishing intensity (Fig. 1B) in the area due to poor fish stocks.

Comparison of the 250 × 250 m gridded SAR with the fishing intensity data reported by ICES on a resolution of c-squares (0.05° x 0.05°; ICES, 2017), which also includes data from fishing fleets belonging to other countries, confirmed that the Swedish fleet's spatial distribution reflected the general distribution of the international fleet. Sites that had 'High' (>6 y^{-1}) and 'Low' (<1 y^{-1}) SAR between 2012 and 2016 were then selected on soft sediment seabeds within a range of similar water depth (49–62 m) and hydrographic conditions (Fig. 1A, Table S1). Secondly, to confirm the High/Low designation of our sampling sites, the swept area was also calculated for the years 2012 – 2019 within a 250 m radius from each sampling site (Fig. 1B). Despite interannual variation and the recent decrease in SAR, the three sites classed as High trawling intensity (A, C, E) have been consistently more trawled than the sites classed as Low (B, D, F) (Fig. 1B). Site positions were (from A to F): 55° 27.889′ N 14° 35.959′ E; 55° 19.979′ N 14° 26.489′ E; 55° 20.928′ N 14° 26.970′ E; 55° 18.416′ N 14° 26.227′ E; 55° 28.367′ N 14° 35.367′ E; 55° 18.297′ N 14° 26.427′ E.

2.3. *Abiotic factors*

Mean dissolved oxygen concentrations (μmol L^{-1}) and salinity in the bottom water at each site were extracted from the relevant grid cells of the Copernicus Marine Environment Monitoring Service (CMEMS)'s Baltic Sea Physics and Biogeochemical Reanalysis products (CMEMS, 2021). This is based on an oceanographic and a biogeochemical model and regular data assimilation from environmental monitoring programmes. Monthly averages were obtained from the water depth closest to each site's depth in each grid cell and a mean for 2016–2019 calculated; bottom water salinity was in the range 13.5–13.8 and bottom water oxygen 204–269 μmol L^{-1} (Table S1).

2.4. *Sampling*

Sampling was carried out at each of the six selected sites (A-F) during a research cruise with Stockholm University's R/V Electra between 15 and 23 May 2019. At each site, a CTD (Seabird 911+ (Seabird

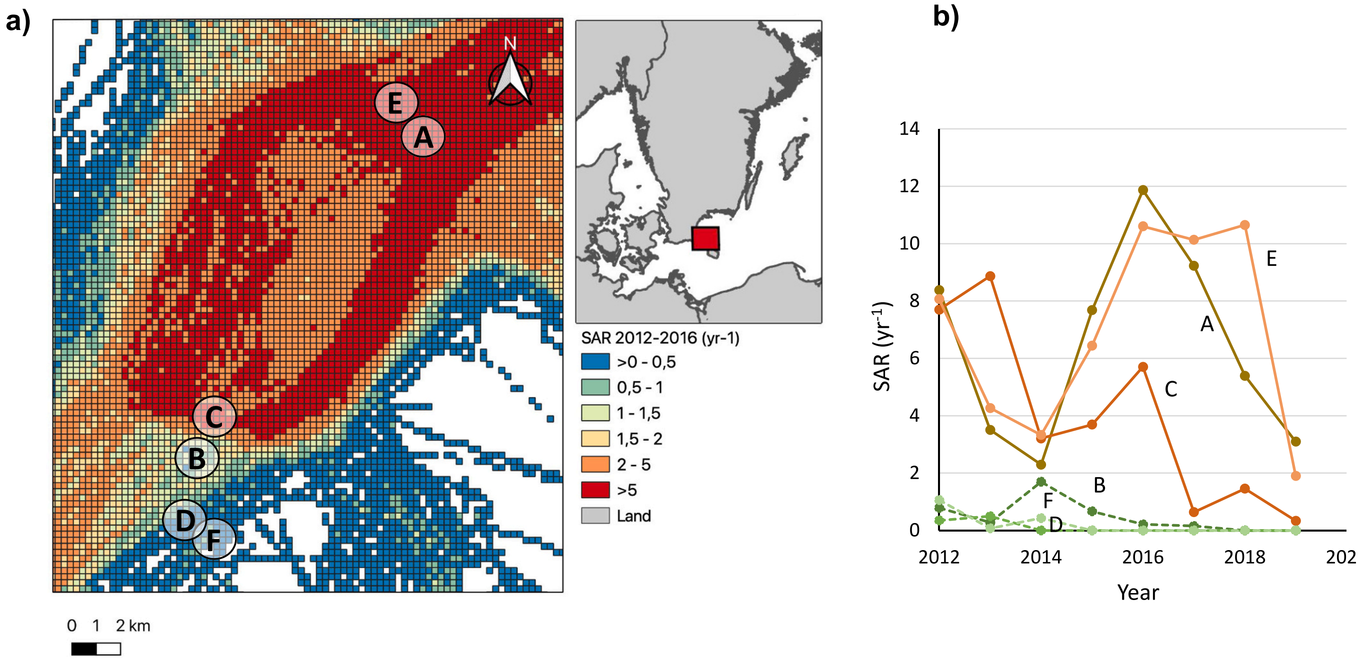

Fig. 1. a) Map of the study area in the southern Baltic Sea, showing the spatial distribution of fishing intensity (average annual trawling intensity (SAR, yr^{-1}, grid cell size 250 × 250 m) of the Swedish fleet for the years 2012–2016) and the positions of the six sample sites in this study (A-F). b) Time trends in SAR (yr^{-1}), 2012–2019, calculated for a 250 m radius around each of the six sampling sites; orange/red = 'High' SAR (sites A, C, E), green = 'Low' SAR (sites B, D, F).

Scientific)) was used for a general characterization of the water column (temperature, salinity, turbidity). About 20 L of bottom water (~0.5 m. a.b.) were pooled from several 5 L Niskin bottle samplers for later use during the incubation experiment (see Section 2.5.3). Six to eight multicorer casts (MUC, K.U.M. Umwelt und Meerestechnik Kiel, with up to 4 cores retrieved per cast, acrylic core tubes with inner diameter of 9 cm, length 60 cm) were deployed. After each cast, the ship was moved c. 50 m in a spiral to avoid re-sampling of the sampled area and to provide some measure of spatial variability. All samples were from seabeds with oxic bottom waters. The sediment cores from each cast were assigned to the different analyses (sediment properties, microbial and meiofauna communities, benthic fluxes (and macrofauna)), avoiding taking multiple replicates of the same sample type from a single cast, thus ensuring true replicates.

2.5. *Sample analyses and measurements*

2.5.1. *Sediment properties*

At each site, four sediment cores (three at site A) were sliced on board immediately after retrieval in 1 cm intervals to the end of the core. Each sediment slice was put in separate plastic Ziploc bags and frozen (−18 °C) until further processing. The thawed samples were homogenized, one 5 mL subsample was taken from each slice and wet, dry (60 °C, 24 h) and ash free (500 °C, 4 h) dry weights were determined. From these weights, water content (% by weight), total organic matter (OM, % by weight) and porosity (% by volume) were calculated. In the data analyses in this paper, the average values from the top 2 cm are used, since this is where the majority of the fauna are present and/or feed (Ankar and Elmgren, 1978; Nascimento et al., 2008). Lastly, subsamples were taken from the top 1 cm of sediment for analysis of the biochemical composition of organic matter and extracellular enzyme activities, and the subsequent calculation of carbon degradation rates.

2.5.2. *Sediment OM content, biochemical composition, and degradation rates*

Concentrations of protein, carbohydrate, lipid and phytopigments and extracellular enzymatic activities in the sediment were determined according to the protocols described in Danovaro (2010), briefly summarised below.

Phytopigments were analysed fluorometrically, after extraction with 90 % acetone, without (chlorophyll *a*) or after (phaeopigment) acidification with 0.1 N HCl (Lorenzen and Jeffrey, 1980). Total phytopigment concentrations were defined as the sum of chlorophyll *a* and phaeopigment concentrations and, once converted into C equivalents using 40 μgC μg phytopigment^{-1} as a conversion factor utilized as an estimate of the organic material of algal origin (Pusceddu et al., 2009a).

Total protein (PRT), carbohydrate (CHO) and lipid (LIP) analyses were carried out spectrophotometrically (Danovaro, 2010) and concentrations calculated using standard solutions of bovine albumin, D+ glucose and tripalmitine, respectively. For each biochemical assay, blanks were obtained using pre-combusted sediments (450 °C for 4 h). Concentrations were converted, into carbon equivalents, using 0.40 and 0.49 and 0.75 mgC mg^{-1} conversion factors, normalized to sediment dry weight (Fabiano et al., 1995). The sum of total protein, carbohydrate and lipid carbon equivalents was reported as biopolymeric C (BPC) (Pusceddu et al., 2009b).

Extracellular aminopeptidase, β-glucosidase and alkaline phosphatase activities, here used as proxies of protein and carbohydrate degradation rates and phosphate liberation rates, respectively, were estimated by the cleavage of fluorogenic substrates (L-leucine-4-methylcoumarinyl-7-amide, 4-MUF-D-glucopyranoside and 4-MUF-P-phosphate, respectively; all from Merck) at saturating concentrations (Danovaro, 2010). Aminopeptidase and β-glucosidase activities (μmol of substrate g^{-1} h^{-1}) were converted into C equivalents using 72 as a conversion factor, and their sum reported as the C degradation rate (μgC g^{-1} h^{-1}) (Pusceddu et al., 2014). The turnover time (in days) of the whole protein (PRT) and carbohydrate (CHO) pools were calculated as the inverse of the ratios of the PRT or CHO degradation rates (i.e. aminopeptidase or β-glucosidase activities) to the whole protein and carbohydrate concentrations (μgC g^{-1}), respectively.

2.5.3. *Benthic fluxes*

Fluxes of O_2, NH_4^+, NO_x^- (sum of NO_3^- and NO_2^-), PO_4^{3-} and dissolved inorganic carbon (DIC) across the sediment-water interface were measured using an incubation experiment on board in a climate room (8 °C) using 6 small cores per site ($n = 5$ at A), subsampled from MUC cores. The incubation temperature was the same as *in situ* water temperature (mean 7.7 °C, SD 0.3 °C, measured with a CTD 0.5 m above the seafloor at the 6 sites). The cores had an internal diameter of 4.6 cm, a length of 30 cm and were half-filled with sediment (exact sediment depth was measured). Overlying water in all cores was replaced with the same *in situ* bottom water collected with Niskin bottles and continuously stirred during incubations using a small magnet in each core, driven by an external rotor. After 2 h acclimation, initial (t_i) samples were taken for analysis of DIC, NH_4^+, NO_x^- and PO_4^{3-} concentrations, by withdrawing 60 mL of overlying water and passing it through a 0.45 μm filter. The water was replaced with more *in situ* bottom water and initial oxygen concentrations (t_i) were measured using a mini-electrode (OX-500, Unisense) and the cores were capped airtight. Additional samples for all nutrients were taken from the reserve *in situ* water for correction of the initial nutrient concentrations after the refill. The duration of the experiment was about 20 h to obtain a decrease in oxygen concentration of approximately 30 % (Dalsgaard et al., 2000); to assess when to end the incubations, we measured oxygen multiple times in a control core run in parallel. After that time (t_f), oxygen concentrations were measured again and samples for the final concentration of the nutrients were taken. DIC samples were stored at 4 °C until analysis using a Multi N/C 3100 (Analytic Jena) at Stockholm University. Samples were acidified with phosphoric acid and produced CO_2 analysed with a nondispersive infrared sensor. Nutrient samples were stored frozen at −18 °C until analysis of dissolved concentrations of NH_4^+-N, (NO_2^- + NO_3^-)-N, and PO_4^{3-}-P on a segmented flow autoanalyzer system (ALPKEM, Flow Solution IV) at Stockholm University.

Oxygen uptake rates and nutrient fluxes (μmol m^{-2} d^{-1}) were calculated using the following equation:

$$\mathrm{Flux} = \left(\left(\mathrm{C_f} - \mathrm{C_i}\right) \times \mathrm{V}\right) / (\mathrm{t} \times \mathrm{A})$$

where C_f and C_i are the final and initial concentrations of the corresponding solute (μM), V is the volume of the overlying water in the core (L), t is the incubation time (h) and A is the sediment surface of the incubation core (m^2).

2.5.4. *Macrofauna*

Macrofauna were extracted from the incubation cores at the end of the incubation experiment by sieving them over a 500 μm mesh sieve. The animals were preserved with 95 % ethanol diluted with 10 % glycerol until taxonomic determination in the laboratory. Sorting and taxonomic identification of the organisms were done with a stereomicroscope with ×10–40 magnification. Each individual was identified to the lowest taxonomic level possible and nomenclature was verified by the World Register of Marine Species (WoRMS Editorial Board, 2022). Dry biomass (gdw) was determined by drying the sorted samples at 60 °C for 24 h. Although the sediment area and volume sampled with these incubation cores is far smaller than that usually used to describe benthic communities, we used these fauna samples in order to directly link the flux measurements to the actual fauna contributing to, or possibly driving, those fluxes. Despite the small sample size, these cores also appear to have captured the majority of the taxa found at each site, as quantified by a van Veen grab also taken at each site (Table S5).

2.5.5. *Bacteria and meiofauna*

At each site, the top 2 cm of four replicate cores were used for the analysis of the microbial and meiofauna community composition. Duplicate 2 g subsamples were taken and stored at −20 °C in 2 mL Eppendorfs for later DNA extraction for the bacterial community analysis. Bulk meiofauna were separated from the remaining 100 mL subsampled sediment by first sieving the sediment on a sterile 40 μm mesh and then separating the meiofauna from this fraction by density extraction with Levasil colloidal silica gel (H.C. Starck) with a specific gravity of 1.3 kg m^{-3} (Nascimento et al., 2012).

2.5.5.1. DNA extraction. For bacterial DNA, we used 0.25 g of sediment for the direct extraction of eDNA using the DNEasy PowerSoil kit (QIAGEN) following the manufacturer's instructions. For the density-extracted bulk meiofauna samples (community DNA), the PowerMAX kit (QIAGEN) was used on the full sample volume (max. 10 mL per sample).

2.5.5.2. Library preparation and metabarcoding. Libraries for 16S and 18S ribosomal RNA were prepared for the sediment eDNA and extracted meiofauna DNA, respectively, following the dual-index amplification methods adapted from Nascimento et al. (2018) and Andersson et al. (2008) and were sequenced by the National Genomics Infrastructure (NGI) in Stockholm, Sweden. PCR1 was carried out using the 18S TAReuk454FWD1/TAReukREV3 primers for meiofauna (Stoeck et al., 2010) and 16S 341F/805R for eDNA Phusion High-Fidelity DNA Polymerase (New England BioLabs) and the following programme: 30 s at 98 °C, followed by 20 cycles of 10 s at 98 °C, 30 s at 55 °C, 30 s at 72 °C (BioRad T100 Thermal Cycler). Amplicons were cleaned through bead purification using MagSI (Magtivio, the Netherlands) beads, followed by PCR2 (indexing) as described by Andersson et al. (2008) to barcode each sample with a unique combination of forward and reverse index sequences to avoid cross-contamination (Esling et al., 2015). The PCR2 thermocycler conditions for both 16S and 18S libraries was: 3 min at 95 °C, 8 cycles of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C, and a final elongation of 5 min at 72 °C. The final barcoded amplification products were then cleaned again using the same method for PCR1. Amplicon concentrations were measured using a Qubit 2.0 Fluorometer (dsDNA BR Assay Kit, Invitrogen), standardized, pooled, and sequenced on an Illumina MiSeq V3 system using a 2 × 300 bp platform.

2.5.5.3. Bioinformatics. Prokaryotic 16S RNA and eukaryotic 18S RNA amplicons were successfully sequenced for all samples. After quality filtering, removing chimeras and merging pair-end reads, a total of 1,138,416 sequences for 16S and 2,702,666 for 18S remained, with an average sequence depth of 45,536 and 108,106, respectively. The 16S dataset was subset to only contain bacteria and the 18S dataset was filtered for meiofauna taxa prior to further analysis, resulting in 7660 and 548 unique ASVs respectively.

Sequence reads were demultiplexed by the sequencing facility using bcl2fastq_v2.20.0.422 from the CASAVA software suite. Further quality filtering and chimera removal was done in R (v4.0.2) using the DADA2 pipeline (Callahan et al., 2016). In more detail, forward and reverse paired-end reads were truncated at 290 and 210 bp respectively, and trimmed at 8 bp using the following parameters: truncLen = c(290,210), maxEE = c(2,2), trimLeft = c(8,8), minFoldParentOverAbundance = 4 and allowoneoff = TRUE. Taxonomic assignment (at the level of family or order) for 16S rRNA amplicon sequence variants (ASVs) was carried out using the SILVA SSU database (r132) and the DECIPHER package (v 2.10.2, Wright, 2016). The ASVs generated by the DADA2 pipeline are higher-resolution alternative to the operational taxonomic units (OTUs) and report the number of times each amplicon is observed per sample (Callahan et al., 2017).

For eukaryotic ASVs, sequences were aligned against the NCBI NT database using BLAST (v2.7.0, Altschul et al., 1990) with an e-value threshold of 0.001. The output file was imported to MEGAN (v 6.14.2, Huson et al., 2016) that links NCBI NT association numbers with taxonomic classifications and uses the lowest common ancestor algorithm to further estimate taxonomic classifications. Singletons (ASVs occurring only once in the dataset) were removed and the final read counts were normalized as relative abundance of each taxa (at the level of family or order) calculated from the proportion of that taxa relation to a total count per sample.

2.6. *Data analyses*

Sedimentary, environmental, ecological, biogeochemical and trawling-related variables may interact in complex ways to influence benthic ecosystem structure and function (see Graphical abstract). In addition, the large number of variables measured means that there is a strong risk of over-parameterisation in the analyses. We dealt with this complexity by breaking the analyses down into a number of stepwise analyses. First, we analysed if trawling and/or site (nested as a factor in 'trawling') affected sediment properties (Section 2.6.1) and the three different benthic communities (macrofauna, meiofauna, bacteria; Section 2.6.2). Since 'site' had a strong effect, we performed a more detailed analysis to determine which environmental variables most affected community structure, with trawling included as a conditioning variable (Section 2.6.3). Lastly, we explored if benthic ecosystem processes were affected by trawling and/or site (Section 2.6.4.a) and by benthic community structure (Section 2.6.4.b). All statistical analyses were carried out using R (v4.0.3 or 4.2.1).

2.6.1. *Effects of trawling and/or site on sediment properties*

To determine whether there were between-site differences in sediment characteristics, and whether trawling intensity also affected these properties, a nested ANOVA (site (A-F) nested in trawling (High/Low)) was performed. Where necessary, sediment parameters were first $\log_{10}$ transformed to achieve normal distributions and homogeneous variance. The sediment parameters included were: % total OM (from loss on ignition), porosity (vol/vol), protein (PRT), lipid (LIP) and carbohydrate (CHO) concentrations (mg gdw^{-1}), biopolymeric carbon (BPC) concentration (mgC gdw^{-1}), chlorophyll-a and phaeopigment concentrations (μg gdw^{-1}).

2.6.2. *Effects of trawling and/or site on bacterial and faunal communities*

A dissimilarity matrix was generated for each of the community datasets with the *vegdist* function of the *vegan* package (Oksanen, 2015) in R (v 4.0.2) using relative read counts with Bray-Curtis distances. To test for differences in community composition through the Bray-Curtis dissimilarity matrix, a permutational multivariate analysis of variance (PERMANOVA) was carried out using the *adonis2* function of the R package *vegan* (Oksanen, 2015). In both analyses, trawling intensity was used as a fixed factor (levels High and Low) and "site" as a nested random factor. In the case of macrofauna, where the PERMANOVA was significant, a Similarity Percentage analysis (SIMPER) was used to identify which species contributed to the differences in community structure between High and Low trawled regions.

2.6.3. *Effects of environmental variables on bacterial and faunal communities*

To analyse the role of environmental variables in structuring benthic bacteria, meiofauna and macrofauna community structure, we used canonical correspondence analysis (CCA). In the fauna data, singletons were removed and relative abundances used, since it is necessary to address differences in sequencing depth between samples in bacteria and meiofauna data; for consistency the same approach was used for macrofauna data. The analyses required paired fauna-sediment data, but since 4 cores were not always retrieved per multicorer cast, we therefore paired each macrofauna, meiofauna or bacteria sample with the geographically closest sediment sample, usually ≤50 m apart.

To identify the environmental variables that best explained community variance, we first reduced the 23 environmental variables into six. Co-correlating or dependent variables were combined into three groups of variables ('Physical', 'Chemical' and 'Pigments') (see Table S1) and followed by a Principal Component Analysis (PCA) to extract the first and second axes (PC1 and 2) of each group of variables (*prcomp* in the R *vegan* package). The PC1 and PC2 axes from each of these three groups were then used as independent latent variables to determine the best explanatory variables that accounted for similarities in the community data using CCA (*cca* function in *vegan*), with trawling (High/Low) as a conditioning variable. The significance of the overall model and the contributing environmental variables was tested with ANOVA (*anova.cca* function in *vegan*).

2.6.4. Factors affecting ecosystem function

The following analyses were run using replicates of fauna and ecosystem process data paired in the same way as described in Section 2.6.3.

a. Exploring whether site and/or trawling affected ecosystem function

The ecosystem processes considered were: extracellular enzyme activities (aminopeptidase, β-glucosidase and alkaline phosphatase); fluxes of oxygen, DIC and nutrients (NH_4^+, NO_x^- and PO_4^{3-}) across the sediment-water interface; and protein (PRT), carbohydrate (CHO) and carbon turnover times and carbon degradation rates. Variables that were not normally distributed and/or did not have homogeneous variance were either $\log_{10}$ transformed or standardized and then Box-Cox transformed before running a nested ANOVA, with site nested in the factor 'trawling intensity' (High/Low).

b. Exploring whether macrofauna community structure affected ecosystem function

In order to remove the effect of rare species in our sediment cores, we included only the taxa that represented >2 % total abundance and >0.02 % of biomass (gdw) in the whole dataset; this left 11 of a total of 18 taxa. Neither the abundance nor the biomass data was transformed. Ecosystem process data was max-min normalized in order to enable comparison across variables with different units (oxygen and nutrient fluxes across the sediment-water interface, extracellular enzyme activities and carbon turnover times).

First, an NMDS of the ecosystem process data was performed, using Euclidean dissimilarity distances, to examine whether sites differed regarding different types or strengths of ecosystem processes. Then, we used the method *bioenv* (*vegan* package) to determine which single species or groups of species best explained these multivariate patterns in ecosystem processes, using faunal data as the explanatory variables. We tested both faunal abundance (using Manhattan index) and biomass data (gdw; Euclidean distances); in both cases Spearman correlations were used. *Envfit* was used to produce vectors of the four taxa identified by *bioenv* (*vegan* package) as best correlated to the ecosystem processes and these were superimposed on the NMDS for visualization purposes.

3. Results

3.1. Sediment properties were affected by between-site variability and trawling

Sedimentary protein (PRT), lipid (LIP), carbohydrate (CHO) and biopolymeric carbon (BPC), as well as chlorophyll *a* and phaeopigments, were all significantly higher at High sites (nested ANOVA, $p < 0.05$, Tables 1, S2). Total OM and porosity were not significantly different between High and Low sites. However, all sediment properties were also significantly related to site (Tables 1, S2). For example, BPC at the three High sites had quite different quality, with Site A having relatively high proportions of PRT (c. 55 %), Site E having a relative high proportion of carbohydrates (c. 68 %) and Site C being intermediate (Tables 1, S1), compared to the other sites. The lowest total OM, BPC and phytopigment concentrations were found at B (Low) and C (High), and the highest at E (High) and F (Low) (Tables 1, S1).

3.2. Trawling affected macrofaunal community composition, but not meiofauna or bacteria

There was no significant effect of trawling on the community composition of bacteria or meiofauna (Fig. 2A, B) (*adonis2*, $p = 0.14$, df $= 1$, $_{\text{pseudo}}F = 1.73$; $p = 0.1$, df $= 1$, $_{\text{pseudo}}F = 0.105$ respectively), but trawling had a significant effect on macrofaunal composition (*adonis2*: $p = 0.039$, df $= 1$, $_{\text{pseudo}}F = 3.04$) (Fig. 2C).

Several species contributed to the overall dissimilarity between High and Low sites (Table S3) but only one, *Halicryptus spinulosus*, was identified by the Similarity Percentage (SIMPER) analysis as contributing significantly to the difference in macrofaunal community structure between High and Low sites (Fig. S1, Table S3); this species was more abundant at sites with high trawling intensity (FDR-adjusted $p = 0.02$, Table S3).

Table 1

Summary of environmental variables and sediment properties. First column refers to the categories of variables in the CCA (3.3). Values are means and standard deviations of $n = 4$ sediment cores, except Site A where $n = 3$. * = significance at $p < 0.05$, n.s. = not significant. Raw data and additional variables are available in Table S1. More details of ANOVA test results are in Table S2.

	Trawling intensity	High				Low			ANOVA significance	
	Site		A	E	C	B	D	F	Trawling	Site
Physical	Organic matter (LOI) (%)	Mean	5.35	10.17	2.97	2.61	6.75	8.86	n.s.	*
		stdev	0.52	1.19	0.29	0.46	2.45	1.78		
	Porosity (vol / vol)	Mean	0.73	0.81	0.61	0.61	0.74	0.78	n.s.	*
		stdev	0.02	0.11	0.03	0.07	0.07	0.08		
Chemical	Protein (mg gdw^{-1})	Mean	11.90	59.35	5.66	3.42	12.07	23.58	*	*
		stdev	3.02	18.88	1.50	0.87	1.88	5.95		
	Carbohydrate (mg gdw^{-1})	Mean	7.13	175.96	6.27	5.72	18.37	53.14	*	*
		stdev	0.72	43.37	0.78	0.72	1.87	7.88		
	Lipid (mg gdw^{-1})	Mean	2.88	4.96	0.73	0.50	2.01	4.26	*	*
		stdev	0.17	1.11	0.16	0.06	0.38	0.71		
	Biopolymeric carbon (mgC gdw^{-1})	Mean	10.85	103.18	5.83	4.34	14.77	36.00	*	*
		stdev	1.61	25.46	1.14	0.60	1.85	3.52		
Pigment	Chlorophyll *a* (μg gdw^{-1})	Mean	14.26	25.05	2.83	1.65	8.53	27.27	*	*
		stdev	2.78	6.86	1.01	0.12	0.44	4.57		
	Phaeopigments (μg gdw^{-1})	Mean	26.02	55.87	10.86	9.37	16.45	48.71	*	*
		stdev	2.14	7.92	3.22	0.42	3.39	9.10		

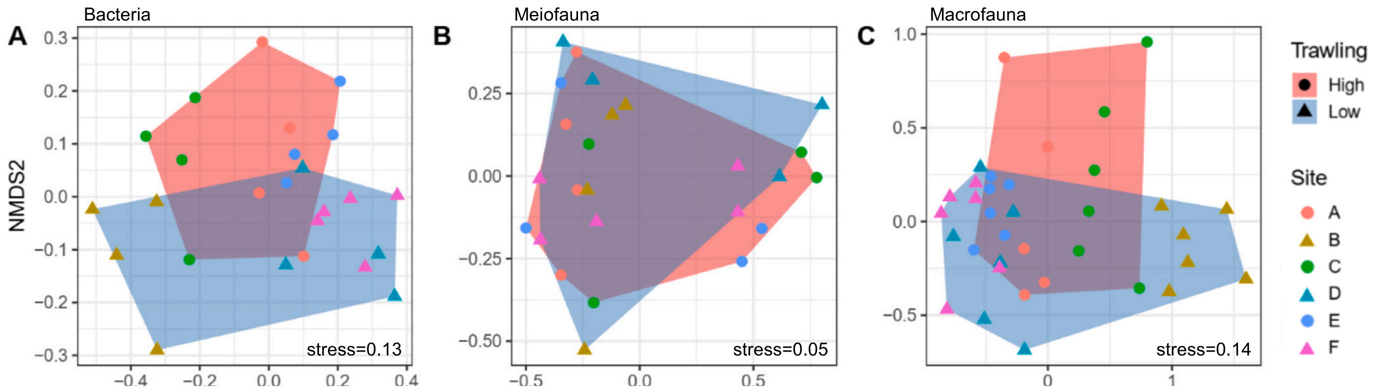

Fig. 2. NMDS of Bray-Curtis dissimilarities based on the relative abundance of all ASVs classified as bacteria (A), meiofauna (B) and macrofauna (C). Each data point represents a replicate at the sampling site. Coloured polygons and points depict high and low trawled sites.

3.3. Environmental variables affected bacterial, meiofaunal and macrofaunal community structure

There was a strong clustering of replicates by site, showing the strong association of bacterial and faunal community structure with site-specific properties (Figs. 2, 3, S1). For example, the macrofaunal communities at sites A and E were dominated by *Macoma balthica*, *H. spinulosus* and *Scoloplos armiger*. A similar community structure was detected at sites D and F, but with higher abundances of *Pontoporeia femorata* and *Bylgides sarsi* compared to A and E (Fig. S1). At sites B and C, *Ampharete balthica*, *Aricidea minuta* and *Astarte borealis* were more abundant compared to the other four sites (Fig. S1).

After removing any effect of trawling (by including it as a conditioning variable in the CCA), environmental variables explained 27 % of the similarity in bacteria, 40 % in meiofauna and 37 % for the macrofauna communities (CCA, Fig. 3). The Physical PC1 axis (derived from bottom water O_2 and salinity, sediment OM, water content and porosity; Table S1) had a significant effect on the microbial, meiofaunal and macrofaunal community structures (*anova.cca*, $p = 0.001$ in all cases; Table S6). This axis seems to reflect the lower water and OM content of the sediments and slightly higher salinity at sites B and C, and the reverse pattern at D and F (see Tables 1, S1).

Both Chemical PC axes were significant for the meiofauna community (*anova.cca*, $p = 0.014$ and 0.046 for PC1 and PC2, respectively), reflecting the large range in BPC concentrations (highest at E/F) and the relative contributions of LIP, PRT and CHO to BPC (high % of CHO at E/F).

For the macrofauna community, in addition to ‘Physical PC1’, ‘Pigment PC2’ was significant (*anova.cca*, $p = 0.013$). The contribution of phytopigments to the total biopolymeric carbon (CCPE/BPC) contributed most to this PC, being highest at site A and lowest at E.

Pigment PC1 was not significant for any of the three communities, but Fig. 3 suggests that different (low) pigment concentrations at sites B and C might play a role.

3.4. Trawling, site-specific factors and macrofauna affected ecosystem function

Extracellular enzyme activities (aminopeptidase, β-glucosidase and alkaline phosphatase activities) were all significantly higher at High sites (nested ANOVA, $p < 0.05$; Fig. 4, Table S7), as was C degradation rate. O_2 and NO_x^- fluxes were significantly higher at Low sites and PO_4^{3-} fluxes were significantly higher at High sites. However, between-site variability in each category was high (Fig. 4, Table S8); site had a significant effect on all tested ecosystem processes except PO_4^{3-} fluxes. PRT, CHO and C turnover times, as well as NH_4^+ and DIC fluxes, were not affected by trawling intensity (Fig. 4, Table S7).

Macrofaunal community composition explained up to c. 50 % of the variation in ecosystem processes. *Macoma balthica* was the single species that best correlated with (i.e. best explained) multivariate patterns in ecosystem processes (*bioenv*: *Macoma* abundance, $r = 0.477$; *Macoma* biomass, $r = 0.264$). However, slightly better models were obtained with

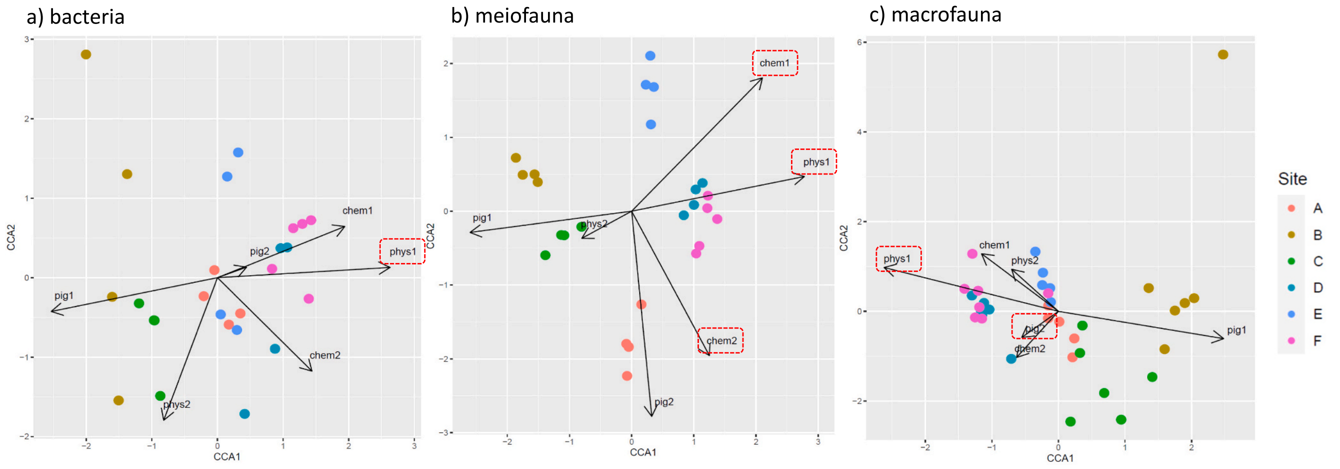

Fig. 3. CCA plots for communities of a) bacteria, b) meiofauna and c) macrofauna, with the contributions of environmental variables to the ordination, expressed as the two main principle components (PC) for sets of physical (phys), chemical (chem) and pigment (pig) variables. PC axes identified as significant (ANOVA, $p < 0.05$) are highlighted with a red box.

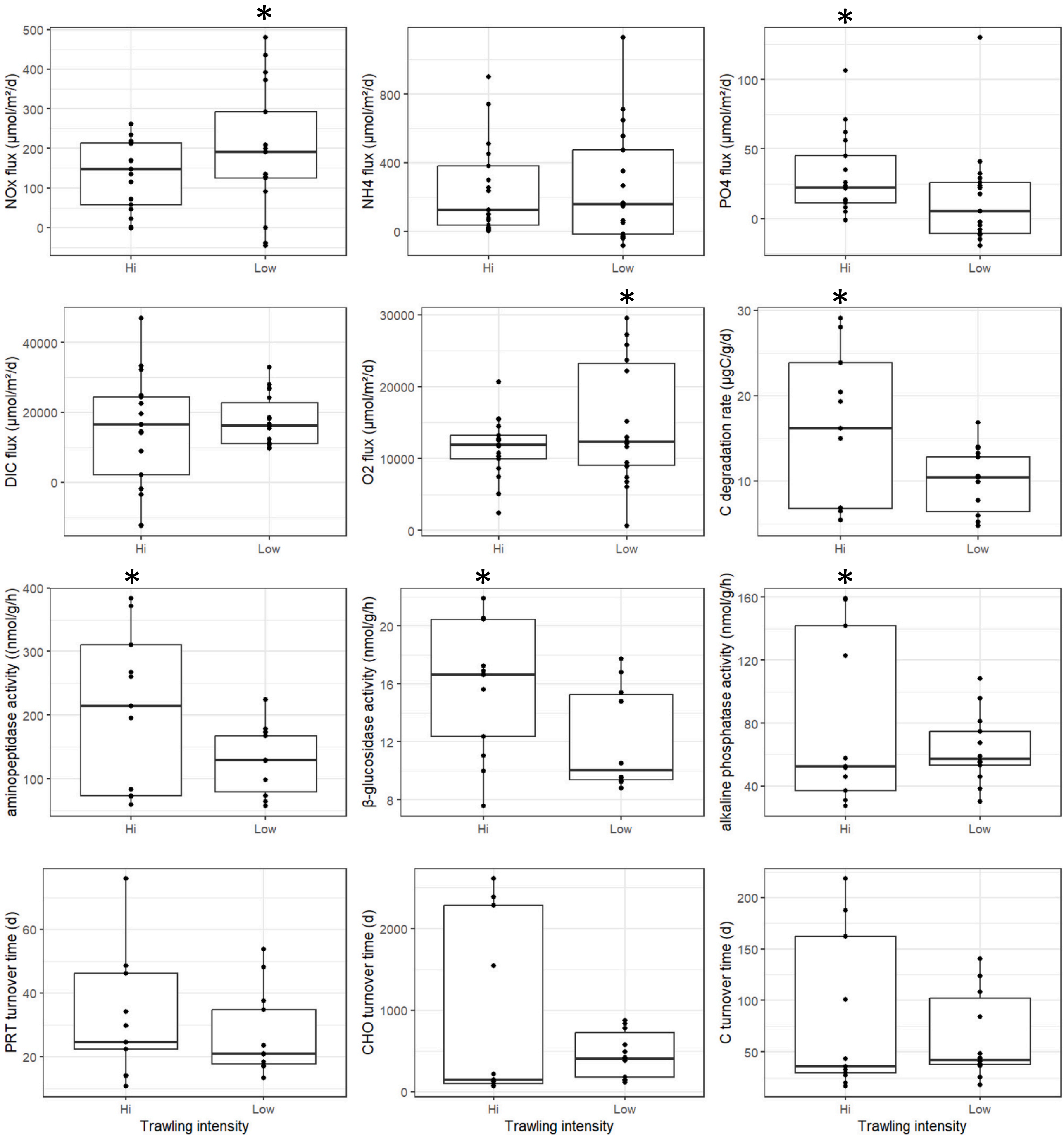

Fig. 4. Ecosystem processes at High and Low trawled sites. Box plots show median with 25th and 75th percentiles, with whiskers extending to the largest and smallest values no further than 1.5 x the inter-quartile range. Significant differences ($p < 0.05$, nested ANOVA) between High and Low are marked with * (see Table S7).

more than this single species: a combination of *Halicryptus spinulosus*, *Scoloplos armiger* and *Pontoporeia femorata* provided the most parsimonious model in terms of biomass (*bioenv*: $r = 0.297$) and the combination of *M. balthica* and *H. spinulosus* in terms of faunal abundance ($r = 0.505$), though the addition of *S. armiger* and *P. femorata* provided abundance models that were as good as for *M. baltica* alone ($r = 0.489, 0.476$) (Table S9).

In general (across all sites and replicates), high numbers and/or biomass of the four taxa identified above were correlated with high rates of extracellular enzyme activities, but longer CHO-, PRT- and C turnover times (i.e. slower turnover) (Figs. 5a, S2a). In addition, these taxa were positively correlated with higher O_2 consumption rates, higher NH_4^+ effluxes from the sediment, and low NO_x^- effluxes. Total biomass of these four taxa also varied widely between sites; for example, sites A and B had low total biomass (Table S4).

4. Discussion

4.1. Sediment properties are affected by between-site variability and trawling

Previous studies of the effects of demersal fishing (hereafter called trawling) on fine-grained sediments have shown a range of results (Martín et al., 2014; Epstein et al., 2022). Some have observed a lower

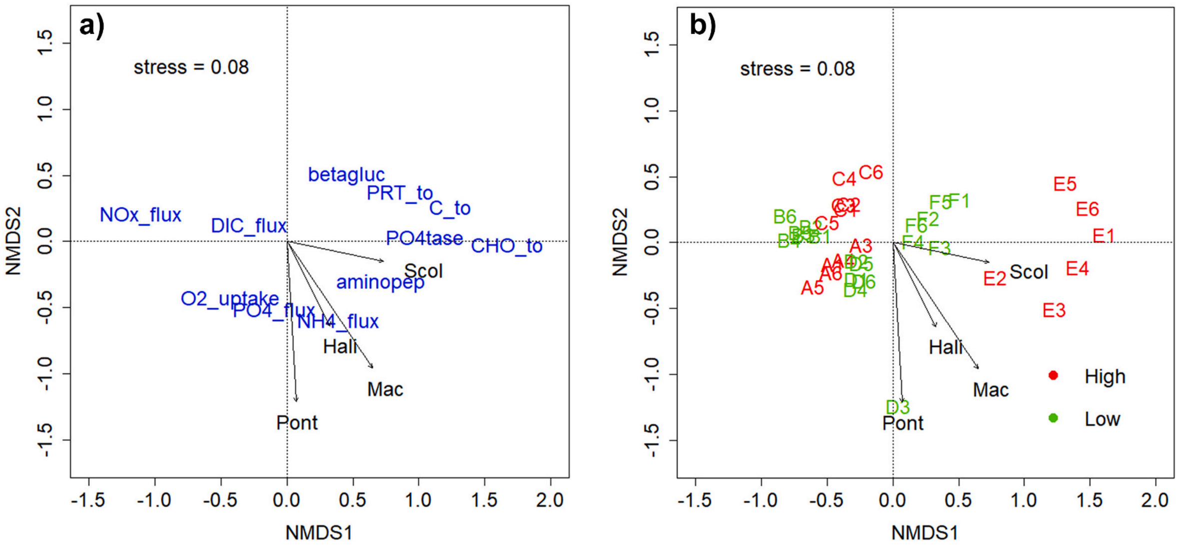

Fig. 5. NMDS of ecosystem processes. Superimposed for visualization purposes are the vectors for the four taxa identified by *bioenv* as best correlated to the ecosystem processes (and fitted here using *envfit*). Vectors are based on faunal biomass (gdw), with rare taxa removed (i.e. those that comprise <2 % of the total abundance and < 0.02 % total biomass in the whole dataset). Hali = *Halicryptus spinulosus*, Mac = *Macoma balthica*, Pont = *Pontoporeia femorata*, Scol = *Scoloplos armiger*. a) shows the ecosystem processes: PRT_to, CHO_to and C_to are turnover times of protein, carbohydrate and carbon; aminopep, betagluc and PO4tase are extracellular activities of aminopeptidase, β-glucosidase and alkaline phosphatase; NOx_flux, NH4_flux, DIC_flux and PO4_flux are the fluxes of those substances across the sediment-water interface and O2_uptake is the consumption of O_2 by the sediment. b) shows the site/sample names; red text = high trawled sites, green = low trawling.

OC content, of a lower food quality (i.e. more refractory), in trawled areas and have attributed this to erosion and/or increased degradation of surficial OC over time through repeated remineralization in chronically disturbed, deep sea areas (Pusceddu et al., 2014; Paradis et al., 2019, 2021). In contrast, studies that measured short-term effects of trawling found higher amounts of OC and higher OC food quality (e.g. higher PRT:CHO ratios) (Pusceddu et al., 2005; Polymenakou et al., 2005), indicating shorter term effects related to the uplift of deeper sediments to the surface by trawling and temporarily elevated degradation and release of labile OC. In our study of chronically trawled areas there were higher amounts of biopolymeric carbon (BPC, the sum of LIP, PRT and CHO) at High sites, although the food quality of this carbon (i.e. % lipids and proteins) varied widely between the studied sites. General patterns related to spatial variation across sites indicated a relationship between sediment type (e.g. water content, particle size and OM content, that all reflect the hydrodynamics of the different sites), and sediment OC food quality (e.g. LIP, PRT, pigments) and OC inputs (Fabiano and Danovaro, 1998; Pusceddu et al., 2009a).

We also observed higher phytopigment concentrations at High sites, in contrast to several field experiments that found sedimentary chl *a* concentrations to be reduced on short time scales due to sediment displacement (Brylinsky et al., 1994; Watling et al., 2001; Tiano et al., 2019). Our results could be due to lower rates of surface sediment processing and reworking by benthic faunal communities at chronically trawled sites (Sciberras et al., 2016); deep-burrowing, gallery-building worms (*Halicryptus spinulosus*, *Scoloplos armiger*) were more common at our High sites (see Section 4.2) and these degrade freshly deposited carbon more slowly than species that bioturbate and feed at the sediment surface (Josefson et al., 2012). If so, this suggests that short term changes in phytopigments may be determined more by physical processes, while long-term chronic effects are mediated by changes in the benthic communities. Alternatively, patchy settling of the spring algal bloom in the area might explain between-site differences. Sediment pigment concentrations in our study area, and at such depths, depend strongly on seasonal OM inputs related to the spring phytoplankton bloom, but spatial and temporal resolution of monitoring data in the area is too coarse to resolve differences in inputs between our six sites. Also, pelagic measurements or satellite data are hard to relate to actual sedimentation on the seafloor, since water currents and stratification affect OM sinking rates in complex ways. From the available information, we know that the spring bloom in this general area of the Baltic Sea peaked in March/April in 2019 (Wesslander et al., 2020; Zettler et al., 2020) and by mid-April/early May sedimentation of fresh OM was already occurring on the seabed (Zettler et al., 2020). This is confirmed by depth profiles of chl *a* at all our sites (data not shown) that indicated high concentrations in the upper 1 cm sediment layer that decreased rapidly down to 4 cm sediment depth. Other authors have suggested that the depauperating effects of trawling on OM content in benthic sediments can be “temporarily or partially abated by the arrival of fresh, high quality OM” (Paradis et al., 2019). This could also be the case at our study sites; C degradation rates were higher at High sites, but none of the measures of C turnover time (that additionally depend on OC concentration) were affected, suggesting the high availability of BPC might mask a more general trawling impact.

4.2. *Benthic fauna community composition is influenced by both trawling and environmental factors*

Trawling had a significant effect on the community composition of macrofauna, but not on meiofauna or bacteria. Environmental variables influenced community composition of all three groups; the group of ‘physical’ variables (comprising 4 year-averaged bottom water salinity and oxygen concentrations, total sediment OM and sediment water content) affected all three taxa groups. In addition, ‘pigments’ (sedimentary phytopigments) affected the macrofauna and ‘chemical’ (i.e. sedimentary biopolymeric C such as protein, lipid and carbohydrate) affected the meiofauna.

4.2.1. *Macrofauna*

Numerous studies have shown that trawling affects seabed macrofauna, with impacts depending on an individual taxon's sensitivity to disturbance and its recovery potential, habitat type, environmental conditions, gear type and fishing history (Kaiser et al., 2006; Hiddink et al., 2017; Sciberras et al., 2018), as well as altered ecological interactions (Sköld et al., 2018; van de Wolfshaar et al., 2020). However, we detected only a weak effect of trawling on the macrofaunal community composition. In terms of potential to recovery from trawl disturbance, most of the macrofaunal taxa are <2 cm in size and have life cycles of <5 y and small, short-lived species have been found to be less affected by physical disturbance than large, slow-growing, longer-lived species (Kaiser et al., 2006; Hiddink et al., 2017). However, it cannot be ruled out that regardless of trawling intensity, decades of chronic disturbance at both High and Low sites has permanently altered benthic communities across the whole area. Since no suitable control areas exist, it is impossible to test this hypothesis in the field.

The only species significantly contributing to the difference in macrofaunal community structure between High and Low sites was the priapulid *Halicryptus spinulosus*, which was more abundant at High sites. This is a common species in the Baltic Sea (Aarnio et al., 1998; Gogina and Zettler, 2010), and in harsh environmental conditions (e.g. fluctuating salinity or low oxygen concentrations) it may be the dominating species in benthic communities (van der Land, 1970). Its burrowing behaviour down to as much as 30 cm depth (Powilleit et al., 1994) may make it fairly resistant to otter trawling disturbance that occurs closer to the sediment surface; trawl doors disturb sediments to 5–20 cm, depending on gear and substrate type, and ground gear only a few cm at most (Ivanović et al., 2011; Bradshaw et al., 2021; Schönke et al., 2022). Although not significant, another deep-burrower, the polychaete *S. armiger*, was also more abundant at the High sites. As well as avoiding direct impacts, deep-burrowing species may experience reduced competition or predation in highly trawled areas if their competitors or predators are more sensitive to disturbance, as has been seen for other resilient species (Sköld et al., 2018; van de Wolfshaar et al., 2020). A recent analysis of 13 case studies of trawling impacts found that despite large differences in environmental settings and benthic communities between cases, deep burrowing taxa were often more common at trawled sites (Beauchard et al., 2023).

As well as general environmental (‘physical’) factors, sediment pigments also explained the variance in macrofauna community structure. Fourteen of the eighteen macrofauna taxa found in this study are obligate or facultative deposit-feeders and these are quick to respond to the fresh input of plankton bloom material that had recently occurred at all sites, particularly at this time of year when growth and reproduction rates are high. This is in contrast to deep sea ecosystems, where fresh pigment deposition does not occur and carbon quality (our ‘chemical’ factors) are more important for the macrofauna (Baldrighi et al., 2013).

4.2.2. Bacteria and meiofauna

There was no clear effect of trawling activity on the structure of meiofaunal or bacterial communities. These groups have the potential to recover more quickly than macrofauna following mechanical disturbances, since their small size may mean that they are displaced or suspended rather than damaged or killed (Schratzberger et al., 2002) and rapidly recolonize the seabed following physical disturbances (Probert, 1984; Schratzberger et al., 2000).

Several other studies have observed no effect of trawling on meiofauna (or nematode) community structure, diversity, abundance or biomass (Schratzberger et al., 2002; Schratzberger and Jennings, 2002; Lampadariou et al., 2005; Ramalho et al., 2020). In shallow shelf seas, such as our study area, trawling impacts on meiofauna may be equal to or less than those caused by seasonal or other environmental variation (Schratzberger and Jennings, 2002; Schratzberger et al., 2002; Lampadariou et al., 2005; Tinlin-Mackenzie et al., 2023), but more pronounced impacts of trawling on meiofauna community structure have been observed in chronically disturbed deep-sea grounds (below 500 m; Pusceddu et al., 2014; Good et al., 2022).

Studies on the effects of trawling on benthic microbial communities are scarce and are mostly based on experimental field studies. While these studies have generally shown some change in community composition, microbial abundance has either been unaffected (Polymenakou et al., 2005; Ramalho et al., 2020) or decreased (Watling et al., 2001; Fiordelmondo et al., 2003) in trawled areas, though these general trends may mask more subtle effects at different seasons (Polymenakou et al., 2005) or different depths in the sediment (Watling et al., 2001). A recent field study of longer term chronic impacts showed that although trawling intensity explained up to 13 % of the variance in bacterial composition, sediment properties, in particular grain size, were more important, both for community structure and diversity (Bonthond et al., 2023).

In our study, the meiofaunal and bacterial community structure was mainly explained by general ‘physical’ environmental variables. It is not possible to pinpoint which of the underlying physical parameters were most important, but differences in characteristics of the sediment and overlying water are known to affect the suitability of the habitat for these organisms (Baldrighi et al., 2013; Rosli et al., 2016; Ramalho et al., 2020). In addition, sediment carbon quality (‘chemical’ variables in the CCA) also explained some variance in meiofaunal community structure (see also Grémare et al., 2002). Carbon quality (i.e. food availability) is crucial to benthic fauna, and it is perhaps surprising that this was not also identified as an important factor for macrofauna and bacteria, as has been seen in other studies (e.g. Baldrighi et al., 2013). Likewise, sediment pigment concentrations have been positively correlated with meiofaunal abundance (Rosli et al., 2016), but in our study pigments only appeared to be related to macrofauna community structure. It is likely that different environmental settings and seasonal variation lie behind the disparity in these findings.

It is worth noting that the meiofauna and bacteria were only identified at higher taxonomic levels (e.g., family/order), potentially making ecological interpretation and inference about their functions difficult. For bacteria in particular, closely related taxa are not necessarily functionally similar and they may also have quite high metabolic plasticity, further complicating our understanding of their role in biogeochemical cycling (Arnosti, 2011). To confidently make ecological inferences from 16S and 18S metabarcoding data there is a need to improve and expand on the taxonomic coverage and resolution for these groups (Andújar et al., 2018). In addition, ecological knowledge of many of these taxa, at any taxonomic level, is often lacking.

Lastly, macrofaunal, meiofaunal and bacterial communities are closely interlinked in a multitude of ways. For example, macrofaunal bioturbation may provide favourable sedimentary conditions for meiofauna and bacteria to thrive, while at the same time predating on these smaller organisms, or interfering with the meiofauna's access to food (Nascimento et al., 2010; Ingels et al., 2014). So, although bacteria and meiofauna may be highly dependent on organic matter quality and quantity, their ability to utilise this resource may be controlled by predation (Aarnio et al., 1998; Fabiano and Danovaro, 1998) or interference competition (Nascimento et al., 2010), potentially masking clear relationships between community structure and environmental conditions.

4.3. Multiple factors affect benthic ecosystem processes

Extracellular enzymes are excreted by bacteria into the environment in order to break down larger molecules into smaller ones that can be taken up by the cells (Arnosti, 2011). Previous studies that have measured short-term effects of physical disturbance and sediment suspension on extracellular enzyme activities (EEA) have commonly seen an increase in aminopeptidase activity, variable responses in beta-glucosidase activities and an eventual decrease in EEA with time after disturbance (Fiordelmondo et al., 2003; Fiordelmondo and Pusceddu, 2004; Polymenakou et al., 2005; Pusceddu et al., 2005). Our study gives a snapshot of EEA in a chronically trawled area and shows significantly higher activities at High sites for all enzymes measured. This may be due to the High sites having significantly more available substrate (CHO, LIP, PRT) (see Section 4.1).

Fluxes of nutrients and oxygen across the sediment-water interface are also partly driven by microbial activity, but also by meiofauna and macrofauna respiration and biogeochemical processes in the sediment.

Thus, oxygen uptake to the sediment might be expected to be high in areas with large amounts of organic substrate and/or as organic matter is remobilised and metabolised in disturbed areas, as we saw in this study, but this relationship is not always clear and may differ between different size fractions of the community (Leduc et al., 2016; Ramalho et al., 2020). There may also be temporal differences, with short term decreases in oxygen consumption due to removal of surface sediment and/or organisms by trawling (Tiano et al., 2019; Morys et al., 2021). Oxygen fluxes and oxygen penetration into the sediment are closely coupled to other biogeochemical fluxes. Sediment-to-water ammonium fluxes are commonly higher in trawled areas and/or directly after trawling (Falcão et al., 2003; Percival et al., 2005; van der Molen et al., 2013; Bradshaw et al., 2021; Morys et al., 2021) due, at least partly, to the removal of surface sediment and release of NH_4-rich porewater. In contrast, we found no effect of trawling on NH_4^+ fluxes in this study. However, our study sampled chronically disturbed areas; responses in NH_4^+ may only be seen for a short time after a disturbance event (van de Velde et al., 2018; Morys et al., 2021). Spatial and temporal variability probably also explain the different effects on NO_x^- and PO_4^{3-} fluxes reported in the literature (Falcão et al., 2003; Morys et al., 2021). Different components of biogeochemical cycles take different amounts of time to reach a new equilibrium after a disturbance event (van de Velde et al., 2018) and there is also small-scale spatial variability in disturbance related to exactly which areas of the seafloor are trawled and by which parts of the gear (Bradshaw et al., 2021; Morys et al., 2021), and macrofaunal sediment mixing may be so extensive as to obscure trawling effects on geochemical profiles (Rooze et al., 2024).

Biogeochemical fluxes can be strongly correlated to the biomass and traits of the benthic fauna through their bioturbation (Mermillod-Blondin and Rosenberg, 2006; Mermillod-Blondin, 2011) and processing of organic matter (Griffiths et al., 2017; Ehrnsten et al., 2019). Any change in macrofauna species composition by physical disturbance may consequently modify ecosystem functions, through indirect impacts on biodiversity or traits (Beauchard et al., 2023; Ingels et al., 2014; Olsgard et al., 2008; Strong et al., 2015). In our study, up to c. 50 % of the variation in the measured ecosystem processes in this study were explained by the abundance or biomass of a combination of four common macrofauna species; *M. balthica* (which was also the single species that best explained the patterns), *H. spinulosus*, *S. armiger* and *P. femorata*. These species were correlated with high EEA, but slow carbon turnover times; this can be explained by an excess of substrate (PRT, CHO) for enzymatic activities at sites (mainly E, F) where these taxa were common. In addition, active surficial bioturbation by *M. baltica* and *P. femorata* and bioirrigation of the deep burrows of *H. spinulosus* and *S. armiger* may have enhanced bacterial activity and NH_4^+, PO_4^{3-} and O_2 fluxes (Mermillod-Blondin and Rosenberg, 2006; Nascimento et al., 2012; Bonaglia et al., 2014). At the other extreme, sediments with very few *M. balthica*, *S. armiger* and *P. femorata* were characterized by low oxygen consumption and low NO_x^- effluxes (or even NO_x^- uptake). Sediments here (sites B, C) had much lower OM and pigment content and had lower water content. Although they were not identified by the *bioenv* analysis as important, the fauna at sites B and C also included larger numbers and/or biomass of the tube-dwelling polychaete worms *Ampharete baltica* and *Polydora* spp. and the bivalve *Astarte borealis*. These taxa live close to, or at, the sediment surface, are not very active bioturbators and are obligate or facultative suspension feeders (Queirós et al., 2013; Wrede et al., 2018), potentially explaining the low rates of most ecosystem processes. These results are in line with the finding of Olsgard et al. (2008) who showed experimentally that a reduced density of trawling-sensitive key bioturbators affected benthic nutrient cycling in a species-specific manner. The disruption or decoupling of fauna-biogeochemical relationships by trawling disturbance has been seen in a few recent field studies. Both Hale et al. (2017) (Irish Sea) and Tsikopoulou et al. (2022) (Mediterranean) concluded that changes in the type or extent of macrofauna bioturbation in trawled sediments led to effects in macronutrient and carbon cycling. Tiano et al. (2022) identified one key species in North Sea sediments, the filter-feeding *Lanice conchilega*, whose reduction by trawling strongly impacted many biogeochemical processes.

4.4. Challenges and limitations of evaluating trawling impacts in a heterogeneous environment

Quantification of trawling intensity is fundamental but often a limitation in these types of studies; due to low spatial and temporal resolution of the underlying data, e.g. 1–2 h between positions of vessels and logbook reporting by trip, and uneven distribution of fishing effort in a given grid cell, calculated values are at best indicators of fishing intensity. Most studies use official swept area ratio (SAR) statistics based on ICES c-squares (grid cell size 0.05° x 0.05° - i.e. c. 17 km^2 at our latitude), which are usually expressed as an average SAR over one year and do not include smaller vessels ($\leq$12 m), since these are not reported in the fisheries vessel monitoring system (VMS). We have a SAR dataset with unusually high spatial resolution (250 × 250 m) which although more precise than the c-square data still suffers from the same issues of spatial and temporal averaging within a cell. However, our sampling strategy was to contrast areas of 'high' and 'low' fishing intensity, which in this area is quite robust to different ways of quantifying fishing intensity. Since trawling by smaller vessels and those from neighbouring countries tend to fish in the same areas, this relative measure of high/low also accounts for more general fishing pressure for which we do not have high resolution data. Ideally, non-trawled areas with comparable environmental settings would provide controls, but such areas do not exist.

As is evident from this study and previous literature, trawling impacts are context dependent. Therefore, it is crucial to specifically quantify and consider relevant variables that might also determine observed differences in, for example, faunal communities or biogeochemical processes. However, this also affects statistical considerations and data requirements; i) the more variables that are measured, the more likely it is that they will correlate with each other and not be independent and ii) with increasing numbers of predictor variables, they will soon outweigh the number of response variables and the number of samples may be insufficient. In our study, we considered 23 variables, necessitating a breakdown of the statistical analyses into a series of several analyses (see Section 2.6), the use of a subset of less correlated variables (Section 2.6.1) and PCA to group variables for use in the CCA (Section 2.6.3). In recent years there has been more of an effort to perform multifactorial analyses and, depending on the study design and nature of the data, authors have used a range of statistical methods, such as; linear mixed effects models (Tinlin-Mackenzie et al., 2023), generalized additive mixed models (GAMMs) (Bonthond et al., 2023; Nielsen et al., 2023), distance-based linear modelling or multivariate multiple regression (distLM) (Leduc et al., 2016; Rosli et al., 2016; Sköld et al., 2018), multivariate generalized linear models (mGLMs) (Bonthond et al., 2023). Rooze et al. (2024) took a more mechanistic biogeochemical approach and Beauchard et al. (2023) used co-inertia analysis (RLQ) and fourth corner analysis to examine how trawling and habitat characteristics affected macrofauna trait composition and ecosystem processes. In all cases, these studies show that environmental variables have the same or greater effect than trawling on benthic ecosystems, but also that trawling effects can be disentangled from environmental effects.

5. Conclusions

The aim of this study was to investigate to what degree chronic bottom trawling disturbance affected benthic ecosystem structure and function, in the context of environmental variability such as physical and chemical sediment properties, using the fishing grounds in the southern Baltic Sea as a case study. In general, environmental variability between sites affected benthic ecology and ecosystem processes more than trawling. We were not able to demonstrate any effect of chronic trawling on meiofaunal and bacterial community structure, perhaps due to these organisms' short life cycles and resilience to physical disturbance. However, highly trawled sites had higher amounts of the labile component of the sediment carbon pool, and higher carbon degradation rates, extracellular enzyme activities, oxygen fluxes and some nutrient fluxes. There were differences in macrofaunal community structure, with deep-burrowing species more common at highly trawled sites. In addition, the abundance and biomass of the four key macrofauna species explained about half of the variation in the measured ecosystem processes. Ecological, biogeochemical and physical variables thus interact in a complex manner, highlighting the complexity of determining cause and effect in a chronically trawled area. However, we have also shown the importance of considering trawling disturbance in the wider context of environmental variability, in order to provide a better understanding of the different forces driving benthic ecosystem structure and function.

CRediT authorship contribution statement

Clare Bradshaw: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Sven Iburg:** Formal analysis, Investigation, Visualization, Writing – original draft. **Claudia Morys:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Mattias Sköld:** Conceptualization, Investigation, Methodology, Visualization, Writing – review & editing. **Antonio Pusceddu:** Conceptualization, Methodology, Resources, Writing – review & editing. **Claudia Ennas:** Investigation. **Patrik Jonsson:** Formal analysis, Methodology, Visualization. **Francisco J.A. Nascimento:** Formal analysis, Methodology, Supervision, Visualization, Writing – review & editing.

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.

Data availability

Supplementary information includes environmental and macrofauna data. Raw DNA sequence data are available in the NCBI Sequence Read Archive (18S data, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1035422, 16S data, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1072011).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171076.

References

Aarnio, K., Bonsdorff, E., Norkko, A., 1998. Role of *Halicryptus spinulosus* (Priapulida) in structuring meiofauna and settling macrofauna. Mar. Ecol. Prog. Ser. 163, 145–153. https://doi.org/10.3354/meps163145.

Aller, R.C., Cochran, J.K., 2019. The critical role of bioturbation for particle dynamics, priming potential, and organic C remineralization in marine sediments: local and basin scales. Front. Earth Sci. 7, 157. https://doi.org/10.3389/feart.2019.00157.

Altschul, S., Gish, W., Miller, W., Myers, Lipman, D. J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Amoroso, R.O., Pitcher, C.R., Rijnsdorp, A.D., McConnaughey, R.A., Parma, A.M., Suuronen, P., et al., 2018. Bottom trawl fishing footprints on the world's continental shelves. Proc. Natl. Acad. Sci. 115, E10275–E10282. https://doi.org/10.1073/pnas.1802379115.

Andersson, A.F., Lindberg, M., Jakobsson, H., Bäckhed, F., Nyrén, P., Engstrand, L., 2008. Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS One 3, e2836. https://doi.org/10.1371/journal.pone.0002836.

Andújar, C., Arribas, P., Yu, D.W., Vogler, A.P., Emerson, B.C., 2018. Why the COI barcode should be the community DNA metabarcode for the Metazoa. Mol. Ecol. 27, 3968–3975.

Ankar, S., Elmgren, R., 1978. The Benthic Macro- and Meiofauna of the Askö-Landsort Area - A Stratified Sampling Survey, 11. Contributions from the Askö Laboratory, University of Stockholm, Sweden, pp. 1–115.

Arnosti, C., 2011. (2011) microbial extracellular enzymes and the marine carbon cycle. Annu. Rev. Mar. Sci. 3, 401–425.

Baldrighi, E., Aliani, S., Conversi, A., Lavaleye, M., Borghini, M., Manini, E., 2013. From microbes to macrofauna: an integrated study of deep benthic communities and their response to environmental variables along the Malta Escarpment (Ionian Sea). Sci. Mar. 77 (4), 625–639.

Beauchard, O., Bradshaw, C., Bolam, S., Tiano, J., Garcia, C., de Borger, E., Laffargue, P., Blomqvist, M., Tsikopoulou, I., Papadopoulou, N.K., Smith, C.J., Claes, J., Soetaert, K., Sciberras, M., 2023. Trawling-induced change in benthic effect trait composition – a multiple case study. Front. Mar. Sci. 10 https://doi.org/10.3389/fmars.2023.1303909.

Bonaglia, S., Nascimento, F.J.A., Bartoli, M., Klawonn, I., Brüchert, V., 2014. Meiofauna increases bacterial denitrification in marine sediments. Nat. Commun. 5, 5133. https://doi.org/10.1038/ncomms6133.

Bonthond, G., Beermann, J., Gutow, L., Neumann, A., Barboza, F.R., Desiderato, A., Fofonova, V., Helber, S.B., Khodami, S., Kraan, C., Neumann, H., Rohde, S., Schupp, P.J., 2023. Benthic microbial biogeographic trends in the North Sea are shaped by an interplay of environmental drivers and bottom trawling effort. ISME Commun. 3, 132. https://doi.org/10.1038/s43705-023-00336-3.

Bradshaw, C., Jakobsson, M., Brüchert, V., Bonaglia, S., Mörth, C.-M., Muchowski, J., Stranne, C., Sköld, M., 2021. Physical disturbance by bottom trawling suspends particulate matter and alters biogeochemical processes on and near the seafloor. Front. Mar. Sci. 8, 683331 https://doi.org/10.3389/fmars.2021.683331.

Braeckman, U., Van Colen, C., Soetaert, K., Vincx, M., Vanaverbeke, J., 2011. Contrasting macrobenthic activities differentially affect nematode density and diversity in a shallow subtidal marine sediment. Mar. Ecol. Prog. Ser. 422, 179–191. https://doi.org/10.3354/meps08910.

Brylinsky, M., Gibson, J., Gordon Jr., D.C., 1994. Impacts of flounder trawls on the intertidal habitat and community of the Minas Basin, Bay of Fundy. Can. J. Fish. Aquat. Sci. 51, 650–661. https://doi.org/10.1139/f94-066.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11, 2639–2643. https://doi.org/10.1038/ismej.2017.119.

Carstensen, J., Andersen, J.H., Gustafsson, B.G., Conley, D.J., 2014. Deoxygenation of the Baltic Sea during the last century. Proc. Natl. Acad. Sci. 111 (15), 5628–5633. https://doi.org/10.1073/pnas.1323156111.

CMEMS, 2021. Baltic Sea Biogeochemistry and Physics Reanalysis. E.U. Copernicus Marine Service Information (CMEMS). Marine Data Store (MDS) doi: 10.48670/moi-00012 and 10.48670/moi-00013. Data downloaded January 2021.

Dalsgaard, T., Nielsen, L.P., Brotas, V., Viaroli, P., Underwood, G., Nedwell, D.B., Sundbäck, K., Rysgaard, S., Miles, A., Bartoli, M., Dong, L., Thornton, D.C.O., Ottosen, L.D.M., Castaldelli, G., Risgaard-Petersen, N., 2000. In: Dalsgaard, T. (Ed.), Protocol Handbook for NICE - Nitrogen Cycling in Estuaries: A Project Under the EU Research Programme: Marine Science and Technology (MAST III). Silkeborg, National Environmental Research Institute, Denmark.

Danovaro, R., 2010. Methods for the Study of Deep-sea Sediments. CRC Press Taylor & Francis Group, Their Functioning and Biodiversity.

Danovaro, R., Gambi, C., Dell'Anno, A., Corinaldesi, C., Fraschetti, S., Vanreusel, A., Vincx, M., Gooday, A.J., 2008. Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. Curr. Biol. 18 (1), 1–8. https://doi.org/10.1016/j.cub.2007.11.056.

Depestele, J., Ivanović, A., Degrendele, K., Esmaeili, M., Polet, H., Roche, M., Summerbell, K., Teal, L.R., Vanelslander, B., O'Neill, F.G., 2016. Measuring and assessing the physical impact of beam trawling. ICES J. Mar. Sci. 73, i15–i26. https://doi.org/10.1093/icesjms/fsv056.

Duplisea, D.E., Jennings, S., Malcolm, S.J., Parker, R., Sivyer, D.B., 2001. Modelling potential impacts of bottom trawl fisheries on soft sediment biogeochemistry in the North Sea. Geochem. Trans. 2, 112. https://doi.org/10.1186/1467-4866-2-112.

Eero, M., MacKenzie, B.R., Karlsdóttir, H.M., Gaumiga, R., 2007. Development of international fisheries for the eastern Baltic cod (*Gadus morhua*) from the late 1880s until 1938. Fish. Res. 87 (2–3), 155–166.

Ehrnsten, E., Norkko, A., Timmermann, K., Gustafsson, B.G., 2019. Benthic-pelagic coupling in coastal seas – modelling macrofaunal biomass and carbon processing in response to organic matter supply. J. Mar. Syst. 196, 36–47. https://doi.org/10.1016/j.jmarsys.2019.04.003.

Eigaard, O.R., Bastardie, F., Breen, M., Dinesen, G.E., Hintzen, N.T., Laffargue, P., et al., 2016. Estimating seabed pressure from demersal trawls, seines, and dredges based on gear design and dimensions. ICES J. Mar. Sci. 73, i27–i43.

Eigaard, O.R., Bastardie, F., Hintzen, N.T., Buhl-Mortensen, L., Buhl-Mortensen, P., Catarino, R., et al., 2017. The footprint of bottom trawling in European waters: distribution, intensity, and seabed integrity. ICES J. Mar. Sci. 74, 847–865. https://doi.org/10.1093/icesjms/fsw194.

Epstein, G., Middelburg, J.J., Hawkins, J.P., Norris, C.R., Roberts, C.M., 2022. The impact of mobile demersal fishing on carbon storage in seabed sediments. Glob. Chang. Biol. 28, 2875–2894. https://doi.org/10.1111/gcb.16105.

Esling, P., Lejzerowicz, F., Pawlowski, J., 2015. Accurate multiplexing and filtering for high-throughput amplicon-sequencing. Nucleic Acids Res. 43, 2513–2524. https://doi.org/10.1093/nar/gkv107.

EU, 2019a. Commission Implementing Regulation (EU) 2019/1248 of 22 July 2019 establishing measures to alleviate a serious threat to the conservation of the eastern Baltic cod (*Gadus morhua*) stock. Off. J. Eur. Union L195/2.

EU, 2019b. Council regulation (EU) 2019/1838 of 30 October 2019 fixing for 2020 the fishing opportunities for certain fish stocks and groups of fish stocks applicable in the Baltic Sea and amending regulation (EU) 2019/124 as regards certain fishing opportunities in other waters. Off. J. Eur. Union L281/1-14.

Fabiano, M., Danovaro, R., 1998. Enzymatic activity, bacterial distribution, and organic matter composition in sediments of the Ross Sea (Antarctica). Appl. Environ. Microbiol. 64, 3838–3845.

Fabiano, M., Fraschetti, S., Danovaro, R., 1995. A three-year time series of elemental and biochemical composition of organic matter in subtidal sandy sediments of the Ligurian Sea (Northwestern Mediterranean). Cont. Shelf Res. 15, 1453–1469.

Falcão, M., Gaspar, M.B., Caetano, M., Santos, M.N., Vale, C., 2003. Short-term environmental impact of clam dredging in coastal waters (south of Portugal): chemical disturbance and subsequent recovery of seabed. Mar. Environ. Res. 56, 649–664. https://doi.org/10.1016/S0141-1136(03)00069-2.

Ferguson, A.J.P., Oakes, J., Eyre, B.D., 2020. Bottom trawling reduces benthic denitrification and has the potential to influence the global nitrogen cycle. Limnol. Oceanogr. Lett. 5, 237–245. https://doi.org/10.1002/lol2.10150.

Fiordelmondo, C., Pusceddu, A., 2004. Short-term response of benthic bacteria and nanoflagellates to sediment resuspension: an experimental study. Chem. Ecol. 20 (2), 107–121. https://doi.org/10.1080/02757540410001667401.

Fiordelmondo, C., Manini, E., Gambi, C., Pusceddu, A., 2003. Short-term impact of clam harvesting on sediment chemistry, benthic microbes and meiofauna in the Goro Lagoon (Italy). Chem. Ecol. 19 (2–3), 173–187. https://doi.org/10.1080/0275754031000119924.

Gogina, M., Zettler, M.L., 2010. Diversity and distribution of benthic macrofauna in the Baltic Sea. Data inventory and its use for species distribution modelling and prediction. J. Sea Res. 64, 313–321. https://doi.org/10.1016/j.seares.2010.04.005.

Good, E., Holman, L.E., Pusceddu, A., Russo, T., Rius, M., Lo Iacono, C., 2022. Detection of community-wide impacts of bottom trawl fishing on deep-sea assemblages using environmental DNA metabarcoding. Mar. Pollut. Bull. 183, 114062 https://doi.org/10.1016/j.marpolbul.2022.114062.

Grémare, A., Medernach, L., deBovée, F., Amouroux, J.M., Vétion, G., Albert, P., 2002. Relationships between sedimentary organics and benthic meiofauna on the continental shelf and the upper slope of the Gulf of Lions (NW Mediterranean). Mar. Ecol. Prog. Ser. 234, 85–94.

Griffiths, J.R., Kadin, M., Nascimento, F.J.A., Tamelander, T., Törnroos, A., Bonaglia, S., Bonsdorff, E., Brüchert, V., Gårdmark, A., Järnström, M., Kotta, J., Lindegren, M., Nordström, M.C., Norkko, A., Olsson, J., Weigel, B., Žydelis, R., Blenckner, T., Niiranen, S., Winder, M., 2017. The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. Glob. Chang. Biol. 23, 2179–2196.

Groenewold, S., Fonds, M., 2000. Effects on benthic scavengers of discards and damaged benthos produced by the beam-trawl fishery in the southern North Sea. ICES J. Mar. Sci. 57, 1395–1406.

Hale, R., Godbold, J.A., Sciberras, M., Dwight, J., Wood, C., Hiddink, J.G., et al., 2017. Mediation of macronutrients and carbon by post-disturbance shelf sea sediment communities. Biogeochemistry 135, 121–133. https://doi.org/10.1007/s10533-017-0350-9.

Hiddink, J.G., Jennings, S., Sciberras, M., Szostek, C.L., Hughes, K.M., Ellis, N., et al., 2017. Global analysis of depletion and recovery of seabed biota after bottom trawling disturbance. Proc. Natl. Acad. Sci. 114, 8301–8306. https://doi.org/10.1073/pnas.1618858114.

Hintzen, N.T., Bastardie, F., Beare, D., Piet, G.J., Ulrich, C., Deporte, N., Egekvist, J., Degel., H., 2012. VMStools: open-source software for the processing, analysis and visualisation of fisheries logbook and VMS data. Fish. Res. 115–116, 31–43. https://doi.org/10.1016/j.fishres.2011.11.007.

Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H.-J., Tappu, R., 2016. MEGAN community edition - interactive exploration and analysis of large-scale microbiome sequencing data. PLoS Comput. Biol. 12, e1004957 https://doi.org/10.1371/journal.pcbi.1004957.

ICES, 2017. HELCOM request on the production of spatial data layers of fishing intensity/pressure. In: ICES Technical Service sr.2017.18. https://doi.org/10.17895/ices.advice.4682.

Ingels, J., Dashfield, S.L., Somerfield, P.J., Widdicombe, S., Austen, M.C., 2014. Interactions between multiple large macrofauna species and nematode communities - mechanisms for indirect impacts of trawling disturbance. J. Exp. Mar. Biol. Ecol. 456, 41–49. https://doi.org/10.1016/j.jembe.2014.03.009.

Ivanović, A., Neilson, R.D., O'Neill, F.G., 2011. Modelling the physical impact of trawl components on the seabed and comparison with sea trials. Ocean Eng. 38, 925–933. https://doi.org/10.1016/j.oceaneng.2010.09.011.

Josefson, A.B., Norkko, J., Norkko, A., 2012. Burial and decomposition of plant pigments in surface sediments of the Baltic Sea: role of oxygen and benthic fauna. Mar. Ecol. Prog. Ser. 455, 33–49. https://doi.org/10.3354/meps09661.

Kaiser, M.J., Clarke, K.R., Hinz, H., Austen, M.C.V., Somerfield, P.J., Karakassis, I., 2006. Global analysis of response and recovery of benthic biota to fishing. Mar. Ecol. Prog. Ser. 311, 1–14. https://doi.org/10.3354/meps311001.

Lampadariou, N., Hatziyanni, E., Tselepides, A., 2005. Meiofaunal community structure in Thermaikos Gulf: response to intense trawling pressure. Cont. Shelf Res. 25, 2554–2569. https://doi.org/10.1016/j.csr.2005.08.016.

Leduc, D., Pilditch, C.A., Nodder, S.D., 2016. Partitioning the contributions of mega-, macro- and meiofauna to benthic metabolism on the upper continental slope of New Zealand: potential links with environmental factors and trawling intensity. Deep-Sea Res. Part Oceanogr. Res. Pap. 108, 1–12. https://doi.org/10.1016/j.dsr.2015.12.003.

Lorenzen, C.J., Jeffrey, S.W., 1980. Determination of chlorophyll in seawater: report of intercalibration tests. In: International Council of Scientific Unions. Scientific Committee on Oceanic Research. Unesco Technical Papers in Marine Science, 35, 20pp.

Martín, J., Puig, P., Palanques, A., Giamportone, A., 2014. Commercial bottom trawling as a driver of sediment dynamics and deep seascape evolution in the Anthropocene. Anthropocene 7, 1–15. https://doi.org/10.1016/j.ancene.2015.01.002.

Mermillod-Blondin, F., 2011. The functional significance of bioturbation and biodeposition on biogeochemical processes at the water–sediment interface in freshwater and marine ecosystems. J. N. Am. Benthol. Soc. 30, 770–778. https://doi.org/10.1899/10-121.1.

Mermillod-Blondin, F., Rosenberg, R., 2006. Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. Aquat. Sci. 68, 434–442. https://doi.org/10.1007/s00027-006-0858-x.

Morys, C., Brüchert, V., Bradshaw, C., 2021. Impacts of bottom trawling on benthic biogeochemistry in muddy sediments: removal of surface sediment using an experimental field study. Mar. Environ. Res. 169, 105384 https://doi.org/10.1016/j.marenvres.2021.105384.

Nascimento, F.J.A., Karlson, A.M.L., Elmgren, R., 2008. Settling blooms of filamentous cyanobacteria as food for meiofauna assemblages. Limnol. Oceanogr. 53, 2636–2643. https://doi.org/10.4319/lo.2008.53.6.2636.

Nascimento, F.J.A., Karlson, A.M.L., Näslund, J., Elmgren, R., 2010. Diversity of larger consumers enhances interference competition effects on smaller competitors. Oecologia 166, 337–347. https://doi.org/10.1007/s00442-010-1865-0.

Nascimento, F.J.A., Näslund, J., Elmgren, R., Naslund, J., Elmgren, R., 2012. Meiofauna enhances organic matter mineralization in soft sediment ecosystems. Limnol. Oceanogr. 57, 338–346. https://doi.org/10.4319/lo.2012.57.1.0338.

Nascimento, F.J.A., Lallias, D., Bik, H.M., Creer, S., 2018. Sample size effects on the assessment of eukaryotic diversity and community structure in aquatic sediments using high-throughput sequencing. Sci. Rep. 8, 11737. https://doi.org/10.1038/s41598-018-30179-1.

Nielsen, J.R., Vastenhoud, B.M.J., Bossier, S., Møhlenberg, F., Christensen, A., Diekman, R., Dinesen, G.E., Eigaard, O.R., Gogina, M., Zettler, M.L., Darr, A., Bastardie, F., 2023. Impacts of habitat-specific benthic fishing compared to those of short-term induced variability by environmental drivers in a turbulent Baltic Sea environment. Fish. Res. 257, 106514 https://doi.org/10.1016/j.fishres.2022.106514.

Oksanen, J., 2015. Multivariate Analysis of Ecological Communities in R: Vegan Tutorial. https://doi.org/10.1016/0169-5347(88)90124-3.

Olsgard, F., Schaanning, M.T., Widdicombe, S., Kendall, M.A., Austen, M.C., 2008. Effects of bottom trawling on ecosystem functioning. J. Exp. Mar. Biol. Ecol. 366, 123–133.

O'Neill, F.G., Ivanović, A., 2016. The physical impact of towed demersal fishing gears on soft sediments. ICES J. Mar. Sci. 73, i5–i14. https://doi.org/10.1093/icesjms/fsv125.

Paradis, S., Pusceddu, A., Masqué, P., Puig, P., Moccia, D., Russo, T., et al., 2019. Organic matter contents and degradation in a highly trawled area during fresh particle inputs (Gulf of Castellammare, southwestern Mediterranean). Biogeosciences 16, 4307–4320. https://doi.org/10.5194/bg-16-4307-2019.

Paradis, S., Goñi, M., Masqué, P., Durán, R., Arjona-Camas, M., Palanques, A., Puig, P., 2021. Persistence of biogeochemical alterations of deep-sea sediments by bottom trawling. Geophys. Res. Lett. 48, e2020GL091279 https://doi.org/10.1029/2020GL091279.

Percival, P., Frid, C., Upstill-Goddard, R., 2005. The impact of trawling on benthic nutrient dynamics in the North Sea: implications of laboratory experiments. Am. Fish. Soc. Symp. 41, 491–501.

Polymenakou, P.N., Pusceddu, A., Tselepides, A., Polychronaki, T., Giannakourou, A., Fiordelmondo, C., et al., 2005. Benthic microbial abundance and activities in an intensively trawled ecosystem (Thermaikos Gulf, Aegean Sea). Cont. Shelf Res. 25, 2570–2584. https://doi.org/10.1016/j.csr.2005.08.018.

Powilleit, M., Kitlar, J., Graf, G., 1994. Particle and fluid bioturbation caused by the priapulid worm *Halicryptus spinulosus* (v. Seibold). Sarsia 79, 109–117.

Probert, P.K., 1984. Disturbance, sediment stability, and trophic structure of soft-bottom communities. J. Mar. Res. 42, 893–921.

Pusceddu, A., Fiordelmondo, C., Danovaro, R., 2005. Sediment resuspension effects on the benthic microbial loop in experimental microcosms. Microb. Ecol. 50, 602–613. https://doi.org/10.1007/s00248-005-5051-6.

Pusceddu, A., Dell'Anno, A., Fabiano, M., Danovaro, R., 2009a. Quantity and bioavailability of sediment organic matter as signatures of benthic trophic status. Mar. Ecol. Prog. Ser. 375, 41–52. https://doi.org/10.3354/meps07735.

Pusceddu, A., Gambi, C., Zeppilli, D., Bianchelli, S., Danovaro, R., 2009b. Organic matter composition, metazoan meiofauna and nematode biodiversity in Mediterranean deep-sea sediments. Deep Sea Res. Part II Top. Stud. Oceanogr. 56, 755–762. https://doi.org/10.1016/j.dsr2.2008.10.012.

Pusceddu, A., Bianchelli, S., Martín, J., Puig, P., Palanques, A., Masqué, P., et al., 2014. Chronic and intensive bottom trawling impairs deep-sea biodiversity and ecosystem functioning. Proc. Natl. Acad. Sci. U. S. A. 111, 8861–8866. https://doi.org/10.1073/pnas.1405454111.

Pusceddu, A., Paradis, S., Moccia, D., Puig, P., Masque, P., Russo, T., et al., 2020. Impacts of bottom trawling on benthic trophic status and meiofauna in a deep-sea sedimentary environment (Gulf of Castellammare, southwestern Mediterranean Sea). In: EGU General Assembly 2020 EGU2020-9804. https://doi.org/10.5194/egusphere-egu2020-9804.

Queirós, A.M., Birchenough, S.N.R., Bremner, J., Godbold, J.A., Parker, R.E., Romero-Ramirez, A., Reiss, H., Solan, M., Somerfield, P.J., Van Colen, C., Van Hoey, G., Widdicombe, S., 2013. A bioturbation classification of European marine infaunal invertebrates. Ecol. Evol. 3 (11), 3958–3985. https://doi.org/10.1002/ece3.769.

Ramalho, S.P., Lins, L., Soetaert, K., Lampadariou, N., Cunha, M.R., Vanreusel, A., et al., 2020. Ecosystem functioning under the influence of bottom-trawling disturbance: an experimental approach and field observations from a continental slope area in the west Iberian margin. Front. Mar. Sci. 7, 457. https://doi.org/10.3389/fmars.2020.00457.

Rooze, J., Zeller, M.A., Gogina, M., Roeser, P., Kallmeyerd, J., Schönke, M., Radtke, H., Böttcher, M.E., 2024. Bottom-trawling signals lost in sediment: a combined biogeochemical and modeling approach to early diagenesis in a perturbed coastal area of the southern Baltic Sea. Sci. Total Environ. 906, 167551 https://doi.org/10.1016/j.scitotenv.2023.167551.

Rosli, N., Leduc, D., Rowden, A.A., Clark, M.R., Probert, P.K., Berkenbusch, K., Neira, C., 2016. Differences in meiofauna communities with sediment depth are greater than habitat effects on the New Zealand continental margin: implications for vulnerability to anthropogenic disturbance. PeerJ 4, e2154. https://doi.org/10.7717/peerj.2154.

Schönke, M., Clemens, D., Feldens, P., 2022. Quantifying the physical impact of bottom trawling based on high-resolution bathymetric data. Remote Sens. 14, 2782. https://doi.org/10.3390/rs14122782.

Schratzberger, M., Jennings, S., 2002. Impacts of chronic trawling disturbance on meiofaunal communities. Mar. Biol. 141, 991–1000.

Schratzberger, M., Rees, H.L., Boyd, S.E., 2000. Effects of simulated deposition of dredged material on structure of nematode assemblages: the role of burial. Mar. Biol. 136, 519–530.

Schratzberger, M., Dinmore, T.A., Jennings, S., 2002. Impacts of trawling on the diversity, biomass and structure of meiofauna assemblages. Mar. Biol. 140, 83–93.

Sciberras, M., Parker, R., Powell, C., Robertson, C., Kröger, S., Bolam, S., Hiddink, J.G., 2016. Impacts of bottom fishing on the sediment infaunal community and biogeochemistry of cohesive and non-cohesive sediments. Limnol. Oceanogr. 61, 2076–2089.

Sciberras, M., Hiddink, J.G., Jennings, S., Szostek, C.L., Hughes, K.M., Kneafsey, B., et al., 2018. Response of benthic fauna to experimental bottom fishing: a global meta-analysis. Fish Fish. 19, 698–715.

Sköld, M., Göransson, P., Jonsson, P., Bastardie, F., Blomqvist, M., Agrenius, S., et al., 2018. Effects of chronic bottom trawling on soft-seafloor macrofauna in the Kattegat. Mar. Ecol. Prog. Ser. 586, 41–55.

Smith, C., Papadopoulou, K., Dafnomili, E., Zivanovic, S., 2016. The impact of trawling on nutrient and oxygen fluxes in the Cretan Sea. In: 8th Panhellenic Oceanography & Fisheries Symposium, 6pp.

Snelgrove, P.V.R., 1999. Getting to the bottom of marine biodiversity: sedimentary habitats ocean bottoms are the most widespread habitat on Earth and support high biodiversity and key ecosystem services. BioScience 49, 129–138. https://doi.org/10.2307/1313538.

Snelgrove, P.V.R., Soetaert, K., Solan, M., Thrush, S., Wei, C.-L., Danovaro, R., Fulweiler, R.W., Kitazato, H., Ingole, B., Norkko, A., Parkes, R.J., Volkenborn, N., 2014. Global carbon cycling on a heterogeneous seafloor. TREE 33 (2), 96–105. https://doi.org/10.1016/j.tree.2017.11.004.

Snelgrove, P.V.R., Thrush, S.F., Wal, D.H., Norkko, A., 2018. Real world biodiversity–ecosystem functioning: a seafloor perspective. TREE 29 (7), 398–405. https://doi.org/10.1016/j.tree.2014.05.002.

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D., Breiner, H., et al., 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol. Ecol. 19, 21–31.

Strong, J.A., Andonegi, E., Bizsel, K.C., Danovaro, R., Elliott, M., Franco, A., Garces, E., Little, S., Mazik, K., Moncheva, S., Papadopoulou, N., Patrício, J., Queirós, A.M., Smith, C., Stefanova, K., Solaun, O., 2015. Marine biodiversity and ecosystem function relationships: the potential for practical monitoring applications. Estuar. Coast. Shelf Sci. 161, 46–64. https://doi.org/10.1016/j.ecss.2015.04.008.

Thrush, S.F., Dayton, P.K., 2002. Disturbance to marine benthic habitats by trawling and dredging: implications for marine biodiversity. Annu. Rev. Ecol. Syst. 33, 449–473. https://doi.org/10.1146/annurev.ecolsys.33.010802.150515.

Thrush, S.F., Ellingsen, K.E., Davis, K., 2016. Implications of fisheries impacts to seabed biodiversity and ecosystem-based management. ICES J. Mar. Sci. 73, i44–i50. https://doi.org/10.1093/icesjms/fsv114.

Thrush, S.F., Hewitt, J.E., Pilditch, C.A., Norkko, A., 2021a. Ecosystem functions and the work of soft sediments. Chapter 9. In: Thrush, et al. (Eds.), Ecology of Coastal Marine Sediments: Form, Function, and Change in the Anthropocene. Oxford University Press, pp. 121–136.

Thrush, S.F., Hewitt, J.E., Pilditch, C.A., Norkko, A., 2021b. The sedimentary environment. Chapter 1. In: Thrush, et al. (Eds.), Ecology of Coastal Marine Sediments: Form, Function, and Change in the Anthropocene. Oxford University Press, pp. 3–17.

Tiano, J.C., Witbaard, R., Bergman, M.J.N., Van Rijswijk, P., Tramper, A., Van Oevelen, D., et al., 2019. Acute impacts of bottom trawl gears on benthic metabolism and nutrient cycling. ICES J. Mar. Sci. 76, 1917–1930. https://doi.org/10.1093/icesjms/fsz060.

Tiano, J.C., Depestele, J., Van Hoey, G., Fernandes, J., van Rijswijk, P., Soetaert, K., 2022. Trawling effects on biogeochemical processes are mediated by fauna in high-energy biogenic-reef-inhabited coastal sediments. Biogeosciences 19, 2583–2598. https://doi.org/10.5194/bg-19-2583-2022.

Tillin, H.M., Hiddink, J.G., Jennings, S., Kaiser, M.J., 2006. Chronic bottom trawling alters the functional composition of benthic invertebrate communities on a sea-basin scale. Mar. Ecol. Prog. Ser. 318, 31–45. https://doi.org/10.3354/meps318031.

Tinlin-Mackenzie, A., Sugden, H., Scott, C.L., Kennedy, R., Fitzsimmons, C., 2023. Trawling for evidence: an ecosystem-based multi-method trawling impact assessment. Fish. Res. 268, 106858 https://doi.org/10.1016/j.fishres.2023.106858.

Tsikopoulou, I., Smith, C.R., Papadopoulou, K.N., Austen, M.C., 2022. Linking species functional traits to specific biogeochemical processes under trawling pressure. Biology 11, 1378. https://doi.org/10.3390/biology11101378.

van de Velde, S., van Lancker, V., Hidalgo-Martinez, S., Berelson, W.M., Meysman, F.J.R., 2018. Anthropogenic disturbance keeps the coastal seafloor biogeochemistry in a transient state. Sci. Rep. 8, 5582. https://doi.org/10.1038/s41598-018-23925-y.

van de Wolfshaar, K.E., van Denderen, P.D., Schellekens, T., van Kooten, T., 2020. Food web feedbacks drive the response of benthic macrofauna to bottom trawling. Fish Fish. 21, 962–972. https://doi.org/10.1111/faf.12481.

van der Land, J., 1970. Systematics, zoogeography, and ecology of the Priapulida. Zool. Verh. 112, 1–118.

van der Molen, J., Aldridge, J.N., Coughlan, C., Parker, E.R., Stephens, D., Ruardij, P., 2013. Modelling marine ecosystem response to climate change and trawling in the North Sea. Biogeochemistry 113, 213–236. https://doi.org/10.1007/s10533-012-9763-7.

Watling, L., Findlay, R.H., Mayer, L.M., Schick, D.F., 2001. Impact of a scallop drag on the sediment chemistry, microbiota, and faunal assemblages of a shallow subtidal marine benthic community. J. Sea Res. 46, 309–324. https://doi.org/10.1016/S1385-1101(01)00083-1.

Watson, R., Revenga, C., Kura, Y., 2006. Fishing gear associated with global marine catches: II. Trends in trawling and dredging. Fish. Res. 79, 103–111. https://doi.org/10.1016/j.fishres.2006.01.013.

Wesslander, K., Viktorsson, L., Thor, P., Nilsson, M., Skjevik, A.-T., 2020. The Swedish National Marine Monitoring Programme 2019. Hydrography, Nutrients, Phytoplankton. Report Oceanography No.69, 2020.

WoRMS Editorial Board, 2022. World Register of Marine Species. Available from. https://www.marinespecies.org. at VLIZ. doi:10.14284/170. Accessed 2022-01-29.

Wrede, A., Beermann, J., Dannheim, J., Gutow, L., Brey, T., 2018. Organism functional traits and ecosystem supporting services – a novel approach to predict bioirrigation. Ecol. Indic. 91, 737–743. https://doi.org/10.1016/j.ecolind.2018.04.026.

Wright, E.S., 2016. Using DECIPHER v2.0 to analyze big biological sequence data in R. The R Journal 8 (1), 352–359.

Zettler, M.L., Kremp, A., Dutz, J., 2020. Biological assessment of the Baltic Sea 2019. In: Meereswiss. Ber., Warnemünde, 115. https://doi.org/10.12754/msr-2020-0115.