



Benthic assemblage responses to different sources of macrophyte detritus using contrasting methodological approaches

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Abstract

Highly productive coastal vegetated habitats export macrophyte subsidies to adjacent shallow bare sediments, delivering detrital matter to a diverse group of co-occurring benthic consumers that rely heavily on these allochthonous food sources. We studied the effects of detrital subsidies composed of three macrophyte species, *Fucus vesiculosus*, *Cladophora glomerata*, and *Zostera marina*, on the composition of shallow benthic bacterial and faunal assemblages. Biodiversity estimates were obtained through traditional (macrofauna) and eDNA (bacteria, meiofauna, and macrofauna) approaches to characterise the benthic assemblages associated with the detritus. Benthic bacterial assemblage changed in response to the availability of different types of detritus. The meiofauna assemblage composition shifted when comparing the macrophyte detritus and the bare sediment over time. The biodiversity of the macrofauna assemblage estimated using the traditional method showed, unlike the eDNA method, a clear response to the presence of macrophyte detritus. Despite the disparity in the results between the traditional and the eDNA approaches, we found similar relationships and trends between the different benthic groups when using macrofauna abundance data from both methods. In general, more degraded and labile detritus with a high diversity of bacteria and meiofauna triggered macrofauna abundance and diversity. Consequently, a multi-approach design can be implemented to better understand the relationships between different benthic assemblages and the influence of macrophyte subsidies on the structure and functioning of shallow soft-sediment habitats. An ecological understanding of the effects of different detritus sources on benthic assemblages is highly relevant due to the predicted global change scenario of decreasing macrophyte diversity, potentially modifying the amounts and types of detritus reaching recipient habitats with cascading effects on detritus-dependent assemblages.

Keyword Bacteria · Baltic Sea · Biodiversity · eDNA · Macrofauna · Meiofauna · Seafloor · Seagrass

Introduction

Most of the macrophyte production from coastal vegetated areas is not directly consumed by herbivores but rather enters detrital pathways as detached organic matter (Krumhansl and Scheibling 2012). Detached macrophyte detritus provides a transient food subsidy in many adjacent marine habitats, structuring benthic communities, shaping food webs and driving secondary productivity beyond their points of origin, particularly in low-productive sedimentary environments with limited sources of nutrients (Lastra et al. 2008; Sävström et al. 2016; de Bettignes et al. 2020; Kahma et al. 2023).

Following deposition on the seafloor, detached macrophyte material is subjected to physical processes such as fragmentation and abrasion that facilitate its breakdown, subsequently undergoing decomposition and remineralisation

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driven by a diverse assemblage of benthic organisms (Norikko et al. 2000; S  wstr  m et al. 2016; de Bettignies et al. 2020). The deposition of detritus material triggers a colonisation process by benthic communities, largely mediated by microorganisms that mineralise organic material, playing a key role in coastal nutrient cycling (Hardison et al. 2010; S  wstr  m et al. 2016; Rodil et al. 2019). The presence of microorganisms also enhances the detrital nutritional quality for higher trophic levels (Hardison et al. 2010; Lavery et al. 2013; Rodil et al. 2019). Most of the studies focusing on the effects of detritus on seafloor biodiversity often involve a single biotic group, either bacteria or metazoans (macro- and meiofauna). A single approach assumes that all taxa respond similarly to macrophyte subsidies and exhibit congruence across different food sources. However, the extent of the response of different biotic groups to various detrital subsidies is unclear. Bacteria, meiofauna, and macrofauna assemblages interact, regulating biodiversity and key ecosystem processes in various marine ecosystems (e.g., Papa-georgiou et al. 2007; Rodil et al. 2015; Duggins et al. 2016). For instance, bacterial degradation of macrophytes makes primary production available to other benthic consumers, and the bacterial assemblage itself may also represent an important contribution to the consumers' diet (Duggins et al. 1989; Norderhaug et al. 2003). Meiofauna is known to stimulate bacterial growth (Coull and Chandler 2001), and benthic macrofauna can control meiofauna populations through predation and resource competition (  lafsson 2003; Nascimento et al. 2011).

In temperate coastal areas, the largest detritus sources contributing to the existing organic pool are composed of different macrophytes, mainly seagrasses and macroalgae (Duarte 2017). Seagrass detritus can positively affect the structuring of benthic assemblages and promote trophic connectivity (Lavery et al. 2013). However, macrophyte detritus often contains a lot of macroalgae, and small epigrazers tend to prefer macroalgae over seagrass due to higher nutritional quality and faster degradation rates (e.g., Crawley and Hyndes 2007; Lavery et al. 2013; Rodil et al. 2015). Ephemeral algae can show higher faunal diversity than some perennial macroalgal species and be the preferred food source of key macroinvertebrates (e.g., *Gammarus* spp., *Idotea* spp.), with more suitable food items (microalgae and bacteria) for macrofauna juveniles (  lafsson et al. 2013; Kahma et al. 2021). Most studies on detritus focus on one type of macrophyte, while a bioavailability comparison of different types of subsidies is rare (e.g., Lavery et al. 2013; Kahma et al. 2021).

In the coastal areas of the Baltic Sea, various habitats contain a large number of coexisting macrophyte species. For instance, aquatic plant meadows in soft-sediment habitats are biomass-dominated by the seagrass *Zostera marina*

(L.). Whilst shallow rocky substrates are biomass-dominated by the perennial canopy-forming macroalgae *Fucus vesiculosus* (L.), shaping monospecific algal beds combined with several ephemeral macroalgal species, such as the green algae *Cladophora glomerata* (L.) (Kahma et al. 2021). Here, we study the decomposition of the abovementioned macrophyte species and their ecological role in shallow soft-sediment benthic assemblages, using a mesh-bag *in situ* experiment. Our study aims to investigate the effects of different types of macrophyte detritus on soft-sediment benthic groups, including bacteria, meiofauna, and macrofauna assemblages. We hypothesise that differences in detritus nutritional composition and degradation rates will drive a preferential association of bacterial and metazoan assemblages, strongest with ephemeral macroalgae, followed by *Fucus*, and weakest with *Zostera*. We also aim to estimate potential relationships among the different benthic assemblages as well as between the benthic assemblages and the biochemical composition of the detritus. We hypothesise that an increase in the abundance and taxonomic diversity of bacterial assemblages will promote increases in the abundance and diversity of metazoan assemblages, with a pronounced effect on macrofaunal assemblages. Conversely, we expect macrofauna to correlate negatively with meiofauna assemblages (  lafsson 2003; Nascimento et al. 2011). The variability of the target responses and the potential relationships can change over time (Kahma et al. 2023). Therefore, we compare the early (a few days) and later (several weeks) stages of the macrophyte degradation process. We employ DNA-based molecular approaches to characterise bacterial and metazoan (meiofaunal and macrofaunal) diversity, complemented by a traditional macrofaunal assessment involving core and mesh-bag sampling, followed by taxonomic sorting and identification. We want to evaluate the potential discrepancies between two contrasting approaches, i.e., DNA-based molecular vs. traditional approach, in generating macrofauna biodiversity estimates.

Material and methods

Field location and experimental design

The field experiment was conducted at Klobbarn (Hanko peninsula, South Finland), a shallow (4 m depth) sandy site (59   49.708' N, 23   10.835' E) adjacent to a large *Zostera marina* (hereafter *Zostera*) meadow, and surrounded by neighbouring rocky shore areas populated with canopy-forming *Fucus vesiculosus* (hereafter *Fucus*) communities and seasonally associated with filamentous algal species dominated by *Cladophora glomerata* (hereafter *Cladophora*). We manually collected large amounts of fresh

Fucus, *Cladophora*, and *Zostera* from the surrounding area. Macrophytes were taken to the laboratory, washed profusely with seawater, and all macroinvertebrates were removed. Macrophytes were weighed and separated in individual experimental mesh bags (50 x 50 cm, 3 mm sieve area) of 250 ± 50 mg wet weight ($n = 4$ replicates per macrophyte species, 24 bags in total) and stored in water in a dark, cold room to trigger the decomposition process for five days (Kahma et al. 2023). In the field, all the mesh bags were randomly interspersed by SCUBA divers in a two-column array (total length 20 m, width 4 m) on the seafloor ($n = 12$ per column) to facilitate the divers' work, encompassing a feasible sampling area (Fig. S1). Each bag was placed 1 to 2 m apart and loosely attached to the seafloor using metal pegs, but allowing a continuous flow under the mesh bag to avoid oxygen deficiency in the sediment. The field experiment started on the 31st of July 2020 and lasted 38 days.

A degradation process of several weeks or even months is required before the incorporation of detritus material starts to show changes in the receiving benthic environments (Kahma et al. 2023). Therefore, we wanted to study the variability of the target responses at the very early stages of the degradation compared to later stages of the degradation process. Hence, after 7 (07.08.20) and 38 (07.09.20) days, four randomly chosen replicate bags of each macrophyte species were collected by enclosing each bag within individual net bags to prevent epifauna from escaping. The surface sediment layer (approximately 1–2 cm) underneath each mesh bag was gently scraped using 45 mL Falcon tubes and stored at -20 °C for biochemical, metazoan (i.e., meio and macrofauna), and bacterial assemblage analyses. Finally, one benthic core ($\varnothing = 5$ cm, 15 cm deep) was collected underneath the experimental bags for macroinfauna assemblage samples. Bare sediment cores ($n = 4$) were also randomly taken each time in the surrounding sediment around the experimental set-up to study the faunal assemblages in nearby bare sediment.

Biochemical composition of the macrophytes: organic matter and C:N ratio

At each sampling time, macrophyte subsamples (± 10 g wet weight, $n = 4$) were randomly excised per replicate mesh bag for biochemical composition analysis, dried (60 °C, 48 h), and frozen at -30 °C until further processing. The total organic matter (OM) of the macrophyte subsamples was measured as the difference between oven-dried macrophytes (60 °C, 48 h) before and after ignition in a muffle furnace at 500 °C for 4 h (ash-free dry mass, AFDM). The nutritional value of the detritus during the decay process was determined through the analyses of the total carbon (TC) and total nitrogen (TN) on subsamples ($n = 4$) previously

oven-dried and finely ground with a mortar and pestle, and measured as the C:N ratio. Total carbon (TC) and nitrogen (TN) content (%) were determined by combustion using a TruSpec CHN analyser (Leco®, MI, USA). Before the experiment started, macrophyte subsamples in fresh natural conditions (no degradation) were analysed for AFDM, TC, and TN as a baseline comparison to the degraded samples.

Bacteria and metazoan assemblages from the seafloor: eDNA and traditional approaches

An eDNA approach on the sediment samples (collected in Falcon tubes, see above) was used to identify the benthic metazoan (i.e., meio- and macroinfauna) and microbial (bacteria) assemblages. The primer pair TAREuk454FWD1/TAREukREV3 (Stoeck et al., 2010) targeting the 18S nSSU gene region, yielding an average fragment size of 421, not including adaptors or barcodes, were used to determine the metazoan diversity. The eDNA bacteria diversity was determined using primer pair S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Klindworth et al. 2013) targeting the 16S ribosomal RNA V3 and V4 gene region and yielding an average fragment size of 417 bp, not including adaptors or barcodes. DNA extractions were performed using the DNeasy Powersoil Kit (Qiagen, 12888–100) following the standard kit protocol. DNeasy Powerclean Pro Cleanup Kit (Qiagen, 12997–50) was used to purify 100 µl of each DNA extract, with subsequent DNA being quality checked on a NanoDrop™ (Thermo Scientific™), yielding concentrations between 5.1–10.5 ng/µl, and stored at -20 °C. Negative reactions of MQH₂O and two elution buffers from DNA extraction and purification were processed alongside the samples. Polymerase chain reaction (PCR) amplification was performed in duplicate with 20 µl reaction mixes prepared on ice. Each reaction contained 10 µl Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific™, F548S), 2 µl each of forward and reverse primers (10 µM), 2 µl DNA and MQH₂O to make up to 20 µl. Thermocycling was run on a Veriti 96-Well Thermocycler (Applied Biosystems) using different programs for metazoan and bacterial reactions. The thermocycler program for metazoan reactions consisted of an initial denaturation step of 98 °C for 10 s, 10 cycles of denaturation at 98 °C for 1 s, annealing at 57 °C for 5 s and extension at 72 °C for 15 s, then 25 cycles of denaturation at 98 °C for 1 s, annealing at 47 °C for 5 s and extension at 72 °C for 15 s. A final extension of 72 °C for 2 min was performed. The thermocycler program for bacterial reactions consisted of an initial denaturation step of 98 °C for 10 s, 20 cycles of denaturation at 98 °C for 1 s, annealing at 55 °C for 5 s and extension at 72 °C for 15 s, and a final extension of 72 °C for 2 min. PCR products were quality checked by gel electrophoresis before duplicate reactions

were pooled. Downstream applications were performed by the DNA Sequencing and Genomics Lab, Institute of Biotechnology, Helsinki Institute of Life Science, University of Helsinki, using the Miseq system (Illumina) and 600-cycle V3 Illumina MiSeq sequencing kit. Quality assessment of raw amplicon reads was done using FastQC v0.11.9 (Andrews 2010), demultiplexing and primer removal using cutadapt v2.1 (Martin 2011), and pair merging using PEAR v0.9.11 (Zhang et al., 2014). Pair concatenation was then performed using cutadapt v2.1 (Martin 2011). Processed sequence reads underwent quality filtering (metazoan: threshold 1; bacteria: threshold 0.5), dereplication, denoising (minsize 8; unoise_alpha 2), length filtering (metazoan: 405–456 bp, bacterial: 400–430 bp), and chimera filtering using Vsearch v2.15.1 (Rognes et al. 2016). After filtering, operational taxonomic unit (OTU) delimitation and read mapping were performed by greedy clustering (97%) using Vsearch v2.15.1 (Rognes et al. 2016). Taxonomic affiliations were made using SILVAngs version: 1.9.10/1.4.9; r138.1 (<https://ngs.arb-silva.de/>). Laboratory controls for eDNA metazoan included numerous sequence reads identified as *Salmo salar* (DNA extraction and purification buffer controls) and a single sequence read of Haplotaxida (DNA purification buffer control). Laboratory controls for eDNA bacteria contained sequence reads from Actinobacteria, Bacteroidia, two OTUs of Gammaproteobacteria, and two OTUs of Cyanobacteria, with all sequences representing a maximum of 4 reads per laboratory control. A traditional macrofauna sampling approach incorporated two techniques to capture both the macroinfauna (benthic core) and epifauna (mesh bags) assemblages. All bags containing the macrophytes and all benthic cores were washed and sieved separately through a 0.5 mm mesh. The retained macrofauna was sorted and identified to the lowest possible taxonomic level in the laboratory using microscopes, and data were established as the number of individuals per core (infauna) and per mesh bag (epifauna). The taxonomy of the meiofauna and macrofauna data was further checked through the World Register of Marine Species web page (WoRMS, 2024).

Statistical analyses

Two-factor non-parametric multivariate analyses of variance (PERMANOVA) were run separately to detect changes in the OM and C:N ratio of the macrophytes (fixed factor: *Fucus*, *Cladophora*, and *Zostera*) over time following the macrophyte degradation process (fixed factor: fresh, early degradation stage at 7 days, and late degradation stage at 38 days). We calculated Euclidean dissimilarity measures based on log (x+1) transformed data using 9999 permutations.

For the eDNA analyses, two matrices with the 18S (metazoan) and 16S (bacterial) sequence information were used to examine changes in the benthic assemblages. A two-factor PERMANOVA was run separately to examine the differences in the bacterial and metazoan assemblages between macrophytes (fixed factor: *Fucus*, *Cladophora*, *Zostera*, bare sediment) over time (fixed factor: early degradation at 7 days and late degradation at 38 days). The data were normalised by presence/absence before being analysed using a Bray–Curtis resemblance matrix (9999 permutations, unrestricted permutation of raw data, Type III SS). Non-metric multidimensional scaling (nMDS) of distances among centroids was used to visualise multivariate patterns in bacteria and metazoan assemblages. The bacterial and metazoan richness was estimated as the total number of unique OTUs. The Shannon–Wiener diversity index, which considers the number of species present and their relative importance within the assemblage, was calculated using the DIVERSE function (PRIMERv7). Changes in the total OTUs (number of taxa) and Shannon diversity index (log-transformed data) for both 18S and 16S sequence information were analysed through a 2-way PERMANOVA (same as above) using Euclidean dissimilarity matrices. Significant effects identified ($p \leq 0.05$) with the PERMANOVAs were further investigated by pairwise comparisons. PERMDISP was used to check whether data showed homogeneity in multivariate dispersion.

For the traditional macrofauna sampling approach, the same PERMANOVA analysis as above was used to detect significant changes in the macroinfauna (benthic core) and epifauna (mesh-bags) assemblages (4th-root transformed data, Bray–Curtis resemblance matrix, 9999 permutations). PERMANOVAs were run separately to detect significant changes in the macrofauna (infauna and epifauna) total abundance, and the number of taxa associated with the treatments over time (7 vs 38 days), using Euclidean dissimilarity measures (log (x+1) transformed data, 9999 permutations). When the number of unique permutations was low (i.e., < 100), the Monte Carlo p-value was used. The benthic taxa that mostly contributed to the similarity among treatments over time were identified using SIMPER analysis (data standardised as relative abundances). All the multivariate analyses were performed using PRIMER7 (Clarke and Gorley 2015).

We used generalised linear models (GLM) to examine the potential relationships between the macrofauna responses of both the eDNA (abundance as sequence reads and OTUs as taxa richness) and the traditional data (macroinfauna per core, epifauna per bag, and taxa richness) to changes in the main bacterial and meiofauna assemblage descriptors (i.e., OTUs and sequence reads), and also to macrophyte changes in the OM and C:N ratio. We applied a Gamma model

distribution with a log-link structure for the raw data of the response variables. We simplified the models by removing non-significant explanatory variables. Akaike's Information Criterion (AIC) and the proportional increase in explained deviance (pseudo- R^2) were used to evaluate each model fit. Multicollinearity was checked with the $\text{vif}()$ function. The normality (Shapiro test) and the potential autocorrelation (Durbin-Watson test) of the residuals were estimated. Models were fitted in R 4.4.2. (R Development Core Team 2024).

Results

Biochemical composition of the macrophyte detritus: Organic matter and C:N ratio

The total OM was higher (pseudo- $F_{2,27} = 90.2$; $p < 0.001$) in the fresh portions of both *Zostera* and *Fucus* compared to *Cladophora* (Fig. 1a). The OM was reduced over time in *Cladophora* and *Zostera* samples (pseudo- $F_{4,27} = 14.2$; $p < 0.001$), while there was no significant change in *Fucus* (Fig. 1a). The C:N ratio was higher in the fresh portions of *Fucus* (38.76 ± 2.24) and *Cladophora* (42.80 ± 3.85) compared to *Zostera* (22.68 ± 0.36) (mean $\pm$ SE), but this relationship changed over time (pseudo- $F_{4,27} = 35.5$; $p < 0.001$). At t_{38} , *Cladophora* showed the lowest C:N ratio (8.81 ± 0.17) compared to *Zostera* (17.79 ± 3.55) and *Fucus* (23.90 ± 1.81) (mean $\pm$ SE) (Fig. 1b). However, the C:N ratio of *Zostera* did not change significantly (ranged from 22.7 to 17.8) over time (Fig. 1b).

Detritus-associated bacteria assemblages using the eDNA approach

The eDNA analysis of the 16S sequence information accounted for 767 total OTUs belonging to 34 different bacterial phyla (Table 1, Table S1). There was an increase in the total number of bacterial OTUs (pseudo- $F_{1,24} = 21.34$; $p < 0.001$) and Shannon diversity (pseudo- $F_{1,24} = 8.10$; $p < 0.001$) over time (Table 1). There was a change in the bacterial taxa (pseudo- $F_{1,24} = 7.3$; $p < 0.001$, Fig. 2a) and

the bacterial phyla assemblages (pseudo- $F_{1,24} = 16.1$; $p < 0.001$; Fig. S2) over time. Here, almost 80 % of the contribution (relative abundance) to the dissimilarity is accounted for by 7 phyla, with >50 % by Cyanobacteria, Actinobacteriota, Bacteroidota, and Proteobacteria (Fig. 3a). The addition of *Cladophora* ($t = 1.34$; $p \leq 0.05$) and *Zostera* ($t = 1.4$; $p \leq 0.05$) treatments changed the bacteria taxa assemblages (pseudo- $F_{3,24} = 1.44$; $p \leq 0.05$) compared to the bare sediment (Fig. 2a). According to the SIMPER analysis, *Cladophora* showed a higher contribution of taxa belonging to the phyla Bacteroidota (10.4%), Proteobacteria (9.6%), Myxococcota (8.7%), and Desulfobacterota (7.3%), and *Zostera* showed a higher contribution of taxa belonging to Bacteroidota (9.5%), Proteobacteria (8.3%), and Planctomycetota (6.6%) compared to bare (Fig. S3). SIMPER showed similar relative abundances between *Fucus* and bare sediment (Fig. S3).

Detritus-associated meio and macrofauna assemblages using the eDNA approach

The eDNA approach (18S sequence analysis) detected 83 metazoan taxa (Table 1). There was a change in the metazoan assemblages over time (pseudo- $F_{1,24} = 2.71$; $p < 0.001$), but no significant change was associated with the different macrophyte treatments (Fig. 2b). We further subdivided the 18S sequence reads into two different matrices, one with the meiofauna (or meiobenthos); i.e., the smallest metazoans (< 500- μm), dominated by harpacticoid and cyclopoid copepods, nauid and enchytraeid oligochaetes, and nematodes, ostracods, gastrotrichs, mites, ostracods, and flatworms (Platyhelminthes) (Table S2). A second 18S matrix was composed of the typical Baltic macrofauna (or macrobenthos); i.e., large metazoans (>500- μm), usually dominated by benthic clams such as *Macoma balthica* and *Cerastoderma edule*, and other typical invertebrate species including different gastropod species (e.g., *Peringia ulvae*), polychaetes (e.g., *Hediste diversicolor*) and insect larvae (e.g., Chironomidae) (Table S3). The two matrices showed 68 meiofauna and 15 macrofauna taxa, respectively (Table 1).

Fig. 1 Plots showing (a) the total organic matter and (b) the C:N ratio of the three treatments (*Fucus vesiculosus*, *Cladophora glomerata*, and *Zostera marina*) over time, following the degradation process (fresh samples, early stage at 7 days and later stage at 38 days). Different letters show significant differences between treatments (uppercase letters) and over time (lowercase letters)

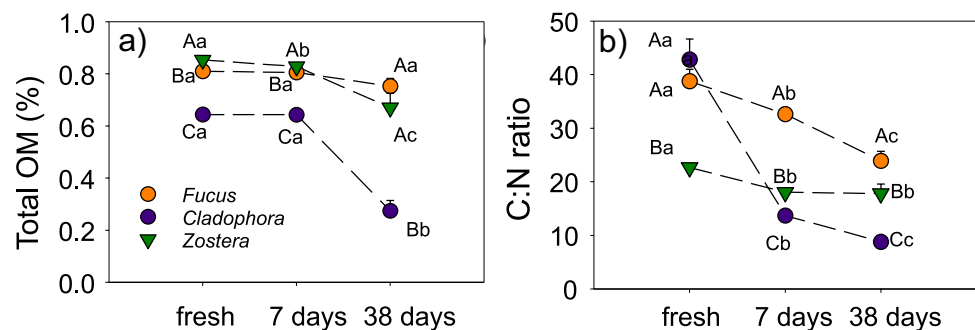


Table 1 Mean ($\pm$ standard error), minimum and maximum and total diversity values for bacteria (16S) and metazoan (18S, meiofauna and macrofauna) measured by eDNA approach across macrophyte treatments (*Fucus vesiculosus*, *Cladophora glomerata*, *Zostera marina* and bare sediment) over time (7 and 38 days). Number of taxa measured as operational technical units (OTUs) and diversity measured as the Shannon diversity index (H' , Napierian logarithm)

eDNA	Variable	# Taxa (OTUs)		Shannon index (H')	
		t_7	t_{38}	t_7	t_{38}
Bacteria (16S)	<i>Fucus</i>	610.8 $\pm$ 12.8	650.0 $\pm$ 9.8	4.67 $\pm$ 0.23	4.95 $\pm$ 0.06
	<i>Zostera</i>	635.5 $\pm$ 9.1	650.5 $\pm$ 8.2	4.81 $\pm$ 0.03	4.95 $\pm$ 0.04
	<i>Cladophora</i>	590.5 $\pm$ 14.6	646.5 $\pm$ 14.2	4.77 $\pm$ 0.03	4.97 $\pm$ 0.03
	Bare	690.8 $\pm$ 9.1	649.5 $\pm$ 11.8	4.79 $\pm$ 0.03	4.90 $\pm$ 0.04
	Time	612 $\pm$ 13.5	649 $\pm$ 10	4.76 $\pm$ 0.11	4.94 $\pm$ 0.04
	min-max	562–653	607–675	3.99–4.94.99.94	4.81–5.08.81.08
	Total	767		-	
Metazoa (18S)	<i>Fucus</i>	32.8 $\pm$ 2.6	30.8 $\pm$ 2.8	1.48 $\pm$ 0.22	1.30 $\pm$ 0.42
	<i>Zostera</i>	39 $\pm$ 4.4	32.5 $\pm$ 5.0	1.76 $\pm$ 0.13	1.35 $\pm$ 0.36
	<i>Cladophora</i>	33.3 $\pm$ 2.5	34.3 $\pm$ 2.2	1.87 $\pm$ 0.10	1.54 $\pm$ 0.22
	Bare	31.5 $\pm$ 3.9	36.8 $\pm$ 4.0	1.52 $\pm$ 0.16	1.61 $\pm$ 0.31
	Time	34.1 $\pm$ 3.4	33.6 $\pm$ 3.5	1.66 $\pm$ 0.16	1.45 $\pm$ 0.31
	min-max	22–45	18–45	0.83–2.15.83.15	0.27–2.23.27.23
	Total	68		-	
Macrofauna	<i>Fucus</i>	7.8 $\pm$ 1.0	6 $\pm$ 0.4	0.8 $\pm$ 0.14	0.64 $\pm$ 0.26
	<i>Zostera</i>	6.8 $\pm$ 0.9	8 $\pm$ 1.1	1.13 $\pm$ 0.18	0.98 $\pm$ 0.26
	<i>Cladophora</i>	5.8 $\pm$ 0.9	7.5 $\pm$ 0.7	1.04 $\pm$ 0.19	0.42 $\pm$ 0.10
	Bare	7.3 $\pm$ 0.8	8 $\pm$ 1.3	0.89 $\pm$ 0.20	1.01 $\pm$ 0.11
	Time	6.9 $\pm$ 0.9	7.4 $\pm$ 0.9	0.97 $\pm$ 0.17	0.76 $\pm$ 0.22
	min-max	4–9	5–11	0.41–1.57.41.57	0.18–1.5.18.5
	Total	15		-	

Non-metric MDS

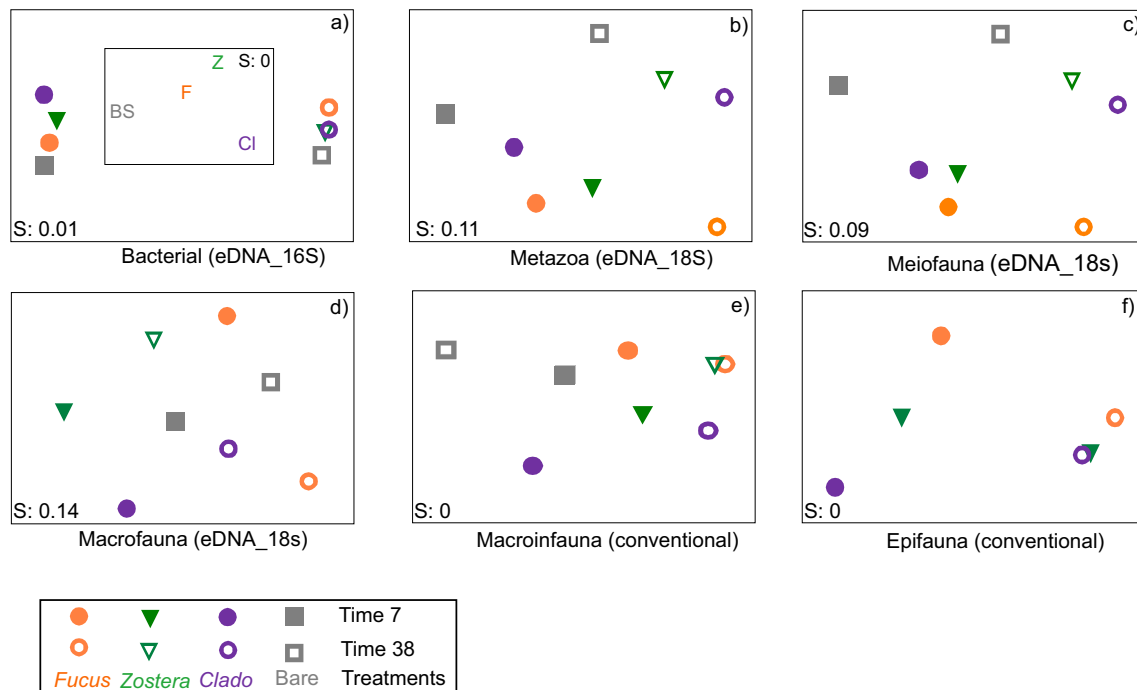
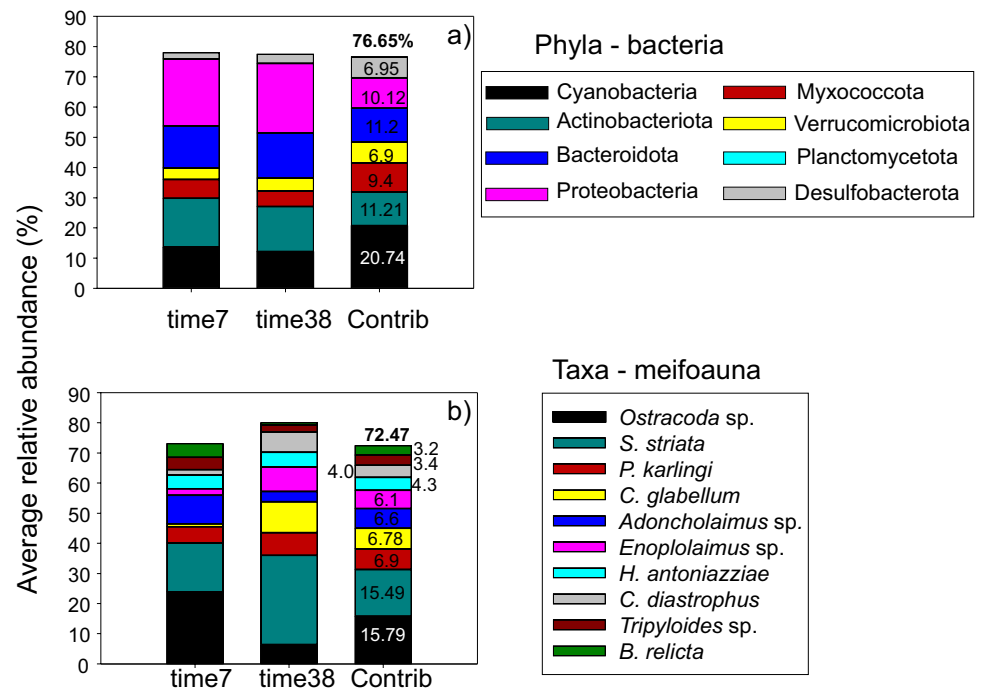


Fig. 2 Non-metric multidimensional scaling (nMDS) for differences in bacterial (a), metazoan (b), meiofauna (c), and macrofauna (d) assemblages using the eDNA approach and macroinfauna (e) and epifauna (f) assemblages using the traditional approach across treatments (*Fucus*

vesiculosus, *Cladophora glomerata*, *Zostera marina* and bare sand) and over time (7 and 38 days). The inner panel in 2a shows the significant difference in bacteria assemblages between *Cladophora/Zostera/Fucus* vs bare sand (BS) during the duration of the experiment

Fig. 3 Contribution (% relative abundance) of the main bacterial phyla (a) and meiofauna taxa (b) over time (7 and 38 days) in the sediments estimated by SIMPER. Total contribution to change between time₇ and time₃₈ in bold



There were no significant changes between the treatments or over time in the total abundance, number of taxa, and Shannon index of both meiofauna and macrofauna (Table 1). There was a change in the meiofauna taxa assemblages over time (pseudo- $F_{1,24} = 2.97$; $p < 0.001$) (Fig. 2c). The relative abundance of the meiofauna phyla was dominated (pseudo- $F_{1,24} = 5.17$; $p \leq 0.05$) by Arthropoda (mainly copepods and ostracods) and Platyhelminthes, especially at t_7 , Annelida, and Nematoda (t_7 and t_{38}) (Table S2, Fig. S4). One taxon of the class Ostracoda, the nematode *Adoncholaimus* sp. and the Platyhelminthes *Bresslauilla relicta* showed the highest abundance contribution at t_7 , while the Ostracoda *Semicytherura striata*, the microsnail *Caecum glabellum*, the nematode *Enoplolaimus* sp., and the oligochaete *Chaetogaster diastrophus* showed the highest relative abundance at t_{38} (Fig. 3b, Table S2). Pairwise comparisons showed significant differences ($p \leq 0.05$) between all the macrophyte treatments and the bare sand (Fig. 2c). There were no significant changes in the macrofauna assemblages (18S, eDNA) (Fig. 2d).

Detritus-associated macrofauna assemblages using the traditional approach

The traditional approach accounted for 13 macrofauna taxa and 1065 individuals, and 18 epifauna taxa and 7701 individuals (Table 2, Table S4). The total abundance of macrofauna was higher (pseudo- $F_{3,24} = 5.72$; $p < 0.01$) in the treatments than in the bare sediment at t_7 , and it was the highest in *Fucus* at t_{38} (Fig. 4a). There was an increase in the

macrofauna abundance in *Cladophora* ($t = 3.76$; $p < 0.01$) and *Zostera* ($t = 2.62$; $p \leq 0.05$) over time (Fig. 4a). There was a significant difference in the number of macrofauna taxa between treatments, but this pattern changed over time (pseudo- $F_{3,24} = 3.35$; $p \leq 0.05$). At t_7 , the number of taxa was lower in *Cladophora* than in *Zostera* ($t = 3.18$; $p \leq 0.05$) and no differences were found among treatments at t_{38} (Fig. 4b). The number of taxa increased over time in *Fucus* ($t = 2.38$; $p \leq 0.05$) and *Cladophora* ($t = 4.36$; $p < 0.01$) treatments (Fig. 4b). The Shannon diversity index was significantly higher in the *Zostera* compared to *Cladophora* ($t = 2.63$; $p < 0.01$) and bare sediment ($t = 2.67$; $p \leq 0.05$) (Fig. 4c).

The macrofauna assemblage differed (pseudo- $F_{3,24} = 3.82$; $p < 0.01$) between treatments and bare sediment (Fig. 2e). The treatments showed a high relative abundance of the oligochaete subclass, the gastropod *P. ulvae*, and the amphipod *Corophium volutator* compared to the bare sand (Fig. 5a-c, Table S5). There was a high relative abundance of the bivalve species *M. balthica* in the bare sediment and *C. glauca* in the *Cladophora* treatment (Fig. 5a-c, Table S5). The macrofauna assemblage differed between *Cladophora* and *Zostera* treatments ($t = 1.63$; $p \leq 0.05$) with high relative abundances of *P. ulvae* and *M. balthica* in the former, and *C. volutator* and oligochaete in the latter (Fig. 5d, Table S5). There were differences in the macrofauna assemblage (pseudo- $F_{1,24} = 3.37$; $p \leq 0.05$) over time (Fig. 2e), with a high contribution of *M. balthica* at t_7 and of *P. ulvae* at t_{38} (Fig. 5e, Table S5). The majority of these taxa were not detected through the eDNA approach, except for *P. ulvae* and *H. diversicolor* (Table S3).

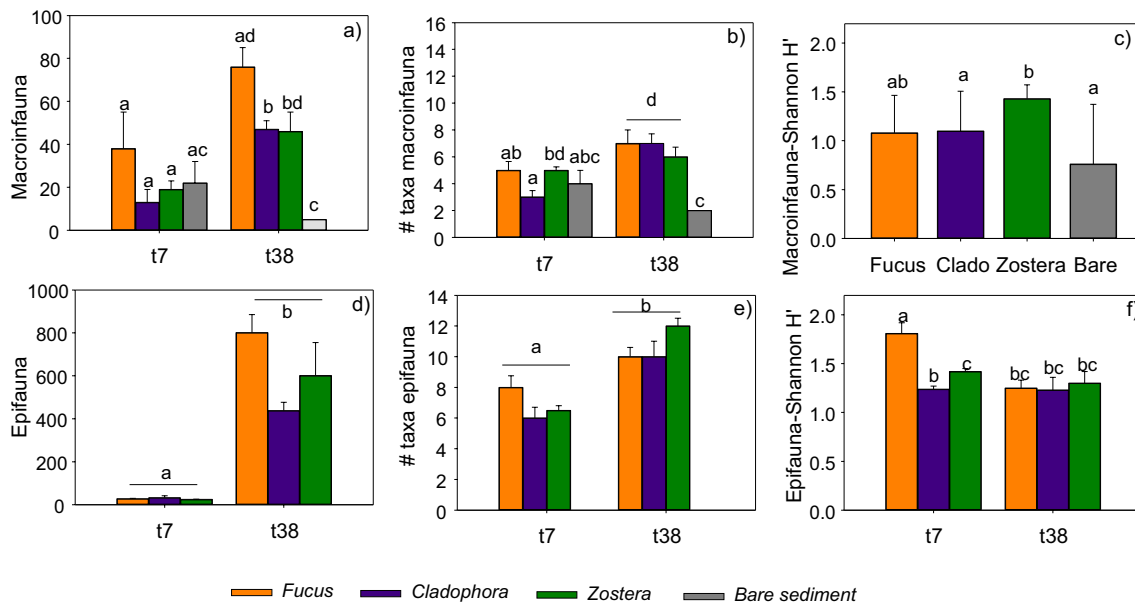


Fig. 4 Plots showing macroinfauna abundance (a), number of taxa (b) and Shannon diversity index (c) per core and epifauna abundance (d), number of taxa (e), and Shannon diversity index (f) per mesh bag

using the traditional approach across treatments (*Fucus vesiculosus*, *Cladophora glomerata*, and *Zostera marina* and bare sand as control) over time (7 and 38 days). Different letters show significant differences

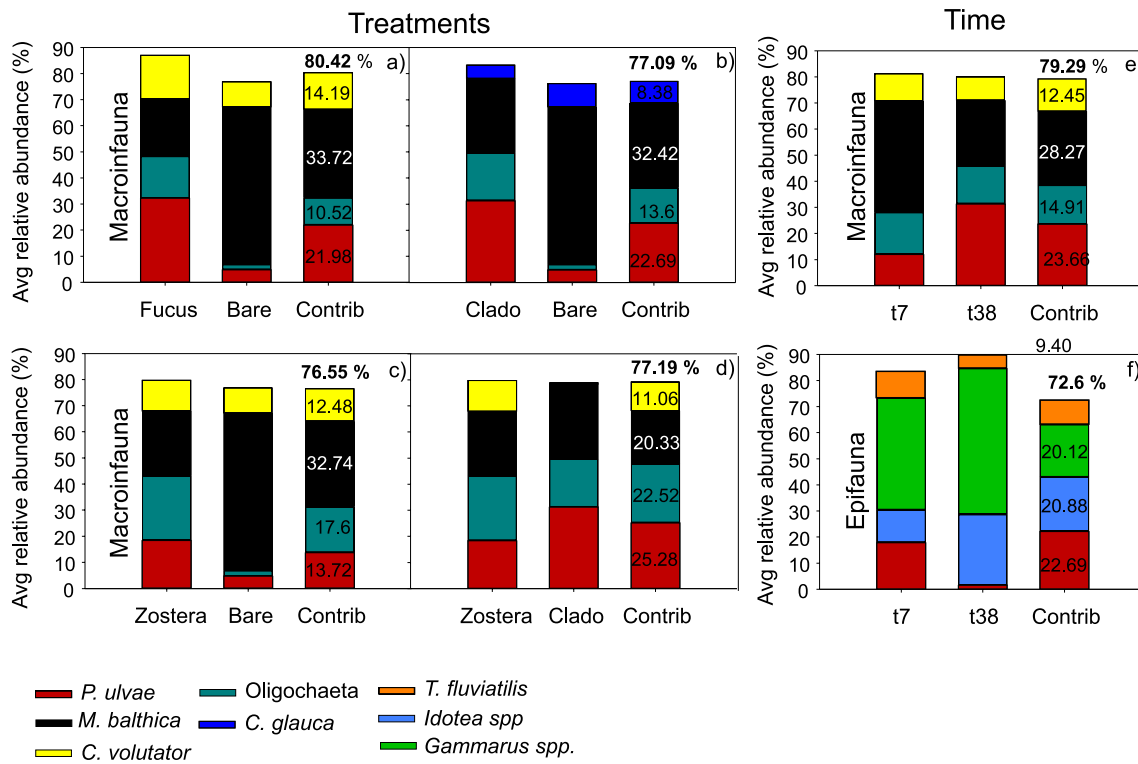


Fig. 5 Contribution (%) of the macroinfauna abundance of the main taxa between *Fucus* and bare sand (a), *Cladophora* and control (b), *Zostera* and control (c) and *Zostera* and *Cladophora* (d) treatments (*Fucus vesiculosus*, *Cladophora glomerata*, *Zostera marina* and bare

sand as control) and over time (7 and 38 days) per core (e), and contribution of the epifauna per mesh bag (f) over time using the traditional approach and estimated by SIMPER. Total contribution to change between treatments and time in bold

Table 3 Summary of the Generalized Linear Models (GLM) relating benthic assemblage variables, measured by the eDNA (16S and 18S) and traditional approaches, and the influence of macrophyte biochemical content (total organic matter and C:N ratio) on the benthic assemblage variables across treatments (*Fucus vesiculosus*, *Cladophora glomerata*, *Zostera marina* and bare sediment) over time (7 and 38 days). The final models followed the most parsimonious GLM based on the Akaike information criterion (AIC). N: relative abundance (eDNA) and counts. Taxa: operational technical units (OTUs, eDNA) and the number of taxa. Macrofauna (macro), meiofauna (meio) and bact (bacteria). Significant results (*p*) are shown in *italics*

Response variable	Coefficients	Estimate	SE	t	<i>p</i>	pseudo-R ²	AIC
N _{macro} (18S, sequence reads) - Fig. 6a-d	Intercept	-15.650	4.385	-3.569	<i>0.002</i>	0.582	419.1
	Taxa _{bact} (16S, OTUs)	0.040	0.007	6.087	<i>< 0.0001</i>		
	N _{meio} (18S, sequence reads)	-0.00003	0.00001	-2.171	<i>0.043</i>		
	C:N ratio	0.107	0.034	3.173	<i>0.005</i>		
	organic matter (%)	-5.432	1.367	-3.973	<i>0.001</i>		
N _{macro} (ind per core) - Figure S4	Intercept	-7.639	2.150	-3.554	<i>0.002</i>	0.677	204.5
	Taxa _{bact} (16S, OTUs)	0.01839	0.00324	5.679	<i>< 0.001</i>		
	N _{meio} (18S, sequence reads)	-0.00002	0.00001	-3.235	<i>0.004</i>		
	C:N ratio	0.056	0.016	3.392	<i>0.003</i>		
	organic matter (%)	-1.594	0.670	-2.378	<i>0.028</i>		
Taxa _{macro} (18S, OTUs) - Fig. 6e-f	Intercept	-0.28	0.82	-0.339	0.737	0.271	122.6
	Taxa _{bact} (16S, OTUs)	0.003	0.001	2.156	<i>0.040</i>		
	Taxa _{meio} (18S, OTUs)	0.012	0.006	2.095	<i>0.045</i>		
Taxa _{epifauna} (mesh bag) - Fig. 6g	Intercept	-1.362	1.059	-1.286	0.212	0.321	108.3
	Taxa _{bact} (16S, OTUs)	0.006	0.0017	3.330	<i>0.003</i>		
N _{epifauna} (mesh bag) - Fig. 6h	Intercept	-13.926	4.966	-2.804	<i>0.010</i>	0.323	316.5
	Taxa _{bact} (16S, OTUs)	0.031	0.008	3.909	<i>< 0.001</i>		

brown macroalgae and seagrasses in coastal areas (Manca et al. 2024), and their potential replacement by turf algae (Filbee-Dexter and Wernberg 2018). These shifts are expected to alter the quantity and composition of detritus reaching recipient habitats, potentially triggering cascading effects on detritus-dependent benthic communities and coastal food webs.

Biochemical composition of the macrophyte detritus: Decomposition and food quality

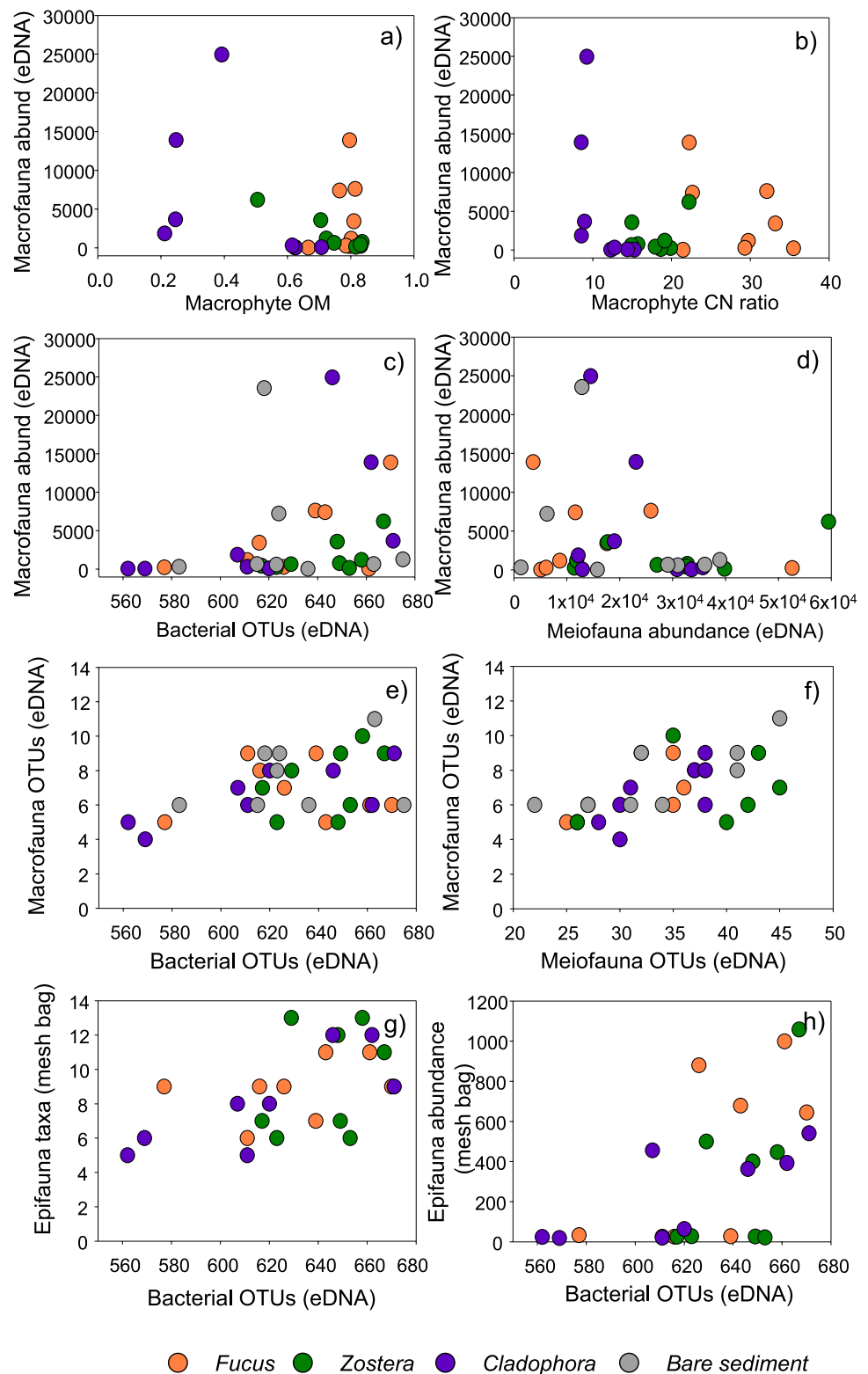
In our study, the OM content (*Zostera* > *Fucus* > *Cladophora*) and the C:N ratio (*Cladophora* = *Fucus* > *Zostera*) of fresh macrophytes were relatively high. These patterns changed over time, with *Cladophora* losing 2.4 of its total, highly labile OM content after 38 days of experimental manipulation, while the more refractory *Fucus* barely changed at all. After 38 days, the C:N ratio of *Cladophora* had decreased by almost a factor of 5 (from 42.8 to 8.81), and the C:N ratio of *Fucus* (38.8) by 1.5, while it barely changed in *Zostera*. By the end of the experiment, the C:N ratio of *Cladophora* indicated highly degraded food ready, not only for microbial colonisation (Norderhaug et al. 2003), but also for macrofauna foraging (Ólafsson et al. 2013; Rodil et al. 2015). After 38 days, the C:N ratio of the more refractory *Fucus* and *Zostera* was still very high (i.e., C:N > 17), representing ratios unfavourable for animals to feed upon (Russell-Hunter 1970). This finding aligns with previous studies, which demonstrate that both *Fucus vesiculosus* and *Zostera*

marina can take a considerable amount of time to degrade and enter the benthic food web (Lavery et al. 2013; Kahma et al. 2023).

Detritus-associated bacteria assemblages using the eDNA approach

Microorganisms drive organic material generation, transformation, and assimilation in coastal environments (Azam and Malfatti 2007; Sävström et al. 2016). However, knowledge of the effects of macrophyte detritus on bacterial assemblages from shallow marine seafloors is sparse. In our study, the addition of macrophyte detritus triggered the presence of bacteria, increasing diversity and modifying the assemblages following macrophyte degradation. Previous studies in aquatic environments have identified that a massive amount of phytodetritus reaching lacustrine sediments can generate benthic cyanobacterial mats (Qi et al. 2020). Our study shows that the accumulation of macrophyte detritus in shallow marine soft sediments can also contribute to cyanobacterial assemblages. This is important because large accumulations of cyanobacterial phytodetritus and opportunistic macroalgae can eventually create eutrophic conditions and major environmental problems in aquatic environments (Wikström et al. 2016; Qi et al. 2020). In the Baltic Sea, eutrophication has resulted in a decrease of perennial macrophyte beds, such as *F. vesiculosus* and *Z. marina*, and the rise of a turbid-water system dominated by pelagic primary production and drifting mats of ephemeral algae (Bonsdorff

Fig. 6 Significant responses of the macrofauna abundance (18S, eDNA) to (a) macrophyte organic matter (%), (b) macrophyte C:N ratio, (c) bacterial OTUs (16S, eDNA), (d) meiofauna abundance (18S, eDNA), (e) bacterial OTUs, and (f) meiofauna OTUs estimated by eDNA, and significant relationships between (g) epifauna taxa (counts per bag) and bacterial OTUs, and (h) epifauna abundance (counts per bag) and bacterial OTUs. Best fit model using Generalised Linear Model analyses (Table 3). Macrophyte treatments: *Fucus vesiculosus*, *Cladophora glomerata*, *Zostera marina* and bare sediment



et al. 1997; Wikström et al. 2016; Kahma et al. 2023). In our study, the presence of detrital particulate and dissolved organic matter triggered a rapid growth of Actinobacteriota, which constitutes one of the most diverse bacterial phyla

and key heterotrophs for carbon mineralisation (Miksch et al. 2021). We found evidence to support the hypothesis that bacterial assemblages change in response to the availability of different types of detritus. For instance, the phyla

Bacteroidota and Proteobacteria showed a higher contribution to sediments associated with *Cladophora* and *Zostera* compared to *Fucus* detritus. Different lineages belonging to these two phyla are known to be active heterotrophic bacteria specialised in OM degradation (Gihring et al. 2009; Lozada et al. 2023).

Detritus-associated meio and macrofauna assemblages using the eDNA approach

We did not detect any significant differences in meiofauna diversity and abundance associated with any type of detritus. However, a clear shift of the meiofauna assemblage associated with the macrophyte detritus was apparent compared to the bare sediment and over time. Overall, the detritus-associated meiofauna was dominated by taxa belonging to the Arthropoda phylum, mainly copepods (harpacticoids) and ostracods, and a broad group of marine worms. Macrophyte detritus is known to be selectively consumed by several species of copepods and ostracods (Harfmann et al. 2019; Hwang et al. 2023). Marine worms, constituted by flatworms (Platyhelminthes), roundworms (Nematoda), and segmented worms (Annelida), can be abundantly found associated with detritus decomposition (Arroyo et al. 2012; Majdi et al. 2014; Hwang et al. 2023). Flatworms can accelerate detritus decomposition in aquatic environments through positive effects on microbial decomposers (Majdi et al. 2014). Some of the most abundant nematode taxa in our study, such as *Adoncholaimus* sp. and *Enoplolaimus* sp., are known to feed on algae, diatoms, bacteria, protists and fungi as well as on detrital sediment rich in microbial activity (Smythe 2015). Over time, there was a higher contribution of *Enoplolaimus* sp., *Semicytherura striata*, and *Chaetogaster diastrophus*. The ostracod *Semicytherura* spp. lives on both marine algae and deposited algal mats on surface sediment (García-Madrigal et al. 2022). The oligochaete *C. diastrophus* feeds primarily on diatoms, but also detritus, mineral particles, and other invertebrates (Smith and Kaster 1986). On the other hand, the macrofauna assemblage detected using the eDNA approach did not show any change among treatments or over time.

Detritus-associated macrofauna assemblages using the traditional approach

By using the traditional approach, we found a clear response in the macrofauna assemblage to the presence of macrophyte detritus. After 7 days, the detritus showed 2 to 3 times higher total abundance and twice the number of taxa than the bare sand, and after 38 days, the abundance was over 100 times, and the number of taxa was 4 to 5 times higher than the bare sand. The detritus showed a high contribution of

opportunistic detritivore taxa, particularly oligochaetes, the gastropod mudsnail *P. ulvae* and the amphipod *C. volutator*. The presence of detritus in shallow soft bottoms typically attracts large numbers of opportunistic detritivores, while suspension feeders are negatively affected by the presence of large amounts of detritus (Norkko and Bonsdorff 1996a,b; Norkko et al. 2000). Marine detritivores are found associated with macrodetritus, contributing significantly to the carbon and nutrient cycles of coastal habitats through the breakdown of organic matter (Norkko and Bonsdorff 1996a,b). The oligochaeta influences decomposition either directly, by consuming detritus or indirectly, by processing material or feeding on the microbial assemblages (Coupland and McDonald. 2008; Godbold et al. 2009). The high contribution of *P. ulvae* after 38 days of the experiment could be related to the high level of decomposition of the treatments, enriching the sediment at the end of the experiment, and the feeding niche preference of this mudsnail for sediments with a diverse microphytobenthic community composed of diatoms, bacteria and associated detritus (Coelho et al. 2011; Araújo et al. 2015). Benthic macroinfauna species differed significantly in their ability to utilise detritus. For instance, *Cladophora* showed a benthic assemblage with a higher contribution of the bivalve *M. balthica* and the mudsnail *P. ulvae* compared to the *Zostera* treatment, with a higher contribution of oligochaetes. Seagrass detritus, such as *Zostera*, can attract large numbers of typical decomposers such as oligochaetes (Beltrán et al. 2020). This can be explained by the faster degradation rates of *Cladophora* compared to the more recalcitrant *Zostera* detritus, resulting in quicker cleaning of the detritus from the sediment, consequently benefiting *M. balthica*. The high degradation rate of *Cladophora* also leads to fast incorporation of detritus into the sediment that might benefit both the facultative sediment/deposit feeder *M. balthica* and the opportunistic *P. ulvae*.

The mobile epigrazers *Gammarus* spp. and *Idotea* spp. showed the highest contribution to the assemblage associated with the detritus, and this contribution increased over time. These two species are known to be strongly associated with macrophyte detritus that may function as an important rafting mechanism for invertebrate species (Norkko et al. 2000; Rodil et al. 2021), as well as a refuge from predation and a trophic resource (Norkko 1998; Kahma et al. 2023). The mobile gastropod *T. fluviatilis* showed a high contribution to the epifauna assemblage associated with the detritus. The increasing contribution of *P. ulvae* to the bare sediment over time was likely due to the increasing degradation of the macrophyte detritus and the transfer of organic matter to the sediment. It seems that macroinfauna such as bivalves may prefer bare sediment instead of algal-covered seafloor,

while mobile epifauna may, to some extent, benefit from detritus accumulation.

Macrofauna assemblage comparison of approaches

The number of macrofauna taxa identified by the eDNA and the traditional approaches was similar. However, the macrofauna assemblage detected with the eDNA was very different from the assemblage identified by the traditional approach. The latter is largely dependent on detection probability, capture efficiency, and taxonomic identification (Tyre et al. 2003; Preston et al. 2024), whilst eDNA introduces further dependencies within the sample processing relating to extraction efficiency, sample interference, and assay sensitivity (Ficetola et al. 2015; Goldberg et al. 2016). Therefore, the dissimilarity in detected assemblages is unsurprising. In eDNA, the non-detection of taxa does not automatically imply their absence, and the common occurrence of false negatives is a characteristic limitation of the method (Roussel et al. 2015; Beng and Corlett 2020). For instance, the eDNA approach failed to detect typical epigrazers and detritus consumers such as *Gammarus* spp., *Idotea* spp., or *T. fluviatilis*. Similarly, a previous study from the same area also indicated poor detection of *Gammarus* spp. and *Idotea* spp. when using eDNA (Preston et al. 2024). Consequently, the impeded accuracy of detection for these species is likely a result of limitations in the process including variable DNA degradation rates, primer bias, PCR inhibition, PCR/sequencing induced errors, pair merging and quality filtering problems, and the quality of reference databases (Ficetola et al. 2015; Harper et al. 2019; Foster et al. 2022). However, the traditional method can also misrepresent taxa, particularly motile, sporadically or rarely occurring small-sized taxa (Eleftheriou 2013; Majaneva et al. 2024). For instance, common macroinfauna in the Baltic Sea, such as *Hediste diversicolor*, were not identified by the traditional method but by the eDNA method. Some phyla, such as Bryozoa or Cnidaria, which are not typically identified through typical core sampling, were identified through the eDNA method.

False positives are also common in eDNA, with a positive detection not always indicating the current presence of taxa, since eDNA can be transported over space or preserved over time (Roussel et al. 2015). False positives may also be introduced to the sequence reads and taxonomic assignment by PCR and sequencing errors, and the quality of reference databases (Ficetola et al. 2015; Foster et al. 2022). Some of the taxa detected by the eDNA approach displayed several dubious macrofauna taxa, such as the gastropods *Ercolania felina* and *Phosinella clathrata*, and the bivalve *Tageus californianus* (Conrad, 1837). The latter is a species of marine clam typically found in the Eastern Pacific, while

E. felina is a typical sea slug in the family Limapontiidae from New Zealand, and *P. clathrata* is a small sea snail in the family Rissoinidae that occurs in the Red Sea, the Indian Ocean, and the Philippines (WoRMS Editorial Board 2024). Similarly, the detection of meiofauna species which are not expected within the Baltic Sea, such as the gastropod *Caecum glabellum* native to Japan or the aquatic acari *Halacaroidea antoniaziaae* from Brazil, is likely a result of some of these issues associated with the eDNA methodology.

In previous eDNA analyses from the same geographic location, using the same primer and target region, detection rates of Mollusca were fairly low (Preston et al. 2024). It thus seems likely that the Mollusca species confirmed present through the traditional method may have been poorly represented or misrepresented in the eDNA due to several limitations of the eDNA method, including DNA degradation and errors in PCR and sequencing. Importantly, the eDNA metabarcoding method, unlike the traditional approach, did not detect any change in the macrofauna assemblage descriptors among detritus treatments or between detritus and bare sediment over time. This is likely partially a response to the fact that sediment-bound DNA can remain detectable for long periods (Turner et al. 2015). Thus, eDNA is good at representing diversity over longer temporal scales, but the limited length of this study likely did not allow an adequate response time for the eDNA pool of metazoan taxa. This is further supported by our observations of rapid bacterial assemblage changes, which would be expected to have higher turnover times. Environmental DNA can also be transported over large spatial scales, consequently representing habitats outside of the immediate sampled area (Deiner et al. 2016). This may have contributed to the homogenised states between and among the detritus and bare sediment treatments, particularly as the distances between treatments were fairly short (~1–2 m). The methods and protocols utilised for sediment eDNA metabarcoding can vary considerably among studies, and standardisation efforts are needed to improve their robustness, comparability, and use within regulatory frameworks (Pawlowski et al. 2022).

Relationships between the different benthic assemblages and the influence of detritus

The negative relationship between macrofauna abundance (eDNA) and the biochemical composition of the macrophyte detritus (Fig. 6) describes the macrofauna colonisation process linked to the quality of food availability. Despite having more organic matter at the beginning of the experiment, it was not until the C:N ratio was low enough to provide palatable food to the macrobenthic assemblage that the abundance of macrofauna increased. A small C:N ratio

suggests more degraded and labile detritus available as a food source to opportunistic consumers (Taylor et al. 2010; Rodil et al. 2015). Macrophyte detritus offers abundant food resources in the form of decaying organic matter but also associated bacteria to opportunistic colonisers, such as *P. ulvae*, or decomposers such as oligochaetes (Godbold et al. 2009; Araújo et al. 2015). This could explain the positive relationships between macrofauna abundance and bacterial diversity, and between macrofauna diversity and bacterial diversity, measured by eDNA (Fig. 6c and e). The increase in bacterial diversity (OTUs) also promoted an increase in the epifauna richness and abundance per mesh bag (Figs. 6g-h). Our study outlines the typical role of macrophyte detritus in structuring biodiversity and subsidising benthic assemblages in different marine environments (e.g., Lastra et al. 2008; de Bettignes et al. 2020); Kahma et al. 2023). Thus, bacteria first colonise the degrading detritus, stimulating the release of organic matter into the sediments, followed by rapid colonisation of a diverse assemblage of meio- and macrofauna that feed back into the microbial assemblage.

The negative relationship between macrofauna and meiofauna abundance is probably a consequence of competition for the same food source, or direct control of meiofauna assemblages through macrofauna predation (Ólafsson 2003; Papageorgiou et al. 2007; Nascimento et al. 2011). The relationship between both assemblages is more likely driven by competition for the same food source than macrofauna predation, since we did not find any significant regression model using meiofauna abundance as a response variable. However, potential predation effects between specific species of both assemblages are also feasible since an increase in meiofauna diversity triggered an increase in macrofauna diversity (Fig. 6f). Despite the discordance between the traditional and the eDNA approach in generating biodiversity estimates, we found similar relationships and trends when comparing macrofauna abundance data from both approaches (Figs. 6 and S5). This means that the eDNA methods can be used successfully to establish relationships between the different benthic components and their relationships with transient macrophyte subsidies. Unravelling the applicability of new technologies and their complementarity with traditional approaches can be used to better understand the relationships between the different benthic assemblages and their role in ecosystem functioning.

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Author contributions Iván Franco Rodil: Writing – original draft, project administration, supervision, methodology, field and laboratory work, funding acquisition, data analysis, and conceptualisation. Roxana Preston: Writing – original draft, methodology, laboratory work, data analysis. Tuomas Kahma: Writing – review and editing, field and laboratory work. Camilla Gustafsson: Writing – review and editing, field work, and conceptualisation. Alf Norkko: Writing – review and editing, field work, methodology, funding acquisition, and conceptualisation.

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Data availability The datasets generated during the current study are available in the supplementary material.

Declarations

Competing interests All authors have no conflicts of interest to disclose. All applicable international, national and/or institutional guidelines for sampling, care and experimental use of organisms for the study have been followed, and all necessary approvals were obtained.

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