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PRACTICAL TOOLS

Methods in Ecology and Evolution BRITISH ECOLOGICAL SOCIETY

A microsensor-based method for measuring respiration of individual nematodes

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Abstract

1. Meiofauna (invertebrates that pass through a 1-mm mesh sieve, but are retained on a 40-μm mesh) represent the most abundant and diverse animal group on Earth, but empirical evidence of their role in benthic respiration, production and carbon cycling across ecosystems is not well documented. Moreover, how meiofauna respond to changing oxygen conditions is poorly understood.
2. We further developed an incubation system, in which oxygen and temperature conditions are easily controlled and single meiofaunal nematode respiration is resolved in glass capillary tubes, using Clark-type oxygen microsensor. We performed the respiration measurements after exposing nematodes to different ambient oxygen concentrations, which resulted in 3–60 μM O_2 during hypoxic and 80–210 μM O_2 during oxic incubations in close proximity to the respective nematodes.
3. Individual nematode respiration rates ranged from 0.02 to 1.30 nmol O_2 ind.$^{-1}$ day^{-1} and were 27% lower during hypoxic than oxic incubations. Rates derived from established allometric relations were on average fourfold higher than our direct measurements.
4. The presented method is suitable for single nematode respiration measurements and can be adapted to a wide range of experimental conditions. Therefore, it can be used to assess meiofauna contribution to ecosystem processes and investigate species-specific responses to changing environmental conditions, for example, oxygen stress, increasing water temperature.

KEYWORDS

Clark-type microsensor, hypoxia, meiobenthos, meiofauna, nematodes, oxygen consumption, respiration

1 | INTRODUCTION

Oxygen respiration is typically measured as a proxy for metabolic or biological activities and express how much an organism contributes to carbon cycling in a given environment. Individual meiofauna (invertebrates that pass through a 1-mm mesh sieve, but are retained on a 40-μm mesh) are large enough to be physically handled, but respiration measurement is challenging due to insufficient sensitivity of standard respirometry approaches (Moens et al., 1996; Moodley et al., 2008). To date, only the manometric Cartesian diver method (Linderstrom-Lang, 1937) has been able to detect single nematode respiration by monitoring density changes associated with oxygen consumption by a nematode inside a sealed glass vessel (Wieser et al., 1974). However, due to its complexity, the Cartesian diver approach has never been used for routine meiofauna respiration measurements. Instead, nematode respiration has been 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 (Moens et al., 1996; 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 IRR as a function of species, age and sex by standard procedures. Moreover, respiration rate may depend on the number of animals incubated in the chamber.

A recently developed microsensor-based nanorespiration system has enabled respiration measurements of sessile microscopic organisms such as individual copepod eggs and bovine embryos. The method is based on the fact that a respiring organism on the bottom of one-end-open tube and a continuous oxygen supply from the overlying water will create a linear concentration gradient between the top and the bottom of the tube after certain incubation time (Hammervold et al., 2015; Lopes et al., 2005; Nielsen et al., 2007). The oxygen flux towards an organism (i.e. respiration rate) can then be calculated based on the slope of the measured gradient. However, this approach has never been used for respiration measurements of mobile organisms that are likely to escape the tubes. Moreover, these previous nanorespiration measurements could not be done under in situ oxygen conditions. This is important because generally oxygen penetrates only few millimetres or a centimetre into the sediment (Glud, 2008). Therefore, plethora of already produced meiofauna respiration data might only poorly reflect in situ respiration rates (Braeckman et al., 2013).

Here we present an improved microsensor-based system for respiration measurements on single meiofaunal specimens, which can distinguish between abiotic conditions and variation of respiration rates among individuals. We applied this method to the most abundant and diverse animal group in aquatic sediments—nematodes, while exposing them to a range of relevant oxygen and temperature conditions (Giere, 2008). To evaluate its applicability, we targeted nematodes with potentially diverse metabolic rates by sampling two sites with naturally contrasting oxygen levels in the Baltic Sea: an oxic (~70% air saturation, ~240 μM O_2 at the sediment–water interface) and a severely hypoxic site (2% air saturation, 13 μM O_2). Finally, all measured IRR were compared to the rates that were derived from widely used theoretical allometric assessments.

2 | MATERIALS AND METHODS

2.1 | Sampling and experimental design

Nematodes were collected at the oxic site (58.81012N, 17.61653E) in November 2019 and June 2020, and at the long-term hypoxic site (59.19086N, 18.60434E) in November 2019 (Table 1).

Bottom water temperature was 5°C in November and 15°C in June.

Right before the experiment, the top 1-cm sediment layer was sliced off and sieved. Individuals retained on a 40-μm sieve were handpicked, photographed for later body length and width determinations (Supporting Information Text S1) and placed into separate capillary tubes filled with twice-filtered in situ water (pore size 0.2 μm). Nematodes were placed into the tubes by gently picking each nematode and transferring it into a submerged tube using a tungsten wire. The tubes were then kept in an aquarium for a 3-hour incubation at 10°C in darkness (Figure 1a,b). Incubations were done at ~210 μM (oxic) and at 60 μM (hypoxic) ambient oxygen concentrations (Table 1), while the concentration on the bottom of the tubes varied depending on animal-specific respiration rate. Thus, based on the observed oxygen profiles, the incubations resulted in steady state oxygen levels ranging from 3 to 60 μM O_2 during hypoxic incubations and from 80 to 210 μM O_2 during oxic incubations at the bottom of the tubes.

2.2 | Incubation system

Capillary tubes containing nematodes were incubated in a 1-L capped plexiglass aquarium (9 × 9 × 16 cm; KreativPlast). The lid was

TABLE 1 Number of nematodes incubated under oxic and hypoxic conditions

Site	Genus	Oxic incubation (ind.)	Hypoxic incubation (ind.)
Oxic	*Paracanthonchus*	9	8
	Sabatieria	9	9
	Desmolaimus	17	19
	Eleutherolaimus	7	6
	Oncholaimidae		1
	Sphaerolaimus	3	3
Hypoxic	*Paracanthonchus*	1	1
	Sabatieria	5	5
	Axonolaimus	2	1
	Leptolaimus	5	4
	Chromadorita	9	7

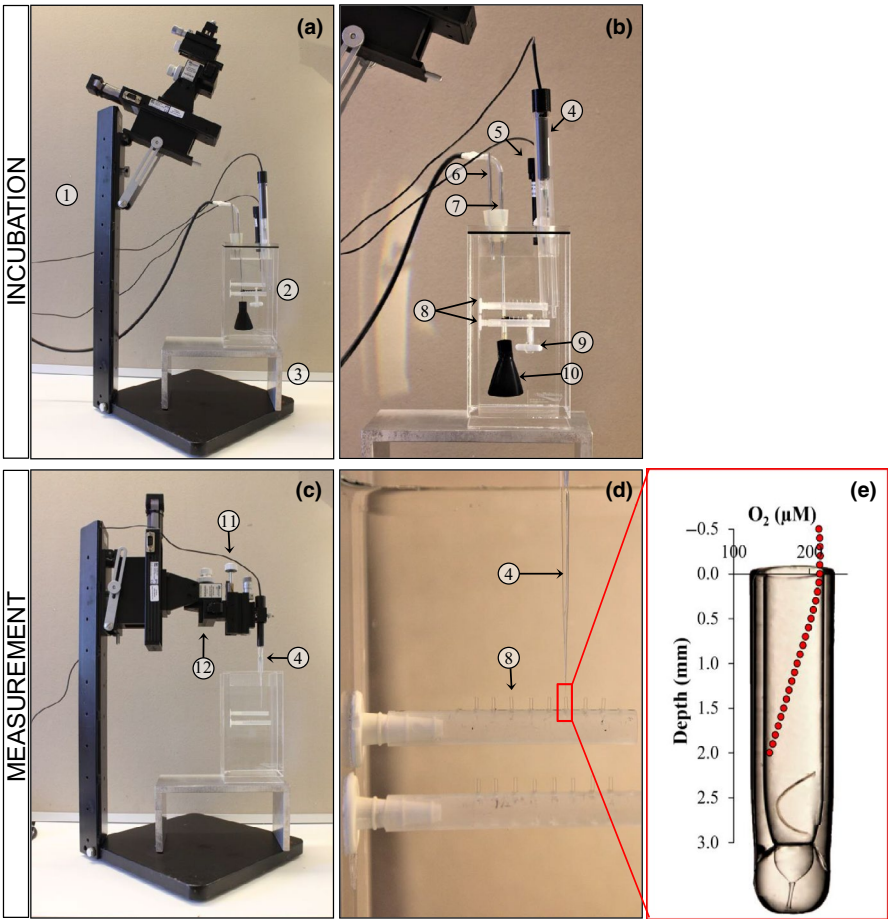

FIGURE 1 Incubation setup (a, b) 1-microprofiling unit, 2-incubation aquarium, 3-elevation stand, 4-oxygen microsensor, 5-temperature sensor, 6-gas outlet, 7-gas inlet, 8-capillary tubes secured on two holders, 9-magnetic stirring bar, 10-covered air stone. Note: 15-L tank and cooling coil are not shown (see Figure S1). Measurement setup (c, d) 11-micromanipulator, 12-motor. Capillary tube with a nematode inside (e). Red dots indicate oxygen concentration obtained from the microsensor readings

equipped with a temperature sensor (TP2000, tip diameter 2 mm, Unisense), oxygen sensor (OX-50, tip diameter 50 µm, Unisense), an air stone and a magnetic stirring bar (Figure 1b). The incubation aquarium was placed in a 15-L tank connected to a thermostatic circulator to control the temperature (LKB Bromma 2219, Haake; Figure S1).

The core of the incubation system is a set of capillary tubes (inner Ø 0.6 mm, length 3 mm, Supporting Information Text S2; VitroTubes™, VitroCom) secured on a plastic holder which can easily be attached and removed from the side wall of the incubation aquarium (Figure 1d). The only source of oxygen to the capillary tubes is the overlying water in the aquarium. The respiring organism on the bottom of the tube acts as an oxygen sink, and thus the diffusion will establish a vertical linear concentration gradient towards the animal (Figure 1e; Nielsen et al., 2007). At steady state, the concentration close to the organism reaches a constant level and the oxygen consumption by the organism equals the oxygen supply.

Right before the measurements, the lid of the aquarium was removed and the oxygen microsensor was mounted on the motorized micromanipulator. The microsensor tip was positioned 0.5 mm above the opening of the tube (Figure 1c,d). The gradient in each tube was then measured at 0.1-mm depth intervals down to 2-mm depth, meaning that approximately 15 s were needed to complete the measurements in one tube by the applied sensor (Figure 1e). Every fourth tube was left empty for parallel blank measurements.

2.3 | Calculation of measured and theoretical IRR

The oxygen flux at steady state (J) was quantified by Fick's first law of diffusion:

$J = -D_{O_2}(dC/dx)$, where dC/dx is the vertical oxygen concentration gradient inside the capillaries from 0.1- to 2-mm depth and D_{O_2} is the molecular diffusivity of oxygen at the specific temperature and salinity. D_{O_2} vues were obtained from Broecker and Peng (1974). IRR was then calculated from the oxygen flux (J) by multiplying it by the cross-sectional area of capillary (A) and was corrected for blank measurements followed by data quality control (Supporting Information Text S3).

Theoretical IRR for all nematodes were calculated as described in Kennedy (1994), by taking into account nematode's body volume, feeding group-specific metabolic constant and metabolic scaling exponent. The calculations are described in Supporting Information Text S4. Theoretical nematode respiration rates represent nematode IRR at 20°C. Thus, the rates were scaled to 10°C, assuming that thermal sensitivity of meiofauna metabolic rates (Q_{10}) is equal to 2 (Braeckman et al., 2013).

2.4 | Supporting model calculations

A mathematical model describing the vertical diffusive oxygen transport in a capillary tube was developed to estimate the time required to establish 97.5% of oxygen gradient at steady state and project ambient oxygen levels around the nematodes. The model provides non-steady state one-dimensional solutions to Fick's second law of diffusion: $dC/dt = D_{O_2}(d^2C/dx^2)$, where C is the oxygen concentration, t is time, D_{O_2} is oxygen molecular diffusivity, x is distance from the top of the tube. This equation was solved numerically using a group of numerical methods called control volume approach (Patankar, 1980), and a separation of the 3-mm-tall water column inside the tube into 100 control volumes. The incubation time required to reach the steady state was estimated for a range of molecular oxygen diffusivities between 1.05×10^{-5} cm^2/s ($T = 0$°C, $S = 35$‰) and 2.75×10^{-5} cm^2/s ($T = 30$°C, $S = 0$‰). These times were independent of nematode metabolism and the ambient oxygen concentration.

3 | RESULTS AND DISCUSSION

The new method can be implemented in any laboratory because all components are commercially available and easy to assemble. In addition, the presented mathematical model can be used as a guide for optimizing the incubation time depending on inner tube diameter or temperature and salinity conditions. For example, the model indicated that at our experimental conditions ($T = 10$°C, $S = 8$‰), 2.6-hr incubation was sufficient to establish 97.5% of the oxygen gradient at steady state (Figure 2a,b), meaning that after this time, measured O_2 gradients remained the same. Furthermore, the model can be used to estimate the O_2 concentration at the bottom of the tube, if an approximate respiration rate is known.

Based on visual inspection of capillary tubes after the measurements, all nematodes remained at the bottom and only five nematodes, in total, escaped the tubes during the incubations. Due to imposed mixing, oxygen concentration outside the capillary tubes was constant, while the profiles within the capillary tubes, containing single nematodes, showed a linear decrease in oxygen concentration (Figure 3). This indicates that the method allows incubating individuals at desired temperature and oxygen conditions. Other water parameters such as pH and salinity can also be easily manipulated. The exact oxygen concentration that animal will experience at steady state can be calculated using the model's results (Figure S2).

Although we detected minor oxygen gradients in a few blank capillaries, overall the oxygen concentration in blanks was near-constant with depth (Figure 3). In a previous work using a microsensor-based method, oxygen gradients were also detected in blank capillaries (Nielsen et al., 2007). Hence we recommend (a) using filtered in situ water; (b) including at least one blank per four samples in each incubation; (c) ensuring that the steady state is reached before the measurements (Figure 2b); (d) using Clark-type microelectrodes (Unisense, DK) with low stirring sensitivity and oxygen consumption; (e) starting the measurements from the opening of the tube towards the bottom avoiding excessive water mixing, and (f) quickly measuring the developed oxygen gradient that is, ≤1 s waiting time between the microsensor steps.

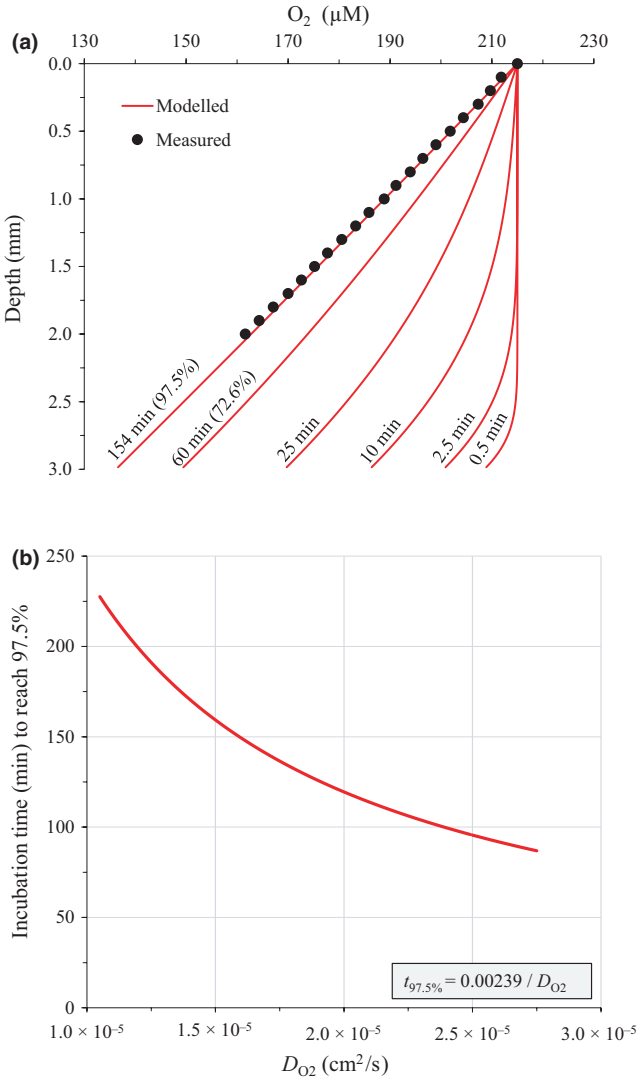

FIGURE 2 (a) An example of modelled (red lines) development of oxygen concentration gradient throughout 154 min incubation of a nematode with a respiration of 1.01 nmol ind.$^{-1}$ day^{-1}. Numbers in parentheses indicate the percentage of oxygen gradient at steady state. Black circles represent the measured oxygen concentration gradient after 3-hr incubation inside a tube containing the nematode at $T = 10$°C, $S = 8$ ‰ and 215 µM O_2 ambient water oxygen concentration. The steepness of the gradients are dependent on nematode metabolism. (b) Time it takes to reach 97.5% of oxygen gradient at steady state when oxygen molecular diffusivity (D_{O_2}) varies from 1.05 to 2.75×10^{-5} cm^2/s ($T = 0$°C, $S = 35$‰ to $T = 30$°C, $S = 0$‰). T = Temperature, S = Salinity. The times are independent of nematode metabolism

We are certain that 0.6-mm diameter of a capillary tube is enough to detect respiration of single meiofaunal organism, but if necessary, the sensitivity of the method could be further increased by using tubes with smaller inner diameter. The oxygen diffusion in narrow tubes is lower, allowing oxygen concentration gradient to

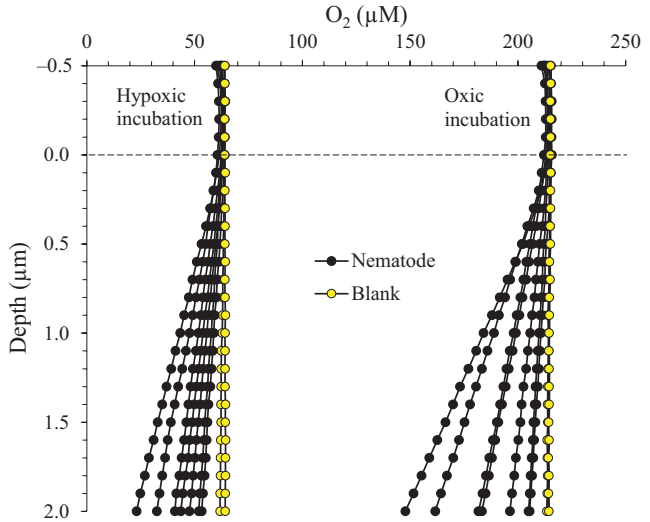

FIGURE 3 Examples of measured oxygen profiles in capillary tubes containing single nematodes (black circles) and in empty tubes without nematodes (yellow circles). Oxygen profiles were measured at steady state under either oxic or hypoxic conditions. The data points from −0.5 mm to 0 mm were measured above the capillary tubes. Horizontal dashed line represents the top of the tubes

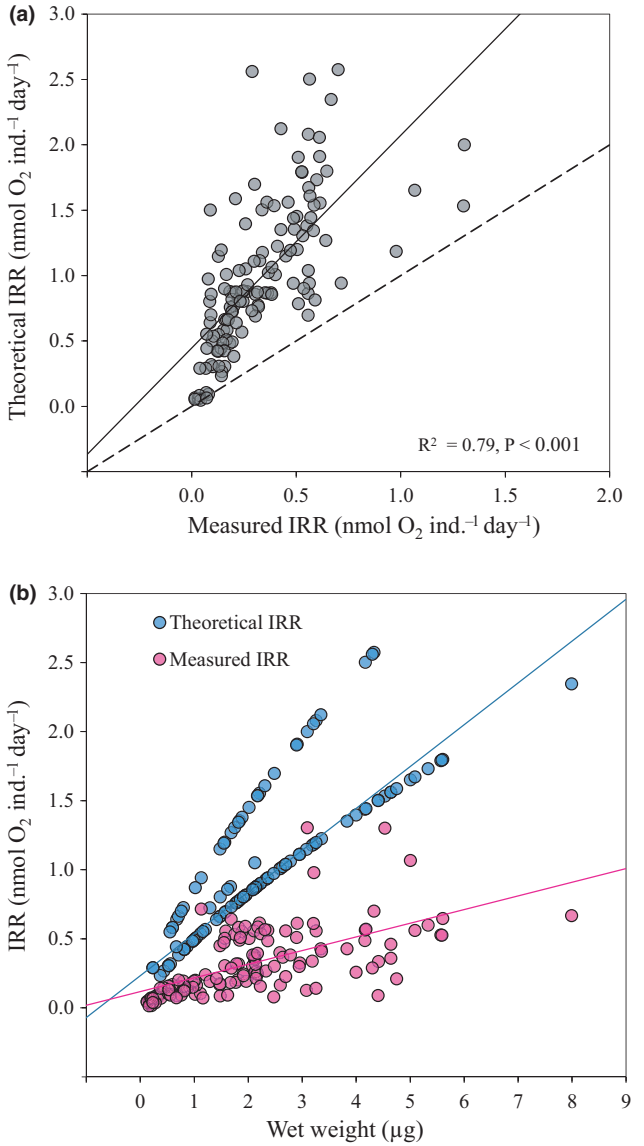

FIGURE 4 (a) Correlation between measured and theoretical individual respiration rates (IRR). Theoretical respiration rate varies depending on nematode body volume and feeding group. Dashed diagonal line indicates a 1:1 relationship between the two rates. (b) Comparison of measured (blue) and theoretical (pink) nematode IRR in relation to body weight. Data from both sampling sites, oxic and hypoxic incubations were combined ($n = 131$)

establish even at low respiration rates. The exact effects of this can be explored by applying the presented mathematical model.

The combined dataset of IRR measured under oxic and hypoxic conditions correlated significantly with theoretical IRR ($R^2 = 0.79$, $p < 0.001$, Spearman correlation; Figure 4a). However, theoretical IRR tended to be on average fourfold higher compared to the measured rates ($V = 8{,}776$, $p < 0.001$, dependent Mann–Whitney U test; Figure 4b). Of note, theoretical IRR coefficients were derived by studies that measured respiration under 100% air saturation (Price & Warwick, 1980), while generally, sediments are not 100% air saturated. In addition, calculations of theoretical IRR are based on feeding group-specific metabolic constants as well as theoretically calculated body volume or mass that often cannot be measured directly. Therefore, it is most likely that the assumptions behind body mass or volume calculations together with oxygen conditions in the ambient water have introduced errors in theoretical IRR and contributed to the observed fourfold overestimation.

The new method was tested on 131 nematodes belonging to nine genera from sediments with contrasting oxygen conditions and with a body mass ranging by almost two orders of magnitude. Moreover, the nematodes were exposed to a range of oxygen levels during incubations. As a result, the respiration rates varied by almost a factor of 90, with the lowest recorded nematode IRR of 0.02 nmol ind.$^{-1}$ day^{-1} (*Leptolaimus*, 0.16-µg wet weight, hypoxic incubation), and the highest – 1.30 nmol ind. $^{-1}$ day^{-1} (*Paracanthonchus*, 3.09-µg wet weight, oxic incubation; Figure 5). Great variation in IRR among individuals is due to both different body sizes and potentially different activity levels, as even similarly sized individuals may have up to threefold difference in respiration rates (Wieser & Kanwisher, 1961). Thus, when measuring single animal respiration, the importance of body mass, physiology and activity levels can be assessed more accurately. We recommend that future studies analyse enough individuals belonging to the same genus or feeding group in order to realize ecologically meaningful comparisons.

Individual respiration rates were only 27% lower under hypoxia than under oxic conditions ($H = 5$, $p = 0.03$, Scheirer-Ray-Hare test). In contrast, *Enoploides longispiculosus* biomass-standardized respiration rates decreased fourfold when oxygen concentration in respiration chambers decreased from 230 µM O_2 (normoxia) to 23 µM O_2 (severe hypoxia) (Braeckman et al., 2013). In the present study, effects of hypoxia on IRR was overall small, most likely because nematodes were incubated under relatively mild hypoxic (60 µM O_2)

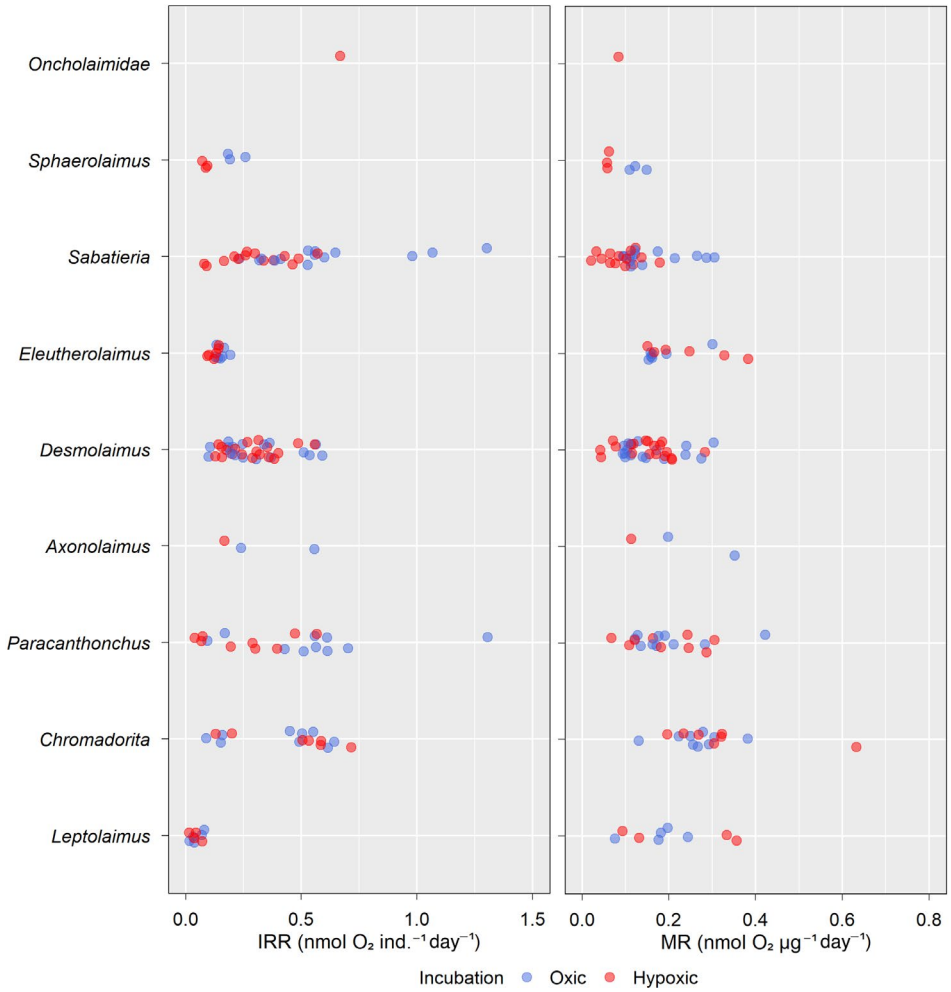

FIGURE 5 Individual respiration rate (IRR) and mass standardized respiration rate (MR) after hypoxic (red) and oxic (blue) incubations. The incubations resulted in oxygen steady state concentrations of 3–60 µM during hypoxic incubations, and of 80–210 µM during oxic incubations at the bottom of the tubes. To reduce the effect of over-plotting, data points were jittered on the *y*-axis, while the *x*-axis position was preserved. Note: different individuals were used for oxic and hypoxic incubations

conditions. In addition, future studies should incubate same individuals under both oxic and hypoxic conditions.

The large variation in IRR at the respective conditions might be related to different tolerances to low oxygen conditions between nematode taxa and traits (Jensen, 1995; Steyaert et al., 2005), feeding group as a result of different lifestyles (Teal & Wieser, 1966) or historical exposure to oxygen stress (Wetzel et al., 2001). Clearly, our method offers the possibility to investigate all of these factors.

Taken together, when measuring respiration of single animals, the effects of changing environmental conditions or intra- and interspecific differences in physiology on respiratory rates can be assessed more accurately, resulting in more realistic estimates of meiofauna contribution to carbon cycling.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

A.M. planned the study, sampled in the field, designed the incubation system, carried out the experiment, analysed data and drafted the manuscript; O.H. assisted with the respiration experiment, planned the study, determined nematode abundance, gave feedback on the manuscript; P.B. developed the mathematical model, gave feedback on the manuscript; E.B. and F.J.A.N. planned the study, sampled in the field, gave feedback on the manuscript; R.N.G. contributed to the development of the mathematical model and manuscript drafting; S.B. conceived the original idea, planned the study, sampled in the field, co-drafted the manuscript.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/2041-210X.13674.

DATA AVAILABILITY STATEMENT

Individual respiration, metabolic rates and nematode body parameters are available on Zenodo https://doi.org/10.5281/zenodo.5041510 (Maciute et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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