UNIVERSITY OF GOTHENBURG

Tiny titans:
Impact of meiofauna diversity and activity on coastal sediment biogeochemistry

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Contents

Abstract

Chemical reactions in marine sediments and the resulting fluxes across the sediment-water interface influence ecosystem functioning, global carbon cycling, and ultimately global habitability. Although previous research has recognized the major role of microorganisms and macrofauna (invertebrates > 1 mm), it still debated whether meiofauna (invertebrates < 1 mm) can make significant contributions to ecosystem functioning due to their small size. This motivated me to estimate meiofauna contribution to total oxygen uptake and methane release from the sediment, and further investigate meiofauna activity and diversity under environmental perturbations, such as microplastic pollution and climate change.

In this thesis, I described a microsensor-based method for respiration measurements, with ability to induce desired experimental conditions (**paper I**). This method revealed that traditional theoretical estimates of respiration can lead to a four-fold overestimation of measured rates. Although respiration rates were highly variable within each meiofauna group, rates were lower (and thus contribution to ecosystem processes was smaller) under hypoxic compared to oxic conditions (**paper II**). Macrofaunal bioturbation significantly enhanced methane release from sediments, but this effect was somewhat offset by meiofauna due to interactions with microorganisms (**paper III**). Bioturbation depth, however, was reduced when communities were exposed to microplastic pollution (**paper IV**) which may affect organic matter mineralization and nutrient fluxes over longer periods. Lastly, climate change is intensifying environmental stressors such as river discharge and coastal erosion, which were shown to affect meiofauna community, but not nematode diversity (**paper V**). In addition, nearshore habitats, which are particularly impacted by these stressors, favored colonizer-dominated nematode communities, whose future dominance may reduce ecosystem stability as river discharge and coastal erosion increase.

Overall, the results provide new insights into meiofauna's role in sediment biogeochemistry by quantifying its contribution to essential ecosystem processes. This thesis presents the first direct measurements of respiration rates for specific meiofauna, the first investigation of macrofauna-

meiofauna-microorganism interaction effects on methane release, impact of microplastics on bioturbation, and the application of molecular tools to study metazoan diversity in Siberian Arctic. The presented findings are especially relevant as growing oxygen-deprived bottoms, intensifying microplastic pollution, and accelerating climate change increasingly threaten marine ecosystems. Such ecosystem-level changes may negatively impact meiofauna and could potentially lead to previously overlooked cascading effects on sediment biogeochemistry.

Keywords: meiobenthos, biogeochemistry, sediment, oxygen uptake, hypoxia, greenhouse gasses, Arctic, metabarcoding, microplastic

Svensk sammanfattning

Kemiska reaktioner i marina sediment och de resulterande flödena över gränssnittet mellan sediment och vatten påverkar ekosystemets funktion, den globala kolcykeln och i slutändan människans välbefinnande. Även om tidigare forskning har erkänt mikroorganismers och makrofaunas (ryggradslösa djur > 1 mm) betydande roll, diskuteras det fortfarande om meiofauna (ryggradslösa djur < 1 mm) kan ge betydande bidrag till ekosystems funktion, primärt på grund av deras lilla storlek. Detta motiverade mig att uppskatta meiofaunas bidrag till det totala syreupptaget och metanutsläppet från sedimentet, och ytterligare att undersöka meiofaunas aktivitet och mångfald under miljöstörningar, såsom mikroplastföroreningar och klimatförändringar.

I denna avhandling beskrev jag en mikrosensorbaserad metod för respirationsmätningar, med möjlighet att inducera önskade experimentella förhållanden (**artikel I**). Denna metod visade att traditionella teoretiska uppskattningar av respiration kan leda till en fyrfaldig överskattning av uppmätta respirationshastigheter. Även om respirationshastigheterna varierade mycket inom varje meiofaunagrupp, var respirationshastigheterna lägre (och därmed bidrog mindre till ekosystemprocesser) under syrefattiga jämfört med syrerika förhållanden (**artikel II**). Dessutom ökade bioturbation av makrofauna avsevärt metanfrisättningen från kustsediment, men denna effekt kompenserades något av meiofauna på grund av dess interaktioner med mikroorganismer (**artikel III**). Bioturbationsdjupet minskade dock vid mikroplastföroreningar (**artikel IV**). Sådan minskning av bioturbationsdjupet under längre perioder kan påverka mineraliseringen av organiskt material och näringsflöden. Slutligen visade sig stressfaktorer på miljön relaterade till klimatförändringar, som flodutsläpp och kusterosion, påverka meiofaunasamhället, men inte nematoddiversiteten (**artikel V**). De kustnära livsmiljöerna, som är främst påverkade av dessa stressfaktorer, gynnade nematodsamhällen med en dominerande andel koloniserande arter, vars framtida dominans kan minska ekosystemets stabilitet när flodutsläpp och kusterosion ökar.

Sammantaget ger denna avhandling nya insikter om meiofaunas roll i sedimentbiogeokemi genom att kvantifiera dess bidrag till väsentliga ekosystemprocesser. Avhandlingen presenterar de första direkta mätningarna av respirationshastigheter för specifik meiofauna, den första undersökningen av makrofauna-meiofauna-mikroorganismers interaktionseffekter på metanfrisättning, påverkan av mikroplaster på bioturbation och tillämpningen av molekylära verktyg för att studera metazoandiversitet i Sibiriska Arktis. Här presenterade resultat är särskilt relevanta eftersom växande syreberövade bottnar, intensifierade mikroplastföroreningar och accelererande klimatförändringar i allt högre grad hotar marina ekosystem. Sådana förändringar på ekosystemnivå kan påverka meiofauna negativt och kan potentiellt leda till tidigare förbisedda kaskadeffekter på sedimentbiogeokemi.

Nyckelord: meiobentos, biogeokemi, sediment, syreupptagning, hypoxi, växthusgaser, Arktis, metabarcoding, mikroplast

List of publications

This thesis is based on the following papers, which are referred to in the text by roman numerals:

I. A microsensor-based method for measuring respiration of individual nematodes. **Maciute A**., Holovachov O., Berg P., Glud R.N., Broman E., Nascimento F.J.A., & Bonaglia S. (2021). *Methods in Ecology and Evolution*.

II. Reconciling the importance of meiofauna respiration for benthic oxygen demand in coastal sediments. **Maciute A**., Holovachov O., Glud R.N., Broman E., Berg P., Nascimento F.J.A., & Bonaglia S. (2023). *Limnology and Oceanography*.

III. Biotic interactions between benthic infauna and aerobic methanotrophs mediate methane fluxes from coastal sediments. Broman E., Olsson M., **Maciute A**., Donald D., Humborg C., Norkko A., Jilbert T., Bonaglia S. & Nascimento F.J.A. (2024). *ISME*.

IV. Microplastic-induced shifts in meiofauna and macrofauna bioturbation and oxygen penetration depth in subtidal sediments. Ridall A., **Maciute A**., Nascimento F.J.A, Bonaglia S. & Ingels J. (2024). *Marine Pollution Bulletin*.

V. Environmental gradients, not geographic boundaries, structure metazoan communities in Siberian seas. **Maciute A**., Broman E., Nascimento F.J.A., Tesi T., Yakushev E., Wild B., Kirillova E., Semiletov I., Gustafsson Ö., Bonaglia S. *Submitted to eDNA*.

My contribution:

I: Planned the study and sampled in the field, together with co-authors. Designed the incubation system, carried out the experiment, analyzed the data, drafted the manuscript, and wrote revisions during review process.

II: Conceived the study idea, together with co-authors. Led sediment sampling, respiration experiments, sediment microprofiling, whole-core sediment incubations, meiofauna size measurements, data analysis and writing of the manuscript. Carried out revisions during the review process.

III: Performed experiments, together with co-authors. Was responsible for sediment microprofiling, oxygen data analysis. Performed dissolved inorganic carbon analysis. Contributed to drafting the manuscript and addressing reviewers' comments throughout the review process.

IV: Contributed to the planning of the study and was responsible for sediment microprofiling. Participated in the experiment. Substantially contributed to the writing and revisions of the manuscript.

V: Planned the expedition, together with co-authors. Sampled in the field for later molecular analyses and sediment microprofiling. Analyzed the data with substantial contribution from Elias Broman and led the writing of the manuscript.

Other papers published during the PhD but not included in the thesis:

- Intracellular nitrate storage by diatoms can be an important nitrogen pool in freshwater and marine ecosystems. Stief P., Schauberger C., Lund M.B., Greve A., Abed R.M.M., Al-Najjar M.A.A., Attard K., Bonaglia S., Deutzmann J.S., Franco-Cisterna B., García-Robledo E., Holtappels M., John U., **Maciute A.**, Magee M.J., Pors R., Santl-Temkiv T., Scherwass A., Sevilgen D.S., de Beer D., Glud R.N., Schramm A., Kamp A. (2022). *Communications Earth and Environment*, 3(1), 1-11. https://doi.org/10.1038/s43247-022-00485-8

- Methane plume detection after the 2022 Nord Stream pipeline explosion in the Baltic Sea. Abrahamsson K., Damm E., Björk G., Bunse C., Sellmaier S., Broström G., Assmann V., Dumitrascu A., **Maciute A.**, Olofsson N., Pourdanandeh M., (2024). *Scientific Reports*, 14(1), p.12848.

https://doi.org/10.1038/s41598-024-63449-2

- Thin-layer capping with granular activated carbon and calcium-silicate to remediate organic and metal polluted harbor sediment–A mesocosm study. Wikström J., Forsberg S.C., **Maciute A.**, Nascimento F.J.A., Bonaglia S., Gunnarsson J.S. (2024). *Science of The Total Environment*, 946, 174263.
  https://doi.org/10.1016/j.scitotenv.2024.174263

Background

Nematodes do not furnish hides, horns, tallow, or wool. They are not fit for food; they do not produce anything fit to eat; neither do they sing or amuse us in any way; nor are they ornamental—in fact, when they are displayed in museums, the public votes them hideous…What claim, then, one may ask, can such beings lay to our attention?"
Nathan Cobb, considered father of nematology

Why study the biological component of sediments?

Oceans cover 71 % of the Earth's surface making it the largest ecosystem. Entire ocean floor is covered in sediment which can contain higher concentrations of nutrients and 10–10.000-fold more microbial cells per unit volume than the overlying water column (Jørgensen & Boetius, 2007). Therefore, out of all habitats on Earth, sediments form the prime site of organic carbon burial and are hotspots of carbon and nutrient cycling. The seafloor landscape that we see today is shaped by bottom-dwelling organisms and without them, benthic ecosystems are fundamentally different – homogeneous and overall, less efficient in processing organic matter (Thrush et al., 2021).

Benthic (bottom) organisms that reside within marine sediments are divided into three major groups depending on their size:

- microorganisms (less than 0.04 mm in size, such as bacteria, protozoans)
- meiofauna (animals smaller than 1 mm but larger than 0.04 mm, as well as foraminifera and juvenile stages of macrofauna: known as "temporary meiofauna"); *size ranges vary across literature
- macrofauna (a few millimeters to several centimeters in size).

These organisms by moving, feeding, and excreting in the sediment change distribution of sediment particles, solutes, and microorganisms (Figure 1). Subsequently, such activities create new niches, and alter rates and pathways of benthic mineralization and inorganic nutrient fluxes (Bonaglia et al., 2014; Nascimento et al., 2012). For example, macrofauna through bioturbation can modify contaminant bioavailability (Remaili et al., 2016), stimulate greenhouse gas fluxes (Bonaglia et al., 2017), and increase denitrification which removes fixed nitrogen, and thus potentially increase

phosphorus retention in the sediment, thereby alleviating eutrophication (O'Meara et al., 2020). All these processes are important for ecosystem services, which are fundamental to human well-being and societal development (Figure 1).

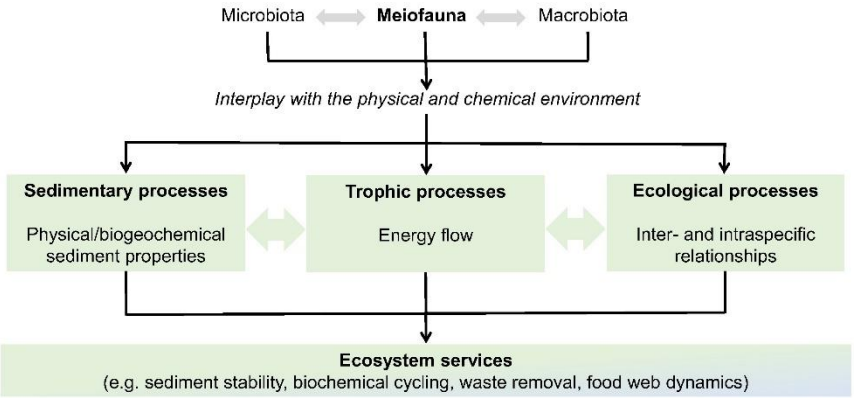

Figure 1. Schematic diagram depicting benthic fauna mediated effects on sediment, trophic and ecological processes from which desired ecosystem services are derived (Schratzberger & Ingels, 2018)

Generally, microorganisms (bacteria, archaea) are considered the key players in biogeochemical cycling due to their high abundance and great variety of metabolic pathways. In addition, some microorganisms are the quickest to respond to a sudden increase of organic matter availability, with prokaryotic abundances doubling within just a few days following carbon enrichment (Manna et al., 2020; Sebastián et al., 2019). Although macrofauna are much less abundant, their larger size makes them relatively important contributors as well. The importance of benthic macrofauna for benthic-pelagic coupling as well as cycling and storage of carbon, nitrogen, phosphorus and oxygen within the sediment has been widely acknowledged (Glud, 2008; Karlson et al., 2007; Politi et al., 2019). Meiofauna, on the other hand, are not as abundant as microorganisms and are smaller than macrofauna. Therefore, the question arises: does relatively high meiofauna abundance translate into significant effects on ecosystem processes? (Schratzberger & Ingels, 2018). To answer this question, data on meiofauna diversity, density, biogeography and its activity is needed.

What exactly is meiofauna?

The term *meiofauna* loosely defines organisms by their size, which you cannot see by naked eye. It is somehow paradoxical that while the sole defining characteristic is their size, meiobenthologists still lack consensus on the precise size limits that define this group today. For example, some studies refer to meiofauna as benthic invertebrates or animals that are smaller than 1 mm (Ballentine & Dorgan, 2024; Broman et al., 2019). Some studies define size range between 0.5 mm and the lower limit set as either 44 μm, 63 μm, 31 μm, 38 μm or 20 μm (de França et al., 2024; Giere, 2008; Ptatscheck et al., 2020a; Sautya et al., 2024). Small juvenile meiofauna and rotifers are often not retained on the 40 μm sieve, leading to underestimation of meiofauna abundance and diversity (Ptatscheck et al., 2020b). Thus, it has been recently suggested that the size range should be 1 mm to at least 20 μm as current definition of meiofauna is not sufficient to encompass the entire spectrum of meiofauna present in a sample (Ptatscheck et al., 2020a).

It is true that size definition depends on study area. For example, deep-sea and extreme environments in general tend to harbor smaller organisms as an adaptation to pressure, low temperature, limited food availability and etc. (Zeppilli et al., 2018). Nevertheless, even within coastal marine studies there is no strict size rage for meiofauna. This is one of the challenges when standardizing meiofauna research protocols. I, throughout my thesis, focus on meiofauna in the size range between 1 and 40 μm, which seems to be the most used range in studies dealing with marine and brackish environments (Giere, 2008). In addition, identification and handling of meiofauna smaller than 40 μm was not possible during my thesis.

Because the body size is the only characteristic defining meiofauna, it is not surprising that at least every third animal on the planet is meiofauna (Van Den Hoogen et al., 2019). Moreover, more than 20 phyla have meiofaunal representatives, making them both abundant and diverse component of aquatic sediments and terrestrial soil communities worldwide. In natural environments, free-living marine nematodes are the most dominant and diverse meiofaunal group (Figure 2). Usually, they account for 70–90 % of meiofauna abundance (Austen, 2004).

Figure 2: Four most observed meiofauna groups throughout the thesis.

Other, common marine meiofauna groups are foraminifera, ostracods, and kinorhynchs (Figure 2). Although copepods were not as abundant in my microscopy samples, they tend to comprise a significant 10 % of the total meiofauna in marine systems (Giere & Schratzberger, 2023). Important to note, some macrofauna qualify as meiofauna during part of their life cycles, and thus are so-called temporary meiofauna. For example, juveniles of polychaetes, oligochaetes, bivalves start their life within the size range of meiofauna but grow into macrofauna as they develop (Giere, 2008).

Where meiofauna can be found?

Everywhere. Perhaps the greatest motivation to study meiofauna stems from their ubiquity. Meiofaunal organisms are found even in the most extreme and unusual environments both in terrestrial and aquatic systems. Devil worms (nematodes) are the deepest-living animals, inhabiting 3.6 kilometers under the Earth surface (Borgonie et al., 2011). Nematodes and water mites have also been found in highly acidic (pH=0–3) bacterial mats (Borgonie et al., 2010), and chemosynthetic methane seep environments (Sergeeva & Gulin, 2007). Some nematodes are even able to survive intracellular ice formation in Antarctica or endure arsenic-rich alkaline lake water (Raymond & Wharton, 2016; Shih et al., 2019). Meiofaunal communities have been recently described on sea turtles' carapaces, travelling across the oceans (Ingels et al., 2020). Even temporary water bodies, such as water retained by plants (Zotz & Traunspurger, 2016) or water-filled tree holes get colonized by numerous meiofauna within a week (Ptatscheck & Traunspurger, 2014). It is noteworthy that not always extreme environments are inhabited by a specific set of species (Vieira &

Fonseca, 2013), and so the mechanisms which allow some meiofauna to tolerate extreme conditions (e.g., low oxygen, high toxic sulfide or arsenic concentrations) remain unclear. We can only assume that life in extreme environments offers favorable trophic conditions and the absence of food competitors.

Such widespread distribution of meiofauna, coupled with its direct benthic development and presumed limited dispersal, have given rise to the "meiofauna paradox" phenomenon. The paradox refers to the puzzling questions: how can so many meiofaunal taxa from distant and isolated areas be so similar? Shouldn't their limited dispersal lead to distinct populations in different habitats? (Cerca et al., 2018). Although various mechanisms have been proposed to explain cosmopolitan distribution of meiofauna, it is most likely that meiofauna paradox does not exist and it was a by-product of taxonomic challenges, sampling biases and the occurrence of cryptic species (Cerca et al., 2018). Nevertheless, the clarification of this debate is a work in progress.

Literature data on meiofauna abundance in marine sediments is too scarce to produce a global meiofauna abundance or biomass map. In addition, the lack of standardized protocols for meiofauna analysis and collection presents a major challenge for integrating diverse meiofauna studies into a broader investigation of meiofauna biogeography (Giere & Schratzberger, 2023). For now, such dataset only exists for soil nematodes (Figure 3), and it shows that soil nematode biomass represents 82 % of total human biomass on Earth (Van Den Hoogen et al., 2019). By combining such biomass data with nematode metabolic activity data, it was shown that nematodes may be responsible for a turnover of 0.14 gigatons of carbon per month, out of which 0.11 gigatons is respired back to the atmosphere as a carbon dioxide (CO_2) (Van Den Hoogen et al., 2019). This means that carbon respiration of all 1 mm-long nematodes is equivalent to 15 % of carbon emissions from fossil fuel use (Van Den Hoogen et al., 2019).

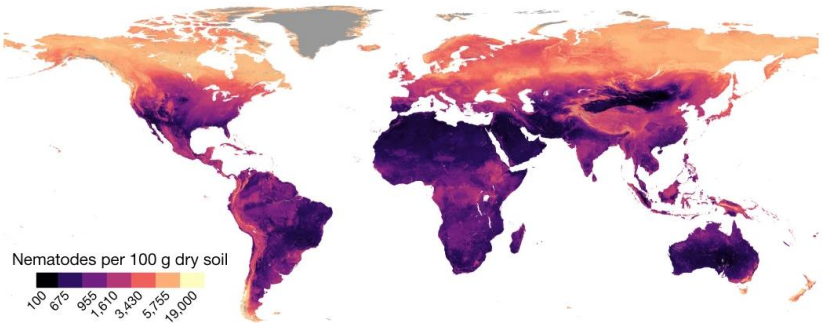

Figure 3: Global soil nematode abundance, in individuals per 100 gram dry soil (Van Den Hoogen et al., 2019). The map was created combining soil sample data from >6000 locations with 73 global environmental covariate data layers (including soil properties, vegetation, climate, etc.) using machine learning

Given that the biomass of nematodes in marine systems is likely higher, it would be interesting to estimate how much marine nematodes contribute to this budget. Yet, in addition to the absence of data on global nematode biomass and diversity, in this thesis (**papers I** and **II**), I addressed several concerns related to the theoretical estimations of marine nematode activity (respiration) used in Van Den Hoogen et al. (2019) study to assess nematode contribution to carbon turnover, which hamper us from making accurate estimations. Nevertheless, the Van Den Hoogen et al. (2019) study revealed an unexpected pattern: the highest nematode abundance is in boreal regions, and not in the tropics. Such enormous dataset shows that namely soil organic carbon content, which is higher in the boreal region, is the key factor regulating nematode abundance (Van Den Hoogen et al., 2019).

In marine sediments, organic carbon content has also been identified as one of the most important factors in shaping meiofaunal community structure and abundance, when oxygen is not limited (Bianchelli et al., 2020; Neira et al., 2001). In addition, other environmental conditions (e.g., depth, pressure, temperature, salinity, dissolved oxygen, physical sediment characteristics) as well as primary production, geographical location and interactions with predators and preys can be even more important than the sediment organic carbon content alone (Broman et al., 2019; Giere & Schratzberger, 2023; Neira et al., 2001). However, interactions between physical, chemical and biological conditions in each marine habitat are

enormously complex, leading to patchy diversity and abundance of meiofauna within each habitat. Relative importance of each of those factors might depend on the ecology and physiology of specific taxa (Giere & Schratzberger, 2023).

Studying systems subjected to anthropogenic pressures adds another layer of complexity. For example, in **paper V**, I aimed to better understand meiofauna diversity and distribution in Siberian Arctic. Studies in such areas are challenging not only because of the remoteness but also due to the habitat instability caused by rapid environmental change. In particular, the Arctic is warming four times as fast as the global average rate (Rantanen et al., 2022), resulting in severe reduction and thinning of sea-ice cover (Selivanova et al., 2024), acidification (Semiletov et al., 2016), increasing coastal erosion, permafrost degradation (Frey & McClelland, 2009), and river discharge (Mann et al., 2022) that bring even more particulate matter, carbon, nutrients, and fresh water to the coastal seas. The interplay between natural and anthropogenic factors becomes crucial in understanding how meiofauna adapt and respond to changing environments. Ultimately, this understanding is essential for predicting the resilience of these communities.

To complicate things even more, the sediment is a three-dimensional habitat. And although we lack the data on horizontal meiofauna distribution across the oceans, a significant effort has been made to better understand vertical meiofauna distribution in the sediments. Meiofauna's miniscule size enables them inhabit space between sediment particles, though some meiofauna such as ostracods, copepods mainly inhabit the sediment-water interface. The highest meiofauna abundance and diversity is often observed at the topmost 0–2 cm muddy, or 0–5 cm sandy sediment layers, and tend to decrease with sediment depth generally down to 10 cm depth (Giere, 2008). Some living meiofauna, such as foraminifera, can be observed at considerable depths of about 50 cm inside the sediment (Choquel et al., 2021). Yet, it is important to note that meiofauna community patterns in the sediments are better explained by changes in redox potential than sediment depth *per se* (Vieira & Fonseca, 2013).

How does meiofauna affect sediment biogeochemistry?

To understand how meiofauna change the sediment, one first needs to understand chemical gradients in the sediment (Figure 4). The most extreme (i.e., steepest) chemical gradient is in the uppermost sediment layer, where the transition between aerobic (oxic) and anaerobic (anoxic) conditions occur (Glud, 2008). This layer typically ranges from only a few millimeters in coastal sediments to several tens of centimeters in the deep-sea, and depends on the sediment organic matter content (Glud, 2008; Kristensen, 2000). All animals prefer to inhabit this oxic, generally thin sediment layer. The sharp oxic/anoxic interface at the end of the oxygen gradient is generally followed by nitrate and later toxic sulfide concentration peaks (Figure 4). Since majority of the sediment volume is anoxic, iron, manganese, and sulphate respiration processes can dominate organic carbon mineralization, and their relative importance may depend on water column depth (Kristensen, 2000).

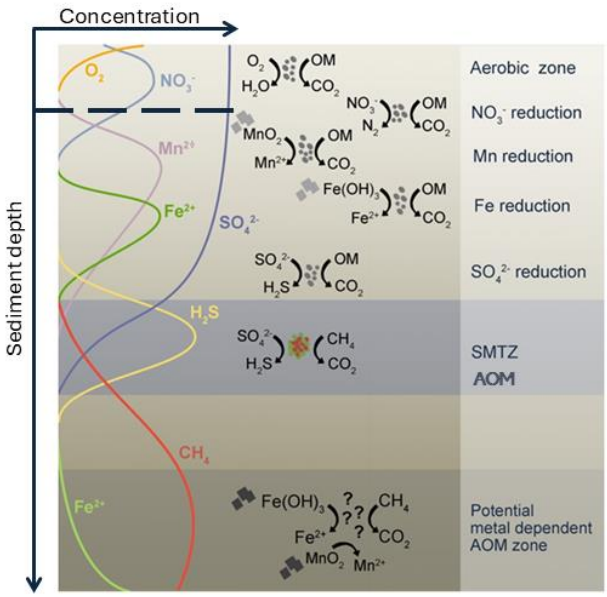

Figure 4. Schematic of vertical distribution and sequential utilization of electron acceptors in marine sediment. The potential metal dependent AOM zone was proposed based on the geochemical data. Sulphate-Methane Transition Zone (SMTZ) where anaerobic oxidation of methane (AOM) is coupled to sulphate reduction. Maximum bioturbation depth indicated as dashed line. Modified from Liang et al. (2019).

Sediments are inhabited by microorganisms that participate in biogeochemical processes e.g., oxygen respiration, nitrification, sulfide oxidation, manganese oxide reduction (Figure 4) (Kristensen, 2000). These microorganisms interact with meiofauna through predator-prey, symbiosis, or commensalism relationships. Thus overall, meiofauna can affect sediment biogeochemistry directly through moving (mixing) in the sediment, respiring, excreting, and indirectly – through interactions with microorganisms and potentially macrofauna.

Meio-bioturbation

Bioturbation is a transport of sediment particles and porewater by animals and plants. I believe, it is one of the most important concepts in coastal biogeochemistry, as it can drastically alter chemical gradients presented in figure 4, stimulate chemical reactions and sediment-to-water fluxes. As mentioned above, animals prefer living in the oxygenated sediment layer, and it is namely bioturbation which can expand this layer significantly.

From the first sight, meiofauna bioturbate sediment to the lesser extent compared to macrofauna. Yet, foraminifera populations have been shown to have high surface sediment reworking (i.e., particle replacement) rates, which were comparable to or even exceeding those of macrofauna populations (Bouchet & Seuront, 2020). Thus, it is reasonable to expect that bioturbation by meiofauna i.e., meiobioturbation can have a significant effect on oxygen penetration depth (OPD) in the sediment. It has been shown that when meiofauna abundance is high (up to 2000 ind. 10^{-3} m^{-2}), meiofauna can deepen oxygen penetration by 62–85 % (Bonaglia et al., 2020; Bonaglia & Nascimento, 2023). But this has only been shown for low-oxygen (hypoxic) sediment (with OPDs of~1.5 mm), devoid of macrofauna (Bonaglia et al., 2020). Similarly, in an experiment, excluding macrofauna, OPD was on average 20 % deeper in sediment containing 30 foraminifera per cm^{-2}, compared to sediment without foraminifera (Langlet et al., 2023). Whereas, in oxygenated sediment (~5 mm OPD) with some treatments having macrofauna, meiobioturbation did not extend the oxic layer in the sediment significantly (Bonaglia et al., 2014). Thus, meiofauna have significant effect on oxygen penetration

depth only in sediments with very shallow oxygen penetration and / or sediments devoid of macrofauna.

Meiobioturbation can also affect the transport of solutes (Bonaglia & Nascimento, 2023). Meiofauna (mainly nematodes and foraminifera) are found even in anoxic sediment layers, meaning that these organisms continuously oscillate between microoxic/anoxic niches and layers. As chemistry between these layers is different (Figure 4), meiofauna act as vertical conveyors between surface and deeper sediment thereby altering vertical chemical gradients, sediment porosity and solute transport compared to uninhabited by meiofauna sediments (Aller & Aller, 1992; Coull, 1999). Particularly, solute transport was enhanced by a factor of 1.5–2.0 (Aller & Aller, 1992; Rysgaard et al., 2000). Meiofauna has also been shown to remove toxic sulfide by introducing oxygen into deeper sediment layers, thereby reducing sulfide fluxes from 8.8 to 0.4 mmol m^{-2} d^{-1} (Bonaglia et al., 2020). Yet again, these examples are from the sediment devoid of macrofauna.

While relative abundance of meiofauna is high in the deep-sea and hypoxic sediment, effects of meiobioturbation are still to be investigated in presence of macrofauna. Although studies investigating meiobioturbation when macrofauna is present are lacking, foraminiferal burrowing depth was shown to be dependent on the foraminifera density (Deldicq et al., 2023). At the highest densities (90 indiv. cm^{-2}), foraminifera *H. germanica* exhibited lower burrowing depth (max depth 1.53 cm), compared to the lower density (30 indiv. cm^{-2}) treatment (max burrowing depth 1.93 cm) (Deldicq et al., 2023). Yet, at the lowest foraminifera densities (10 indiv. cm^{-2}), the burrowing depth did not increase more (max depth 1.87 cm) (Deldicq et al., 2023). This was explained by intra-specific competition for trophic resources and space at the highest densities, which might hamper the individual feeding rate and crawling behavior, and thus will ultimately have implications for vertical solute transport in the sediment (Deldicq et al., 2023).

Due to limited research, our understanding of meiobioturbation effect on solute transport remains incomplete, highlighting the need for further studies to elucidate this complex process, as pointed out by Bonaglia and Nascimento (2023). Such studies would benefit the most from application

of planar oxygen optodes, which visualize two-dimensional oxygen distribution over an area of sediment (Glud et al., 1996). The method has been successfully used to demonstrate macrofauna bioturbation and bioirrigation patterns, small-scale spatial and temporal oxygen heterogeneity, as well as oxygen microdynamics around burrows (Volkenborn et al., 2007; Wenzhöfer & Glud, 2004). However, the planar oxygen optode system needs to be optimized for investigating meiobioturbation, as this will require significant increase in system's magnification and resolution.

Respiration

Meiofauna consume oxygen and produce carbon dioxide through respiration. How much meiofauna contributes to total sediment oxygen uptake (TOU) or carbon cycling is difficult to estimate due to methodological constraints. This challenge arises from insufficient sensitivity of standard respirometry approaches for measuring respiration. The respiration rates that we know today are mainly from pioneer respiration studies using sophisticated Cartesian divers (discussed below) or theoretical calculations of respiration rates (Kennedy, 1994; Linderstrøm-Lang, 1937). Based on which, nematode contribution to total sediment oxygen uptake ranges from 0.5 to 14 % (De Bovée et al., 1996; Soetaert et al., 2009). Whole meiofauna community accounts for 5 % of TOU in Arctic fjords (Kotwicki et al., 2018), 1–22 % in the deep-sea (Baguley et al., 2008; Heip et al., 2001; Shimabukuro et al., 2022), and equal meiofauna and macrofauna contributions account for a total of 12 % on New Zealand's continental margin (Leduc et al., 2016). Question remains, however, how accurate these estimates are i.e., how well theoretical respiration rate calculations and respiration rate measurements in closed vials are supported by empirical evidence? This knowledge gap was addressed in **papers I** and **II**.

In addition to oxygen respiration, it has been demonstrated that certain benthic foraminifera can respire nitrate (denitrify) in deep anoxic sediment layers (Risgaard-Petersen et al., 2006). For example, denitrification by *Nonionella sp. T1* benthic foraminifera species can account for up to 50–100 % of the porewater nitrate loss from the sediment below oxic bottom

waters in Gullmar Fjord (Choquel et al., 2021). Therefore, the impact of these foraminifera is particularly significant to sediment biogeochemistry, especially considering this species is non-indigenous to the area and already dominate local foraminifera communities, with a relative abundance ranging from 15 and 72 % in the Gullmar Fjord (Polovodova et el., 2023). It is important to note, however, that foraminiferal denitrification can be of little importance in deep ocean margin sediment. It has been shown that, despite the fact that nitrate stored inside foraminifera represented 80 % of the total benthic nitrate, foraminiferal denitrification was responsible only for ~4 % of the sediment nitrogen (N_2) production (Glud et al., 2009).

Excretion

Meiofauna excrete nitrogen and phosphorus-rich organic compounds (Schratzberger & Ingels, 2018) and thus have a potential to directly affect sediment chemistry. Aquatic organisms, excrete urea, uric acid and ammonium – the major nitrogenous waste product as it can diffuse across membranes and is quickly washed away because it is soluble in water (Edwards et al., 2023). Ammonium can be assimilated by other organisms, sequestered onto sediment particles, and used for nitrification or anammox processes (Bonaglia & Nascimento, 2023).

Unfortunately, just like in the case of respiration measurements, we lack sensitive methods to measure meiofaunal excretion. To my knowledge, there are two studies investigating marine meiofaunal excretion rates. One study estimated excretion rates of copepods inhabiting rockpools to be 1.0504 – 1.3237 μg N mg^{-1} dry weight h^{-1} (Harris, 1973). These rates have been suggested to be on a higher end, as rock pools could be classified as an extreme environment and be inhabited by organisms with higher metabolic rates (Gray, 1985). The second study investigated meiofauna (nematodes, copepods, polychaetes, turbellarians) in coral sediments, and reported excretion rates of 0.621 μg N mg^{-1} dry weight h^{-1} (Gray, 1985), with no estimates of how much it contributes to total nitrogen sediment pool. For comparison, terrestrial bacterivorous nematodes on average excrete about 100 times less nitrogen per unit of biomass (0.006

μg N mg^{-1} h^{-1}, assuming that dry weight is approximately 25 % of the wet weight (Lang & Russell, 2022).

Grazing on bacteria

For the most part, however, it is probably not the meiofauna themselves who significantly and directly alter sediment biogeochemistry but microbial communities in presence of meiofauna. There are several suggested mechanisms by which meiofauna potentially stimulate activity of microbial communities (Coull, 1999):

- meiofauna mechanically break down large detrital particles thereby making them more available to microbial digestion.
- meiofauna directly excrete nutrients (metabolic waste containing nitrogen and phosphorus) for microbial use.
- meiofauna produce slime/mucus which attracts and sustains bacterial growth.
- meiofaunas' grazing on bacteria helps to keep bacterial growth in the log phase, and, therefore, the bacteria metabolize faster.
- meiofauna, by vertically migrating through the redox zone, supply their chemosynthetic ectosymbionts with essential substrates.

It has been shown that meiofauna (at abundance of 784±133 ind. 10^{-3} m^{-2}) led to doubled denitrification rates (Bonaglia et al., 2014). Potentially, excretion of nitrogen-rich mucus provided ammonium for nitrifiers, and nitrate to heterotrophic denitrifiers, thereby stimulating microbial activity (Bonaglia et al., 2014). Similarly, organic matter mineralization, was doubled when meiofauna abundances were 585±89 ind. 10^{-3} m^{-2}, and while underlying mechanisms could not be identified in the study, it has been speculated that both meiobioturbation and grazing on bacteria played a significant role (Nascimento et al., 2012).

Diffusive oxygen uptake (DOU) is mainly driven by microbial mediated aerobic respiration and oxidation of reduced constituents from anaerobic mineralization processes (Glud, 2008). To minor extent, respiration of protozoans and meiofauna may also contribute directly to the DOU, but in theory, the main mechanism through which meiofauna could affect DOU is their interactions with microorganisms. Such indirect effect of

meiofauna on DOU has been investigated in two studies (Bonaglia et al., 2014; Langlet et al., 2023), and briefly reviewed in Bonaglia et al. (2020). Both studies suggest that meiofauna may lower DOU rates, with explanations provided through two different mechanisms. Because Langlet et al. (2023) observed positive correlation between sediment organic carbon content and bacterial richness, authors assumed that foraminifera by feeding reduced the amount of labile organic carbon in the sediment which in turn lowered bacterial richness resulting in reduced DOU rates. Whereas Bonaglia et al. (2014) speculated that meiofauna predation on aerobic bacteria and protozoans may have led to the relative reduction in DOU rates.

However, these explanations remain speculative. This is because the top-down effects of meiofauna grazing on microorganisms require further investigations. Although meiofauna is capable eating its body weight equivalent in microorganisms each day, microorganisms have high turnover rates (5.5–30 h) (Montagna, 1984). Preliminary results suggest that the hourly meiofauna grazing rates correspond to only 3 % of the standing stock of bacteria (Montagna, 1984). Yet, it is noteworthy that bacterial communities are continuously replenished by constant bacterial growth. In addition, while meiofauna grazing rates remained nearly the same between winter and summer, microorganism activity differed by 4 to 5 times between the seasons (Montagna, 1984). This indicates that bacterial activity was primarily influenced by abiotic mechanisms, rather than meiofauna grazing. Therefore, although many studies suggest that meiofauna grazing could stimulate microorganism activity, none of them provide direct evidence. This knowledge gap is mainly due to the previously used labor-intensive methods required to count microbial cells. However, the use of ^{14}C-labeled bacteria as a tracer of ingestion in addition to quantitative PCR (qPCR) technique offers a more efficient and accurate approach for quantifying microbial populations, and ingestion rates potentially addressing this limitation.

An opposite, but perhaps more likely, relationship between meiofauna and DOU was found in the deep-sea (Shimabukuro et al., 2022). Shimabukuro et al (2024) documented a strong correlation between DOU and abundance of meiofauna (Figure 5). It was suggested that the underlying reason for this relationship is that DOU serves as a reliable proxy for

microbial activity and growth, which is the primary food sources for the dominant bacterivorous meiofauna. The direct contribution of meiofauna through respiration accounted for only a small percentage of the total benthic O_2 consumption rate (Shimabukuro et al., 2022). Therefore, this study demonstrates that food availability was the primary factor regulating the abundance and biomass of meiofauna. Moreover, it shows that meiofauna had minimal impact on both sediment O_2 consumption rate and microbial biomass, which were constrained by the availability of labile organic material (Shimabukuro et al., 2022). These relationships, however, may depend on meiofauna feeding guild as well as sediment organic carbon content and quality.

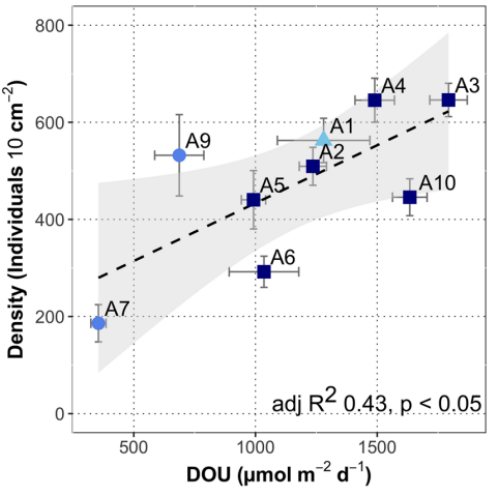

Figure 5. Regressions of meiofauna density against diffusive oxygen uptake (DOU). Light blue triangle – bathyal depth; blue circles – abyssal depths; dark blue squares – hadal depths. Figure from Shimabukuro et al. (2022).

Because DOU provides insights into the rate of organic matter mineralization, it is crucial to analyze microbial communities and explore their diversity and abundance in relation to sediment organic matter content and quality, and meiofauna abundance, in order to better understand benthic carbon cycling.

How does meiofauna respire?

A significant part of this PhD thesis is about meiofauna respiration and its oxygen requirements to survive (**papers I** and **II**). This is an important

topic because just like all animals, meiofauna (except some foraminifera) require oxygen to survive. Yet, at the same time, it is known that some meiofauna inhabit deep anoxic sediment layers (Powell, 1989). Also, meiofauna are the only animals found in the so-called dead zones – bottoms deprived of oxygen for prolonged periods of time (Broman et al., 2020b).

Meiofauna do not have specialized respiratory organs, instead they take up oxygen and release carbon dioxide through their body surfaces (Giere, 2008). Small meiofauna size results in high surface area-to-volume ratio, which allows them to exchange gases efficiently with their environment. As a result, their respiration rates tend to be relatively high, especially compared to larger animals (Gerlach, 1971). For example, it has been suggested that meiofauna has a metabolism five times more active than that of macrofauna (Gerlach, 1971). In addition to small body size, meiofauna must be thin enough to supply their tissues with oxygen through oxygen diffusion (Powell, 1989). This is especially important in low oxygen environments. Braeckman et al. (2013) defined "critical thickness" as the minimum nematode body width, keeping their tissues oxygenated (Figure 6A). Confirming that thinner nematodes are more adapted to oxygen-poor areas or deeper sediment layers.

Anoxia in marine sediments is often accompanied by the presence of toxic hydrogen sulfide (H_2S) (Figure 4) and therefore some nematodes harbor symbiotic sulfur-oxidizing bacteria on their cuticles, that can may protect nematodes from the toxic H_2S (Bellec et al., 2019) (Figure 6B). Yet, cuticular symbionts potentially increase meiofauna body thickness and thus substantially decrease diffusion of oxygen through the meiofauna body wall. This is an interesting problem in meiofauna inhabiting low oxygen environments. For now, we are only starting to describe meiofauna symbionts (Bellec et al., 2019). How meiofauna meet their oxygen requirements is unclear, yet intriguing to explore.

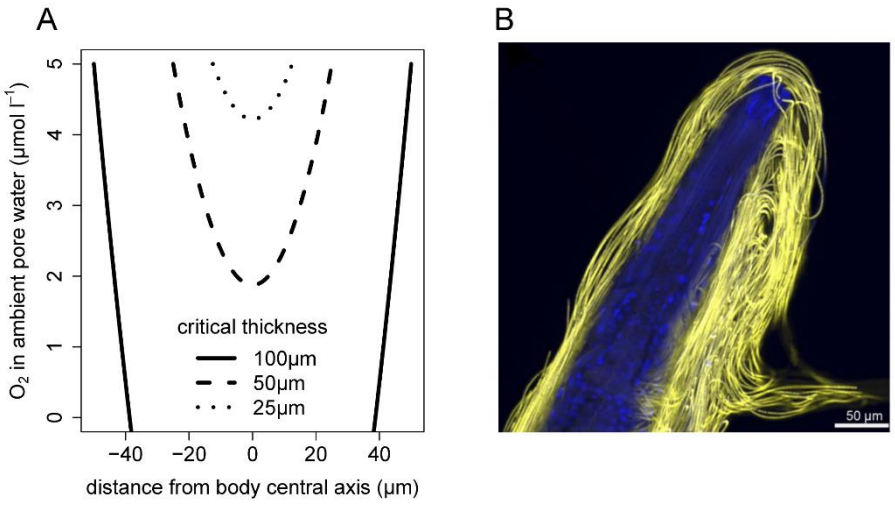

Figure 6. Oxygen concentration in ambient pore water and modelled body wall as a function of nematode body radius in suboxic conditions by Braeckman et al. (2013)(A), anterior region of nematode *M. albidus* (in blue) colonized by Eubacteria (in yellow) from Bellec et al. (2019)

Knowing a respiration rate of an organism can give an indication about its metabolic requirements to survive and an estimate of how much an organism contributes to total sediment oxygen uptake. Thus, respiration rate can answer questions at both an individual and ecosystem levels. Not surprisingly, there have been previous attempts to measure respiration rate of meiofauna. The very first attempts were made by applying Cartesian diver method, which is based on density changes associated with oxygen consumption by an organism inside a sealed glass vessel (Linderstrøm-Lang, 1937). However, due to very low respiration rates by small organisms, incubations needed to last up to 30 hours to observe density changes. Moreover, the method itself was extremely complicated and required to manufacture sealed vessels with several microscopic compartments: one for an animal and other for chemicals and gasses (Welsh, 2016). Those were the reasons why the method has never been used for routine meiofauna respiration measurements. Instead, nematode respiration was measured by pooling up to several hundreds of individuals in an enclosed respiration chamber and then recalculating obtained data to an individual respiration rate (IRR) or to respiration per microgram of biomass (Moodley et al., 2008). By doing so, oxygen consumption of

microscopic specimens can be detected when using regular polarographic or optic oxygen sensors, yet it is difficult to accurately assess respiration as a function of species, age, and sex. Moreover, respiration rate may depend on the number of animals incubated in the chamber.

Respiration can also be derived from empirical allometric relations based on biomass (or volume) and metabolic intensity of meiofauna (Mahaut et al., 1995) – a method I refer to as the calculation of theoretical respiration rate in **papers I** and **II**. It is typically expressed mathematically as: $Respiration = a \times X^b$ (or when log-transformed $log_{10}IRR = a + b \times log_{10}X$, where X represents biomass (or volume), and a and b are regression coefficients related to organisms' biology (i.e., metabolic intensity, behavior, feeding guild) (Kennedy, 1994). However, to my knowledge there are no estimates available to assess how well the theoretical respiration rate calculations align with the measured rates.

Overall, meiofauna constitute a significant part of seafloor biomass and play an important role in benthic metabolism. However, respiration measurements are limited, and the methods used are diverse together complicating comparison or upscaling and thus it is difficult to draw a precise picture of meiofauna role in benthic respiration. In addition to understanding how meiofauna interact with their surrounding sediment, it is increasingly important to examine how human-induced environmental changes (e.g., microplastic pollution) influence these interactions. Anthropogenic factors introduce new variables that may disrupt natural processes, compelling us to investigate their impact on interstitial communities and sediment dynamics concurrently.

How does microplastic pollution affect meiofauna?

About 8 million tons (equivalent to 800 Eiffel towers) of plastic waste finds its way into the ocean every single year, and there is evidence of increasing quantities over time (Napper & Thompson, 2020). Plastic particles that are between 5000 and 1 μm in size are called microplastics. Microplastics enter the ocean as either primary microplastics, which are intentionally manufactured at a small size, or secondary microplastics, which are generated from the breakdown of larger plastic items (Napper &

Thompson, 2020). Global studies on microplastics concentration in the sediment are lacking but one is clear – concentrations tend to be higher in sediments than in the water column (Hoseini & Bond, 2022; Maes et al., 2017; Sagawa et al., 2018).

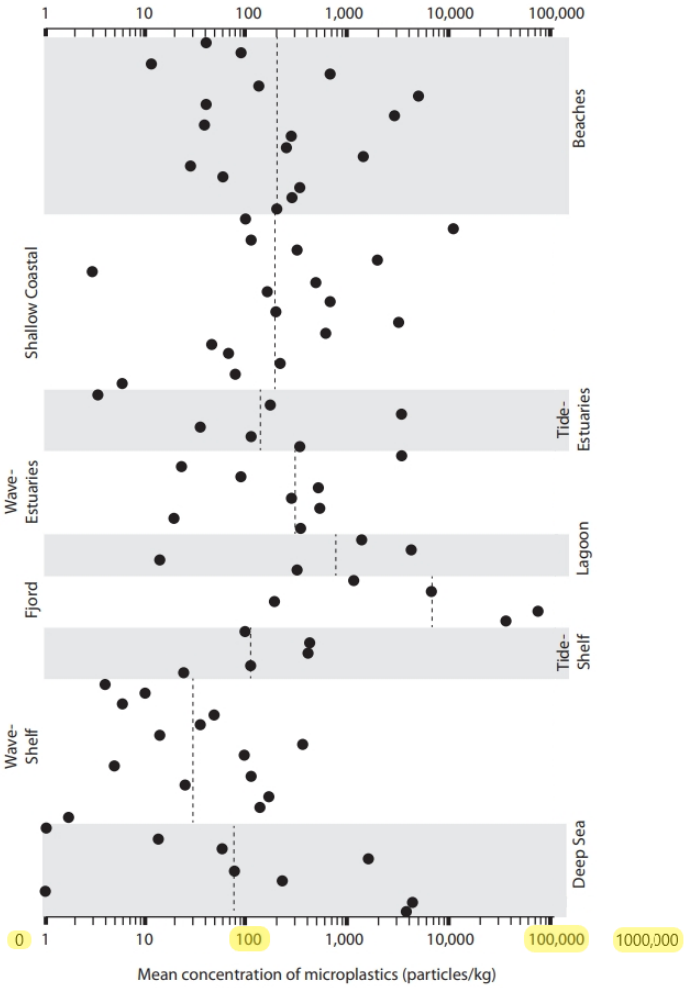

Figure 7. Log of mean concentrations of microplastics particles per kilogram of dry sediment reported in 80 studies for different sedimentary environments: sandy beaches on exposed coasts; shallow water depositional environments including tidal flats and embayments; tide-dominated estuaries and deltas; wave-dominated estuaries and deltas; lagoons and coastal lakes; fjords; tide-dominated continental shelves; wave-dominated continental shelves; and deep-sea environments. The vertical dashed lines are median values for each environment. Figure modified from Harris (2020). The yellow-highlighted x-axis values show microplastic concentrations added to sediment mesocosms for each treatment in the experiment presented in **paper IV**.

Reported microplastic concentrations in the sediment range over five orders of magnitude (Figure 7) (Harris, 2020). Based on literature review, median microplastics concentration is the highest in fjords at 7000 particles kg^{-1} dry sediment, followed by 300 in estuarine environments, 200 in beaches, 200 in shallow coastal environments, 50 on continental shelves and 80 particles kg^{-1} for deep-sea environments (Harris, 2020). In **paper IV**, which investigated the effects of microplastics on fauna in subtidal sediments, the maximum observed microplastics concentration in the area was around 90±30 particles kg^{-1} dry sediment (Ridall & Ingels, 2022). Thus, the 10^2 microplastic particles kg^{-1} dry sediment concentration represented current microplastics levels in the investigated subtidal sediment in **paper IV**. Whereas medium and high concentration treatments (10^4 and 10^6 particles kg^{-1} dry sediment, respectively) were used to assess the potential impact of future microplastics concentrations on fauna.

Meiofauna can ingest microplastics, raising concerns about the potential transfer of these microplastics to higher trophic levels, as many larger organisms rely on meiofauna as a food source (de França et al., 2024; Gusmão et al., 2016; Ridall et al., 2023). Yet, meiofauna also egest the microplastics, and the time between ingestion and egestion can be less than an hour for some nematodes and copepods or at least be similar to that of natural dietary materials (Au et al., 2015; Fueser et al., 2020; Li et al., 2020). Therefore, whether meiofauna can truly ingest large quantities of microplastics and transfer them to higher trophic layers is still unclear. This potentially depends on microplastics concentration in the sediment, type of microplastics, exposure time, and retention time (time between ingestion and egestion) (Au et al., 2015).

The existing literature on microplastics effects is limited and report highly variable outcomes. For example, microplastics can alter meiofauna community composition and reduce their abundance (Lagos et al., 2023), with mixed effects on nematode diversity – ranging from reductions (Bellakhal et al., 2023) to no impact (Rauchschwalbe et al., 2022). In another study, no significant effects were observed on the abundance and biomass of meiofauna (nematodes, rotifers, oligochaetes, gastrotrichs, nauplii), while harpacticoid copepod abundance and biomass were affected (Rauchschwalbe et al., 2022). From a functional perspective, microplastic

pollution combined with sediment organic enrichment were shown to increase abundance of opportunistic nematodes and copepods and alter the trophic structure by increasing relevance of epistrate-feeder nematodes (Corinaldesi et al., 2022).

These community-level changes could be due to changed texture of the sediment after microplastics deposition, toxic effects after ingestion of microplastics, or via absorption of leached chemicals from microplastics through cuticle in soft-bodied organisms or foraminifera (Figure 8) (Bouchet et al., 2023; Corinaldesi et al., 2022; Lei et al., 2018). In addition, ingested microplastic beads have been shown to damage guts of terrestrial nematodes (Lei et al., 2018), though no physical damage from the passage of microplastic fibers through the gut was observed for meiofaunal annelids (Gusmão et al., 2016).

An interesting observation has been recently made using epifluorescence microscopy, which shows that microplastic particles were affixed to locomotory appendages and exterior of copepods, turbellarians, water mites, tardigrades and nematodes which may disturb their locomotion, for instance, through a reduction in swimming speed (de França et al., 2024). While direct evidence is lacking, the logical extension of such reduced meiofauna mobility (e.g., meiobioturbation) suggests significant implications for sediment biogeochemistry. Similarly to these results, benthic foraminifera have been shown to incorporate microplastics to their tests, potentially exposing them to the leaching of harmful chemicals from microplastics (Bouchet et al., 2023). Study investigating the toxicity of microplastic (polypropylene) leachates showed no effect on foraminifera locomotion speed and direction, nor respiration rates (Langlet et al., 2020). However, as the authors suggest, the observed lack of effect on foraminiferal activity may be due to the relatively short-term (10 hours) exposure to leachates (Langlet et al., 2020). In addition, accumulated microplastic particles inside symbiont-bearing foraminifera test decreased photosynthetic area of their symbionts (Zientek et al., 2024). Moreover, the presence of microplastic particles led to reduced carbon and nitrogen stable isotope uptake in *A. lobifera* foraminifera, which indicates inhibition of inorganic carbon and nitrogen assimilation (Zientek et al., 2024).

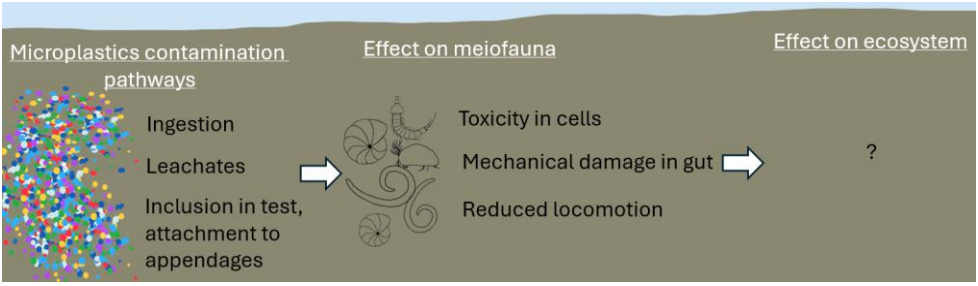

Figure 8. A scheme illustrating the pathways through which microplastics impact meiofauna and the potential meiofaunal responses to these effects. Adapted from (Bouchet et al., 2023)

Overall, there are very few studies on the effects of microplastics on meiofauna (Giere & Giere, 2019). In addition, the heterogeneity among studies in terms of targeted species, type of plastic polymer, sizes and concentrations of plastic particles, exposure time and measured parameters complicates our understanding of how microplastics alter meiofaunal communities (Bouchet et al., 2023). However, since microplastics have been shown to adversely affect photosynthesis in meiofaunal symbionts, reduce carbon and nitrogen assimilation, and even potentially hinder locomotion, it suggests that there may be potential cascading effects from meiofauna to sediment biogeochemistry due to microplastics pollution.

Aims of the thesis

The overarching aim of this thesis was to advance our understanding of meiofauna ecology and its role in sediment biogeochemistry. By combining experimental approach and field investigations, this thesis aimed to investigate the physiological responses of meiofauna to hypoxia, to examine meiofauna interactions with other sediment-dwelling organisms and ultimately to quantify meiofauna contributions to total oxygen uptake and methane release from sediments. Furthermore, this thesis aimed to address the effects of anthropogenic impacts, including climate change-related stressors and microplastic pollution, on meiofauna activity and diversity. More specifically, the aims were as follows:

Paper I: To present and validate a method for measuring respiration of individual nematodes under controlled oxygen, temperature, and salinity conditions. To compare directly measured respiration rates with those derived from established allometric relations.

Paper II: To investigate whether meiofauna respiration rates are similar among dominant meiofauna groups inhabiting marine and brackish sediments. To assess whether meiofauna respiration is significantly reduced under hypoxic conditions. To examine the mass scaling of meiofauna respiration.

Paper III: To experimentally investigate whether and how macrofauna and meiofauna influence methane fluxes, methane oxidation rates, and abundance as well as activity of aerobic methanotrophs in coastal sediments.

Paper IV: To investigate microplastics effect on interstitial fauna bioturbation, diversity, and abundance as well as oxygen penetration depth and diffusive oxygen uptake in subtidal sediment.

Paper V: To quantify the relative importance of environmental variables that drive meiofauna diversity and distribution in three Siberian seas. In addition, to investigate meiofauna vertical distribution within the sediment.

Methods employed in the thesis

In this section, I provide a review of the key methodologies used in the studies included in this thesis, along with their working principles. For detailed description of procedures and experimental protocols, readers are directed to the respective papers.

High-resolution microprofiling

Microsensors have been applied in all papers of my thesis. In **papers II**, **III**, and **IV**, I have performed sediment microprofiling and in **papers I** and **II** – I used microsensors to measure respiration of individual meiofauna. Microsensors are small, needle-shaped and sensitive electrodes, allowing measurements of fine-scale concentration gradients in water and sediments (Figure 9A), or sediment-like substrates. Some microsensors are thinner than human hair, with a tip diameter of 1–100 µm (Revsbech, 2021). Therefore, the sensors are only minimally invasive and have a very high vertical resolution (typically 50–100 µm). When mounted on an automated micromanipulator, microsensors can detect even the tiniest sample heterogeneities e.g., pH and oxygen gradients inside guts of only a few millimeters-long copepods (Tang et al., 2011).

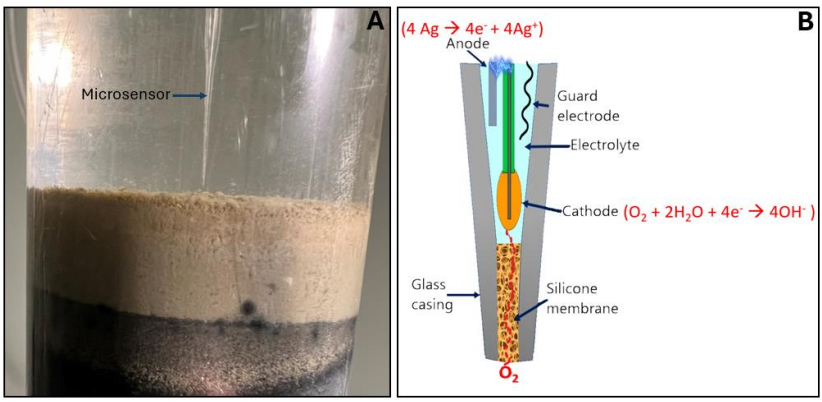

Figure 9. Sediment microprofiling (A), Detailed view of the sensor tip (B). The occurring reactions are shown in red. Electrons are produced by anode in the bulk electrolyte, while the oxygen is reduced at the polarized (-0.8V) cathode (measuring electrode) in the microsensor tip. © Modified from Unisense

Thus, microsensors can give insight into unknown yet biogeochemically active environments. As a result, they have gained a lot of attention, leading to production of microsensors used for O_2, N_2O, H_2S, NO, H_2, CO_2 as well as pH, and redox measurements.

The most important parts of the oxygen microsensors are gold-coated cathode, the gas permeable silicone membrane and silver anode (Figure 9B). Measuring principle is based on the reduction of diffusing O_2 on the cathode, which had been negatively polarized (Revsbech, 1989). Electrons for O_2 reduction are supplied by the silver anode. This electron supply from anode to cathode results in a current, which can be measured and related to the O_2 concentration (Revsbech, 1989). Oxygen microsensors typically have a detection limit of 0.1 μM, and a lifetime of more than a year (Revsbech, 2021).

From the concentration profiles it is also possible to get both volume specific O_2 consumption rates, and the diffusive oxygen uptake (DOU) through the integration of volume-specific rates. This can be done by assuming Fickian steady-state diffusion using the profile interpretation software PROFILE (Berg et al., 1998). Such estimated O_2 uptake rate will only represent activity of microbial communities and chemical oxidation of reduced compounds.

Whole-core incubation for flux measurements

Intact whole-core incubation experiments were carried out in **paper II** for estimations of total sediment O_2 uptake, and in **paper III** for O_2, DIC, CH_4 sediment-to-water flux measurements. Estimation of such fluxes requires the incubation of a known area sediment together with a known volume of overlying water. The theory behind such estimations is that the concentration of a solute of interest will be produced or consumed by the sediment community in a linear manner within a given time (approximately several hours for coastal sediment) (Dalsgaard et al., 2000). Therefore, overlaying water must be sampled at least at two (beginning and end of incubation) or more time points throughout the incubation period.

Finally, when concentrations of solutes are known, solute exchange per m^2 sediment surface per day (J_{solute}, mmol m^{-2} d^{-1}) is determined from the slope

of a linear regression of solute against time (days): $J_{solute} = (Ce - Cs) \cdot h / t$, where *Ce* and *Cs* are end and start concentration in µM (µmol dm^{-3} = mmol m^{-3}), *h* is the height of the water column above the sediment in meters, and *t* is incubation time in days. Such estimated solute exchange rate will represent activity of the whole sediment community: macrofauna, meiofauna, and microorganisms (Glud, 2008).

Metabarcoding

To determine microbial metazoan diversity, eDNA metabarcoding approach was used in **paper V**. Diversity patterns and ecology of meiofauna remain poorly understood because the studies are often constrained by time- and expertise-demanding morphology-based traditional species identification. Although protocols are still being optimized, DNA metabarcoding is a powerful tool to eventually overcome this stumbling block.

Metabarcoding is barcoding of DNA to simultaneously identify multiple taxa (i.e., species composition) within a sample. Metabarcoding is possible due to the variance of short barcode regions across taxonomic groups. For meiofauna, I used primers that target eukaryotic, the most variable V4 region of small-subunit 18S ribosomal RNA gene (Stoeck et al., 2010). These primers were shown to be suited for meiofauna, and commonly used to study eukaryotes, especially for amplicon-based studies using sequencing platforms like Illumina (Gielings et al., 2021; Stoeck et al., 2010). The primers had short synthetic DNA sequences (adapters) attached to their 5' ends.

After the DNA extraction, the first round of polymerase chain reaction (PCR) is performed to amplify the targeted (V4) region and flank it with the two adapter sequences. Then, PCR product is cleaned by removing residual primers, dNTPs, and primer-dimers. The second round of PCR is performed using primers that that bind to overhangs (part of adapters) added during the first round of PCR. This step allows attachment of unique index combinations to each sample, enabling multiple samples to be pooled together and then bioinformatically separated afterwards. Before sequencing, the amplicons were cleaned-up again using magnetic beads that selectively keep only longer fragments of DNA (>100 bp).

Finally, the samples were sequenced using Illumina pair-end sequencing with a read length of 250 bp. However, while metabarcoding is increasingly applied in meiofauna research and has revealed interesting patterns, each step of metabarcoding workflow is a potential source of bias (Gielings et al., 2021).

Bioinformatics

Amplicon-specific error-correction methods are used for separating biological variation from amplicon sequencing errors, which is a challenging task. To process my amplicon data, I used and improved Divisive Amplicon Denoising Algorithm within an open-source R package DADA2 (Callahan et al., 2016). DADA2 was used because it is tailored for the Illumina platform, and also the entire pipeline consisting of filtering, dereplication, sample inference, chimera identification, and merging of pair-end reads can be run within R (Callahan et al., 2016).

First, to remove low quality reads, sequences were trimmed, and quality threshold score was set to perform filtering. Using obtained filtered high-quality reads, error rates were then estimated for forward and reverse reads. The estimated error model was then used to correct errors in amplicon sequence variants (ASVs). Subsequently, merging of forward and reverse sequences was performed to finally produce the sequencing table that contains ASVs and their abundance (counts) in each sample. During sample preparation for sequencing, two or more different fragments can be joined incorrectly, forming so called chimeras that must be removed in the next step. Chimeras were removed from the sequencing table using "consensus" method which was set to define sequences as chimeras if they were at least four times the abundance of its parent sequences.

The final step in bioinformatics is taxonomic assignment. Today there are well-known databases consisting of sequenced 18S rRNA genes such as most commonly used SILVA and NCBI GenBank or nucleotide databases (NT) to assign sequences to meiofaunal taxa (Gielings et al., 2021). Using only one database, however, might not be enough to annotate the taxonomy to all ASVs for microscopic metazoans (Broman et al., 2019; Gielings et al., 2021). Therefore, I used a combination of SILVA (Huson et al., 2016) and NCBI NT (Altschul et al., 1991) databases. First, I used

SILVA database in DADA2 to create taxonomy table which I joined with counts. I then converted the taxonomy table to a FASTA file and ran BLAST (Basic Local Alignment Search Tool) search. By doing so, I could compare sequences in the FASTA file with the sequences in the NCBI NT database. Only the best (highest scoring) hit for each query sequence was returned.

Based on obtained NCBI accession numbers, I retrieved nematode taxonomy in the MEGAN6 ("MEtaGenome ANalyzer") software (Huson et al., 2016). MEGAN6 takes all the BLAST hits along with their taxonomic information and constructs a taxonomic tree. Then, using a key feature of MEGAN6 – Lowest Common Ancestor (LCA) algorithm – it is possible to estimate the lowest possible ancestor among all the matched taxa which helps to obtain more accurate taxonomy.

Meiofauna isolation from the sediment

Two different meiofauna isolation techniques were used in this thesis. In **papers I** and **II**, I used the most straightforward method to isolate meiofauna from the sediment – sieving. This was done by passing small amount of sediment through a 40-µm sieve to retain meiofauna, the sediment was always washed using *in situ* water. Sieving method was chosen as it was the gentlest, least stressful isolation technique, which was important for later respiration measurements.

In **papers III** and **IV**, density extraction was used to isolate meiofauna from the sediment. This technique eliminates the labor-intensive and tedious process of looking for animals in the fine sediment. Because I had no option to bring the Levasil colloidal solution to the USA, Ludox® HS40 colloidal solution was used instead. Whereas in **paper III** – Levasil® 200A 40 % colloidal solution was used. Despite the different solutions used, the working principle is the same: meiofauna can be separated from the sediment particles based on their different densities. Meiofauna have a lower density (specific gravity around 1.5), compared to sediment (specific gravity 2.5–2.8) (Burgess, 2001). Thus, when a sample is mixed with the colloidal solution, meiofauna will float to the top and heavier sediment will sink to the bottom.

The extraction procedure using the two colloidal solutions is also similar: sediment sample is first mixed with the colloidal solution and allowed to settle. Ludox protocol includes an additional step of centrifugation to enhance the settlement. Afterwards, the top layer containing meiofauna is decanted and sieved, using a 40 μm sieve. Process is then repeated 2–3 times. It is important to note, however, that Levasil is often preferred over Ludox solution because it is not toxic to fauna (Näslund et al., 2010). This is especially important if live animals are to be used in experiments. In addition, if the animals are alive during extraction, it is advisable to anesthetize (e.g., using $MgCl_2$) them to prevent them from swimming against the density forces. This is sufficient to relax the fauna without any adverse effects after several minutes' treatment (Somerfield & Warwick, 2013).

Main results and discussion

Paper I

A microsensor-based method for measuring respiration of individual nematodes

I present a microsensor-based method, which is sensitive enough to measure respiration rate of an individual meiofaunal nematode down to 0.16 µg wet weight (equivalent to 0.03–0.08 % of a single eyelash weight). Novelty of the study is that microsensors were adapted to measure respiration of mobile organisms, that in theory could easily escape the sample tubes. Most importantly, the setup allows to incubate organisms at desired temperature, oxygen, and salinity conditions, allowing for more accurate assessment of *in situ* respiration rates.

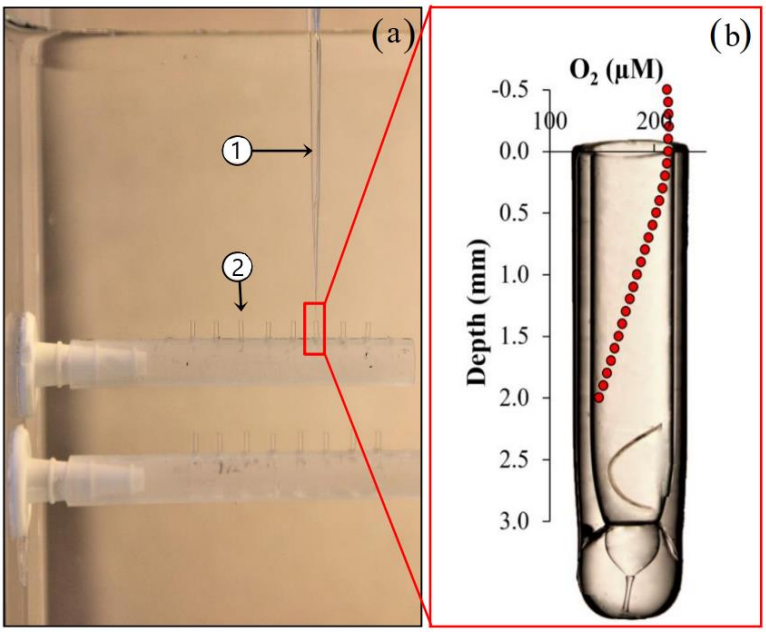

Figure 10. Measurement setup. (a) 1 – oxygen microsensor, 2 – capillary tubes secured on two holders. (b) Capillary tube with a nematode inside. Red dots indicate oxygen concentration obtained from the microsensor readings

The method utilizes Clark-type oxygen microsensors with glass capillary tubes containing individual nematodes (Figure 10A, B). The respiration is determined as oxygen flux towards an organism, which is calculated based on oxygen concentration gradient established between the top and the bottom of the tube (Figure 10B).

To validate this method, I tested it on 131 nematodes belonging to nine genera and with nematode body mass ranging almost two orders of magnitude. The nematodes were also with potentially diverse metabolic rates because they were sampled at oxic and severely hypoxic sediment. Finally, all measured rates were compared to the rates that were derived from widely used theoretical allometric assessments.

Although nematodes are among the most active meiofauna, 96 % of nematodes remained at the bottom of the tubes during the incubation. This suggests that even mobile organisms can be incubated in open tubes and thus the same approach can be used to measure respiration of all meiofauna. Obtained oxygen profiles indicated that the method worked as oxygen concentration inside the capillary tubes was constant and the profiles within the capillary tubes, containing single nematodes, showed a linear decrease in oxygen concentration, indicating oxygen consumption by a nematode. Whereas, in blank capillaries, the oxygen concentration was near constant with depth under both oxic and hypoxic conditions. Thus, the method allows for controlled variations in oxygen, temperature, and salinity during the incubations. This means researchers can simulate environmental changes such as hypoxia, temperature fluctuations, or salinity shifts and observe how nematodes and potentially other meiofauna respond in terms of respiration. This provides a basis for replacing speculations with quantitative predictions about how nematodes will be affected by environmental stressors at an individual level.

After incubating batches of individuals, Braeckman et al. (2013) found that nematodes decreased their respiration by four-fold under hypoxic conditions. In contrast, in **paper I**, we observed a 27 % reduction in individual respiration rates under hypoxic conditions. Thus, these results were lower than anticipated, likely due to the relatively mild hypoxia we induced (60 μM O_2), as we aimed to understand nematode responses without inducing severe stress. Additionally, since we incubated different

individuals under oxic and hypoxic conditions, future studies should focus on exposing the same individuals to varying experimental conditions for more accurate comparisons.

Finally, although measured and calculated respiration rates correlated, calculated theoretical rates tended to be on average four-fold (4±2) higher. Likely such overestimation stems from assumptions behind the body mass calculations and most importantly – the use of theoretical respiration coefficients derived under full-air saturation conditions, which do not reflect the actual oxygen conditions in the natural sediment. Or generalized temperature coefficient (Q10), which quantifies the rate of change in a biological process with temperature. This is an important result, because studies often rely on such theoretical calculations (Baguley et al., 2008; Heip et al., 2001; Kotwicki et al., 2018; Leduc et al., 2016; Shimabukuro et al., 2022). Thus, previously reported meiofauna respiration rate values are probably overestimated, particularly in systems with low ambient oxygen concentrations in the sediment.

Overall, this method provides precise and real-time data on nematode metabolic responses to varying environmental conditions. Although not as pronounced as expected, nematodes significantly reduced their respiration rates under hypoxic conditions. Consequently, while nematodes are known to inhabit hypoxic areas (Broman et al., 2020a; Sergeeva et al., 2021), their direct contribution to total oxygen uptake through respiration is likely negligible in these environments.

Paper II

Reconciling the importance of meiofauna respiration for benthic oxygen demand in costal sediments

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I applied the method presented in **paper I** to measure respiration of 10 groups of meiofauna under oxic and severely hypoxic conditions. The novelty of the study is that respiration rates of certain meiofauna groups (kinorhynch, juveniles of priapulid, starfish, and trumpet worm) were measured for the first time. In addition, we investigated the response of all meiofauna to severely hypoxic conditions and re-assessed the widely used respiration rate-biomass allometric equations using our measured

meiofauna respiration rates. This study differs from **paper I** in several key aspects: we focused on meiofauna from three distinct coastal sites and induced more severe hypoxia (~30 µM O_2), during hypoxic incubations. Most importantly, we incubated the same individuals under varying oxygen levels to accurately assess the impact on each individual.

Although larger meiofauna like ostracods and juvenile macrofauna (i.e., temporary meiofauna) had the highest individual respiration rates, nematodes and foraminifera – due to their relatively high abundances – were the most significant contributors to sediment oxygen uptake. Together they contributed by 3 % and at extremely high abundances (>3000 ind. 10 cm^{-2}) by 30 % to the total sediment oxygen uptake. A 30 % contribution may be an extreme value; more realistically, meiofauna are likely to contribute about 3 % in most systems. This indicates that at least nematodes and foraminifera should be included in any sediment oxygen uptake estimations.

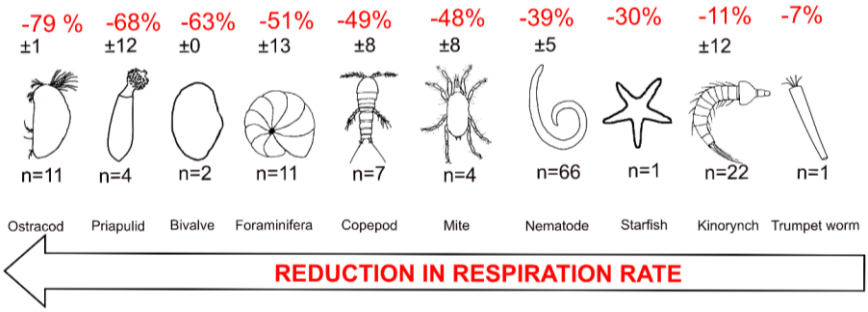

Figure 11. Reduction in respiration rates expressed as a percentage ±SE (%) reduction of respiration rate under hypoxic conditions (% reduction = (oxic respiration – hypoxic respiration)/ oxic respiration * 100). Under ambient ~30 µM O_2 concentration, animals were experiencing 18±1 µM O_2. From left to right: ostracod, priapulid, bivalve, foraminifera, copepod, mite, nematode, starfish, kinorhynch, trumpet worm. Numbers below the organisms indicate the number of incubated individuals.

Our results suggest that biomass was an important factor in defining individual meiofauna respiration rates. However, this relationship became less significant under hypoxic conditions, when meiofauna reduced their respiration rates (Figure 11). Ostracods decreased their respiration the most (by 79±1 %), compared to foraminifera (by 51±13 %) and to nematodes (by 39±5 %) (Figure 11). Variations in tolerance to hypoxia

may be linked to the ecology of different meiofauna groups (Lasserre, 1976). For example, copepods and ostracods primarily inhabit the sediment-water interface, where oxygen levels are higher than those found in the deeper layers of sediment occupied by nematodes and other burrowing meiofauna. However, this alone does not explain the pattern observed in Figure 11. In addition, three individuals – one foraminifer, one nematode, and one kinorhynch – that exhibited typical respiration rates under oxic conditions, significantly reduced their rates under hypoxic conditions, showing a more pronounced decline compared to their respective foraminifera, nematode, and kinorhynch counterparts. This indicates that the response to hypoxia can differ at the individual level, although the reasons for this variation remain unclear.

This experiment represents a response of meiofauna to short-term (several hours) hypoxia. Larger nematodes may enter a dormant state to conserve energy when exposed to hypoxia (Atkinson, 1973); however, this strategy is only effective for approximately 24 hours (Atkinson, 1973). Indeed, based on my observations, meiofauna, especially copepods, were inactive after hypoxic incubations but consistently recovered once returned to oxygenated water after the experiment. This suggests that meiofauna may reduce their movements as a coping mechanism, at least temporarily. In contrast, 14 % of meiofauna increased their respiration rates under hypoxia, as an indication of stress. Unfortunately, the individuals exhibiting this increase did not belong to a specific meiofauna group, making it difficult to explain. However, it is evident that such a response would likely go undetected in setups where batches of individuals are incubated together.

In addition to investigating meiofauna respiration rates and response to hypoxia, this paper examines coefficients related to the mass scaling of meiofauna respiration and their metabolic intensity. Specifically, the coefficients a and b are used in allometric scaling equations to predict meiofauna respiration rates. While coefficient a varies depending on meiofauna activity, coefficient b is well-established and typically approximates 0.75 for meiofauna and poikilotherms (Banse, 1982; Heip et al., 1985). Although our estimated mean b coefficient (b=0.72) was similar to the established value (b=0.75), we observed a large variation in b

coefficient between meiofauna groups. The coefficient for shell-bearing foraminifera and ostracods deviated the most from the established 0.75 value. Potentially, more sophisticated methods such as estimations of shell thickness are needed instead of a simple uniform ratio between shell and biomass to obtain reliable biomass estimations. Coefficient *a*, representing metabolic intensity, was the highest for ostracods (0.08), while copepods (-0.87) and kinorhynchs (-0.83) exhibited the lowest rates.

Coefficient *a* values have been previously reported for copepods, nematodes, ostracods, and foraminifera from various habitats, water mites, oligochaetes, polychaetes, and gnathostomulids (Figure 12). To compare our findings with previously published coefficients *a* and *b*, we converted our respiration rates and biomass data to match the units and experimental temperatures used in earlier studies (assuming Q10 = 2) and then extracted the coefficients *a* and *b* for specific groups of meiofauna (Figure 12). In general, the coefficients established in the literature were not significantly different from those found in this study, indicating that meiofauna in this study had comparable metabolic rates to those from other habitats when exposed to similar ambient oxygen and temperature conditions. Therefore, the coefficients presented here can be applied to estimate meiofauna respiration rates across different habitats, though temperature must be carefully considered. For example, deep-sea organisms are known to have very low metabolic activity. However, when accounting for temperature, copepods from coastal sediment in this study exhibited strikingly similar metabolic intensity (coefficient a, Figure 12). This was not the case when comparing copepods from this study to those inhabiting brackish pond (Figure 12). The sediment in the brackish pond consisted of fine sand with large amounts of detritus, which may have limited oxygen availability. This limitation could have influenced their respiration rates under fully oxic conditions, as noted in Herman and Heip (1983) study.

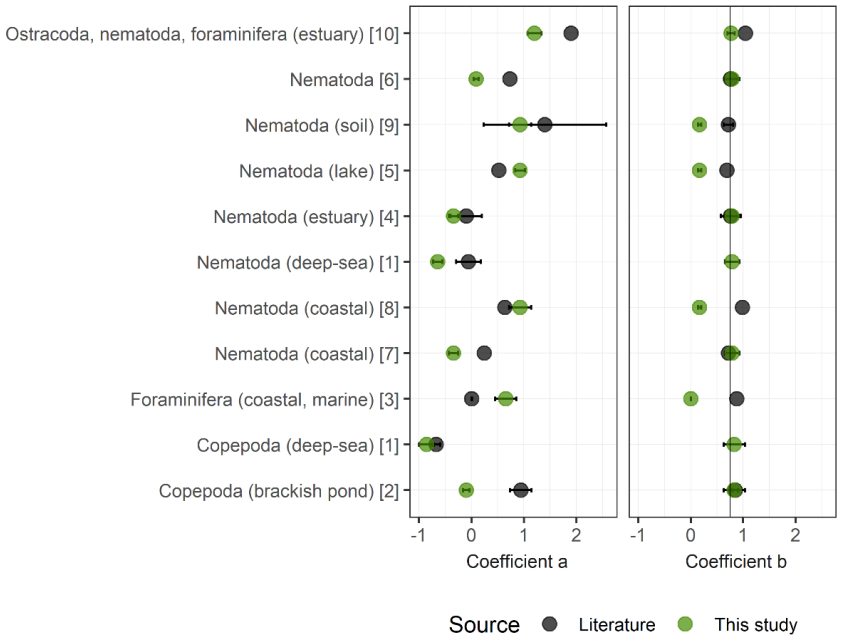

Figure 12. Comparison of established regression coefficients *a* and *b* (±SD, when available) between this study (green) and literature data (black). Each row represents a different study: [1](Shirayama, 1992), [2](Herman & Heip, 1983), [3](Geslin et al., 2011), [4](Warwick & Price, 1979), [5](Schiemer & Duncan, 1974), [6](Banse, 1982), [7](Kim & Shirayama, 2001), [8](Ott & Schiemer, 1973), [9](Klekowski et al., 1972), [10](Moodley et al., 2008) defined for meiofauna across ecosystems. The vertical line represents *b* coefficient of 0.75, which is currently used in all meiofauna studies (Banse, 1982; Heip et al., 1985).

Overall, juvenile macrofauna and larger ostracods exhibited the highest respiration rates and showed the greatest reduction under hypoxic conditions. Despite lower respiration rates per individual, nematodes and foraminifera made the most significant contributions to sediment oxygen uptake, owing to their high abundances. Interestingly, the allometric equation coefficients were not dependent on habitat type and varied significantly across and within meiofauna groups. This suggests that factors beyond biomass and ambient oxygen conditions influence respiration rates, though the specific drivers remain to be investigated.

Paper III

Biotic interactions between benthic infauna and aerobic methanotrophs mediate methane fluxes from coastal sediments

In this paper, we explore how interactions between benthic infauna (macro- and meiofauna) and aerobic methanotrophs (methane-consuming bacteria) regulate methane emissions from coastal environments. The novelty of the study lies in its focus on how microscopic biotic interactions influence methane fluxes. For this purpose, we performed whole-core sediment incubations consisting of five treatments with varying fauna compositions and abundances:

- (HM) – cores containing high meiofauna abundance, and no macrofauna
- (HMM) – cores containing high meiofauna abundance and macrofauna
- (LM) – cores containing low meiofauna abundance and no macrofauna
- (LMM) – cores containing low meiofauna abundance and macrofauna
- (CTRL) – unmanipulated sediment cores, containing natural infaunal communities

The main measured parameters were CH_4 fluxes, CH_4 oxidation rates, abundance, diversity of methanotrophs and a number of RNA transcripts attributed to genes found in methanotrophic bacteria. Sediment oxygen penetration depth (OPD), sediment organic matter content, and fluxes of dissolved inorganic carbon (DIC) and oxygen were also measured in this experiment. Finally, Structural Equation Modelling (SEM) approach was employed to assess the intricate relationships among multiple measured variables, capturing both direct and indirect effects on methane dynamics in coastal sediments.

Our results show that macrofauna increased CH_4 sediment-to-water fluxes by 2 to 6 times (Figure 13A). Although the bioturbation rate of macrofauna was not directly measured, macrofauna biomass was identified as a significant factor in the SEM analysis for methane release from the sediment. The observed increase in CH_4 fluxes is thus potentially due to

macrofaunal bioturbation which accelerated CH_4 transport to the water column thereby reducing the porewater CH_4 concentrations. As a result, this in turn lowered the activity (e.g., oxidation rate) of aerobic methanotrophs (Figure 13B). This may explain why the highest methanotrophic activity (oxidation rates) were observed in the sediment containing the lowest fauna abundance (Figure 13B). In addition, some methanotrophs may prefer environments with very low oxygen levels (sub-micromolar O_2) (Steinle et al., 2017). Consequently, macrofaunal bioturbation could have reduced the relative abundance of methanotrophs by altering their habitats within specific layers of the oxygen-methane transition zone, similarly to findings observed in lakes (Mayr et al., 2020).

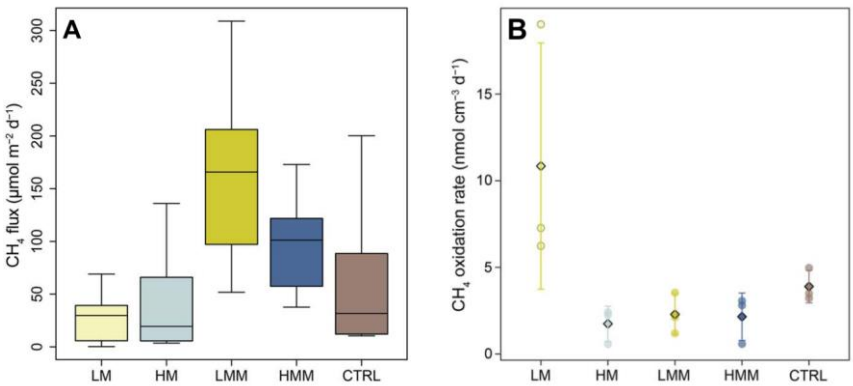

Figure 13. CH_4 sediment-to-water fluxes for each treatment (n = 7 per treatment, except HMM n = 6) (A) and CH_4 oxidation rates inside the sediment (B) based on injecting $^{14}CH_4$ tracer in the top 0–2 cm sediment layer (n = 3 per treatment). The dots denote individual incubated sediment cores, diamonds denote mean values, and the error bars show SD.

Unlike macrofauna, meiofauna exhibited a distinct effect on methane release, demonstrating a more nuanced role in regulating methane fluxes. Methane fluxes were lower in sediments with high meiofauna abundance, as seen when comparing HMM to LMM treatments and HM to LM, (Figure 13A). The SEM analysis further supported these findings by revealing a significant negative relationship between meiofauna abundance and CH_4 flux; however, the precise mechanism by which meiofauna interact with and potentially stimulate methanotrophs in this context remains unclear.

Overall, there is previous evidence of macrofauna contributing directly to methane emissions through their symbionts, which inhabit their anoxic guts, and indirectly through bioturbation (Bonaglia et al., 2017; Politi et al., 2023). Yet, this is the first time we demonstrate that meiofauna had a negative effect on methane release from the sediment to the water column. However, the exact meiofauna role in this study is challenging to disentangle, probably because meiofauna interacts with both macrofauna and microorganisms in complex ways. The findings of this study suggest that sediment biodiversity is important for controlling methane emissions and changes in benthic communities due to environmental stressors (e.g., pollution, climate change) could alter methane dynamics in unpredictable ways.

Paper IV

Microplastic-induced shifts in meiofauna and macrofauna bioturbation and oxygen penetration depth in subtidal sediments

In this paper, we investigated current and two projected levels (Jambeck et al., 2015) of microplastic pollution on meiofauna and macrofauna bioturbation, along with its effects on oxygen penetration depth (OPD) and diffusive oxygen uptake (DOU). The novelty of the study is that vast majority of microplastic exposure studies use macrofauna, whereas we investigated meiofauna as well. Therefore, this is the first study to evaluate changes in faunal community behavior combined with oxygen dynamics in response to microplastic pollution.

This study employs mesocosm incubations. Unlike in **paper III**, we did not manipulate fauna abundances. Instead, we exposed macro- and meiofauna natural communities to three different microplastics concentrations:

- (Low) – mesocosms containing microplastics at the currently observed concentration (10^2 particles kg^{-1} sediment dry weight)
- (Medium) – mesocosms containing microplastics at the concentrations predicted for 50 years from now (10^4 particles kg^{-1} sediment dry weight)

- (High) – mesocosms containing microplastics at the concentrations predicted for 100 years from now (10^6 particles kg^{-1} sediment dry weight)
- (Control) – mesocosms with no added microplastics

Fauna bioturbation was tracked using luminophores (i.e., glow-in-the-dark particles), while oxygen penetration depth was measured using microsensors every three days over the two-week incubation experiment. The deepest sediment layer containing luminophores, was used as indicator of the maximum bioturbation depth. In addition, bioturbation potential was calculated based on fauna present in the sediment.

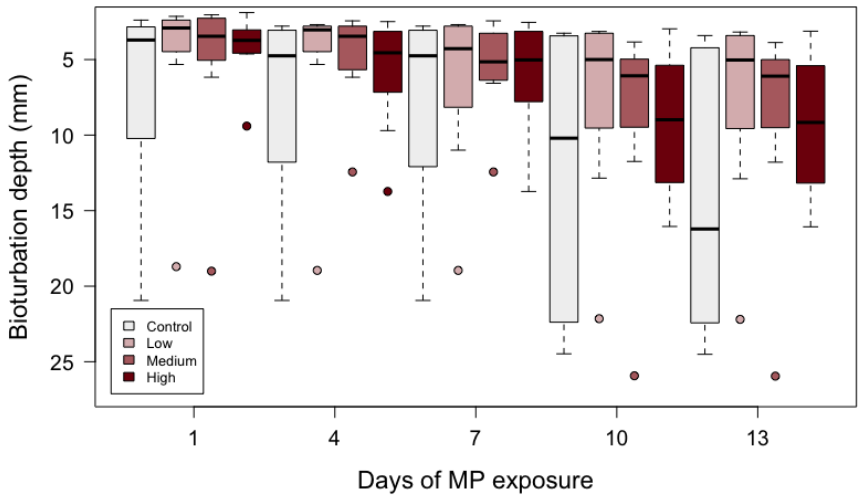

Figure 14. Community bioturbation depth in sediment exposed to microplastics and controls over time (in days). Control treatment contained 0 mg mL-1, low treatment 0.0001288 mg mL-1, 0.01288 mg mL-1, and 1.288 mg mL-1 respectively). Error bars denote standard error.

We observed a clear negative effect of microplastic pollution on bioturbation depth. Bioturbation depth was highly variable in control mesocosms, where microplastics were not added (Figure 14). This variability likely reflects natural bioturbation depth variation in absence of microplastics pollution. Such natural variability was diminished significantly in all mesocosms containing microplastics (Figure 14). In addition, bioturbation depth was significantly lower at low and medium microplastic pollution levels (Figure 14). The relationship between the

microplastic pollution levels and bioturbation depth is not straightforward, however. Mesocosms exposed to high microplastic pollution levels exhibited deeper bioturbation depth compared to low and medium microplastic treatments (Figure 14). It is thus likely that meiofauna bioturbated deeper into the sediment, as an evasion strategy in response to high microplastic pollution levels.

The impact of microplastics on oxygen penetration depth was insignificant, although showing slight average increases due to microplastic contamination. Likewise, there was no evidence that microplastics influenced the rate of diffusive oxygen uptake. While both macrofauna and meiofauna bioturbation can influence oxygen penetration depth and possibly diffusive oxygen uptake, the impact of bioturbation on diffusive oxygen uptake can vary depending on the type of organic matter present in the sediment (Kristensen, 2000), which was not investigated in this study. The disconnect between our bioturbation depth results and benthic oxygen dynamics is likely due to microbial activity and processes not detected by luminophore method. However, it is important to note that the average abundance of meiofauna and macrofauna individuals in the mesocosms was low (116.94±14.47). Therefore, even though the luminophore method was sensitive enough to detect behavioral changes in fauna activity due to microplastics, the effects of these behavioral changes were potentially not enough to be translated into significant impacts on DOU or OPD. Moreover, microplastics had no effect on meiofauna abundance, diversity, or evenness. This lack of impact may be attributed to the already low starting diversity and abundance within the mesocosms. Thus, the effects of microplastic pollution may be more pronounced in sediments with higher abundances and diversity of fauna, particularly among deep-burrowing species.

Overall, the results suggest that microplastic pollution significantly altered faunal behavior in terms of their bioturbation depth, and therefore have a potential to significantly alter coastal sediment biogeochemistry. Even more importantly, this study highlights the need to consider the environmental and ecological context when addressing the effects of microplastics on benthic ecosystems. It suggests that further research in sediments with more abundant faunal communities is essential, as certain

density of organisms may be necessary to observe measurable impacts on sediment oxygen dynamics.

Paper V

Environmental gradients, not geographic boundaries, structure metazoan communities in Siberian seas

During the last large-scale international expedition in Siberian Arctic (ISSS-2020), we were fortunate to collect sediment samples for meiofauna diversity analysis. Previous studies have shown that the diversity of macrofauna is the highest in Kara Sea, decreases in Laptev Sea and is the lowest in East Siberian Sea, as environmental conditions become increasingly harsh. Indicating that the largest of seas in the region – East Siberian Sea – is also the most vulnerable ecosystem to climate change. However, meiofauna distribution in Siberian seas is poorly understood, leaving a significant gap in our understanding of the true vulnerability of the seas. The novelty of the study lies in our application of sediment eDNA metabarcoding to investigate metazoan distribution and diversity in Siberia, making it the first time such approach was used there. To explain potential patterns in meiofauna distribution, we used an array of ancillary water column and surface sediment parameters published in previous study from the same expedition (Wild et al., 2023). In addition, we performed sediment oxygen microprofiling onboard, which not only introduced an additional environmental variable but also provided a unique insight into biogeochemical activity of Siberian shelf sediment.

We did not observe clear differences in chemical and hydro-physical data from bottom water and surface sediment across the three seas. However, cluster analysis indicated that the sampled 20 stations fall into two main groups: nearshore stations (cluster 1), and offshore stations (cluster 2). Significant differences in $\delta^{13}C$ values, salinity, turbidity, and temperature between the two clusters, indicated that nearshore stations were under effect of coastal erosion and riverine discharge. It is well documented that coastal erosion and river discharge have significantly increased in the Siberian Arctic due to climate change, resulting in substantial impacts on

the chemistry and biology of the seas (Mann et al., 2022; Nielsen et al., 2024; Nielsen et al., 2022; Vedenin et al., 2018).

Based on obtained 18S rRNA sequences, meiofauna diversity was similar between the three Siberian seas. The overall meiofauna community diversity, indicated by the Shannon index, was significantly higher at nearshore stations compared to offshore stations, but did not differ for nematodes (Figure 15A). Species richness, as indicated by the Chao1 index, showed no significant differences between nearshore and offshore stations for either nematodes or the meiofauna community as a whole (Figure 15B).

Maturity index of nematode community was significantly lower at nearshore stations (Figure 15C). This indicates presence of more colonizer-dominated communities at nearshore stations, compared to offshore stations which had more persister-dominated communities with longer life span, lower reproduction rates and potentially overall larger individuals. Although colonizer species are generally more resilient to disturbances, their dominance may lead to decreased ecosystem stability in the future. With future increases in river discharge and coastal erosion, we expect colonizer-dominated communities to expand, affecting ecosystems in all three studied seas.

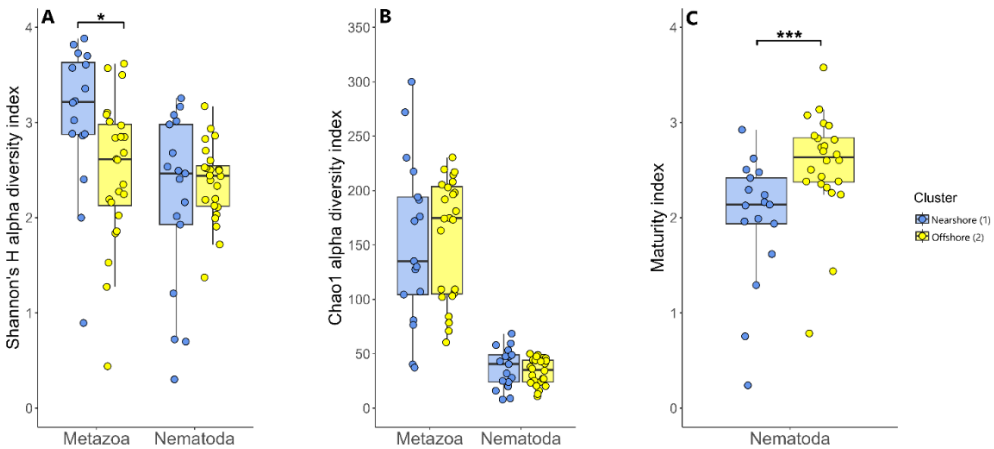

Figure 15. Boxplot of Shannon's H alpha diversity (A), Richness (B), and maturity of nematode community (C) indexes following clusters defined in Figure 2 (same colors)

By using molecular methods, we revealed organisms that have been missed using traditional sampling and isolation methods. For example, *Molgolaimus*

nematodes have been previously associated with deep cold waters of the Siberian shelf, whereas we observed high *Molgolaimus* relative abundance at shallow (14 m depth) and relatively warm (2 °C) station, indicating broader tolerance of this nematode genus than previously thought. We, however, acknowledge the effort needed to improve assignment of species during bioinformatics pipelines, as most nematode genera were unclassified (16±18 %), which could be due to a combination of factors, including limitations of the 18S rRNA marker, gaps in reference databases used for taxonomical assignments, and presence of novel or undescribed genera (Gielings et al., 2021).

The obtained 53 porewater oxygen concentration profiles, confirm that coastal Siberian seas are very biochemically active marine environments (Anderson et al., 2011). That is because despite bottom water being well oxygenated at all stations and experiencing low temperatures, the oxygen gradients were comparable to those in temperate marine systems, where oxygen is rapidly consumed within the top few millimeters or a centimeter (Glud, 2008), similarly to our observed average 0.9±0.3 cm oxygen penetration depth. To our knowledge, only two other studies have performed sediment oxygen microprofiling on Siberian shelf, but while they do have some data on shallow (<50 m depth) stations, their sampling did not include coastal areas (Boetius & Damm, 1998; Brüchert et al., 2018). Therefore, this is the first study to present data on coastal oxygen profiles and diffusive oxygen uptake, volume-specific O_2 consumption in the region and highlights the need for more studies in coastal, unexplored areas.

Overall, future studies investigating benthic communities should take seasonal differences into account, as the outflow of Siberian rivers exhibits significant seasonal and inter-annual variability (Semiletov et al., 2000; Xie et al., 2023). Finally, global warming is predicted to increase riverine run-off (Mann et al., 2022) and it will likely have significant impacts on at least metazoan community alpha and beta diversities, and maturity of nematode communities.

Conclusions and future perspectives

In this thesis, I provide evidence that meiofauna is important to oxygen and methane cycling, but their activity and diversity are impacted by environmental disturbances of increasing severity such as microplastics pollution and global-warming related stressors like coastal erosion, river discharge and spreading of oxygen-deprived bottoms.

In **paper III**, we show that meiofauna is not just a smaller version of macrofauna, as they can have different effects on sediment biogeochemistry. Specifically, we demonstrate that macrofauna significantly increased methane emission from the sediment, whereas meiofauna potentially mitigated it. This shows that interactions between macrofauna-meiofauna-bacteria are complex and that changes in faunal communities will potentially have cascading effects on CH_4 emissions from the sediment.

In **paper II**, we demonstrate that under oxic conditions, meiofauna community (~750 indiv. 10 cm^2) solely by respiring could be contributing by 3 % to total sediment oxygen uptake. However, this percentage may be drastically lower in oxygen-deprived areas because meiofauna reduce respiration rates under hypoxic conditions. Similarly, under oxic conditions, meiofauna biomass is a significant factor in defining their respiration rate. However, the relationship between biomass and respiration rate becomes weak under low-oxygen conditions. In addition, commonly used theoretical calculations of meiofauna respiration rates overestimated the measured rates four-fold. Therefore, these findings should motivate meiobenthologists to quantify the respiration rates at *in situ* oxygen conditions using the method described in **paper I**. Herein reported respiration rates, especially under hypoxic conditions, are relevant as coastal hypoxia have been spreading since the 1960s (Diaz & Rosenberg, 2008).

The other two key stressors on marine systems are microplastics pollution and with global warming accelerating stressors, that were investigated in **papers IV** and **V**. In **paper IV**, we show that even at current microplastic pollution levels, microplastics restrict faunal communities from exhibiting

their natural bioturbation depth, ultimately resulting in overall reduced bioturbation depth. Whereas in **paper V**, increasing coastal input through erosion and river discharge, was found to be significant in shaping meiofauna diversity as well as defining nematode community maturity index.

There are numerous research questions that can be built upon the results presented in this thesis. The microsensor-based method now opens doors for investigating sulfide oxidation by meiofauna-associated symbionts by simply replacing oxygen microsensor with commercially available H_2S sensor and inducing euxinia in the incubation aquarium. Similarly, potential nitrous oxide emission by meiofauna could now be measured using N_2O microsensors. Moreover, such measurements could be combined with molecular investigations looking at gene expression and meiofauna-associated microbial assemblages in relation to measured rates. There is also a pressing need to quantify meiofauna nitrogen excretion, so that we have a better idea of meiofauna contribution to cycling of all key elements, not only oxygen and carbon discussed in this thesis.

Majority of the future investigations will require method development and optimization, indicating a long road ahead. However, this should be an interest to most researchers in the field, as it is not just about meiofauna – it is the overall sediment biogeochemistry, which ultimately impacts us all through health and functioning of marine ecosystems.

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