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Final report

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ABSTRACT

Oceans are becoming louder (currently 2- to 10-fold the sound levels of pre-industrial times) and marine soundscapes are becoming increasingly dominated by anthropogenic low-frequency noise (LFN) from shipping and operational offshore windfarms, among others. Many marine organisms use sound directly (through hearing) or indirectly (e.g. through particle vibrations for benthic species) to communicate with conspecifics and/or obtain information about the environment (e.g. on presence of predators). It is therefore plausible that anthropogenic noise may interfere with such animal 'small-talk' and hamper the survival, partner localization, fitness etc… of organisms involved. Against this background, UGhent and AWI Bremerhaven set out to investigate whether and how LFN affects the abundance, diversity and activity of benthic organisms across a range of size classes, from macrofauna over meiofauna to microbial assemblages. We used a controlled laboratory experiment with a fully crossed factorial design to investigate whether LFN, directly or indirectly via interference with the behaviour of the dominant key bioturbator *Hediste diversicolor* (ragworm), would affect the abundance, taxonomic diversity and functional profile of micro- and meiofaunal assemblages, and whether such effects would cascade through to key ecosystem functions such as sediment aerobic metabolism, organic matter content and ammonium fluxes. Such cascading effects of a changing sound- and vibroscape on benthic communities and their associated ecosystem functioning had not yet been investigated prior to this study. A one-week exposure to continuous LFN at a frequency of 110 Hz increased biodiffusive particle displacement at the sediment-water interface by threefold and boosted aerobic sediment metabolism at ca. the same magnitude as do burrowing ragworms. However, when exposed to noise, ragworms reduced their movement and shifted to feeding on food at the sediment surface, thereby losing a considerable part of their key burrowing activity and hence of their stimulatory effect on aerobic sediment organic matter mineralization, on the survival of meiobenthic nematodes and amphipods, and on the diversity and activity of the sediment microbiome. LFN thus clearly had major indirect effects on meio- and microbenthic organisms. In addition, while LFN exposure *per se* did not significantly affect microbial taxonomic composition and diversity, it did cause significant functional shifts in the microbial community. These shifts at least partly counteracted the positive effects of *Hediste* on the survival of meiobenthic fauna and on sediment ecosystem functions such as organic matter mineralization and ammonium sequestration, which, when extrapolated to regional scales and beyond, is likely to cause significant impacts on source-sink dynamics in coastal sediments and on eutrophication status of the overlying waters. Collectively, our results highlight the key role of biological particle displacement (i.e. bioturbation) for seabed biodiversity and ecosystem functioning, especially when organic matter mineralization is increased under noise pollution. We also demonstrate that a mere focus on direct impacts of LFN on organismal activity cannot paint a correct picture of impacts on the community level, particularly with respect to ecosystem functioning. Overall, our study demonstrates how the hidden and distant implications of low-frequency noise sources, such

as shipping and offshore windfarms, can affect the ecology and functioning of the largest habitat on Earth.

Keywords: low-frequency noise; vibroscape; bioturbation; ragworm; sediment microbiome; microbiome functional profile; ecosystem functioning; indirect effects.

1. INTRODUCTION

Natural phenomena, such as wave action and animal behaviours, create an acoustic underwater world that provides essential sensory information for many marine organisms (Slabbekoorn, 2010). Any sound that alters, or interferes with, this sensory information can be considered as ‘noise’ (de Soto, 2016). Anthropogenic underwater noise (AUN) is becoming increasingly prevalent throughout the world’s oceans, and potentially affects many aquatic species that rely on sound as a signal for intra- and/or interspecific communication, orientation and foraging (Duarte et al., 2021). In most seas, the major sources of noise pollution include shipping, military sonars and oil exploration and exploitation, and increasingly also the construction and operation of offshore windfarms (OWF). Sounds generated by human activities can be divided into high-intensity, impulsive noise (e.g. pile-driving, underwater blasting) and low-frequency stationary or slow-moving noise (LFN, e.g. tidal and wind turbines, shipping), and have made the underwater soundscape of seas and oceans 2 to 10 times louder than in pre-industrial times (Frisk, 2012).

The construction phase of an OWF produces major but short bursts of impulsive sound, the effects of which on the underwater soundscape have been reasonably well investigated. Much less is, however, known on the effects of continuous operational noise of OWF (10 – 2000 Hz; Dannheim et al., 2019) and the noise produced by shipping on marine life. This especially applies to lower trophic levels and sediment-inhabiting organisms, both with respect to their autecology and to their interactions with other organisms. If we want to properly understand and – if needed – manage the impacts of OWF on marine ecosystems, this aspect needs to be investigated alongside much more widely studied impacts such as artificial reef effects and the creation of no-take zones. Knowledge on the effects of noise on benthic invertebrates and microbiota, as well as on ecosystem functioning, remains extremely scant, and the ORCHESTRA project set out to create such a fundamental knowledge base through a combination of laboratory-based as well as field observations and experiments. Approaches on behaviour and fitness of individual species, their interactions with other species and their effects on key ecosystem processes (e.g. bioturbation, predation) and functions (e.g. sediment oxygen consumption, biogeochemical fluxes) have been combined and integrated within the ORCHESTRA project as a whole, and within the tasks led by UGent – Marine Biology specifically. Thé main integration experiment of ORCHESTRA was a task led by UGent – Marine Biology and aimed to investigate if and how LFN affects the activity of the key bioturbator *Hediste diversicolor* (the ragworm), and if and how this cascades down to ecosystem functions such as sediment nutrient and oxygen fluxes through the direct and indirect effects of modified *Hediste* behaviour on the composition, diversity and activity of meio- and microbial communities in the sediment.

2. MOTIVATION AND OBJECTIVES OF THE PROJECT

Soft-sediment invertebrates make up a significant part of the marine biomass and are pivotal players in governing sediment biodiversity and ecosystem functioning. Especially the burrowing and ventilating activities of macrofauna create niches for smaller invertebrates and microbes, and are crucial in stimulating seabed biogeochemical cycling by increasing the aerobic activity of microbial decomposers, thus facilitating the resupply of nutrients back to primary producers. Here we examine how LFN interferes with the activity of a key benthic bioturbator species (ragworm, *Hediste diversicolor*), its interactions with sediment microbes and micro-invertebrates, and sediment functioning using experimental manipulation of ragworm-inhabited sediment communities and non-destructive tracers of invertebrate behavior. We hypothesized that the activity of ragworms would be affected by substrate vibrations, with cascading effects on how LFN modifies survival of small invertebrates, the activity of microbial communities, and (aerobic) sediment metabolism. While Solan et al. (2016) pioneered along the lines of our aims, such an all-encompassing, integrative study of environmental effects of an entire benthic community across size classes (from macrofauna down to the sediment microbiome) is highly novel and we anticipate that its results will challenge our ambition of obtaining comprehensive and applicable descriptors of the ‘status’ or danger level of ecosystems under noise and other stressors.

3. METHODOLOGY

All data reported here are based on a laboratory experiment conducted (March–April 2024) to assess the effects of low-frequency noise (LFN) on bioturbation by *Hediste diversicolor* (henceforth referred to as *Hediste*) and on associated ecosystem properties and processes[1,2].

Field sampling

Sediment cores (10 cm inner diameter, 33.5 cm height) were prepared from sieved (mesh openings 1 mm diam.) and homogenized muddy sediment (157 µm modal particle diam., 23% silt fraction) collected from a Wadden Sea tidal flat near Bremerhaven (53◦32′17.7′′ N, 8◦34′33.8′′ E). Each core was filled to a standardised sediment height of 17 cm. Cores were then placed in recirculating aquaria and incubated for four weeks under controlled laboratory conditions to allow the communities to stabilise prior to experimental manipulation. Ragworms (*Hediste*) were subsequently collected from the same site.

1 We only report on the results of the final experiment, while it is evident that this successful experiment took extensive prior testing and optimization trials. One pilot experiment – albeit using a different target species – resulted in the publication of Wang et al. (2024) (see list of publications resulting from this project).
2 The bioinformatic analysis of the microbial community data was largely established during another pilot study that was published by Guden et al. (2024) (see list of publications resulting from this project).

Experimental design

To test the effects of low-frequency anthropogenic noise and the bioturbating *Hediste*, we conducted a fully controlled laboratory experiment using 16 circular aquaria (60 cm diam., 47.5 cm height; volume 75 L (Figure 1)). Each aquarium contained five cylindrical sub-tanks (plexiglass cores; 10 cm inner diameter, 33.5 cm height) to allow for replication within treatments (n = 8). All aquaria were supplied with aeration via oxygen pumps to maintain normoxic conditions in the overlying water column. Water temperature was kept constant at 10°C, which is representative of *in situ* temperatures in the German Wadden Sea at the time of sediment and *Hediste* collection, but was primarily chosen to minimise thermal variability and thereby avoid temperature as a confounding factor. An internal recirculating system (TECO) provided cooling by circulating cold water through submerged coils in each aquarium.

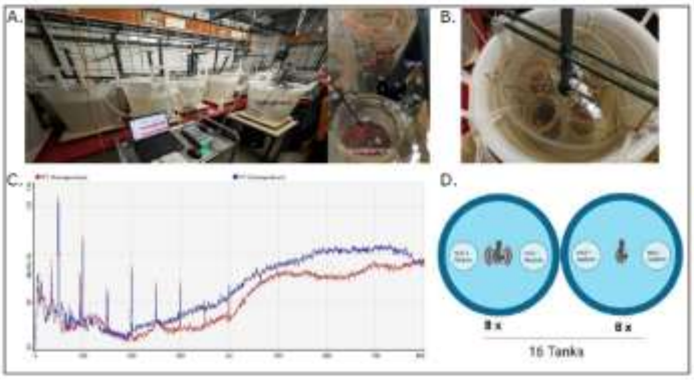

Fig. 1. Experimental setup and acoustic characterization of low-frequency noise treatments. (A) Experimental room containing 16 large tanks (75 L) arranged in two parallel rows, each housing four cylindrical sub-tanks and cooling coils connected to an external chiller. (B) Close-up of a tank showing the suspended noise egg, hydrophone, and accelerometer positioned above the sub-tanks. (C) Representative frequency spectra comparing noise treatment (blue) and control (red) tanks, showing a dominant peak at 110 Hz characteristic of low-frequency anthropogenic noise. (D) Schematic overview of the experimental design: eight tanks exposed to sound and eight silent controls. Each tank contained two mud-only sub-tanks and two sub-tanks with mud plus four individuals of the bioturbator *Hediste*.

Low-frequency sound exposure was generated using custom-built “noise eggs” (de Jong et al., 2017). One noise egg was placed centrally in each aquarium (including control aquaria) to ensure identical physical structure across treatments. In noise treatments, noise eggs continuously emitted low-frequency sound with a dominant peak at ca. 110 Hz (Fig. 1), mimicking anthropogenic low-frequency noise conditions. In control aquaria, identical devices were deployed but remained inactive (no sound emission). Acoustic conditions were characterised daily using a calibrated hydrophone for sound pressure measurements and an underwater accelerometer for particle motion (Fig. 1). All active noise eggs produced a consistent peak around 110 Hz. Additional minor peaks reflected tank- and sensor-position specific resonances and did not differ systematically among treatment replicates. Sound treatment aquaria exhibited sound levels ca. three times higher (in decibels) than controls across the low-frequency band of interest. To minimise background acoustic noise, air conditioning units in the experimental room were turned off during the exposure period. Each aquarium was placed on three 40 mm thick rubber foam pads mounted on a wooden plate to reduce vibration transmission between bench and aquaria and to limit cross-tank coupling.

In treatments with macrofauna, four adult *Hediste* individuals were introduced per sub-tank. To quantify feeding activity, each sub-tank also received 0.1 g lyophilised and resuspended ^{13}C- and ^{15}N-labelled microalgae *Chlamydomonas reinhardtii* (>98% atom% ^{13}C and ^{15}N; Silantes). At the same time, luminophores were added to each core to quantify particle mixing (bioturbation). Cores were incubated for an experimental time span of 7 days. The experimental design comprised two crossed fixed factors: noise exposure (Noise: present vs. absent) and bioturbation (*Hediste* absent vs. *Hediste* present), with a single sediment type (mud), resulting in four treatments (each with 8 replicates)3:

(1) sediment without noise and without macrofauna (No*Hediste*_NoNoise);

(2) sediment with noise but without macrofauna (No*Hediste*_Noise);

(3) sediment with *Hediste* but without noise (*Hediste*_NoNoise); and

(4) sediment with *Hediste* and noise (*Hediste*_Noise).

This design allowed us to disentangle direct noise effects on ecosystem functioning and on microbial communities from indirect effects mediated by the activity of bioturbating macrofauna, as well as to assess interactive effects, i.e. effects of having both noise and *Hediste*.

Measurements of Hediste *activity and ecosystem functioning*

Feeding activity of ragworms was estimated from the uptake of stable-isotope labelled algal food as described above. Pumping activity of *Hediste* was quantified by measuring porewater oxygen fluctuations adjacent to individual tubes using microsensors. Pumping rates were derived from the frequency of oxygen concentration peaks over time and compared between control and noise conditions.

Ecosystem functioning was assessed based on measurements of sediment community oxygen consumption (SCOC) and nutrient fluxes after 6 days of experimental incubation using closed-chamber incubations. Noise and particle motion were measured via hydrophone (AquarianScientific) and accelerometer (Brüel & Kjaer), respectively. Particle mixing was quantified through an accelerometer (Brüsediment profile imaging) under UV light.

3 *Parallel experimental setups including sandy sediment with* Hediste *and muddy sediment with* Corophium volutator *were attempted but excluded due to unsuitable conditions (lack of burrowing and high mortality, respectively).*

Microbial analysis

Sampling, RNA extraction and library preparation

At the end of the exposure period, sediment cores for microbial (bacterial) analyses were sampled from each sub-tank separately. Three sediment subsamples per sub-tank were collected using sterile, cut-off 10 mL syringes (1.4 cm diam.) inserted to a depth of 6 cm. Syringe placement (radial position and distance from core walls) and sampling order were standardised across all treatments to minimise spatial and handling bias. These subsamples were homogenised per sub-tank, immediately frozen at −80°C, and transported on dry ice to Ghent University. Upon arrival, samples were stored at −80°C until RNA extraction. RNA extraction was performed using the PowerSoil Total RNA Kit (Qiagen) according to the manufacturers' protocol, with minor adaptations for marine sediments where necessary (Guden et al., 2024). Residual DNA was removed from the RNA extracts using the DNase Max Kit (Qiagen). DNA contamination was checked by PCR on DNase-treated extracts.

RNA quantity and purity were assessed spectrophotometrically, and integrity was evaluated with an RNA integrity number (RIN), which typically ranged between 8 and 9. Ribosomal RNA (rRNA) was depleted from total RNA using QIAseq FastSelect 5S/16S/23S (Qiagen), followed by library preparation with the NEBNext Ultra II Directional RNA Library Prep Kit (New England Biolabs), following the manufacturers instructions. Directional (strand-specific) libraries were indexed, pooled, and sequenced on an Illumina platform using 2 × 150 bp paired-end runs, with a target yield of ca. 60 million paired-end reads per sample (30 million reads per direction). These metatranscriptomic datasets formed the basis for all downstream taxonomic and functional analyses of the active microbial communities.

Bioinformatic analyses

The bio-informatics pipeline we used was coined from Guden et al. (2024), with some modifications, and is summarized in figure 2. By basing our analyses on RNA instead of DNA, we are sure that all taxonomic units we encounter were metabolically active at time of sampling. Genera with fewer than 20 total reads across all samples or present in fewer than 10% of samples were excluded from analysis.

We first analysed the taxonomic composition and diversity of the active microbial communities based on ribosomal RNA (rRNA) and tested whether these differed among treatments. We analysed both alpha (i.e. within-sample) and beta (i.e. across-sample) diversity. Alpha diversity was quantified using the following metrics: observed richness, Chao1 estimator, Shannon-Wiener diversity, Simpson index. The first two estimate richness *per se*, i.e. the number of observed or expected taxa, independent of the relative abundances of the taxa. The Simpson index assesses evenness, which focuses on the distribution of the individuals across taxa, and the Shannon-Wiener diversity combines both richness and evenness aspects. Beta diversity was estimated based on Bray-Curtis distances derived from a (dis)similarity analysis among samples: Samples that differ more from eachother have higher Bray-Curtis distances.

We then used the non-rRNA fractions (essentially mRNA) for a transcriptomic analysis that allows to address the functional profiles of the active bacterial communities at time of sampling. The messenger RNA's essentially tell us what proteins are being expressed by the microbial community, and these enzymes can be compared with functional libraries containing all known information on the roles of particular proteins/enzymes in different functional pathways. This information provides us with a key to link changes in microbial community composition and/or diversity to measured shifts in ecosystem functions (SCOC, nutrient fluxes etc...).

Meiofaunal analyses

Meiofauna – largely dominated in these sediments by nematodes – were quantified using standard procedures of extraction from the sediment using density-centrifugation in Ludox HS40 and counting under a stereomicroscope (Moens and Beninger, 2018). We also aimed to describe taxonomic composition and species diversity of meiofauna using metabarcoding of the nuclear 18S rDNA and mitochondrial CO I sequences (Derycke et al., 2010). Unfortunately, DNA extractions consistently failed to yield DNA of sufficient quality for sequencing. Hence, we only obtained total abundance data.

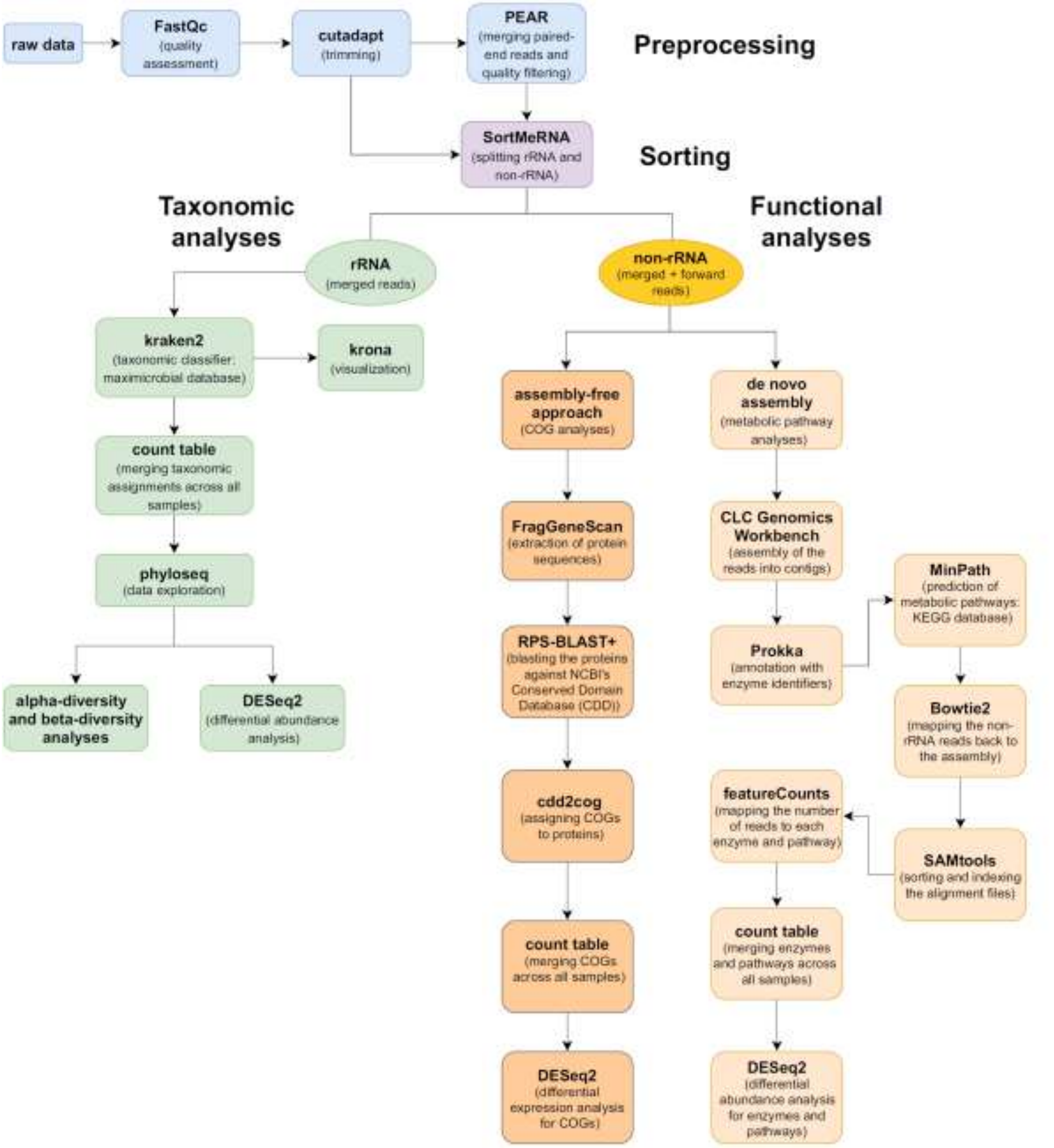

Figure 2. Diagram of the bioinformatics workflow to investigate the taxonomic (based on rRNA) and functional (based on mRNA) profiles of the active bacterial communities in the sediment samples (taken from Guden et al., 2024).

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

UGent activities in ORCHESTRA were largely concentrated on **work package 2: Laboratory studies on individuals, populations and communities**. The aim of WP2 was to make an inventory of the influence of low-frequency noise (LFN) on a suite of species, species interactions and, through cascading effects, on ecosystem functioning.

The results of trials evaluating the responses of individual species highlight that a large number of taxa displayed negative responses to LFN regarding physiological parameters, development and/or behavior. UGent – Marine Biology contributed to the study on the tube building sand mason, *Lanice conchilega*, a sessile polychaete. **This species showed reduced tube ventilation rates under exposure to LFN** (Wang et al., 2024). However, in this report, we focus on our work on another, albeit mobile and burrowing polychaete, the ragworm *Hediste diversicolor*.

Surface sediment mixing and ragworm behavioral plasticity

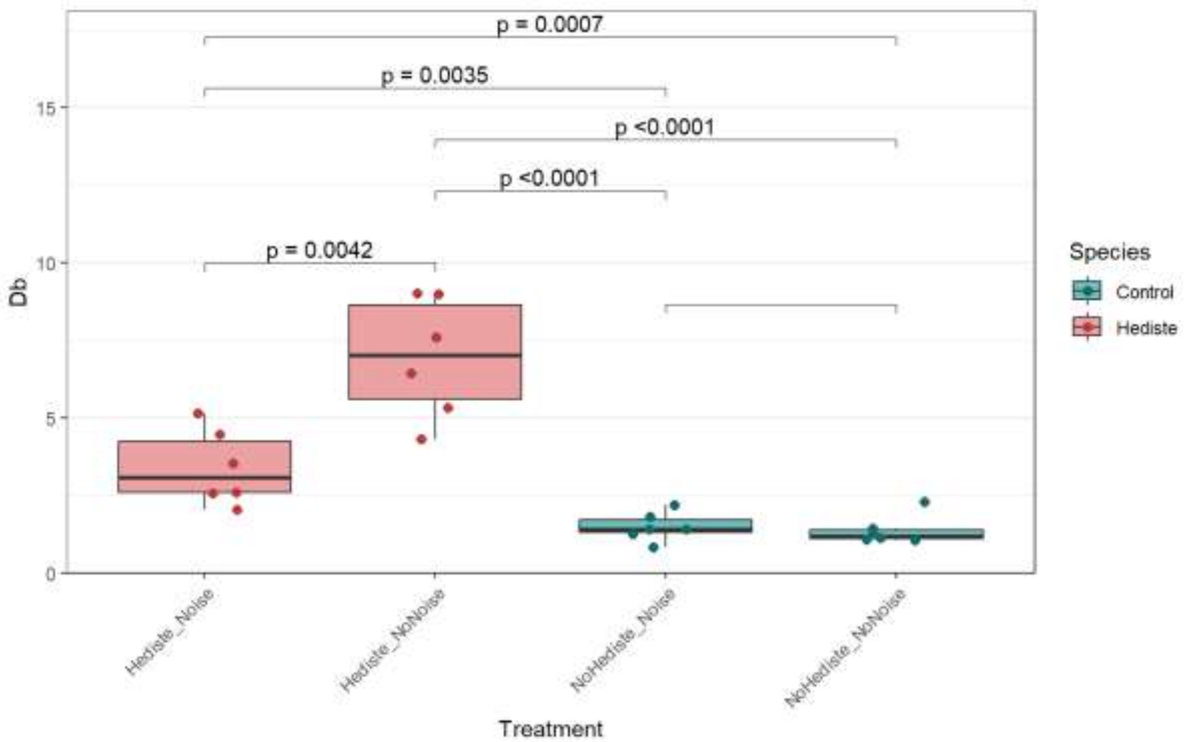

Figure 3. Biodiffusion rates in treatments with and without ragworms (*Hediste*) exposed to noise vs no-noise conditions. Noise is continuous low-frequency noise. Data shown are based on at least 5 independent replicates per treatment. Significant differences among treatments are shown with p-values above the respective boxes. **Hediste *has a major positive effect on biodiffusion rates in sediments. Noise alone also has a positive effect on biodiffusion, but much less pronounced than* Hediste*. Noise interferes with* Hediste *activity, leading to a partial cancelling of the positive* Hediste *effect under LFN exposure.***

Ragworms increased sediment mixing rates through bioturbation (associated with burrow construction, maintenance and ventilation), **but LFN slowed down the rate of this biodiffusive transport** by 51 % (noise x ragworm: p = 0.0074; Fig. 3). In the absence of *Hediste*, LFN also increased biodiffusive particle transport (p = 0.0418) compared to sediment without *Hediste* and without noise, but to a much shallower depth than did *Hediste*. This is likely a direct consequence of noise-induced sediment particle vibrations. LFN also increased variability in ragworm-mediated burial of surface material; such increased variability often reflects stress conditions. Reduced ragworm locomotion corroborates LFN effects found in amphipods; this was suggested to represent a plastic response to minimize detection by predators whose vibration signatures may resemble those associated with LFN (Terschek et al., 2025; Wang et al., 2026).

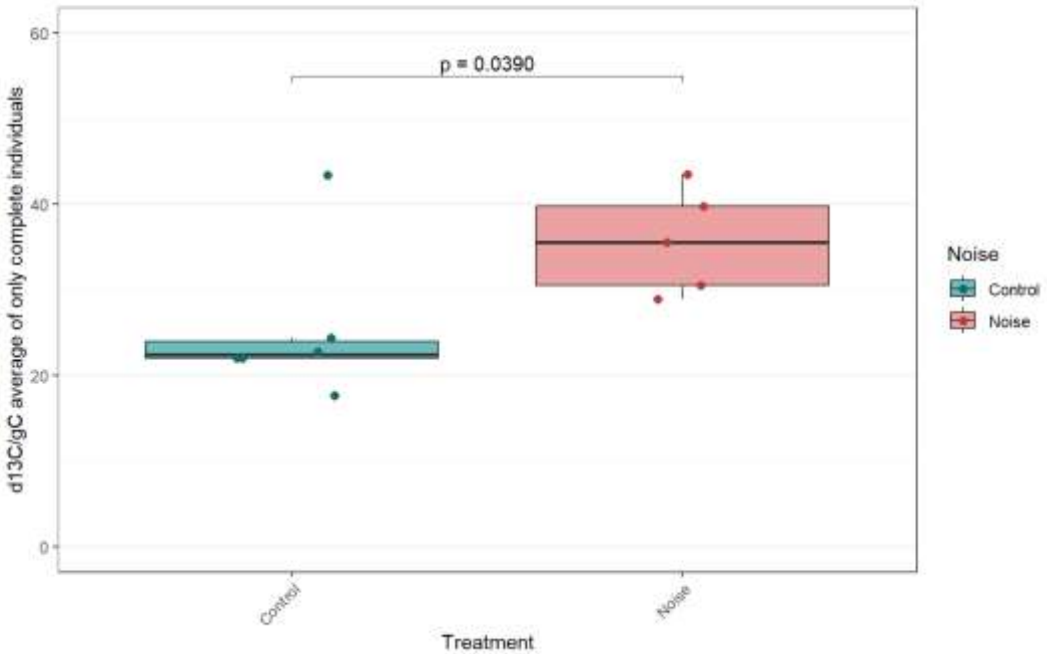

Figure 4. Uptake of algal carbon by *Hediste* in treatments with and without noise. Uptake is expressed here as $\delta^{13}C$ in tissues of (intact) specimens of *Hediste* sampled from sediments which received ^{13}C-enriched algae as a potential food source. Data on at least 5 independent (i.e. stemming from different replicate cores) specimens are included. ***LFN caused a significant increase in feeding on microalgae by* Hediste.**

LFN also increased ragworm consumption of surface algae (Fig. 4). Several non-mutually exclusive mechanisms may have contributed to this unexpected result. First, substrate-borne vibrations may have improved the availaiblity of algal food to ragworms through increased diffusive particle displacements at the sediment surface as a consequence of LFN-induced particle vibrations. Alternatively, LFN may have enhanced feeding as a strategy to cope with noise stress, and/or it may have reduced the availability of other prey such as nematodes and amphipods. The latter is, however, unlikely, since LFN alone did not have significant effects on prey numbers (example for nematodes in Fig. 5). Finally, we observed an increase in mucus production in treatments with *Hediste* exposed to LFN compared to non-exposed controls.

This may indicate a partial shift to more filter feeding activity in noise-exposed ragworms. Filter feeding may have trapped and retained algae into ragworm burrows, improving their availability to the ragworms, particularly given their reduced locomotion when exposed to noise stress.

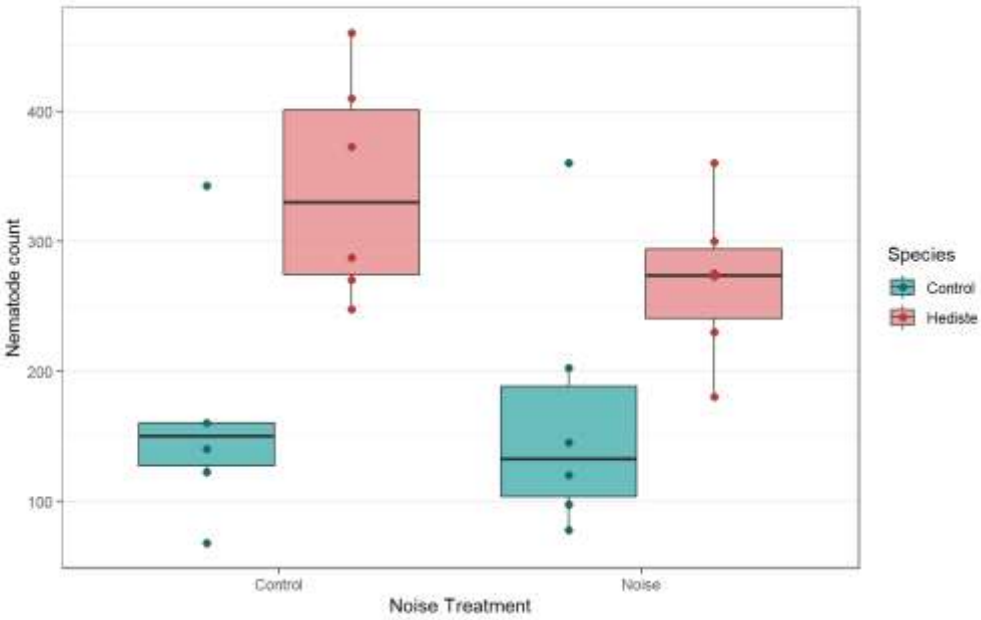

Figure 5. Nematode abundance (ind./10 cm^2) in treatments with and without noise and with and without *Hediste* present. Data shown are based on at least 5 independent replicates per treatment. **Hediste *presence clearly increases the abundance of nematodes by almost twofold, whereas noise alone did not significantly affect nematode abundance.***

Interactive effects of LFN and Hediste *on meio- and microbial communities*

By comparing the response of both the meiofauna and microbial community to LFN in the presence and absence of *Hediste diversicolor*, we investigated how possible LFN-induced macrofauna behavioural shifts mediated LFN effects on the meiofauna and microbial community (Van Colen et al., in prep). LFN did not directly affect population densities of nematodes (Fig. 5) and small (< 1 mm) individuals of *Corophium volutator* (data not shown). However, because LFN reduced bioturbation in *H. diversicolor*, it also lowered the bioturbation-mediated facilitative effect of *H. diversicolor* on the survival of nematodes (by 21 %) and of *C. volutator* (by 45 %). Indeed, a reduction in ragworm locomotion comes at the expense of particle mixing and burrow ventilation, ecosystem processes known to vastly promote survival of invertebrates, particularly in oxygen-depleted sediments like those used in our experiment. Indeed, ragworms promoted survival of amphipods and nematodes, but LFN reduced this facililation (Fig. 5). In other words**, *Hediste* only partly buffered negative LFN effects on meiofaunal abundances.**

Hediste exerted a pronounced influence on microbial community structure: its presence consistently increased alpha diversity (except evenness)(Fig. 6A), reshaped beta diversity, and drove extensive taxonomic turnover (Fig. 6B). By contrast, **LFN alone did not significantly alter diversity nor taxonomic composition of microbial communities** (Fig. 6A,B).

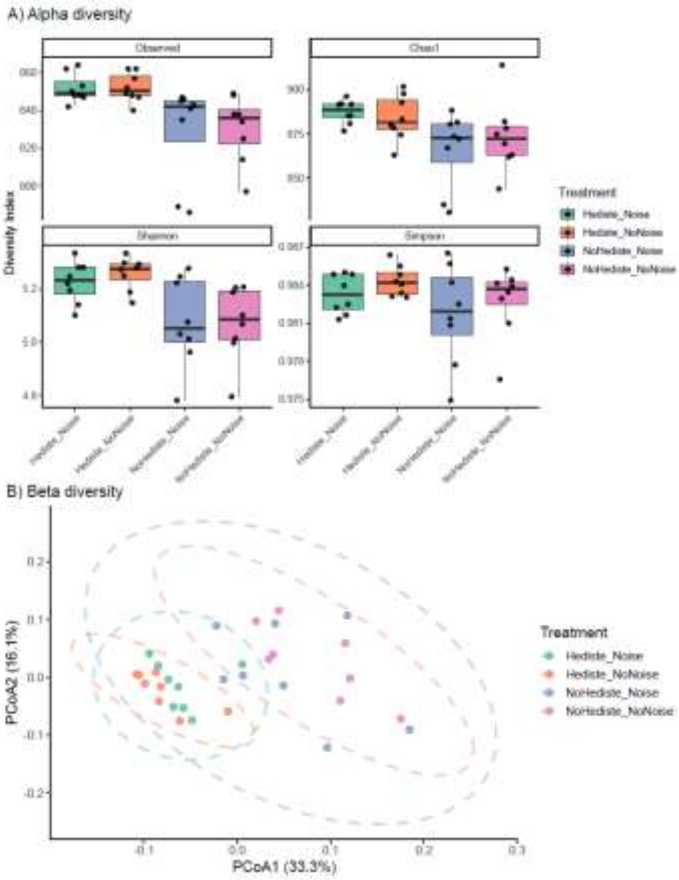

Figure 6. Alpha and beta diversity of active microbial communities across experimental treatments. (A) Alpha diversity metrics at the microbial genus level: observed richness, Chao1 estimated richness, Shannon diversity and Simpson's evenness for four treatment groups (see M&M). Boxplots show diversity distributions with overlaid sample points. ***Presence of* Hediste *consistently increased taxonomic richness and diversity, of microbial communities, but not evenness. LFN alone did not significantly affected any measure of microbial alpha diversity.*** (B) Beta diversity visualized using Bray-Curtis PCoA. Points represent individual samples; ellipses denote 95% confidence intervals per treatment. Much like for alpha diversity, **Hediste *was the main driver of differences in beta diversity among treatments, while LFN alone did not measurably contribute to variability among treatments.***

Interactive effects of LFN and Hediste *on the functional properties of microbial communities*

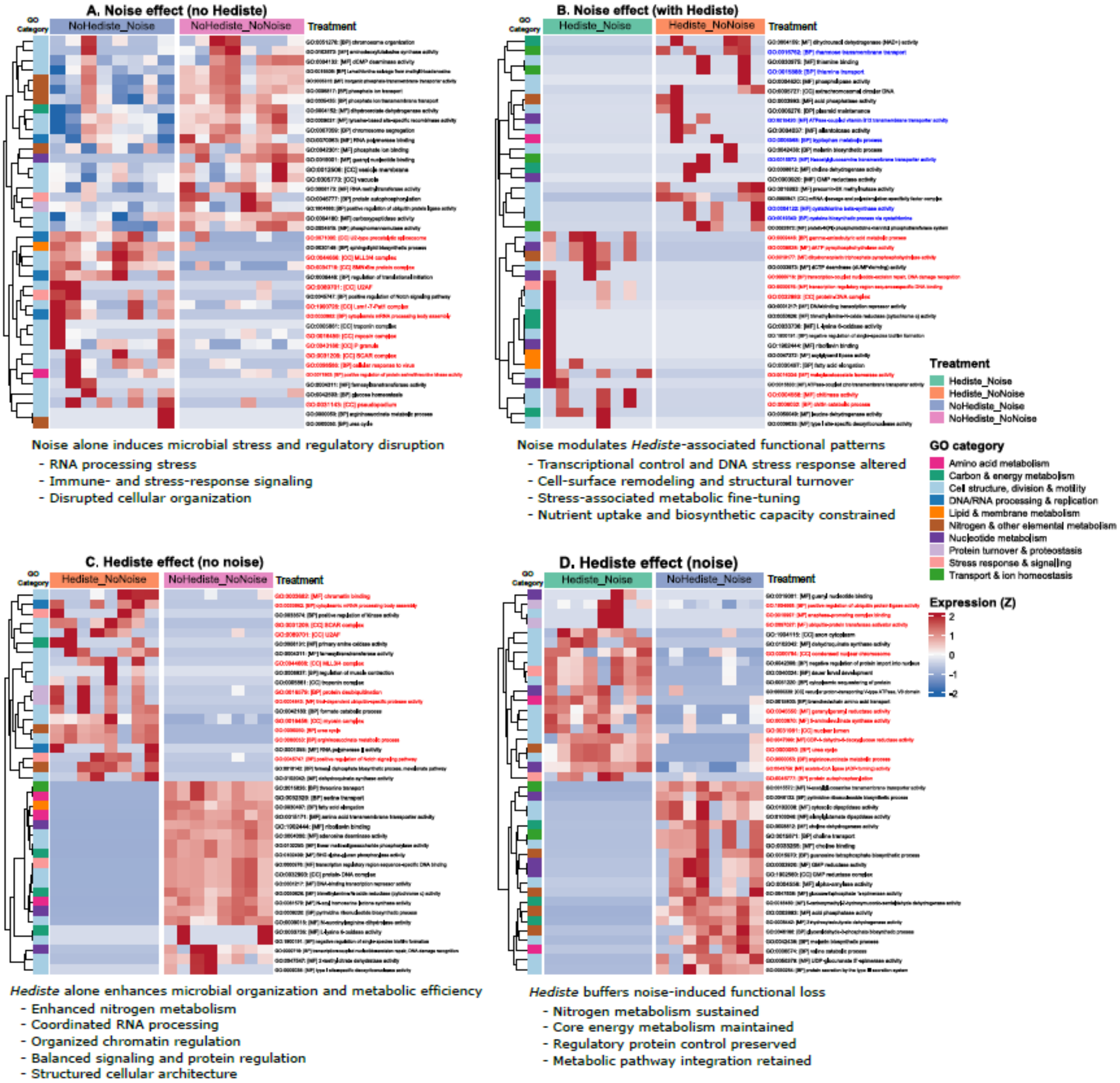

Figure 7. Functional responses of active microbial communities to noise and *Hediste*. Heatmaps showing the 20 most upregulated and 20 most downregulated GO terms per contrast (FDR < 0.05). Rows represent GO terms (BP, MF, CC); columns represent samples grouped by treatments. Values are Z-score scaled VST expression, with hierarchical clustering applied within panels. ***Main observations are summarized in the figure.***

While it did not significantly affect microbial taxonomic composition and diversity, **LFN did trigger a pronounced functional reorganization in non-bioturbated sediments**, stimulating pathways related to amino-acid metabolism, chromatin and RNA processing, and stress-response systems, while suppressing nutrient transport, cofactor biosynthesis and central metabolic functions (Fig. 7A,B). The presence of *Hediste* markedly enhanced microbial metabolic capacity and enriched pathways involved in carbon degradation, nitrogen and sulphur transformations, and membrane-associated processes. ***Hediste* also buffered several**

noise-induced functional disruptions, maintaining elevated biosynthetic capacity and partially restoring metabolic breadth (Fig. 7C,D). **Buffering was, however, not complete**: Enhanced expression of stress-related genes and reductions in nutrient-cycling pathways persisted under noise exposure even in the presence of *Hediste*. **LFN thus modified species interactions between size groups in the marine benthos.**

Collectively, these results demonstrate that LFN primarily acts as a functional rather than taxonomic stressor in coastal sediments, and that macrofaunal bioturbation provides resilience but not immunity to this kind of acoustic disturbance.

Interactive effects of LFN and* Hediste *on ecosystem functioning

Of course, the above build-up evidently leads to thé key question: do the observed effects of LFN on *Hediste* and on meio- and microbial communities translate into effects on ecosystem functioning? We have demonstrated above that LFN affects the behaviour of *Hediste*, which is reflected in rates of bioturbation and biodiffusion. This in turn negatively affects abundance and/or diversity of meio- and microbiota, which tend to benefit from a normal burrowing activity of *Hediste*, transporting oxygen into the sediment. It also leads to different functional activity patterns of microbial communities as described above. But does that translate into measurable differences in ecosystem functioning (EF)? We quantified EF using sediment oxygen consumption (SCOC), ammonium release and sediment organic carbon concentration. In this report, we focus on SCOC and sediment OC concentration.

LFN and ragworms both increased sediment aerobic metabolism (noise: $p = 0.0141$; ragworm: $p = 0022$). However, **the stimulatory effect of ragworms was largely lost when exposed to LFN** (Fig. 8). If aerobic metabolism increases, organic carbon (OC) concentration is expected to decrease. Indeed, **in the absence of ragworms, LFN decreased OC concentration, and *vice versa*, in the absence of LFN, *Hediste* decreased OC concentration** (Fig. 9). This suggests that a purely physical organic matter mixing stimulates microbial functioning at a similar magnitude as do ragworm-mediated particle displacements. However, the effect of LFN was counteracted by the presence of ragworms, resulting in an antogonistic LFN x ragworm effect on sediment organic carbon stocks (noise x ragworm: $p = 0.0435$)(Fig. 9). Similar antagonistic effects of LFN and *Hediste* were also observed on ammonium fluxes: LFN increased ammonium release from sediments, whereas *Hediste* stimulated sedimentary ammonium oxidation, inhibiting a build-up of ammonium and thereby counteracting eutrophication. In fact, solute fluxes show that these **sediment communities shift from a sink to a source for water column nitrate when exposed to LFN – likely resulting from a slowdown in coupled nitrification-denitrification, a process known to occur most efficiently in strongly bioturbated sediments.**

These results highlight the complex interplay of noise-induced sediment vibrations, bioturbation, and microbial functioning that underpin the impact of noise pollution on ecosystem functioning.

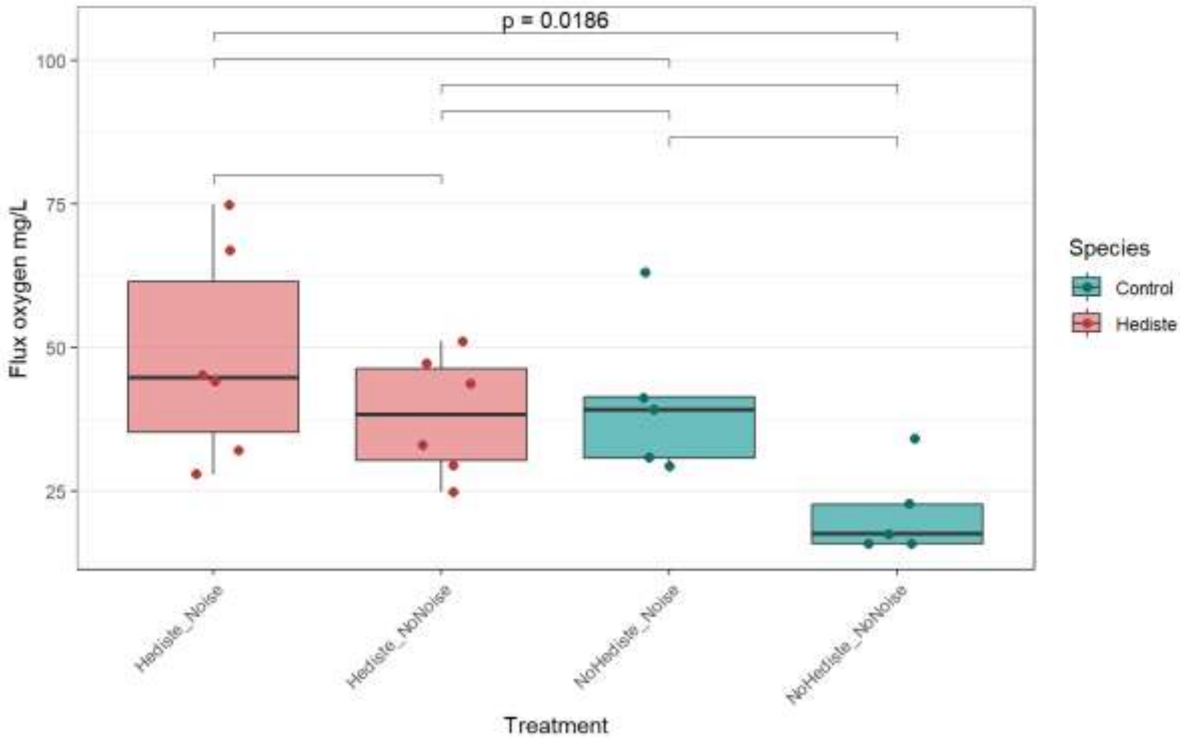

Figure 8. Sediment community oxygen consumption in four treatment groups (see M&M). ***LFN stimulates oxygen consumption to a similar extent as does*** **Hediste*****, but these effects are not straightforwardly cumulative.***

We conclude that integrating microbial functional responses with macrofauna-mediated sediment processes is essential for an accurate prediction of how changing underwater soundscapes may reshape benthic biogeochemistry and ecosystem functioning. Overall, our study demonstrates how the hidden and distant implications of low-frequency noise sources can affect seafloor ecology. Future studies therefore should not neglect investigations into the vibrational consequences for the ecology of the largest aquatic habitat on Earth.

The results of ORCHESTRA highlight several priorities for future research. In particular, there is a need for experiments designed to derive exposure–response relationships that enable the identification of biological thresholds relevant for MSFD assessments. Such relationships should not, however, be limited to single model species in an otherwise completely controlled environment. **Future research should also investigate ecosystem-level responses of noise exposure and of key functional species and their consequences through potential cascading effects.** In addition, LFN effects on species and ecosystems should not be investigated in isolation, but **in combination with other stressors,** since it is clear from the ORCHESTRA project that important ecosystem consequences can follow from relatively marginal fitness or behavioral impacts of exposure on particular species.

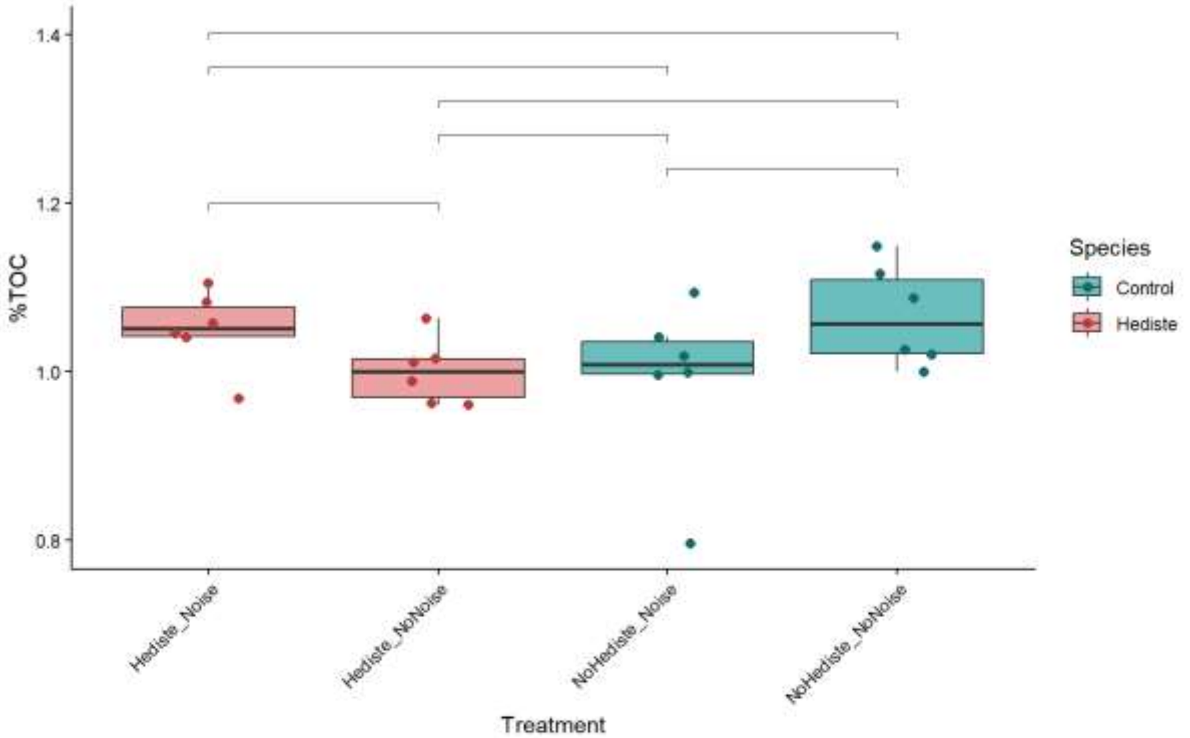

Figure 9. Total organic carbon content (TOC) expressed as a % of sediment dry weight in four treatment groups (see M&M). In the absence of *Hediste*, aerobic sediment metabolism is higher under LFN (fig. 8), leading to a lower extant TOC. Similarly, in the absence of LFN, *Hediste* stimulates aerobic sediment metabolism and decreases TOC. However, ***LFN disrupts the behaviour of*** **Hediste** ***that normally leads to its stimulatory effect on SCOC, and thus partly annihilates the otherwise negative effect of*** **Hediste** ***on TOC.***

5. DISSEMINATION AND VALORISATION

Dissemination

Within the ORCHESTRA project, an entire work package (WP6) was devoted to stakeholder involvement as a key to our dissemination and valorisation strategy. UGent – Marbiol was only involved to a limited extent in this WP, hence much of the below outreach and valorisation comes from other project partners (but some UGent examples are also included).

The project successfully set up pathways for the involvement of different stakeholders from the public and wider scientific community to national and international working groups at the science-policy interface (e.g. BLANO, ICES) as well as the relevant state authorities (NPV-SH, LfU). An overview of target groups is presented below, followed by a brief description of specific examples of how the consortium members reach these audiences.

Target groups

- *Public*
    - Open days, public lectures, experiments for pupils from local schools, social media
- *Scientific community*
    - Publications, conference participations, workshop, social media
- *Policy stakeholders*
    - National and international working groups at the science-policy interface (e.g. BLANO), TG Noise, ICES
- *Technological stakeholders*
    - Press releases, workshop: https://www.uni-kiel.de/en/research/priority-research-areas/kiel-marine-science/20240926-engineering-meets-ecosystem

To engage the ***public***, the project consortium members conducted outreach activities for school children, provided on-site information to tourists in Büsum, and supported student participation in initiatives such as "Schüler experimentieren" and "Jugend forscht." One supervised student even won both the regional competition in 2023 and the state competition in 2024. ORCHESTRA partners also participated in public outreach events, including Maritime Week Bremen (2023), Open lecture series University of Hamburg (2023), Open Days at BAH Helgoland (2023, 2025), and the European Research Night in Chioggia (2025).

In coordination with other JPI Oceans projects, researchers from the University of Padua presented the goals of the JPI Underwater Noise call and related projects (ORCHESTRA, Diaphonia, DeuteroNoise) to citizens in Chioggia during the "Spritz di Scienza" seminar series. This event was particularly relevant as Chioggia hosts one of the largest fishing fleets in the Adriatic, where underwater noise impacts are of strong local interest.

In addition to the evident ways (publications, conference presentations) of targeting the ***scientific community***, ORCHESTRA members took several initiatives to engage students and young researchers. A seminar at the University of Padua (November 2023) introduced students in Environmental Sustainability and Education to the work of ORCHESTRA and related JPI Oceans projects. PhD students also contributed through a half-day practical course and a lecture at the Coastal Summer School Helgoland 2024. Several Master's theses were also embedded within the project. Specifically for the work reported here, we involved both a master and bachelor thesis student (Ilan Slangen and Charlotte Dixon, respectively) in the data analysis (I.S.) and laboratory processing (C.D.) of the microbial and meiofaunal samples, and a PhD student (Marie Cours) in the design and execution of the experiment and the analysis of meiofauna samples. All three are currently being involved in the publication process of the experimental results.

Further, from 17–19 November 2025, the Underwater Acoustics Workshop 2025 was held at the Research Technology Centre West Coast (FTZ), Kiel University, with 27 participants. The workshop fostered exchange between early-career and senior scientists and concluded with a visual synthesis of approaches to evaluate anthropogenic sound impacts on marine life. This event was partially funded through the FYORD Event Grant 2025 awarded to Fabio Viotti and Saskia Kühn. Complementing the workshop, a one-day Underwater Acoustic Course was held at Kiel University on 20 November 2025.

A second workshop "Engineering meets Ecosystem: Developing a Roadmap for Future International Collaboration between Marine Renewable Energy and Marine Ecosystem Researchers", co-organized by the ORCHESTRA partner FTZ, mainly targeted ***technological stakeholders*** from Ocean renewable energy and engineers and aimed at strengthening synergies and collaboration. Among the speakers were the ORCHESTRA members Shenghao Wang (AWI), Karen de Jong (IMR), Fabio Viotti and Saskia Kühn (CAU); Katja Heubel (FTZ) was one of the co-organizers.

At the ***science-policy interface***, consortium members contributed ORCHESTRA results to national and international working groups, including several ICES groups (e.g., WGSHIP, WGOWDF, WGMBRED), the TG Noise working group, and Germany's BLANO Noise group meetings. Moreover, the coordinator of ORCHESTRA, Maarten Boersma, presented major JPIO results in a talk titled "Anthropogenic Noise and Marine life: should we worry? (and the answer is yes)" at the UNOC, Nice, France, on board the METEOR in June 2025. Simultaneously, a video showcasing ORCHESTRA as well as other projects relevant to the UN Ocean Decade projects was aired (https://www.youtube.com/watch?v=LYEyRnuti0A) during UNOC.

In addition to targeted events, ‘permanent’ **communication platforms** were developed and maintained throughout the project. These included a project website (*http://www.ORCHESTRA-jpi-oceans.de/*) as well as social media platforms (*https://www.linkedin.com/company/98253454/admin/feed/posts/*, *https://www.instagram.com/ORCHESTRA_jpi_oceans/*). A twitter account (*https://twitter.com/ORCHESTRA_JPI*) was discontinued after repeated blockage via twitter under accusations of being a fake account.

Press releases by the ORCHESTRA consortium included the following:

- Engineering meets Ecosystem”, website University Kiel, *https://www.uni-kiel.de/en/research/priority-research-areas/kiel-marine-science/20240926-engineering-meets-ecosystem*
- “Welche Folgen hat der Lärm im Meer für das Ökosystem”, website University Kiel, *https://www.uni-kiel.de/de/forschung/forschungsschwerpunkte/kiel-marine-science/20230426-laerm-im-meer* (article in German)
- “Unterwasserschall stört Meeresorganismen bei der Nahrungsaufnahme”, website University Kiel, *https://www.uni-kiel.de/de/detailansicht/news/137-auswirkungen-unterwasserschall-nahrungsnetz* (article in German)
- “Underwater noise disturbs feeding behavior of marine organisms”, website University Kiel, *https://www.uni-kiel.de/en/details/news/137-auswirkungen-unterwasserschall-nahrungsnetz*
- “ORCHESTRA - Auswirkungen von Unterwasserlärm auf die Meeresumwelt”, website University Kiel, *https://www.uni-kiel.de/de/forschung/forschungsschwerpunkte/kiel-marine-science/aktuelles/newsletter/content/news/ORCHESTRA-auswirkungen-von-unterwasserlaerm-auf-die-meeresumwelt* (article in German)
- “Unterwasserschall stört Meeresorganismen bei der Nahrungsaufnahme”, website gwf Wasser - portal for water and wasterwater technologies, *https://gwf-wasser.de/forschung-und-entwicklung/unterwasserschall-stoert-meeresorganismen-bei-der-nahrungsaufnahme/* (article in German)
- “Unterwasserlärm stört wichtige Winzlinge”, website Bild der Wissenschaft, *https://www.wissenschaft.de/erde-umwelt/unterwasserlaerm-stoert-wichtige-winzlinge/* (article in German)
- “Hva betyr lyd fra havvind-anlegg for livet i havet?”, website Havforskningsinstituttet, *Hva betyr lyd fra havvind-anlegg for livet i havet? | Havforskningsinstituttet (hi.no)* (article in Norwegian)

- "Havvind: Før en stor havvind-utbygging, må vi finne ut hva konstant lavfrekvent støy betyr for livet i havet", website Stavanger Aftenblad, *Havvind: Før en stor havvind-utbygging, må vi finne ut hva konstant lavfrekvent støy betyr for livet i havet (aftenbladet.no)* (article in Norwegian)

Valorisation

ORCHESTRA substantially advanced knowledge on the effects of anthropogenic underwater noise (AUN) on marine invertebrates, a group that had previously received little attention in underwater noise research despite representing the majority of marine biodiversity and playing key roles in ecosystem functioning. At the start of the project, empirical evidence on the sensitivity of invertebrates to underwater sound was scarce and fragmented. ORCHESTRA addressed this knowledge gap through an integrated experimental programme combining laboratory and field studies. Through this, and specifically through the results of the experiment reported here, ORCHESTRA work demonstrated that noise-induced changes in invertebrate behaviour cascade down into community composition, abundance, diversity and activity of meio- and micro-organisms, with reverberating effects the functions and services provided by the benthic ecosystem. As such, the results of ORCHESTRA support the implementation of the Marine Strategy Framework Directive (MSFD), Descriptor 11 (Underwater Noise), by expanding the currently limited evidence base on how anthropogenic sound affects marine organisms and the ecosystem processes and functions they affect.

Beyond its scientific contributions, the project has potentially important implications for society and the economy. Maritime activities such as shipping, offshore renewable energy development and coastal infrastructure increasingly contribute to a noisy underwater environment. By providing empirical data on the biological consequences of such noise, ORCHESTRA supports evidence-based management of marine environments, thereby contributing to the sustainable development of marine industries while safeguarding ecosystem functioning.

Together, these activities strengthened the science–policy interface and supported the integration of scientific knowledge into marine environmental management, particularly in relation to the MSFD.

It is also noteworthy that C.V.C and T.M. (UGent – Marine Biology), in collaboration with scientists of the Institute of Marine Research, Austevoll Research Station in Norway, secured 100,000 Eur funding from EMBRC-Belgium through Joint Development Action JDA ID 3184 to further develop sound sensing equipment and experimental aquaria for noise impact experiments in the laboratory (title of the project: "A research infrastructure for chronic noise pollution studies in coastal waters and sediments.").

6. PUBLICATIONS

- Guden, R.M., Haegeman, A., Ruttink, T., Moens, T. and Derycke, S. (**2024**). Nematodes alter the taxonomic and functional profiles of benthic bacterial communities: a metatranscriptomic approach. *Molecular Ecology*, 33: e17331. https://doi.org/10.1111/mec.17331 .
- Guden, R.M., Slangen, I., Van Colen, A., Dixon, C., Cours, M., Beermann, J., Wrede, A. and Moens, T. (**in prep.**). Anthropogenic low-frequency noise reshapes active sediment microbiomes and disrupts bioturbation-mediated processes. *(A complete draft can be obtained from T.M. upon request).*
- Van Colen, C., Guden, R.M., Beermann, J., Cours, M., Slangen, I., Strand, E., Wang, S.V., Boersma, M., Moens, T. and Wrede, A. (**in prep.**). Vibrational pollution: how low-frequency noise interacts with benthic soft-sediment ecosystem functioning.
- Wang, S.V., Van Colen, C. and Beermann, J. (**2024**). Noisy waters hamper the pumping behavior of the polychaete *Lanice conchilega*. In: Popper, A.N., Sisneros, J., Hawkins, A.D., Thomsen, F. (eds) The effects of noise on Aquatic life. Springer, Cham. https://doi.org/10.1007/978-3-031-10417-6_180-1 .

7. ACKNOWLEDGEMENTS

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ANNEX I - REFERENCES

- Dannheim, J. et al. (2019). Benthic effects of offshore renewables: identification of knowledge gaps and urgently needed research. *ICES J. Mar. Sci.,* 17: 1092-1108.
- de Jong, K., Schulte, G. & Heubel, K. U. (2017). The noise egg: A cheap and simple device to produce low-frequency underwater noise for laboratory and field experiments. *Methods Ecol. Evol*., 8: 268–274.
- Derycke, S., Vanaverbeke, J., Rigaux, A., Backeljau, T. & Moens, T. (2010). Exploring the use of cytochrome oxidase c subunit I (COI) for DNA barcoding of free-living marine nematodes. *PLoS One,* 5: e13716.
- De Soto, N.A. (2016). Peer-reviewed studies on the effects of anthropogenic noise on marine invertebrates: From scallop larvae to giant squid. Adv. Exp. Med. Biol., 875: 17-26.
- Duarte, C.M. et al. (2021). The soundscape of the Anthropocene ocean. Science, 371: e583.
- Frisk, G.V. (2012). Noiseonomics: The relationship between ambient noise levels in the sea and global economic trends. Sci. Rep., 2: e437.
- Guden, R.M., Haegeman, A., Ruttink, T., Moens, T. & Derycke, S. (2024). Nematodes alter the taxonomic and functional profiles of benthic bacterial communities: a metatranscriptomic approach. *Mol. Ecol.*, 33: e17331.
- Moens, T. & Beninger, P.G. (2018). Meiofauna: An inconspicuous but important player in mudflat ecology. Chapter 5 in: Beninger, P.G. (ed.), Mudflat Ecology. Aquatic Ecology Series 7. Springer Nature, Switzerland: 91-147.
- Slabbekoorn, H. (2010). Anthropogenic noise: impacts on animals. In: Breed, M. & Moore, J. (ds.), Encyclopedia of Animal Behavior. Elsevier: 82-88.
- Solan, M., Hauton, C., Godbold, J.A., Wood, C.L., Leighton, T.G. & White, P. (2016). Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep., 6: e20540.
- Terschek, A., Tremblay, N., Wang, S.V. & Beermann, J. (2025). Anthropogenic low frequency sound affects metabolic pathways in marine benthic crustaceans. *Mar. Biol.*, 172: e67.
- Tremblay, N., Juma, A.G., Herstoff, E.M., Meunier, C.L. & Boersma, M. (2024). Low-frequency noise affects development and movement patterns of a calanoid copepod. *Limnol. Oceanogr.*, 70: 1, pp- 100-112.
- Wang, S.V., Terschek, A., Tremblay, N., Boersma, M., Wrede, A. & Beermann, J., (2026). Noisy waters affect feeding and metabolic enzyme activities of amphipod mesograzers. Mar. Poll. Bull., 222: e118764.
- Wang, S.V., Van Colen, C. & Beermann, J. (2024). Noisy waters hamper the pumping behavior of the polychaete *Lanice conchilega*. In: Popper, A.N., Sisneros, J., Hawkins, A.D., Thomsen, F. (eds), The effects of noise on Aquatic life. Springer, Cham..