LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 68, 2023, 1895–1905

doi: 10.1002/lno.12393

Reconciling the importance of meiofauna respiration for oxygen demand in muddy coastal sediments

Adele Maciute [1]*, Oleksandr Holovachov [2], Ronnie N. Glud [3,4], Elias Broman [5,6], Peter Berg [7], Francisco J. A. Nascimento [5,6], Stefano Bonaglia [1,3]

[1]Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden
[2]Department of Zoology, Swedish Museum of Natural History, Stockholm, Sweden
[3]HADAL, Nordcee & DIAS, Department of Biology, University of Southern Denmark, Odense, Denmark
[4]Department of Ocean and Environmental Sciences, Tokyo University of Marine Science and Technology, Tokyo, Japan
[5]Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm, Sweden
[6]Baltic Sea Centre, Stockholm University, Stockholm, Sweden
[7]Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia, USA

ABSTRACT

Meiofauna, organisms smaller than 1 mm, are the most abundant and diverse invertebrates inhabiting the world's ocean floor but their contribution to benthic oxygen demand is still poorly constrained. This knowledge is crucial for understanding seabed respiration, global marine carbon, and oxygen cycles, which are relevant to all nutrient cycling and energy flows in the ecosystem. It is common to predict meiofauna respiration based on their biomass or volume, which are difficult to quantify, and thus meiofauna are rarely included in biogeochemistry studies. In addition, it is still unknown how well the generalized allometric relations describe all meiofauna respiration. Therefore, we used a novel approach specially developed for single meiofauna respiration measurements to derive the respiration rates of 10 meiofauna groups in two marine and one brackish coastal muddy environments under oxic and hypoxic conditions, representing natural sediment conditions. Our estimates suggest that large ostracods and juveniles of macrofauna (e.g., bivalves, trumpet worms, and priapulids) had the highest individual respiration rates. Meiofauna community as a whole contributed 3–33% to sediment oxygen uptake. However, the most important contributors to the overall sediment oxygen uptake were nematodes and foraminifera which had lower respiration rates but were highly abundant. Therefore, out of more than 22 meiofauna phyla, we recommend that nematode and foraminifera respiration, which contributes 3–30% (total 3–33%) to sediment oxygen uptake, should be taken into consideration in any estimations of benthic oxygen and carbon cycles.

Marine sediments are globally important for carbon and nutrient cycling. Understanding the cycling rates, pathways and contributors is essential for predicting and protecting the functioning of marine ecosystems. Total oxygen uptake (TOU) is an excellent proxy for the total carbon mineralization rate in marine sediments as it encompasses both the aerobic respiration and the reoxidation of most constituents from the anaerobically mediated carbon degradation (Glud 2008). Macrofauna (invertebrates > 1 mm) mediated oxygen (O_2) uptake is often readily quantified from the difference between the TOU and diffusion-mediated O_2 uptake (DOU), which is derived from high resolution vertical porewater O_2 profiles (Glud 2008). This value includes the macrofauna respiration as well as the microbial and chemical O_2 consumption associated with irrigation and ranges from ~ 50% in coastal settings to insignificant levels in the deep-sea (Glud 2008; Jørgensen et al. 2022). Macrofauna respiration can be also separately derived from individual respiration rate (IRR) measurements or empirical allometric relations between biomass and O_2 requirements (Mahaut et al. 1995). The latter is typically expressed mathematically as: $\text{Respiration} = a \times X^b$ (or when log-transformed $\log_{10}\text{IRR} = a + b \times \log_{10} X$), where X represents biomass, and a and b are regression coefficients related to

*Correspondence: adele.maciute@gu.se

Additional Supporting Information may be found in the online version of this article.

Author Contribution Statement: A.M. and S.B. conceived the ideas. A.M. led the writing of the manuscript with substantial contributions from R.N.G., F.J.A.N., P.B., O.H., S.B., A.M. and P.B. analyzed the data. A.M., S. B., E.B., and O.H. sampled in the field and carried out the experiments

organisms' biology (i.e., metabolic intensity, behavior, and feeding guild) (Kennedy 1994).

Although meiofauna (invertebrates < 1 mm) respiration is included in the TOU, it is rarely quantified separately. Therefore, their relative contribution to the benthic O_2 demand is typically poorly constrained. This is a large knowledge gap in benthic ecology because Nematoda alone is the most abundant meiofauna group which can reach densities of up to 84 million individuals per m^2 of sediment (Lambshead 1993; Coull 1999), suggesting that they may consume a high amount of oxygen. In addition to nematodes, 21 other phyla have meiofaunal representatives, making them an abundant and diverse component of aquatic sediments and terrestrial soil communities worldwide (Balsamo et al. 2012; Van Den Hoogen et al. 2019). Moreover, previous studies have shown that meiofauna by moving, feeding, and excreting in the sediment change distribution of particles, solutes, and microorganisms (Schratzberger and Ingels 2018). Subsequently, these activities can double sediment denitrification rates (Bonaglia et al. 2014), increase by 50% organic matter decomposition rates (Nascimento et al. 2012), oxygen penetration depth (Bonaglia et al. 2020), and sulfide removal rates in the sediment (Bonaglia et al. 2020). Moreover, foraminifera were the first marine eukaryotes found to perform complete denitrification (Risgaard-Petersen et al. 2006), and were shown to account for 50–100% of nitrate loss from Gullmars Fjord sediments (Choquel et al. 2021). Thus, meiofauna can play an important role in coastal ecosystem functioning.

Yet, because most of the respiration measurement techniques either have low detection sensitivity or cannot simulate *in situ* oxygen conditions, we are still lacking data on meiofaunal community respiration rates, hindering estimations of their direct contribution to sediment oxygen uptake. Even though meiofaunal respiration can be determined using empirical allometric relations, concerns exist about the adequacy of such estimations, mainly due to the use of generalized coefficients for all meiofauna groups, which do not consider *in situ* oxygen conditions (Braeckman et al. 2013; Maciute et al. 2021). In addition, because macrofauna abundance is severely decreased under low oxygen conditions, meiofauna are often the only animals present in hypoxic areas (≤ 2 mg O_2 L^{-1} or ≤ 63 μM O_2 L^{-1}; Broman et al. 2020). Yet, it is unknown how meiofaunal contribution to sediment oxygen uptake is affected by hypoxia, which is an increasing problem in marine ecosystems around the world (Diaz and Rosenberg 2008).

To address these knowledge gaps, in this study, we for a first time measured IRRs of meiofauna belonging to 10 different taxonomic groups using a microsensor-based method described in Maciute et al. (2021). The goal was to test whether meiofauna respiration rates are: (1) similar among dominant meiofauna groups inhabiting marine and brackish systems, and (2) if meiofauna respiration is significantly reduced under hypoxic conditions. This knowledge is important as it will facilitate and enhance the precision of current and future estimations of meiofauna respiration and their contribution to TOU. Finally, herein provided IRRs make a basis for re-assessing the widely used respiration rate-biomass allometric equations.

Methods

Sampling

Sediment samples were collected at a brackish site (salinity 6) in the Baltic Sea (58°48′36.4320″N, 017°36′59.5080″ E) and at two marine sites (salinity 33): a coastal and a fjord site, in the Skagerrak, North Sea (58°16′50.94″N, 11°30′30.96″ E, and 58°15.053″N, 11°25.645″ E, respectively) in June 2021 (Fig. 1). At the time of sampling, bottom water temperature was 7–8°C, the bottom water oxygen was 9 mg L^{-1} (i.e., 72% and 83% air saturation, respectively) at sites (40–47 m depth).

At the brackish site, sediment was collected using a sediment multicorer (201 cm^2 per core), while at the marine sites—a box corer (900 cm^2). At each site, sediment was subsampled with five acrylic core liners (Ø 5 cm, $h = 30$ cm) for TOU incubations, and eight liners (Ø 5 cm) for microprofiling and single individual incubations. In addition, ~ 20 L of bottom water was collected at each site for incubations and meiofauna sieving. Collected sediment cores were brought back to the laboratory into a temperature-controlled room (10°C) and submerged in aerated *in situ* water.

Meiofauna respiration measurements

Respiration measurements were started the day after sampling. Employed experimental setup and workflow are described in Maciute et al. (2021). Briefly, respiration was measured by incubating single meiofaunal organisms in glass capillary tubes. This method is based on the fact that a respiring organism on the bottom of a one-end-open tube and a continuous oxygen supply from the overlying water in an incubation aquarium will develop a linear oxygen concentration gradient between the top and the bottom of the tube after a certain incubation time (Maciute et al. 2021). The developed oxygen gradient was then measured using an oxygen microsensor (OX-50, Unisense, Denmark) to calculate IRRs, using Fick's first law of diffusion: $J = -D \times dC/dZ$, where D is the oxygen diffusion coefficient at a given temperature (Broecker and Peng 1974), and dC/dZ is measured oxygen gradient inside the capillary tube.

We measured respiration of meiofaunal organisms that were isolated from the top (0–1 cm) sediment layer using a 40-μm sieve. Meiofauna body surfaces were not sterilized, as this would involve a high risk of injuring or severely stressing the animals, with presumable impact on their respiration rates. Any contribution from naturally occurring microbes at the surface of the animals is thus included in the measurements (as would any internal microbiomes). However, the method is not sensitive enough to detect minor respiration of prokaryotes present on body surfaces.

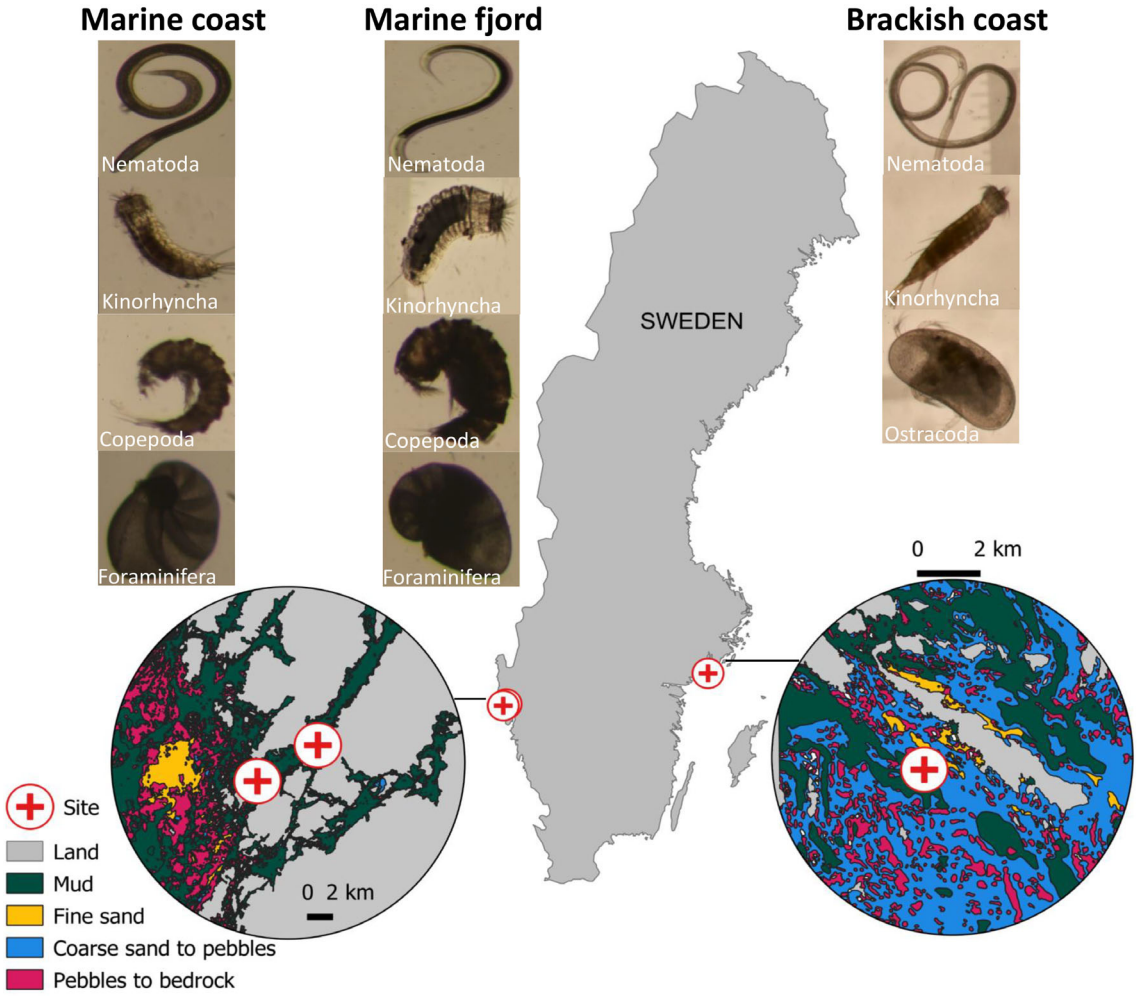

Fig. 1. Map of the three sampling sites: brackish coast on the Swedish east coast and marine coast and fjord on the Swedish west coast. Different colors in inset maps indicate sediment type. Photos of the most common meiofauna groups are presented above the inset maps.

All animals were first photographed for later body length and width determinations using the image-processing program ImageJ (Schneider et al. 2012). The animals were then incubated in either 3 or 4-mm capillary tubes, together with blanks (tubes not containing animals) in *in situ* water at 10°C. To study low oxygen effects at an individual level, we used the same individuals for oxic (~ 280 μM ambient O_2) and hypoxic (~ 30 μM ambient O_2) incubations. At our experimental conditions, the required incubation time was either 3 or 5 h, depending on the capillary tube length as explained in Maciute et al. (2021). After the incubations, measurements of oxygen gradients inside the capillary tubes were started, during which the software-driven micromanipulator was set to start 500 μm above the tubes and continue down to 2 mm depth performing the measurements at 100 μm depth increments with 1 s delay at each depth before a measurement.

Obtained dominant meiofauna respiration rates were normalized to their biomass (μg wet weight) by first calculating body volume and then multiplying it by an average density (1.13 g cm^{-3}; Feller and Warwick 1988). Body volumes of kinorhynchs, nematodes, copepods, and ostracods were calculated as Body volume $= L \times W^2 \times C$, where L and W is body length and width (mm), respectively, and C is the meiofauna group-specific conversion factor (Somerfield et al. 2005). In the case of foraminifera, body volume was estimated by using the best resembling geometric shape, a spheroid prolate: Shell volume $= 4/3\pi \times (W/2)\text{^}2\,(L/2)$. The 75% of the estimated shell's volume was then used as cytoplasmic volume (e.g., biovolume) (Hannah et al. 1994).

Total sediment oxygen uptake

At each site, five cores with a typical sediment height of ~ 13 cm and one core containing only in situ water, were fitted with small stirring magnets and submerged in aerated *in situ* water inside an incubation barrel with an inbuilt central magnetic motor. The cores were then left for a ~ 15 h equilibration period at 10°C.

TOU rates were defined by recording oxygen concentrations in the overlying water at two-time points: immediately after capping the cores and after an incubation time of 11–16 h using a non-invasive fiber optic oxygen meter (Firesting, PyroScience Sensor Technology). One oxygen optode sensor spot was attached to each airtight transparent lid so that it was exposed to the well-mixed overlying water in the cores. The optode sensor spots were calibrated in air-saturated water and in anoxic water, which was prepared using sodium dithionite. To make sure that the oxygen does not decrease below 20% of the initial concentration, one optode fiber was left connected to the lid of one of the cores throughout the incubations.

Assuming the linear decline in oxygen, the TOU (mmol m^{-2} d^{-1}) was calculated as $TOU = (C_e - C_s) \times h/t$, where C_e and C_s are end and start oxygen concentrations in μM, h is the height of the water column in meters and t is incubation time in days. Finally, the TOU values were corrected to the TOU value in the blank.

Diffusive sediment oxygen uptake

Sediment oxygen microprofiles were determined in three sediment cores per site. Microprofilling was performed at 10°C using Clark-type oxygen microelectrode (OX-50, Unisense, Denmark). The measurements were started at 1.2 cm above the sediment–water interface and continued down to anoxic layers at 100 μm depth increments. Obtained oxygen profiles were analyzed using PROFILE software, which by assuming steady-state conditions, provides the best fit to the measured oxygen concentration and estimates the O_2 flux as a function of depth (Berg et al. 1998).

Statistical analysis

Data was tested for normality with Shapiro–Wilk test and for homoscedasticity with Levene's test using R software. A two-way ANOVA, nonparametric ScheirerRayHare test was used to examine differences in rates with meiofauna as a group and treatments as factors. To get allometric equations, regression was performed on log-transformed respiration and biomass data. Regression coefficients a and b established for meiofauna in this study were compared with previously reported coefficients using Kruskal-Wallis test. The results are presented as averages ± SE unless otherwise stated.

Results and discussion

Respiration rates are similar among meiofauna groups

Measured respiration rates of 134 individuals cover 10 groups of meiofauna and indicate that meiobenthic organisms have oxic respiration rates ranging between 0.03 and 4.91 nmol O_2 ind^{-1} d^{-1} at 10°C (Fig. 2, Supporting Information Fig. S2). Relatively high respiration rates (≥ 2 nmol O_2 ind^{-1} d^{-1}) were common for larger meiofauna such as ostracods (3.0 ± 0.2 nmol O_2 ind^{-1} d^{-1}, $n = 12$), a few large nematodes and some macrofauna juveniles such as priapulids (2.4 ± 0.7 nmol O_2 ind^{-1} d^{-1}, $n = 4$), a trumpet worm (1.7 nmol O_2 ind^{-1} d^{-1}), and bivalves (1.6 ± 0.2 nmol O_2 ind^{-1} d^{-1}, $n = 2$; Fig. 2, Supporting Information Fig. S2). These oxic respiration rates should represent *in situ* rates because oxic incubation conditions at 280 μM O_2 resulted in oxygen concentrations of 210 ± 4 μM around animals (throughout the text: ±SE; $n = 134$), while *in situ* oxygen concentration at the sediment surface was ~ 216 μM O_2 (Supporting Information Fig. S1). Following the oxic respiration measurements, meiofauna individuals were incubated in severely hypoxic ambient water (~ 30 μM O_2), which resulted in an average O_2 concentration of 18 ± 1 μM ($n = 130$) at the position of investigated animals, and respiration was then re-measured (hypoxic rates are presented below).

Statistical analysis was done for the five most abundant meiofauna groups in our samples (nematoda, kinorhyncha, foraminifera, copepoda, and ostracoda), which had more than 3 individuals present at each site. Within the five dominant groups, nematodes and ostracods had the highest IRRs compared to other meiofauna groups (ScheirerRayHare test, $p < 0.001$) (Fig. 2). Previous literature reports similar values to our measured nematode IRR (0.02–1.30 nmol O_2 ind^{-1} d^{-1}) when using the microsensor-based method (Maciute et al. 2021), but also significantly higher respiration rates when nematodes were measured in batches (3.1–5.5 nmol O_2 ind^{-1} d^{-1} at 10°C, when assuming Q10 = 2) (Moodley et al. 2008). Interestingly, terrestrial nematode *Caenorhabditis elegans* respiration rates (0.9–1.8 nmol O_2 ind^{-1} d^{-1} at 10°C, when assuming Q10 = 2) are comparable to our aquatic nemtode IRR (Moens et al. 1996).

Foraminifera respiration rates can differ substantially depending on their test shapes. In our study we incubated the most dominant test shape–spheroid prolate, and thus our results may be biased towards such foraminifera. Yet, previously determined foraminifera IRR are in line with our results and range between 0.1 and 0.7 nmol O_2 ind^{-1} d^{-1} (Geslin et al. 2011; Cesbron et al. 2016; Deldicq et al. 2021), although *Ammonia beccarii* foraminifera species which had four times higher biovolume compared to herein presented foraminifera, had respiration rates as high as 5.65 nmol O_2 ind^{-1} d^{-1} (Geslin et al. 2011). Whereas in our study, the highest measured rate was 4.91 nmol O_2 ind^{-1} d^{-1} for an ostracod. In a study by Moodley et al. (2008), high foraminifera rates (2.5–2.6 nmol O_2 ind^{-1} d^{-1} when assuming Q10 = 2) were measured, while ostracods had the lowest rates (0.6–0.7 nmol O_2 ind^{-1} d^{-1} when assuming Q10 = 2). These highly variable rates support the idea that meiofauna respiration rates are similar among meiofauna groups. Therefore, observed changes in meiofauna community structure cannot provide insights about the subsequent consequences on sediment oxygen uptake.

Nematodes have the highest biomass-specific respiration rates

In theory, correcting respiration rates to the biomass or volume of an organism enables more accurate cross-taxon

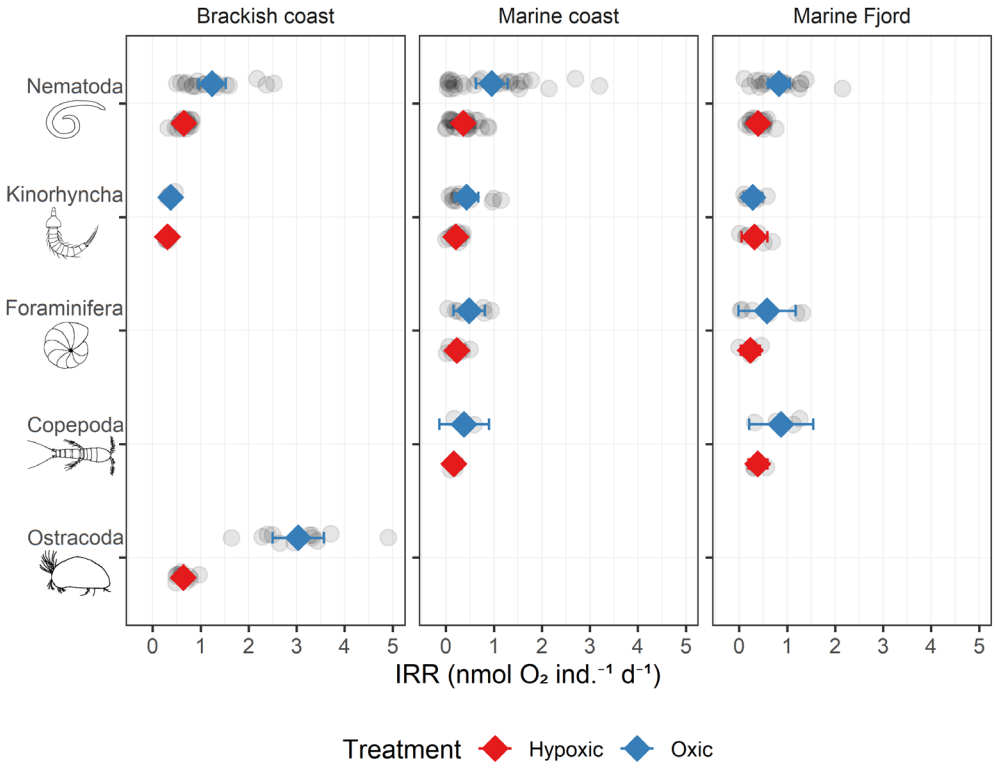

Fig. 2. IRR of five meiofauna groups collected at three sites. Red and blue diamonds are average rates under hypoxic and under oxic conditions, respectively (error bars represent ± SE, ($n > 3$). The same individuals were used for oxic and hypoxic incubations. Gray circles indicate individual data points. Drawings of animal taxa are stylized and not to scale.

comparisons of respiration and will show true differences in meiofauna metabolism. The metabolic, or biomass-specific rate (MR) of nematodes was the highest (0.2 ± 0.03 nmol O_2 μg^{-1} d^{-1}, $n = 68$; ScheirerRayHare test, $p < 0.0001$). Whereas, ostracod MR (0.1 ± 0.02 nmol O_2 μg^{-1} d^{-1}, $n = 12$) were similar to foraminifera (0.05 ± 0.04 nmol O_2 μg^{-1} d^{-1}, $n = 12$) and copepod (0.1 ± 0.01 nmol O_2 μg^{-1} d^{-1}, $n = 8$) rates (ScheirerRayHare test, $p > 0.05$). For all remaining meiofauna groups, the MR ranged between 0.001 and 0.8 nmol O_2 μg^{-1} d^{-1} (Fig. 3).

Comparison of our MR with reported in literature rates is complicated, due to different measures of weight which is expressed as either wet, dry weight or carbon weight, or volume. Nevertheless, it has been suggested that there is a correlation between MR and the size of nematode buccal cavities, which represent feeding guild (Wieser and Kanwisher 1961; Teal and Wieser 1966). In particular, nematodes having large buccal cavities (e.g., nonselective deposit feeders) ingest a large amount of particles, which requires active roaming, high feeding activity, and therefore high oxygen consumption rates (Wieser & Kanwisher 1961; Teal and Wieser 1966). In contrast, nematodes with small buccal cavities (e.g., selective feeders or epigrowth feeders) most likely ingest a smaller amount of higher nutritional quality organic material and require less oxygen to process it (Wieser and Kanwisher 1961). This suggests that nematode contribution to TOU is higher at sites with high abundances of non-selective or deposit feeders. On the basis of this theory, some nematode allometric equations include feeding guild-specific coefficients (Kennedy 1994). In an earlier study, however, no correlation between nematode biomass-specific rate and feeding type nor genus was observed (Maciute et al. 2021). Therefore, the use of such pre-defined coefficients might introduce false variation, when estimating nematode IRR based on allometric equations. In the current study, we show that nematodes, as a meiofauna group, have the highest biomass-specific rates, indicating that they play a significant role in energy flow and nutrient cycling compared to other meiofauna groups in marine and brackish systems.

Meiofauna significantly decrease respiration rates under hypoxia

Average individual and biomass-specific respiration rates of all meiofauna were significantly lower under hypoxic than oxic conditions (ScheirerRayHare test, $p = 0.0001$; Figs. 2, 3). However, we emphasize that estimations based on batches of individuals or average rates should be interpreted with caution as not all individuals expressed reduced respiration under hypoxic conditions. For instance, when looking at the individual level, we observed that 14% of meiofauna increased respiration rates under hypoxia, meaning that most meiofauna

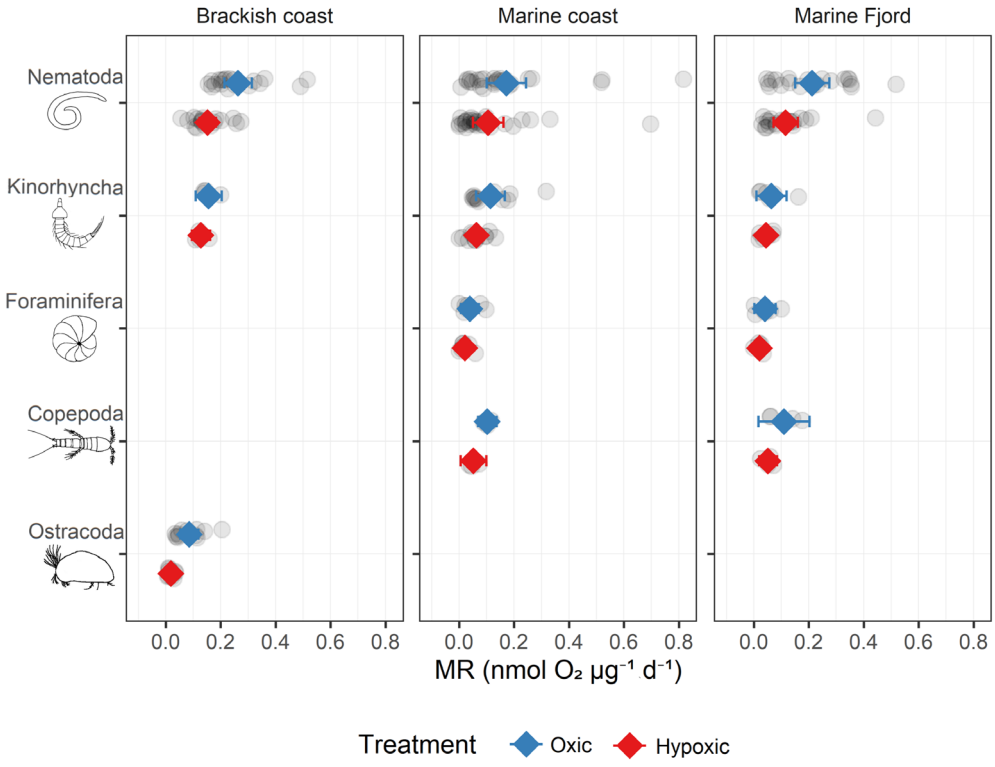

Fig. 3. Individual respiration rates per microgram WW of biomass (MR) of five meiofauna groups collected at three sites. Red and blue diamonds are average rates under hypoxic and oxic conditions, respectively (error bars represent ± SE, ($n > 3$). The same individuals were used for oxic and hypoxic incubations. Gray circles indicate individual data points. Drawings of animal taxa are stylized and not to scale.

(86%) decreased their metabolism. The increase was minor but for one copepod the rate tripled under hypoxia, indicating stressful conditions that in a long term would potentially lead to death (not shown in Fig. 4).

Regarding taxon-specific resilience to variable oxygen conditions, previous study based on incubations of batches of nematodes, ostracods, gastropods, foraminifera in closed chambers report significantly reduced respiration rates under severe hypoxia (Braeckman et al. 2013). It has been shown that one nematode species (*Enoploides longispiculosus*) was the most sensitive to hypoxia (67–83% decrease in respiration), followed by foraminifera (*Ammonia beccarii*; 44–61%), and unidentified species of ostracods (9–42%; Braeckman et al. 2013). One batch of ostracods increased respiration by 16% (Braeckman et al. 2013). Yet, our results show a different trend from the one described above. In particular, ostracods decreased their respiration the most (79 ± 1%), compared to nematodes and foraminifera (Fig. 4). Generally, nematodes are

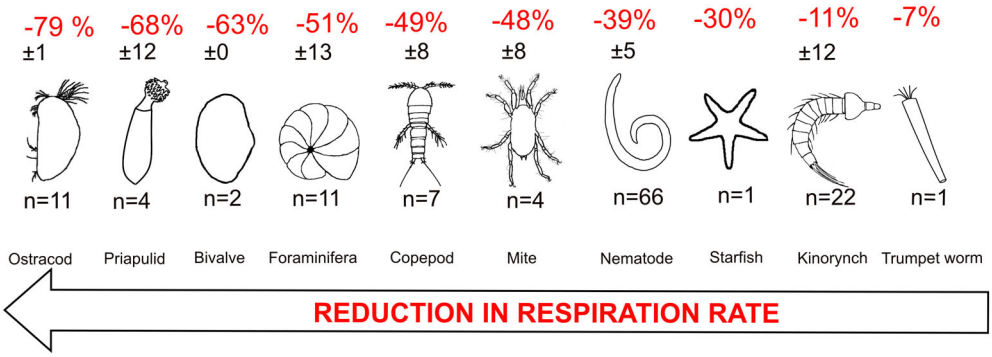

Fig. 4. Reduction in respiration rates expressed as a percentage ± SE (%) reduction of respiration rate under hypoxic conditions (% reduction = [oxic respiration – hypoxic respiration]/oxic respiration * 100). Under ambient ~ 30 μM O_2 concentration, animals were experiencing 18 ± 1 μM O_2. Numbers below the organisms indicate the number of incubated individuals.

considered the most resistant animals to hypoxia, while copepods are the most sensitive ones (Modig and Ólafsson 1998). Nematodes belonging to genus *Sabatieria* have been found in oxygen-deprived areas and deep (3–4 cm depth) sediment layers (Steyaert et al. 1999; Broman et al. 2020). *Sabatieria* has also been shown to be resistant to experimentally induced anoxic conditions at least for 14 days (Steyaert et al. 2007). Although the exact mechanisms of adaptation of *Sabatieria* to low oxygen conditions remain unclear, it seems that vertical distribution in the sediment is dependent on food availability, rather than on oxygen concentration in the sediments (Steyaert et al. 1999).

However, the responses might be species-specific, as indicated by the high standard error and the fact that 14% of individuals increased respiration rates (Fig. 4; Modig and Ólafsson 1998). We also observed three individuals (one foraminifera, one nematode, and one kinorhynch) (Fig. 5) that had unexceptional respiration rates under oxic conditions, but under hypoxic conditions, they lowered their respiration rates substantially compared to all other meiofauna. This shows that response to hypoxia can vary on an individual level as well, while reasons behind this are yet unknown.

Different levels of tolerance to hypoxia are potentially related to the ecology of each meiofauna group (Lasserre 1976). Copepods and ostracods spend most of their time at the sediment–water interface, where the oxygen concentration is higher compared to the concentration in deeper layers of the sediment inhabited by nematodes and other burrowing meiofauna. In addition, because meiofauna lack respiratory organs, they rely exclusively on molecular oxygen diffusion, and thus individuals with a high surface-to-volume ratio might have higher chances of surviving severely hypoxic events (Wetzel et al. 2001). Some, most often large, nematodes enter inactive state to conserve energy (Atkinson 1973). Yet, this coping mechanism would only help for ~ 24 h (Atkinson 1973). During our experiments too, meiofauna seemed quiescent after exposure to hypoxia. Detailed research is required to evaluate the true nature of meiofauna oxygen preferences by investigating their vertical migration along oxygen gradients.

Allometric equation coefficients are not dependent on habitat type

For the five dominant meiofauna groups, log respiration was plotted against the log biomass to give regression equations in the form of $\log_{10}\mathrm{IRR} = a + b \times \log_{10}\mathrm{Biomass}$, where coefficient a is a constant that represents the metabolic rate of an organism with a body mass of one unit, while coefficient b is an exponent that determines the scaling relationship between metabolic rate and body mass (Glazier 2005) Logarithmically transforming variables in a regression model is a common way of presenting meiofaunal respiration rates (Warwick and Price 1979; Herman and Heip 1983; Shirayama 1992).

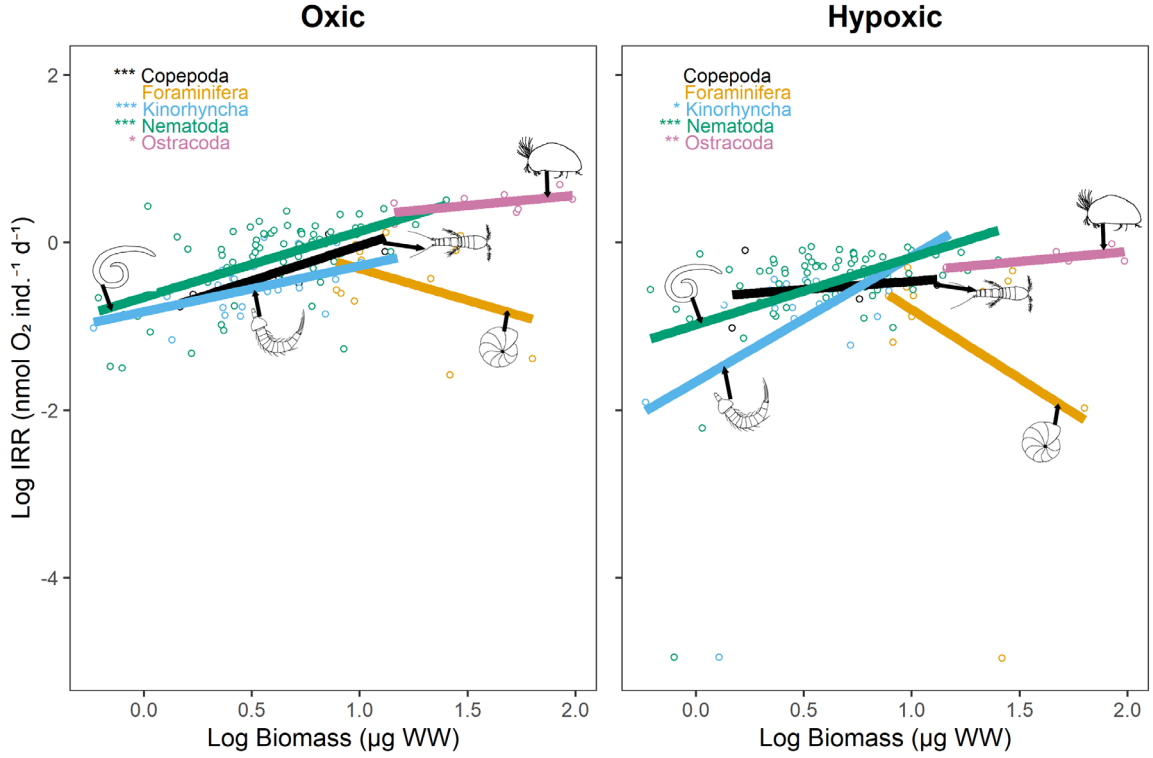

Fig. 5. The relationship between the log-transformed meiofauna individual respiration rate and log-transformed biomass for five meiofauna groups under oxic (left) and hypoxic (right) conditions. The same individuals were used for oxic and hypoxic incubations. Significance codes are displayed as a series of stars (0***, 0.001**, and 0.05*). Regression coefficients are provided in Supplementary Table S1.

As expected, our results suggest that biomass was an important factor in defining the IRR for copepods ($p = 0.006$), kinorhyncha ($p = 0.009$), nematodes ($p < 0.0001$), and ostracods ($p = 0.03$) (Fig. 5, Supplementary Table S2). A significant relationship between log-transformed biomass and respiration was not observed for foraminifera ($p = 0.2$), which might be related to the artifacts of biomass estimations of shell-bearing meiofauna (Moodley et al. 2008). While a positive relationship is generally observed for all meiofauna groups, only two studies out of five have reported a positive relationship between foraminifera cell volume and respiration (Geslin et al. 2011). Furthermore, we demonstrate that under hypoxic conditions, the relationship between biomass and individual respiration became less significant for all non-shell-bearing meiofauna (Fig. 5).

The most common way of estimating meiofauna respiration is using allometric scaling relationship which poses assumptions related to the two applied coefficients and estimations of biomass. The data plotted in Fig. 5 provide regression equations for indirect estimations of meiofauna respiration. Regression coefficient b defines the rate of change of log-transformed metabolism with body weight, and its expected range is 0.6–0.9 (Lasserre 1976). In our significant regression equations, the average established b coefficient for all meiofauna groups ($b = 0.72$, Supplementary Table S2) was very similar to that commonly used for meiofauna and all poikilotherms ($b = 0.75$) (Banse 1982; Heip et al. 1985). We, however, observed a large variation in b coefficient between the groups. The coefficient for shell-bearing organisms (foraminifera $b = -0.75$, ostracoda $b = 0.24$) deviated the most from the established 0.75 value. Therefore, 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.

The highest significant metabolic intensity at 10°C was observed for ostracods ($a = 0.08$), and the lowest for copepods ($a = -0.87$) and kinorhyncha ($a = -0.83$) (Supplementary Table S2). Coefficient a values have previously been established for copepoda, nematoda, ostracoda, foraminifera from various habitats (Fig. 6), and also water mites, oligochaetes (Banse 1982), polychaetes (Price and Warwick 1980), and gnathostomulids (Schiemer 1973). To compare our results with previous published coefficients a and b, our respiration rates and biomass data were converted to match the units and experimental temperatures (assuming Q10 = 2) used in previous studies (Fig. 6). Overall, in literature established b and a coefficients were not significantly different from coefficients of this study (Kruskal-Wallis test, $p = 0.2677$ and $p = 0.2675$,

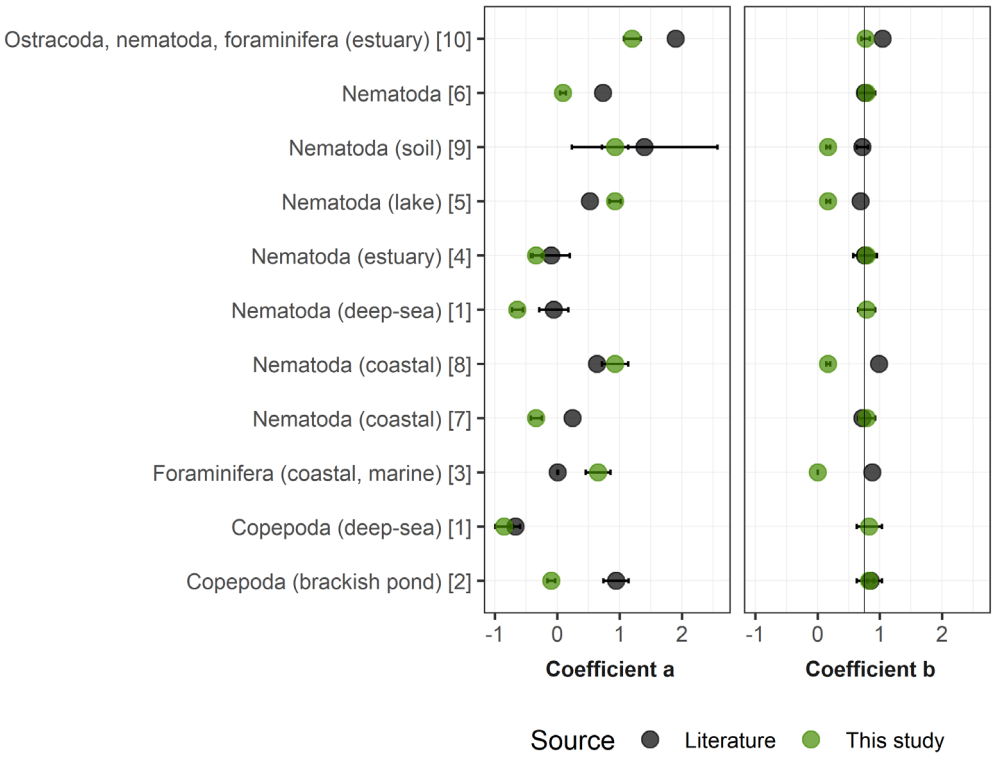

Fig. 6. Comparison of established regression coefficients a and b (± SD, when available) between this study (green) and literature data (black). Each row represents a different study: [1] Shirayama (1992), [2] Herman and Heip (1983), [3] Geslin et al. (2011), [4] Warwick and Price (1979), [5] Schiemer and Duncan (1974), [6] Banse (1982), [7] Kim and Shirayama (2001), [8] Ott and Schiemer (1973), [9] Klekowski et al. (1972), and [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).

respectively). This indicates that meiofauna from marine coastal habitats have similar metabolic rates as meiofauna from other habitats when organisms are exposed to the same oxygen and temperature conditions. For example, deep-sea (> 1000 m depth) copepods had very similar coefficient *a* values compared to the ones established for copepods collected at 40 m depth (this study) (Fig. 6). Therefore, previously developed regression coefficients can be used for allometric meiofauna respiration estimations regardless of the study site. In the case of nematodes, however, the use of feeding guild-specific coefficients *a* might lead to the four-fold overestimation of meiofauna respiration (Maciute et al. 2021). While it is reasonable to think that different feeding types of nematodes have different respiration rates and express different sensitivity to hypoxia, there are only few investigations of this in the literature and the limited amount of data in our investigation did not allow for further exploration of type-specific coefficients.

Nematodes and foraminifera are the most important contributors

In the present study, emphasis has been placed on the determination of meiofauna respiration rates under oxic and hypoxic conditions. While literature data of meiofauna abundances and our defined TOU rates (Supplementary Table S2) allowed for approximate estimates of meiofauna contribution to total sediment oxygen uptake at three study sites. Assuming meiofauna abundances at the brackish Baltic Sea site were as reported by Ólafsson and Elmgren (1997) in the same study area, meiofauna respiration represents 33% of TOU. Based on meiofauna abundance data reported by Josefson and Widbom (1988) in the same marine fjord, meiofauna accounts for 3% and 4% of TOU in coastal and fjord sediments, respectively. This extremely large range of contribution is thus driven by the conspicuous difference in meiofauna abundance between coastal sites.

Nematodes in general and, in marine habitats, foraminifera are recognized as dominant meiofauna groups due to their high abundances (Giere 2008). Accordingly, in our study nematodes and foraminifera contributed the most to the sediment oxygen uptake. Previously reported nematode contribution range from 0.5 to 14% (De Bovée et al. 1996; Soetaert et al. 2009). Our results are within this range, showing that the highest contribution was always associated with nematode community: 30% on the brackish coast, and 2–3% at the two marine sites. In our study, foraminifera were the second most important contributor to TOU (1% at both marine sites). While on the brackish coast, ostracods contributed by 2%, and copepods by 0.5%. Other, less abundant meiofauna groups such as kinorhyncha, water mites, and bivalves contributed the least (< 0.3%).

Similar estimations of meiofauna respiration have mainly been done based on allometric relations, which showed that the whole meiofauna community accounted for 5% of TOU in Arctic fjords (Kotwicki et al. 2018), 12% on New Zealand's continental margin (Leduc et al. 2016), and 1–22% in the deep-sea (Heip et al. 2001; Baguley et al. 2008; Shimabukuro et al. 2022). Macrofauna juvenile (i.e., temporary meiofauna) abundance is seldom reported together with permanent meiofauna abundance. As such most studies only report data on copepoda, ostracoda, foraminifera, and nematoda groups, somewhat hindering the evaluation of macrofaunal juvenile contribution to TOU. In the literature, however, 1–2 ind 10 cm^{-2} abundance of bivalvia spats is considered to be high (Strasser et al. 1999) and thus although macrofaunal juveniles respiration was high (> 2 nmol O_2 ind^{-1} d^{-1}), their significance is still lower than abundant smaller meiofaunal organisms.

We conclude that the extent of meiofauna contribution to oxygen uptake heavily depends on meiofauna abundance, which varies substantially (spanning 2 orders of magnitude) in coastal systems (Soetaert et al. 2009). Nevertheless, our study provides clear evidence that meiofauna contribute significantly to oxygen uptake and thus to benthic biogeochemical cycles. Because of their high abundance and biogeochemical contribution, we recommend that future studies will report at least nematode and foraminifera density and respiration. Since marine and brackish sediments cover almost 70% of our planet, meiofauna should be recognized for their pivotal role in the global oxygen and carbon cycles.

Data availability statement

All data from this study are available as Supplementary Material. Individual respiration, metabolic rates and meiofauna body parameters are available on Zenodo DOI: 10.5281/zenodo.8047551.

References

Atkinson, H. 1973. The respiratory physiology of the marine nematodes Enoplus brevis (Bastian) and E. communis (Bastian) I. the influence of oxygen tension and body size. J. Exp. Biol. **59**: 255–266.

Baguley, J. G., P. A. Montagna, L. J. Hyde, and G. T. Rowe. 2008. Metazoan meiofauna biomass, grazing, and weight-dependent respiration in the northern Gulf of Mexico deep sea. Deep-Sea Res. II Top. Stud. Oceanogr. **55**: 2607–2616.

Balsamo, M., F. Semprucci, F. Frontalini, and R. Coccioni. 2012. Meiofauna as a tool for marine ecosystem biomonitoring. Mar Ecosyst **4**: 77–104.

Banse, K. 1982. Mass-scaled rates of respiration and intrinsic growth in very small invertebrates. Mar. Ecol. Prog. Ser. **9**: 281–297.

Berg, P., N. Risgaard-Petersen, and S. Rysgaard. 1998. Interpretation of measured concentration profiles in sediment pore water. Limnol. Oceanogr. **43**: 1500–1510.

Bonaglia, S., F. J. Nascimento, M. Bartoli, I. Klawonn, and V. Bruchert. 2014. Meiofauna increases bacterial denitrification

in marine sediments. Nat. Commun. **5**: 5133. doi:10.1038/ncomms6133

Bonaglia, S., J. Hedberg, U. Marzocchi, S. Iburg, R. N. Glud, and F. J. Nascimento. 2020. Meiofauna improve oxygenation and accelerate sulfide removal in the seasonally hypoxic seabed. Mar. Environ. Res. **159**: 104968.

Braeckman, U., J. Vanaverbeke, M. Vincx, D. van Oevelen, and K. Soetaert. 2013. Meiofauna metabolism in Suboxic sediments: Currently overestimated. Plos One **8**: e59289. doi:10.1371/journal.pone.0059289

Broecker, W. S., and T. H. Peng. 1974. Gas-exchange rates between air and sea. Tellus **26**: 21–35. doi:10.3402/tellusa.v26i5.9869

Broman, E., S. Bonaglia, O. Holovachov, U. Marzocchi, P. O. Hall, and F. J. Nascimento. 2020. Uncovering diversity and metabolic spectrum of animals in dead zone sediments. Commun Biol **3**: 1–12.

Cesbron, F., E. Geslin, F. Jorissen, M.-L. Delgard, L. Charrieau, B. Deflandre, D. Jézéquel, P. Anschutz, and E. Metzger. 2016. Vertical distribution and respiration rates of benthic foraminifera: Contribution to aerobic remineralization in intertidal mudflats covered by Zostera noltei meadows. Estuar. Coast. Shelf Sci. **179**: 23–38.

Choquel, C., and others. 2021. Denitrification by benthic foraminifera and their contribution to N-loss from a fjord environment. Biogeosciences **18**: 327–341.

Coull, B. C. 1999. Role of meiofauna in estuarine soft-bottom habitats. Austral J Ecol **24**: 327–343.

De Bovée, F., P. Hall, S. Hulth, G. Hulthe, A. Landen, and A. Tengberg. 1996. Quantitative distribution of metazoan meiofauna in continental margin sediments of the Skagerrak (northeastern North Sea). J Sea Res **35**: 189–197.

Deldicq, N., D. Langlet, C. Delaeter, G. Beaugrand, L. Seuront, and V. M. Bouchet. 2021. Effects of temperature on the behaviour and metabolism of an intertidal foraminifera and consequences for benthic ecosystem functioning. Sci. Rep. **11**: 1–14.

Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science **321**: 926–929.

Feller, R., and R. Warwick. 1988. Energetics. Smithsonian Institution Press.

Geslin, E., N. Risgaard-Petersen, F. Lombard, E. Metzger, D. Langlet, and F. Jorissen. 2011. Oxygen respiration rates of benthic foraminifera as measured with oxygen microsensors. J. Exp. Mar. Biol. Ecol. **396**: 108–114.

Giere, O. 2008. Meiobenthology: The microscopic motile fauna of aquatic sediments. Springer Science & Business Media.

Glazier, D. S. 2005. Beyond the '3/4-power law': Variation in the intra-and interspecific scaling of metabolic rate in animals. Biol. Rev. **80**: 611–662.

Glud, R. N. 2008. Oxygen dynamics of marine sediments. Mar Biol Res **4**: 243–289.

Hannah, F., R. Rogerson, and J. Laybourn-Parry. 1994. Respiration rates and biovolumes of common benthic foraminifera (protozoa). J Mar Biol Assoc UK **74**: 301–312.

Heip, C., M. Vincx, and G. Vranken. 1985. The ecology of marine nematodes. Oceanogr Mar **23**: 399–489.

Heip, C., and others. 2001. The role of the benthic biota in sedimentary metabolism and sediment-water exchange processes in the Goban spur area (NE Atlantic). Deep-Sea Res. II Top. Stud. Oceanogr. **48**: 3223–3243.

Herman, P., and C. Heip. 1983. The respiration of five brackish-water harpacticoid copepod species. J. Exp. Mar. Biol. Ecol. **71**: 249–256.

Josefson, A., and B. Widbom. 1988. Differential response of benthic macrofauna and meiofauna to hypoxia in the Gullmar Fjord basin. Mar. Biol. **100**: 31–40.

Jørgensen, B. B., F. Wenzhöfer, M. Egger, and R. N. Glud. 2022. Sediment oxygen consumption: Role in the global marine carbon cycle. Earth Sci Rev **228**: 103987.

Kennedy, A. D. 1994. Carbon partitioning within meiobenthic nematode communities in the Exe estuary, UK. Mar Ecol Prog Ser **105**: 71–78. doi:10.3354/meps105071

Kim, D., and Y. Shirayama. 2001. Respiration rates of free-living marine nematodes in the subtidal coarse-sand habitat of Otsuchi Bay, northeastern Honshu, Japan. Zool Sci **18**: 969–973.

Klekowski, R., L. Wasilewska, and E. Paplinska. 1972. Oxygen consumption by soil-inhabiting nematodes. Nematologica **18**: 391–403.

Kotwicki, L., K. Grzelak, K. Opaliński, and J. M. Węsławski. 2018. Total benthic oxygen uptake in two Arctic fjords (Spitsbergen) with different hydrological regimes. Oceanologia **60**: 107–113.

Lambshead, P. 1993. Recent developments in marine benthic biodiversity reserch. Oceanis **19**: 5–24.

Lasserre, P. 1976. Metabolic activities of benthic microfauna and meiofauna, p. 95–142. *In* I. N. McCave [ed.], The benthic boundary layer. Springer.

Leduc, D., C. A. Pilditch, and S. D. Nodder. 2016. Partitioning the contributions of mega-, macro-and meiofauna to benthic metabolism on the upper continental slope of New Zealand: Potential links with environmental factors and trawling intensity. Deep-Sea Res. I Oceanogr. Res. Pap. **108**: 1–12.

Maciute, A., O. Holovachov, P. Berg, R. N. Glud, E. Broman, F. J. Nascimento, and S. Bonaglia. 2021. A microsensor-based method for measuring respiration of individual nematodes. Methods Ecol Evol **12**: 1841–1847.

Mahaut, M.-L., M. Sibuet, and Y. Shirayama. 1995. Weight-dependent respiration rates in deep-sea organisms. Deep-Sea Res. I Oceanogr. Res. Pap. **42**: 1575–1582.

Modig, H., and E. Ólafsson. 1998. Responses of Baltic benthic invertebrates to hypoxic events. J. Exp. Mar. Biol. Ecol. **229**: 133–148.

Moens, T., A. Vierstraete, S. Vanhove, M. Verbeke, and M. Vincx. 1996. A handy method for measuring meiobenthic respiration. J. Exp. Mar. Biol. Ecol. **197**: 177–190.

Moodley, L., M. Steyaert, E. Epping, J. J. Middelburg, M. Vincx, P. van Avesaath, T. Moens, and K. Soetaert. 2008. Biomass-specific respiration rates of benthic meiofauna: Demonstrating a novel oxygen micro-respiration system. J. Exp. Mar. Biol. Ecol. **357**: 41–47.

Nascimento, F. J., J. Näslund, and R. Elmgren. 2012. Meiofauna enhances organic matter mineralization in soft sediment ecosystems. Limnol. Oceanogr. **57**: 338–346.

Ólafsson, E., and R. Elmgren. 1997. Seasonal dynamics of sublittoral meiobenthos in relation to phytoplankton sedimentation in the Baltic Sea. Estuar. Coast. Shelf Sci. **45**: 149–164.

Ott, J., and F. Schiemer. 1973. Respiration and anaerobiosis of free living nematodes from marine and limnic sediments. Neth J Sea Res **7**: 233–243.

Price, R., and R. Warwick. 1980. The effect of temperature on the respiration rate of meiofauna. Oecologia **44**: 145–148.

Risgaard-Petersen, N., and others. 2006. Evidence for complete denitrification in a benthic foraminifer. Nature **443**: 93–96.

Schiemer, F. 1973. Respiration rates of two species of gnathostomulids. Oecologia **13**: 403–406.

Schiemer, F., and A. Duncan. 1974. The oxygen consumption of a freshwater benthic nematode, Tobrilus gracilis (Bastian). Oecologia **15**: 121–126.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH image to ImageJ: 25 years of image analysis. Nat. Methods **9**: 671–675.

Schratzberger, M., and J. Ingels. 2018. Meiofauna matters: The roles of meiofauna in benthic ecosystems. J. Exp. Mar. Biol. Ecol. **502**: 12–25.

Shimabukuro, M., D. Zeppilli, D. Leduc, F. Wenzhöfer, P. Berg, A. A. Rowden, and R. N. Glud. 2022. Intra-and inter-spatial variability of meiofauna in hadal trenches is linked to microbial activity and food availability. Sci. Rep. **12**: 1–11.

Shirayama, Y. 1992. Respiration rates of bathyal meiobenthos collected using a deep-sea submersible SHINKAI 2000. Deep Sea Res A Oceanogr Res Pap **39**: 781–788.

Soetaert, K., M. Franco, N. Lampadariou, A. Muthumbi, M. Steyaert, L. Vandepitte, E. van den Berghe, and J. Vanaverbeke. 2009. Factors affecting nematode biomass, length and width from the shelf to the deep sea. Mar. Ecol. Prog. Ser. **392**: 123–132.

Somerfield, P. J., R. M. Warwick, and T. Moens. 2005. Methods for the study of marine benthos, v. **3**. Wiley-Blackwell, p. 229–272.

Steyaert, M., N. Garner, D. van Gansbeke, and M. Vincx. 1999. Nematode communities from the North Sea: Environmental controls on species diversity and vertical distribution within the sediment. JMBA **79**: 253–264.

Steyaert, M., L. Moodley, T. Nadong, T. Moens, K. Soetaert, and M. Vincx. 2007. Responses of intertidal nematodes to short-term anoxic events. J. Exp. Mar. Biol. Ecol. **345**: 175–184.

Strasser, M., M. Walensky, and K. Reise. 1999. Juvenile-adult distribution of the bivalve Mya arenaria on intertidal flats in the Wadden Sea: Why are there so few year classes? Helgol Mar Res **53**: 45–55.

Teal, J. M., and W. Wieser. 1966. The distribution and ecology of nematodes in a Georgia salth marsh. Limnol. Oceanogr. **11**: 217–222.

Van Den Hoogen, J., and others. 2019. Soil nematode abundance and functional group composition at a global scale. Nature **572**: 194–198.

Warwick, R., and R. Price. 1979. Ecological and metabolic studies on free-living nematodes from an estuarine mud-flat. Estuar Coast Mar Sci **9**: 257–271. doi:10.1016/0302-3524(79)90039-2

Wetzel, M. A., J. W. Fleeger, and S. P. Powers. 2001. Effects of hypoxia and anoxia on meiofauna: A review with new data from the Gulf of Mexico. Coast Estuar Sci **58**: 165–184.

Wieser, W., and J. Kanwisher. 1961. Ecological and physiological studies on marine nematodes from a small saltmarsh near woods hole, Massachusetts. Limnol Oceanogr **6**: 262–270.

Acknowledgments

The authors acknowledge financial support from the Swedish Research Council Formas (grant no. 2017-01513 to S.B.). The Swedish Environmental Protection Agency's Research (grant no. NV-802-0151-18 to F.J.A.N) in collaboration with the Swedish Agency for Marine and Water Management. The European Commission through HADES-ERC (grant no. 669947), Danish National Research Foundation through the Danish Center for Hadal Research, HADAL (grant no. DNRF145), and the US National Science Foundation (grant no. OCE-1851424). They also acknowledge the Askö and Kristineberg marine station staff for the help during sampling, and Irina Polovodova Asteman and Phoebe O'brien for assistance in foraminifera picking.

Conflict of Interest

All authors declare that they have no conflict of interest.

Submitted 25 October 2022
Revised 08 June 2023
Accepted 15 June 2023

Associate editor: Christopher Edward Cornwall