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Short-term CO_2 exposure and temperature rise effects on metazoan meiofauna and free-living nematodes in sandy and muddy sediments: results from a flume experiment

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1. Abstract

Global concern over increasing CO_2 emissions, and the resultant CO_2 driven temperature rises and changes in seawater chemistry, necessitates the advancement of understanding into how these changes will affect marine life now and in the future. Here we report on an experimental investigation into the effects of increased CO_2 concentration and elevated temperature on sedimentary meiofaunal communities. Cohesive (muddy) and non-cohesive (sandy) sediments were collected from the Eden Estuary in St. Andrews, Scotland, UK, placed within a flume setup and exposed to 2 levels of CO_2 concentration (380 and 750 ppmv, current at the time of the experiment, and predicted CO_2 concentration by 2100, respectively) and 2 temperature levels (12 °C and 16 °C, current in-situ and predicted temperature by 2100, respectively). We investigated the metazoan meiofauna and nematode communities before and after 28 days of exposure under these experimental conditions. The most determinative factor for abundance, diversity and community structure of meiofauna and nematodes was sediment type: on all levels, communities were significantly different between sand and mud sediments which agrees with what is generally known about the influence of sediment structure on meiofaunal organisms. Few CO_2 and temperature effects were observed, suggesting that meiofauna and nematodes are generally much less responsive than, for instance, microbial communities and macrofauna to these environmental changes in estuarine environments, where organisms are naturally exposed to a fluctuating environment. This was corroborated by the observed effects related to the different seasons in which the samples were taken from the field to run the experiment. After 28 days, meiofauna and nematode communities in muddy sediments showed a greater response to increased CO_2 concentration and temperature rise than in sandy sediments. However, further study is needed to investigate the underlying mechanisms and meiofauna species-specific resilience and responses to ocean acidification and warming, and their interactions with other biota, to understand what such changes may mean for meiofauna communities and the ecosystem processes and functions they contribute to.

2. Introduction

In the past 800k years CO_2 concentrations in the atmosphere have remained in the range of 172-300 parts per million by volume (ppmv) (Luthi et al., 2008). Since the start of the industrial era atmospheric CO_2 concentrations have increased dramatically and are currently exceeding 400 ppmv (parts per million in volume), with predictions of between 550 and 900 ppmv by 2100 (Cao and Caldeira, 2008; Hoegh-Guldberg and Bruno, 2010; IPCC, 2014). Of all the anthropogenic CO_2 emitted into the atmosphere, about 30 % has been absorbed in the surface ocean (<200 m) so far (Sabine et al., 2004). As this CO_2 is absorbed, it changes the seawater carbon chemistry and reduces pH in a process that is called "ocean acidification" (Gattuso and Hansson, 2011). Ocean acidification is already occurring (e.g. Caldeira and Wickett, 2003) and is predicted to worsen in the near and distant future with a reduction from pre-industrial pH levels of 8.2 to a projected global average of 7.8 in 2100 (Branch et al., 2013; IPCC, 2014). Ocean acidification, however, is a spatially variable phenomenon that is strongly modified by local conditions, particularly in coastal areas, which could lead to local ocean acidification hot spots. The accumulation of CO_2 in the atmosphere also increases the natural greenhouse effect and continues to drive global warming (IPCC, 2014). As a result, each of the last three decades has been successively warmer at the Earth's surface than any preceding decade since 1850, and whilst surface temperature increases of up to 4.8 °C are predicted by 2081-2100 (IPCC, 2014), the temperature in the top 100m of the ocean is expected to increase by 0.6 to 2.0 °C by 2100 (Collins et al., 2013). A variety of biological responses to ocean acidification and warming have been measured across a range of taxa, with substantial negative effects on individual responses (such as survival, calcification, growth and reproduction), ecological interactions (such as trophic relationships and organism behaviour) and community characteristics (such as abundance, diversity, production and biomass) (Alsterberg et al., 2013; Danovaro et al., 2004; Gingold et al., 2013; Kroeker et al., 2010). However, significant variation in the sensitivity of marine organisms is observed with for instance calcifying organisms being generally more susceptible to pH reductions than non-calcifying organisms, and pH reductions and temperature increases having different effects depending on the developmental stage or even sex (Ellis et al., 2017) of the organisms being studied and the gradients and shifts of complex ecological interactions (Ingels et al., 2012; Kroeker et al., 2010).

Marine organisms will be faced with a wide range of stressors in our future oceans, and it is therefore imperative that experimental approaches include multiple stressors in their designs to assess species, community, and ecosystem-level responses. Biological responses to ocean acidification and warming (OAW) will depend on physiological trade-offs or energy allocation to sustain the performance, survival and fitness of organisms (Brown et al., 2004; Findlay et al., 2011; Pörtner, 2008; Queirós et al., 2015). However, information from studies focusing on particular species or life-stages of certain species is - although crucial in documenting autecological processes and responses - insufficient to predict future change on the level of ecosystems considering the wide range of trophic and non-trophic interactions between species (Russell et al., 2011). Therefore, approaches that focus on groups of marine organisms that have ecological and functional significance are needed to document the effects and responses to OAW at a community level (Riebesell et al., 2010). In addition to the requirement for more studies integrated over various levels of biological organisation (Ingels et al., 2012), there is also an urgent need for more studies that cover the additive and synergistic effects of ocean acidification and warming occurring simultaneously (Pörtner, 2008).

The meiofauna comprises the small-sized organisms (generally between 32-63 µm and 1 mm; the lower size limit varies in the literature) in the benthos, whose morphology, physiology and life history characteristics have evolved to exploit the interstitial sedimentary space. They occur in often

high abundance and diversity in sediments worldwide, and are phyletically very diverse (Balsamo et al., 2012). The most abundant metazoan meiofaunal organisms in marine sediments are consistently the nematodes and copepods, with nematodes having colonized virtually every moist habitat that can sustain metazoan life. Meiofauna are important contributors to the physical, chemical and biological properties of sediments, the resilience of those sediments, and their role in benthic food webs has been amply documented (Schratzberger and Ingels, 2017). We can therefore consider them as an important ecological component of benthic ecosystems and alterations to their communities as a consequence of OAW may give much needed insights in to how benthic ecosystem structure and function may respond in future oceans.

Metazoan meiofauna generally comprise non-calcifying, infaunal invertebrates with low mobility. They are naturally exposed to large fluctuations of pore water pH and CO_2 concentrations and are therefore considered likely to be more tolerant than other animal groups to higher CO_2 concentrations (Gattuso and Hansson, 2011; Widdicombe et al., 2011) and temperature (Giere, 2009; Moens et al., 2013), particularly in shallow-water subtidal and intertidal coastal environments (Giere, 2009). This (assumed) tolerance, in addition to increased logistic effort associated with multiple stressors and necessary replication, as well as the required meiofaunal taxonomic expertise, may explain why relatively few studies so far have documented the effects of OAW on metazoan meiofauna (Hale et al., 2011; Meadows et al., 2015; Sarmento et al., 2016). That being said, there are several studies that have investigated OA or increased CO_2 concentration as a single stressor on metazoan meiofauna or nematodes (Barry et al., 2004; Carman et al., 2004; Dashfield et al., 2008; Ishida et al., 2013; Ishida et al., 2005; Kurihara et al., 2007; Takeuchi et al., 1997; Thistle et al., 2005; Widdicombe et al., 2009; Widdicombe et al., 2011). Some of these studies, however, have focused on effects associated with injected CO_2 or simulated CO_2 leakage or release in the context of Carbon dioxide Capture and Storage (Barry et al., 2004; Carman et al., 2004; Schade et al., 2016; Thistle et al., 2005) rather than ocean acidification in a climate change context. Effects of rising temperatures on metazoan meiofauna and nematodes in the context of climate change have equally been covered in some detail in existing literature (e.g. Gingold et al., 2013; Ingels et al., 2012). The OAW effects reported in these studies vary, depending on whether species or communities were studied, and which ontogenic stage was considered, and of course whether single stressors or multiple stressors were applied. Notably the influence of ecological interactions such as those between the macrofauna and meiofauna (trophic and competitive interactions), but also between individual species, on stressor responses creates a complex view of community dynamics in response to OAW and requires further study.

In the present study we report on the responses of meiofauna and nematode communities to OAW in a flume experiment where we exposed two types of intertidal sediment communities (muddy and sandy) to increased temperatures (+4 °C) and CO_2 concentrations (750 ppmv) in a fully crossed design. We sieved the sediments to exclude macrofauna, and hence were able to test more direct OAW effects on meiofauna in the absence of macro-meiofauna interactions. We used intertidal sediments, in which meiofauna organisms experience temperature and pH variations, amongst others, over tidal and diurnal cycles and while they may be well adapted to cope with such fluctuations, they may also be already pushed to the physiological edge of their tolerances. The main aim of our study was therefore to assess whether OAW has an effect on meiofauna and nematodes on a community level in intertidal sandy versus muddy sediments. Little is understood on how infaunal responses to OAW may differ between different sediment types despite the acknowledgement that benthic communities in different sediment types are distinct from each other. In addition, recent reviews document meiofauna and nematode responses to OAW and that the meiofauna (in particular foraminifera, nematodes, copepods and ostracods) are useful in detecting and monitoring environmental change and anthropogenic impacts (Zeppilli et al., 2015). Scientific endeavours to assess responses of marine ecosystems to naturally occurring and

anthropogenically induced stressors are currently hampered by a lack of 1) understanding of which taxa will be affected in marine communities and their importance for ecosystem functioning, 2) multistressor experiments which can indicate complex changes and biological interactions on a community and ecosystem scale, and 3) understanding how different marine habitats respond differently to these stressors. Addressing these three issues will improve our ability to accurately assess climate change effects and help predict ecosystem shifts across entire systems (Queirós et al., 2015; Russell et al., 2011; Zeppilli et al., 2015). It is in this context that we addressed the following questions with regards to meiofauna responses to OAW: 1) Does OAW affect meiofauna and nematode communities in shallow-water sediments?; 2) If there are OAW effects, do these effects differ between sandy and muddy shallow-water sedimentary environments?; 3) If there are OAW effects, does warming and ocean acidification together affect meiofauna and nematode communities more strongly than either stressor separately? The hypotheses associated with these questions are H1: "OAW affect meiofauna and nematode communities in terms of abundance, diversity and evenness"; H2: "Meiofauna and nematode community responses to OAW are different in muddy versus sandy sediments"; and H3: "ocean acidification and warming together affect the meiofauna and nematode communities more strongly than either stressor in isolation".

3. Material and Methods

3.1. Experimental setup

3.1.1. Sediment collection

Two different sediment types were collected from the Eden Estuary near St Andrews, Fife, Scotland over four campaigns (October 2011; April 2012; June 2012 and July 2012). Cohesive surface sediment (<63 µm) was collected from intertidal mudflats (56° 21.9 N, 2° 50.883 W), and permeable sediment was collected from the West Sands bank near the mouth of the estuary (56° 22 N, 2° 49 W). Surface sediment (the top oxic layer as visually determined by the sediment colour change of the suboxic layer) was collected in the field, by hand, to a depth of no more than 2 cm. This sediment was placed directly into food-grade buckets and returned to the laboratory for sieving. All sediment was sieved (500 µm for cohesive; 1 mm for permeable) in a seawater bath (UV sterilised, 10 µm filtered, salinity ~35) to remove macrofauna and larger shells and stones, and was left to settle for 48 hours prior to removal of the supernatant. This allowed the finer particles of the sediment, along with the meiofauna, to settle. Each sediment type was homogenised and added to custom-built flume tanks, to a depth of 10 cm (Fig. 1). Seawater (1 µm filtered, UV treated, and salinity maintained at 35 through a brine tank set up) was carefully added to each tank and left for a further 48 hours before it was replaced with new seawater (UV treated, 1 µm filtered, ~35 psu). Each tank was then bubbled with ambient air (380 ppmv) for 72 hours prior to implementation of the experimental CO_2 and temperature regimes.

3.1.2. Flume tanks and environmental regimes

Each sediment type filled three flume tanks (L 120 cm x W 30 cm x H 30 cm; approx. 3.24×10^4 cm^3 volume and approx. 10 cm height) per campaign (n=4), with a continuous, recirculating and unidirectional flow of overlying water over the sediment surface (PISCES SC50 water pump, flow rate ~6 cm/s^{-1}) for the duration of the experiment (28 days). All six flume tanks in each campaign were subjected to a 12-hour light/dark cycle (Osram daylight tubing L3677, T8, 36 watts, 1200 mm long; two per tank). Two temperature (12 °C, 16 °C) and two CO_2 regimes (380 ppmv, 750 ppmv) were used in a fully-crossed factorial design to provide 3 replicate flume tanks per unique treatment (n=4), with replication spread temporally over campaigns (Fig. 1). CO_2 was bubbled into the water column to reach equilibrium and concentrations in each tank were monitored using a Li-Cor 820 CO_2

gas analyser (Biogeosciences). Each tank was also individually aerated to achieve oxygen saturation. Temperature was controlled by submerged titanium heaters with a digital regulating unit (Aqua Medic T-meter) for the duration of the experiment.

3.1.3. Carbonate system analysis

Water samples were taken weekly in light and dark conditions for total alkalinity (TA, 30 ml) and DIC (12 ml) and poisoned with 50 µl saturated $HgCl_2$ solution. Samples were stored in acid-washed, rinsed, capped glass bottles and refrigerated (4°C) prior to analysis. TA samples were analyzed using an automatic potentiometric 196 titrator (888 Titrando, Metrohm, Switzerland) with Tiamo® V 2.1 software. A three-point calibration was performed using buffer solutions pH 4, 7 and 9 (Metrohm UK Ltd.) prior to analysis. The precise volume of HCl acid added was plotted against pH, and this curve was then logged to produce a straight line. The gradient of this line was used to calculate TA (Dickson et al., 2007). Certified CO2 reference material (Andrew G. Dickson, Scripps Institution of Oceanography, California, USA) was used to monitor the sampling accuracy of the titrator (Dickson et al., 2003). DIC was determined using a CM140 Total Inorganic Carbon Analyzer (UIC Inc, USA) following Dickson *et al.* (2007). Blanks were run at the start of each analysis to calibrate the machine and to determine the carrier gas carbon content. Seawater standards of known concentration where then also run through the DIC machine to ascertain precision and accuracy within ± 0.01 mmol l^{-1}. Prior to each analysis a standard solution of sodium bicarbonate (NaHCO3), made to known concentrations, was run until a precision of 0.03 % deviation was achieved from three consecutive samples. IAPSO seawater samples (commercially available) were also routinely run through the machine to check accuracy.

3.1.4. Nutrient analysis

Water samples were collected weekly from each flume using 50 ml syringes and a 0.45 µm filter, and were stored in clean 45 ml centrifuge tubes for freezing at -20°C. Samples were defrosted prior to analysis, and gently mixed through manual inversion to reduce saline stratification in the sample tubes. An auto-analyser (LaChat 8500 Flow Injection) analysed four nutrients from each sample in triplicate: ammonium (NH_4), phosphate (PO_4), nitrite + nitrate (NO_2 + NO_3) and Silicate (Si). Low nutrient concentration seawater (salinity 35) was used for standard preparation and machine calibration.

3.2. Sampling

Sediment samples were taken towards the middle of the flume using 4 x 10 mL syringes (1.4 cm diameter; 6-7 cm deep) at T0 and T28 for each flume and campaign. Syringes were frozen at -20 °C to allow for different types of analyses. In the laboratory, the sediments of the four syringes per sampling point were pooled (6.158 cm^2 surface area) and left to thaw in 4 % formaldehyde in order to avoid degradation of the meiofauna during thawing. Pooling of the four syringes was conducted to remove meiofaunal spatial heterogeneity in the flume sediments. The pooled samples were then washed through a 1 mm sieve onto a 63 µm sieve. To extract the meiofauna from the sediment fraction, the material that remained on the 63 µm sieve was thoroughly mixed using a plastic paddle with Ludox TM-50 (specific gravity 1.15) in 500ml glass beakers and left for 40 minutes to enable density separation to occur. This process was repeated 3 times (Somerfield and Warwick, 1996) whereby each time the supernatant Ludox containing the meiofauna organisms was decanted and washed. The final washed and extracted sample was then stored in 75 % Industrial Methylated Spirit until further analysis. From each sample, a subsample of between 20 % or 30 % of total sample volume was taken and meiofauna major taxa were counted under stereoscopic microscope using (Higgins and Thiel, 1988). All Nematodes (or 100 if subsample contained more) were picked out and

mounted on glass slides for genus identification under compound microscope (Somerfield and Warwick, 1996) using appropriate reference materials (Platt and Warwick, 1983, 1988; Warwick et al., 1998). While the protozoan group Sarcomastigophora were counted (they include Foraminifera, flagellates and radiolarians) we have limited our results and discussion to the metazoan meiofauna because they are ecologically and biologically very different to metazoans and we have not identified the different taxa within the Sarcomastigophora. The use of the term meiofauna in the rest of the study refers to metazoans only. A total of 11,441 meiofauna (6,146 nematodes) individuals were identified for this study.

3.3. Data processing and statistical analysis

Density and diversity: Meiofauna abundance values were calculated as total sample abundance converted to number of individuals per 10 cm^2. Diversity was calculated as number of major taxa (meiofauna) and number of genera (nematodes), Shannon-Wiener's diversity index and Pielou's evenness index (using PRIMER v7, Clarke and Gorley, 2015). Graphs and non-metric MDS plots (Clarke and Gorley, 2015) were used to visualize sampling and experimental results. Univariate tests for differences (density, number of taxa, Shannon diversity and Pielou's evenness) were conducted with non-parametric analyses of variance (PERMANOVA) using Primer v7 (Clarke and Gorley, 2015) and the add-on PERMANOVA+ (Anderson et al., 2008). Because of the complex design of the experimental setup we applied a 4-way PERMANOVA test (fixed Factors [levels]: Temperature [12°C, 16°C], CO_2 concentration [380, 750 ppmv], Time [day 0, day 28], Sediment [sand, mud]) on the meiofauna and nematode data, followed by 3-way PERMANOVA tests on split sediment-type data (i.e. sand, mud; fixed Factors [levels]: Temperature [12°C, 16°C], CO_2 concentration [380, 750 ppmv], Time [day 0, day 28]) and 2-way PERMANOVA tests on split sediment type-time data sets (Sand-T0, Sand-T28, Mud-T0, Mud-T28; fixed Factors [levels]: Temperature [12°C, 16°C], CO_2 concentration [380, 750 ppmv]). In the few cases where the number of permutations was <100 we used Monte Carlo values, and the Estimated Components of Variation were sometimes used to interpret the size of the effects.

Communities: The multivariate meiofauna and genus matrices were also subjected to multivariate statistics using Primer v7 (Clarke and Gorley, 2015) and the add-on PERMANOVA+ (Anderson et al., 2008). Here we also applied a 4-way PERMANOVA test (Factors [levels]: Temperature [12°C, 16°C], CO_2 concentration [380, 750 ppmv], Time [day 0, day 28], Sediment [sand, mud]) on the meiofauna and nematode community data, followed by 3-way and 2-way tests on split data as done for the abundance and diversity data, with factors and levels as identified above. The meiofauna and nematode community data were standardised and transformed prior to analysis (meiofauna: 4^{th} root, nematodes: sq. root) to account for sample size differences (20 % vs. 30 %) and downweight the influence of numerically dominant major taxa/nematode genera. Bray-Curtis similarity was used for both meiofauna and nematode community data. Significance was assessed as $p<0.05$. Non-metric MDS plots were created to accompany the non-parametric tests. Cluster analyses (including SIMPROF test at 5 % significance) were performed to analyse significant groupings of samples which were then superimposed on nMDS outputs.

The PERMANOVA tests were used to assess treatment differences as well as assessing the nature of the differences of each factor and their potential interactions. These analyses were followed by pairwise comparisons where significant differences occurred. PERMDISP analyses were performed where appropriate to identify whether significant PERMANOVA results were caused by differences in location in Bray-Curtis (multivariate) or Euclidean (univariate) space or the homogeneity of dispersion of the samples within group, or a combination of both (Anderson et al., 2008). Differences in the multivariate dispersion of assemblage data may indicate stress in the observed communities, and can contribute to our understanding of how communities react to temperature and CO_2

concentration increases in our case (Anderson et al., 2008). Although increased variability may be an artefact caused by low abundance in samples (causing the resemblance measure to vary to a much greater extent compared to high abundance samples), the application of standardisation (transforming absolute abundance into relative abundance) renders the test more useful for assessing stress in communities, although caution with interpreting the results is recommended (Anderson et al., 2008).

The potential effects of the different times at which sampling for the experiment occurred and the six different flumes that were used were assessed by means of 2-way PERMANOVAs, and accompanying pairwise tests where necessary (Factors [levels]: Campaign, Ca [2, 4, 5, 6]; Flume, Fl [1, 2, 3, 4, 5, 6]). These 2-way tests were performed on meiofauna abundance, diversity and community data and nematode diversity and community data, and were repeated for the full data (sand and mud together), sand and mud separately, and each of the Sand-T0, Sand-T28, Mud-T0, Mud-T28 data sets. In some cases, and because of the design, there was no replication between crossed factor levels, causing the exclusion of interaction terms in the PERMANOVA or even rendering tests invalid; this has been indicated in the test result tables and its implications have been considered in the interpretations of the results.

4. Results

4.1. Environmental variables

The pH level was 7.90 ± 0.022 (standard error) and 8.03 ± 0.024 for the control treatments (380 ppmv), 12 °C and 16 °C, respectively. The pH level for the high-CO_2 treatments was 7.86 ± 0.023 and 7.77 ± 0.031 for 12 and 16°C, respectively. Total alkalinity (TA) and DIC were slightly higher in the muddy sediments (TA = 2.8 mmol/kg, DIC 2.6-2.7 mmol/kg) than in the permeable sandy sediments (TA = 2.6-2.7 mmol/kg, DIC 2.3-2.5 mmol/kg) throughout the experiment. This is due to the fact that there are higher benthic respiration rates in the muddy flumes compared to the sandy flumes, which in turn would have stimulated $CaCO_3$ dissolution (release of CO_2 from respiration increase the $CaCO_3$ dissolution). The $CaCO_3$ dissolution generated both DIC and TA; Nutrients showed overall higher concentrations in the muddy sediment compared to the sandy sediment (except for phosphate). Nutrient, DIC and TA data are presented in Table 1. We observed that DIC levels where highest in elevated CO_2 treatments (750 ppmv), which was expected due to the addition of CO_2 compared to the 380 ppmv treatments. This was not reflected in TA, as CO_2 invasion only affects DIC and not TA.

4.2. Meiofauna abundance, diversity and community structure

4.2.1. Meiofauna abundance

Meiofauna abundance was highly variable with a minimum of 75.8 and maximum of 9,841.6 ind. 10 cm^{-2} and averaging 1,735.3 ± 2,136.5 ind. 10 cm^{-2}. A 4-way PERMANOVA test on meiofauna abundance indicated only significant sediment-type differences (p=0.001) caused by a combination of differences in dispersion (greater abundance variability in the muddy sediments, PERMDISP, p=0.007) and actual abundance differences (greater abundance in muddy sediments, Fig. 2; Table S1). Sandy sediments contained on average 374.4 ± 586.8 ind. 10cm^{-2} whilst muddy sediments contained 3,096.1 ± 2,263.2 ind. 10 cm^{-2}. Despite an average decrease in abundance between day 0 and day 28 for both sandy and muddy sediments (Fig. 2A), high variability in recorded values rendered the time effect insignificant (p=0.109). Further evidence for this is provided by the PERMDISP analysis between sediment-time groups (p=0.001), with the high abundance variability in mud and sand sediments at day 0 and day 28 causing an increased dispersion effect. As average

abundance decreases throughout the duration of the experiment for both sediment types we also see a decrease in the variability of meiofauna. The only treatment effect observed was for CO_2 exposure in the mud samples after day 28 (Table S1, Fig. 2B), with significantly lower abundance in the 750 ppmv samples compared to the 380 ppmv samples. This density decrease was caused by a reduction across several taxa, with major decreases in numbers of nematodes, copepods, and ostracods (Fig. 2C-D).

4.2.2. Meiofauna diversity

In terms of diversity, a wider range of effects was observed. The number of major meiofauna taxa or taxon richness (S) ranged between 1 and 8, averaging 3.5 across all samples, and samples were dominated by Nematoda (84.4 %) and Copepoda (11.3 %), whilst Sarcomastigophora and Oligochaeta each represented just over 1 %, and Acari, Bivalia, Gastrotricha, Kinorrhyncha, Ostracoda, Rotifera and Turbellaria were each present in much lower abundance (<1 %). Taxon richness was significantly higher in muddy compared to sandy sediments (p=0.001, Fig. 3) with no dispersion differences (PERMDISP, p=0.749); sandy sediments contained between 1 and 7 taxa, averaging 1.8 ± 1.4 taxa per sample, whilst muddy sediments contained between 3 and 8 taxa, averaging 5.3 ± 1.8 taxa per sample. A number of 2-factor interactions were significant (Ti x Se, Ti x CO_2, Se x Te). Pairwise testing for these interactions and further investigation using 3-way PERMANOVA tests (Ti, Te, CO_2) on sandy and muddy sediment sample groups separately revealed: [1] a time effect (day 0 vs. day 28) in sandy but not in muddy sediments; [2] a severe sediment effect at day 0 as well as day 28; [3] some evidence for a time effect in the CO_2 control (380 ppmv) and enriched (750 ppmv) samples (borderline p-values of 0.051, and 0.081, respectively); [4] a difference between day 0 samples in the 380 ppmv and 750 ppmv treatment (p=0.037); [5] a clear sediment effect in both 12°C and 16°C treatments (p=0.001), and [6] a temperature effect in muddy sediments at day 0 (p=0.014). Taking all these test results together (including the accompanying PERMDISP results), they suggest that [1] there are substantial differences between starting communities mostly owing to sediment differences and variability of taxon presence, [2] sediment differences may obscure other treatment effects, and [3] there is some effect due to the time spent in the flume setup (Table S1). Observations [1] and [2] were confirmed by a significant temperature effect on diversity at day 0 (p=0.025) and not day 28 (p=0.3) in muddy sediments despite the fact that there had not been substantial exposure to higher temperatures yet at day 0 (pairwise comparisons in 3-way PERMANOVA (Ti, Te, CO_2) in muddy sediments), and the significant difference between CO_2 treatments at day 0 (p=0.039) and not day 28 (p=0.356) in sandy sediments prior to exposure (pairwise comparisons in 3-way PERMANOVA (Ti, Te, CO_2) in sandy sediments). The idea that time spent in the flume has an effect on meiofauna taxon richness was evidenced by the significant dispersion effects observed when comparing day 0 and day 28 in both sandy and muddy sediment groups (PERMDISP: p=0.015 and 0.001, respectively), without finding significant temperature or CO_2 effects.

Pielou's evenness was not a discriminative measure since none of the statistical tests revealed any significant differences. Some of the tests were hampered by the low number of taxa in some of the samples making the calculation of Pielou's index invalid, whilst other tests revealed no significant differences using the various PERMANOVA designs.

Four-way PERMANOVA testing on the Shannon diversity measure indicated a significant sediment-type difference across all time, temperature and CO_2 exposure groups (p=0.001) which was not caused by dispersion differences (PERMDISP: p=0.136). This difference can be clearly observed in Fig. 3. No other main-factor significant differences in the various tests were observed (Table S2).

4.2.3. Meiofauna community structure

Four-way PERMANOVA testing indicated significant sediment-type differences across all groups (p=0.001) and several significant interactions (Table S1). Sediment type differences were caused in part by differences in dispersion with clearly greater community variability in the mud samples compared to a closer resemblance between sand samples (PERMDISP: p=0.006; nMDS in Fig. 4). Pairwise testing for the significant Time x Sediment interaction revealed a borderline difference between day 0 and day 28 for mud samples (p=0.06) and slightly greater community differences between sand and mud samples at day 28 (p=0.004) compared to day 0 (p=0.004). Pairwise comparisons within the Time x CO_2 interaction indicated a significant difference between 380 ppmv and 750 ppmv at day 0, suggesting the difference resulted from differences between the starting communities and not the different CO_2 exposure. Pairwise tests for the significant Sediment x Time interaction was caused by significant differences between sediment types at 12°C and 16°C (p=0.001 for both) and not the community differences between 12°C and 16°C in each sediment type (p=0.25 and p=0.238). The significant Time x Sediment x Temperature interaction was caused by sediment-type differences and greater community variability in the 16°C group for both sand and mud samples (p=0.015 and 0.05, respectively). Separating the mud and sand data and performing 3-way PERMANOVA tests did not add anything new to these interpretations, apart from confirming a difference in day 0 communities (for instance between 380 ppmv and 750 ppmv in sandy sediments, p=0.03, Table S1). Taking the sediment-type-time data as separate sets, and conducting 2-way PERMANOVA tests, did not add anything new to what is reported above.

4.3. Nematode diversity and community structure

4.3.1. Nematode diversity

A total of 39 nematode genera were identified, averaging 12.2 ± 4.5 genera per sample, with genus richness ranging between 4 and 19 in samples. Nematode genus richness differed significantly between sediment types in the 4-way PERMANOVA test (p=0.001, Table S2, also see Fig. 5, 6). When splitting the data into mud and sand groups, 3-way PERMANOVA tests revealed a time effect across the mud samples (day 0 vs. day 28, p=0.037). Further testing revealed a significant effect of CO_2 exposure level in the mud samples at day 28 (pairwise testing in 3-way PERMANOVA, p=0.04; 2-way test at day 28, p=0.041; Fig. 6). At day 28, mud samples exposed to 380 ppmv averaged 14.2 ± 2.3 genera, compared to 16.8 ± 1.5 genera in the 750 ppmv treatment (Fig. 6).
PERMANOVA testing on Pielou's evenness index (J') indicated significant differences between sediment types (p=0.018) and a CO_2 exposure effect after 28 days in the mud samples (2-way PERMANOVA test, p=0.035, Table S2). The CO_2 exposure difference in evenness of mud samples at day 28 was more severe at 16 °C (p=0.005), whilst a temperature effect was observed in the 380 ppmv mud samples at day 28 (p=0.024).

PERMANOVA testing using Shannon Wiener index (H') showed a significant sediment-type effect (p=0.004) and an interaction effect between sediment type and CO_2 exposure level (p=0.046). Pairwise testing showed that the CO_2 exposure effect was only observed in the mud samples (p=0.034). This observation was confirmed with the 3-way test on the mud sample group (CO_2 effect, p=0.039) and the 2-way test on mud samples at day 28 (CO_2 effect, p=0.005). In addition, mud samples exposed to 380 ppmv changed significantly in terms of Shannon diversity between day 0 and day 28 (p=0.005).

4.3.2. Nematode community structure

Whilst for the meiofauna community structure the mud samples showed greater heterogeneity compared to the sand samples, using nematode genera data the opposite pattern was observed (Fig.

7). The nMDS plots in Fig. 7 with superimposed SIMPROF analyses presents clearly the greater variability in nematode communities in sand samples compared to the mud samples.

The 4-way PERMANOVA test on the nematode community indicated a clear sediment-type and time effect (p=0.001 and 0.044, respectively). Fig. 8 shows the relative abundance of genera (10 most important genera in terms of relative abundance) and gives an idea of the genus composition in sand and mud samples, with mainly *Metachromadora* dominating the sand samples and *Anoplostoma*, *Metachromadora* and *Ptycholaimellus* dominating the mud samples. PERMDISP analysis indicated that the differences between sediment types is in part caused by the differences in dispersion of mud vs. sand samples in Bray-Curtis space (p=0.001), i.e. communities were more heterogeneous in the sand samples compared to the mud samples. The time effect was consolidated in the mud sample data (3-way PERMANOVA, Time: p=0.022). There were no signs of a temperature or CO_2 exposure effect. This suggests that time spent in the flume may have had an effect on the nematode communities, particularly in the mud sediments, regardless of the different treatment levels.

4.4. Experimental controls – Campaign (sampling time) and Flume effects

For each of the meiofauna and nematode abundance, diversity and community data sets, 2-way PERMANOVA tests were carried out to assess the effects of campaign (time at which the samples were taken before transfer to the flume) and the flumes (six flume tanks were used). Results of these tests can be found in Table S3 (meiofauna) and Table S4 (nematodes).

For the meiofauna data, very few significant differences for the main factors were observed. For meiofauna Pielou evenness, there was a significant effect of Campaign and Flume (p=0.001, 0.005, respectively) which was not caused by differences in homogeneity of dispersions (PERMDISP, p=0.824, 0.713 for factors Campaign and Flume, respectively). The fact that no treatment effects on meiofauna Pielou evenness were found (see above), suggests that only effects caused by differences in flume setup and/or the time of sampling (Campaign) were present. Subtle Campaign effects were observed on meiofauna Shannon diversity and community structure for mud samples on day 28 (Table S3) suggesting the time of sampling before starting the experiment may have interfered with the treatment effects observed after 28 days of experiment as presented in section 4.1.1-4.1.3.

For the nematode full data set (both sand and mud samples together), clear Campaign and Flume effects were observed (Table S4) for all diversity descriptors (genus richness (S), Pielou's evenness, Shannon diversity), but only Flume effects for community structure. Further Flume effects were also observed on nematode genus richness and Shannon diversity, when only sand samples were considered, whilst Campaign effects were significant for Pielou evenness in sand samples and sand samples at day 0, and for community structure in mud samples and mud samples at day 0. This suggests the time of sampling prior to the experiment (Campaign) and the different flumes may have had an additive effect to the treatment effects observed (Time, CO_2, Temperature). Notably the significant interaction terms (Ca x Fl) and pairwise differences suggest that there were differences between particular pairs of campaigns, flumes and levels of one factor (campaign or flume) within each level of the other factor (flume or campaign). For instance, the pairwise test for the factor Campaign on nematode genus richness using the full data set (mud and sand samples together) showed that Campaign 2 and 4 did not differ, and the same was true for Campaign 5 and 6, whilst pairwise comparisons between Campaigns of these two groups (i.e. 2 vs. 5, 2 vs. 6, 4 vs. 5, 4 vs. 6) were significant at the $p<0.05$ level. Pairwise comparisons for the factor Flume on nematode genus richness (again using mud and sand sample data together) indicated that Flume 1 was significantly different from all other Flumes. When we performed pairwise comparisons for nematode Pielou evenness we observed that Campaign 5 was different to all others, and that Flume 1, again, was different from all other Flumes. For Shannon diversity, Campaign 6 was different to all other

campaigns and Flume 1 stood out in contrast to the other Flumes. Finally, using nematode community structure, it was again Flume 1 that differed significantly, but this time only compared to some of the other Flumes.

This of course creates a complex picture of potential interactions between the effects caused by the experimental protocol (factors Campaign and Flume) and the treatment effects (Temperature, CO_2), but also the duration of the experiment (i.e. day 0 vs. day 28). However, we can say with some confidence that for the meiofauna results, this will have made little or no difference in interpreting the treatment effects. For the nematodes, some caution is warranted since the effects of Campaign and Flume are more obvious, and this across the range of community descriptors. At the same time, however, such effects are only apparent when mud and sand samples are considered together, potentially indicating the importance of the differences between sand and mud samples. In addition, when investigating the differences between Campaigns and Flumes, it becomes clear that the differences are caused by a limited set and not the full set of samples within each Campaign or Flume that differ significantly from the samples grouped within the other Campaigns or Flumes in what is in essence a type of outlier effect on the overall result. This implies that Campaign and Flume effects are not uniform across all the samples considered, and reduces the potential additive influence of Campaign and Flume effects on the Temperature and CO_2 effects.

5. Discussion

Ocean acidification and warming are expected to have a range of impacts on marine species, populations and communities (Pörtner, 2008; Pörtner et al., 2004; Widdicombe, 2009; Widdicombe et al., 2011). While the majority of meiofauna and nematode OAW studies have detected limited impacts of changes that lie within the expectations of global warming within the next century or so, there are well-founded concerns about the potential impacts on organismal physiology and energy allocation under increased levels of stressors (Pörtner, 2008; Pörtner and Farrell, 2008). Here we report on the community level responses of meiofauna and nematodes to a 4 °C increase (12 vs. 16 °C) and increased CO_2 concentration (380 vs. 750 ppmv). Our interpretations are therefore limited to assessing differences and variability on the community level, which give insights into the trade-off between species or groups of species, and potentially the underlying causes of community changes. The sediments used in our experiment were sieved to exclude macrofauna, and our experimental setup precludes any influence from pelagic organisms, such as phytoplankton communities. It could be that the biggest impacts in meiofauna from OAW is or will be from changes in the communities with which they interact strongly; e.g. macrofauna (predation, competition for food, etc.) or phytoplankton (e.g. changes to the type and quality of organic matter). We therefore note that our study focuses more on the direct impact of OAW on the meiofauna themselves, and the indirect impacts resulting from interactions with microbial and microphytobenthos communities.

5.1. Meiofauna

Without doubt the most important driver for the abundance, diversity and community differences we observed was sediment type, and this both at the beginning (day 0) and the end (day 28) of the experiment, and across the different temperature and CO_2 treatments. Muddy sediments were characterised by much higher meiofauna abundances than the sandy sediments (Fig. 2), an observation that can potentially be related to the higher density of microbial food sources in muddy sediments compared to sandy sediments (Currie et al., unpublished data). Higher respiration rates in the muddy sediments as indicated by DIC results are supported by higher meiofauna abundance in those sediments and the higher abundance of bacterial, archaeal, and cyanobacterial 16S rRNA genes in muddy sediments compared to sandy sediments (Currie et al., unpublished data). Sediment type and immediately related parameters such as grain size, surface area, porosity and permeability are key factors since they determine the physical and chemical environment of the interstitial space

inhabited by the meiofauna and affects food availability for the meiofauna. Whilst mesobenthic species moving between the sand grains prefer coarse sands, endo- and epibenthic species will generally prefer fine to silty sediments (Giere, 2009). It is therefore not surprising that meiofauna tend to be more sensitive to changes in sediment composition than the macrofauna (Heip et al., 1985; Warwick and Buchanan, 1970), which makes them a useful faunal component to detect benthic environmental change in an OAW experiment using different sediment types. The 11-year meiobenthos study by Coull and co-authors (1985) in which they investigated subtidal estuarine muddy and sandy sites in South Carolina, USA, showed that variability in meiofauna abundance at the mud site was twice that of the sand site they studied. This corroborates our observation that meiofauna abundance in the mud samples varies much more compared to abundance in the sand samples (Fig. 2). This pattern was also visible in the meiofauna community variability as shown in the nMDS of Fig. 4, with greater dispersion of the mud samples compared to the sand samples in Bray Curtis resemblance space. However, considering the seasonal and interannual abundance variability Coull et al. (1985) had found and as reported in the review by Giere et al. (2009), we were surprised to see no campaign effect (i.e. seasonal) on meiofauna abundance, particularly in the mud samples where highest and consistent seasonal variability was expected (Table S3). Despite abundance peaks of over 5,000 ind. 10 cm^{-2} in mud samples of Campaign 4 (April) and peaks of over 7,000 ind. 10 cm^{-2} in mud samples of Campaign 5 (June), the large differences between mud sample abundances resulted in a lack of significant Campaign differences and may be related to spatial variability of meiofauna across the samples. For instance, meiofauna and nematodes are known to have aggregated distribution patterns in virtually all marine habitats with patch sizes smaller than 5 cm in diameter, mainly in response to microtopographic irregularities and aggregate distribution of food sources (Moens et al., 2013 and references therein). It therefore meets expectations that the spatial scale of cm to m is generally found to be the most important source of variability for meiofauna organisms (Fonseca et al., 2010; Ingels and Vanreusel, 2013; Moens et al., 2013; Rosli et al., 2016; Vieira and Fonseca, 2013).

Aside from the sediment effect, there was also a significant CO_2 exposure effect on meiofauna abundance in the mud samples at day 28, which is visualised in the box-whisker plot in Fig. 2. This suggests a moderately negative impact on meiofauna abundance in muddy sediments. When investigating each major taxon, it became apparent that the density decrease was caused by a reduction across most taxa, with major decreases in numbers of nematodes, copepods, and ostracods (Fig. 2). This pattern is in line with our expectations based on the likely metabolic cost of coping with high CO_2 concentration, and potentially the resulting reduction in fitness and survival. Some studies have shown that the meiofauna community may exhibit significant mortality following CO_2 exposure; e.g. decreased meiofauna abundance associated with a rapid pH drop of ~1.5 in deep-sea sediments in Carman et al. (2004) and Barry et al. (2004) or meiofauna abundance decrease under increased CO_2 concentration conditions of 20,000 ppmv at 2,000 m water depth in the Kumano trough off Japan (Ishida et al., 2005). Ishida et al. (2013), on the other hand, reported no abundance decline under increased CO_2 exposure at 400 m depth in a Norwegian Fjord, but slow decomposition rates in deep cold waters (low microbial activity) may have caused an overestimation of meiofauna abundance in high-CO_2 treatments in the absence of specific staining to detect live/dead ratios (Carman et al., 2004). Explanations for abundance effects other than mortality may include physiological processes and behaviour including escaping the unfavourable high-CO_2 conditions in the case of relatively mobile organisms such as copepods (Thistle et al., 2007). Observations of CO_2 effects on meiobenthos in the deep sea, however, may not be comparable to shallow-water observations owing to the likely adaptation of deep-sea organisms to environmentally stable conditions and therefore increased sensitivity to environmental change compared to their shallow-water counterparts. Hale et al. (2011) and Meadows (2015), for instance, reported unaffected or even increased nematode abundance under high-CO_2 conditions, likely as a result of reduced macrofaunal competition and predation, whilst Schade et al. (2016) and Kurihara et al.

(2007) found no nematode and meiofauna abundance response to high CO_2 treatments (1,500-24,400 µatm CO_2 (Schade et al., 2016); 2,000 ppmv above the 380 ppmv CO_2 control level (Kurihara et al., 2007)). At the same time, Schade et al. (2016) reported that the abundance of non-dominant, calcifying meiofauna (gastropods and ostracods) declined in response to high CO_2 concentration, whilst the non-calcifying gastrotrichs increased in abundance in response to very high levels of CO_2 concentration. Meadows et al. (2015) reported that other meiofauna groups such as copepods, copepodites and amphipods decreased in abundance in low-pH treatments, suggesting that different meiofauna taxa and even different species within the same group (e.g. nematodes in Takeuchi et al., 1997; copepods in Thistle et al., 2006) have different tolerances to CO_2 exposure. It is important to note here that one should distinguish between pH reductions or CO_2 concentrations that can be associated with ocean acidification and those associated with the simulation of point-source leakage in the context of Carbon Capture and Storage (CCS). Most, if not all, studies investigating CO_2 impacts on meiofauna abundance report significant effects only when applying severe pH reductions in experimental setups (e.g. >1 pH unit change, or even an incredible pH 5.5-6 in Takeuchi et al. (1997)) and are not comparable to pH reductions of 0.1 or 0.2 units as is the case here. Reductions in pH of more than 1 unit are not associated with ocean acidification as predicted under climate change scenarios, and suggest that meiofauna in general are not affected in terms of abundance as a result of short- to long-term exposure to ocean acidification. This is another reason why deep-sea studies are difficult to compare to shallow-water studies; so far deep-sea studies have mainly addressed the impacts of potential CO_2 storage or leakage on benthic assemblages in CCS contexts instead of looking at ocean acidification impacts.

Going back to the results of the present study, we need to consider the fact that the sediments were sieved to remove macrofauna before incubation in the flume system. A decrease in meiofauna abundance as a result of CO_2 exposure is therefore likely a direct effect and not an indirect macrofauna effect caused through reduced interactions with potentially more severely affected macrofauna (Dashfield et al., 2008). Indirect effects, however, may have resulted through altered meiofauna-microbial interactions and may play an important role in our results. Microbial data from our experiment (Currie et al., unpublished data) indicate significant negative CO_2 effects on bacterial, archaeal and cyanobacterial abundance (as assessed through 16S rRNA gene abundance) in muddy sediments after 28 days in the experiment. Considering microbiota are important food sources for meiofauna (e.g. Montagna, 1984) and particularly nematodes it is likely that a reduction in available food may have had consequences for meiofauna abundance. We also have to consider the fact that a negative CO_2 effect on meiofauna abundance was only observed in the muddy sediments (and not the sandy sediments) which corresponds with the substantial microbial abundance decline observed only in muddy sediments (Currie et al., unpublished data). This supports the likelihood of an indirect CO_2 effect on meiofauna abundance through a microbe-meiofauna relation, most likely a trophic interaction. In addition, we have to consider a potential indirect trophic effect on the meiofauna through a change in abundance and community structure of the microphytobenthos which are prevalent in intertidal muddy sediments. In the same experiment cyanobacterial/chloroplast 16S rRNA gene abundances were reduced and microbial community structure was altered under increased CO_2 in the muddy sediments, yet, gene abundance increased in the higher temperature treatment alone (Currie et al., unpublished data). Microphytobenthos is a well-known food source for different meiofauna (Lebreton et al., 2012; Montagna et al., 1995; Rzeznik Orignac et al., 2008) in intertidal and other coastal marine systems and observations from a previous experiment indicate that there may be a tight correlation between meiofauna and microphytobenthos responses to OAW (Unpublished meiofauna data from another experiment, (Tait et al., 2015)). Trophic restructuring of the meiofauna community (and subsequent diversity changes) in response to a change in microphytobenthos abundance and community structure is a likely scenario since variation in trophic meiofauna types and their abundance has been closely linked with microphytobenthos consumption in intertidal systems (Montagna et al., 1995; Rzeznik-Orignac and Fichet, 2012). It is

very likely that meiofauna abundance was reduced in response to reduced microphytobenthos availability as food source in the high CO_2 treatments. Alternatively, or additionally, the fact that a CO_2 effect occurred only in muddy sediments may be linked to different types of assemblages in the two sediment types and hence potentially different organism tolerance levels, carbonate chemistry, diffusion, and permeability variability between both sediment types.

In our experiment, cyanobacterial/chloroplast 16S rRNA gene abundance increased in the higher temperature treatment (Currie, unpublished data), yet no significant meiofauna response is observed for temperature alone. Considering meiofauna life cycles can be shortened under higher temperatures one may expect meiofauna abundance to increase in the high temperature treatment (Giere, 2009), particularly in the case of nematodes (Gerlach and Schrage, 1971; Heip et al., 1985; Hopper et al., 1973; Vranken et al., 1988; Warwick, 1981). However, the relatively short period (4 weeks) of our experiment may have been too short for such an effect to show given meiofauna reproductive cycles can vary between a couple of days and more than two months depending on the taxon and environmental conditions (Giere, 2009). In their experimental work on biofilm production and macrofaunal grazing during five weeks of OAW conditions, Russel and colleagues (2013) found that whilst primary production increased, consumer grazing decreased, suggesting increased energy expenditure of the consumer in response to their higher metabolic requirements under OAW. Such a scenario does not seem applicable to the meiofauna in our higher temperature treatments, suggesting that the intertidal meiofauna used here may experience relatively little additional metabolic cost under such conditions. Considering intertidal fauna experience extreme temperature gradients under natural conditions, our 4°C temperature increase may have had little effect.

As mentioned at the beginning of this section, meiofauna taxon richness and Shannon diversity differed significantly between sand and mud samples, with consistently higher major taxon richness and Shannon diversity in muddy sediments compared to sandy sediments (Fig. 6). This supports the idea that sediment type and meiofauna diversity is linked as has been reported in several other studies (Giere, 2009 and references therein). The consistency of the sediment type differences across meiofauna diversity measures and across experimental treatments highlights the importance of sediment characteristics in determining the type of meiofauna communities that reside in them and the diversity they exhibit.

The CO_2 and temperature effects on meiofauna taxon richness for day 0 in sand and mud samples, respectively, and the CO_2 effect on meiofauna community structure in day 0 sand samples (Table S1) may be caused by differences between the starting communities or pre-treatment of the sediments (irrespective of sediment type) and does not represent a true CO_2 or temperature effect as a result of the different experimental treatments. Indeed, these differences occur at day 0, before exposure was initiated. Such initial distinction between assemblages is likely representative of assemblage differences as observed in the field, and potentially relates to differences associated with the month in which the samples were taken. Results from the Campaign x Flume statistical tests on meiofauna confirm this to some extent, with a significant Campaign effect on Pielou evenness (sand samples), and Shannon diversity and community structure for day 28 mud samples (Table S3), implying that initial diversity and community differences may persist to some extent through 28 days of experimental exposure. Further evidence for high initial variation can be provided by comparing the standard deviations of the respective diversity measures between day 0 and day 28 samples for each sediment type (each sediment type taken separately to remove the overwhelming sediment type effect): a clear pattern of “shrinking variation” of the meiofauna diversity measures over the duration of the experiment is apparent. This is illustrated by the decreasing size of standard deviation bars of the diversity values over time in Fig. 3. We have presented this pattern more clearly by plotting the size of the standard deviations of each meiofauna diversity measure in Fig. 9, as it clearly shows that variation decreases as communities and assemblages are exposed to 28 days

of experimental conditions. It is remarkable that, despite the differences in absolute values of diversity measures between sandy and muddy sediments (cf. size of the bars in Fig. 3), the variation – as judged by standard deviation - in these values is in fact very similar. This suggests that the observed "shrinking variation" occurs irrespective of the sediment type, and likely irrespective of the season or month in which the samples were taken from the field.

5.2. Nematodes.

As was the case for the meiofauna, the most pronounced difference for nematode diversity and community structure was caused by sediment type. This is clearly shown in Fig. 5-8 (arguably most clearly in the separation between sand and mud samples in the nMDS in Fig. 7). Although nematode density tends to increase in finer sediments such as mud and fine silt, diversity has been reported to be higher in coarser sediments (Heip et al., 1985; Steyaert et al., 1999). This pattern has been ascribed to increased microhabitat heterogeneity thought to be available in the latter (Heip et al., 1985) which could support more diverse communities with more closely related species co-existing (Steyaert et al., 1999). In sediments finer than about 120 μm a true interstitial fauna is lacking and a poorer burrowing fauna remains. However, such reasoning omits the potential of meiofauna and in particular nematodes to manipulate the fine sediments by means of their own bioturbation activities and causing increased microhabitat heterogeneity as well as the stimulation of biogeochemical processes (Bonaglia et al., 2014). In addition, fine sediments are usually associated with greater concentrations of nutrients contained between the fine grains and may promote coexistence of nematode genera and species. Moreover, the manipulation of the sediments prior to our experiment by means of sieving has excluded the presence of macrofauna and hence their contribution to microhabitat heterogeneity. Our results clearly suggest a higher nematode diversity in muddy sediments compared to sandy sediments, in favour of the idea that higher food availability in muddy sediments allows for more diverse nematode assemblages. This is supported by the enhanced levels of nutrients that were found in muddy compared to sandy sediments in our experiment. Despite muddy sediments being more diverse in our study, community variability was much greater in sandy sediments compared to muddy sediments (Fig. 7). The main reason for this may well be related to the much lower numbers of organisms recovered from the sand samples compared to the mud samples. The Bray-Curtis similarity measure we used is very sensitive when comparing low-abundance samples (e.g. comparing samples with one individual each can result in 100 % or 0 % similarity), and disparity between samples with low numbers of individuals may therefore become inflated compared to samples with higher abundance (Clarke et al. 2006). Regardless of what caused the greater variability of nematode communities in sandy sediments compared to muddy sediments, it is clear that they are indeed very different. The nMDS plots in Fig. 7 show a high-level distinction between nematode communities from both sediment types. Sediment characteristics such as organic matter content, porosity, permeability, grain size, etc. are key factors in determining the community that is present in different sediment types. We will not go into much detail on which genera were present in sandy versus muddy sediments in our experimental samples, but our data corresponds with findings of several other studies and support the distinction between sediment types based on nematode communities (e.g. Somerfield and Warwick, 1996). For instance, chromadorid genera (particularly *Metachromadora*) dominate sandy sediments much more than muddy sediments (Fig. 8), and typical genera such as *Anoplostoma* and *Ptycholaimellus* are prevalent in the muddy sediments (e.g. Heip et al., 1985; Moens et al., 2013; Netto and Gallucci, 2003; Yodnarasri et al., 2008 to name but a few). Our results also support the notion that nematodes appear very sensitive to even slight changes in sediment composition (discussed extensively in Giere, 2009; Heip et al., 1985).

Focusing on temperature and CO_2 treatments, there are some significant differences that emerge from our statistical testing. A significant CO_2 effect is observed for nematode genus richness and

Pielou evenness in mud samples of day 28 (higher values in 750 ppmv treatment), and for Shannon-Wiener diversity in mud samples and mud samples of day 28 (higher values in 750 ppmv treatment; Fig. 6, Table S2). So it appears that the increased CO_2 exposure used in our experiment is able to influence nematode diversity, but only in muddy sediments, and does so by increasing diversity. Despite the fact that it is generally acknowledged that nematodes are good indicators of any kind of disturbance or environmental change (Balsamo et al., 2012), we did not expect nematode diversity to be influenced - let alone increase - by CO_2 exposure. Meadows et al. (2015) reported no significant nematode diversity effect in their various pH treatments, nor did Schade et al. (2016) and Dashfield et al. (2008). Although nematode diversity effects have generally not been observed in high-CO_2 experiments to date, community differences in response to high CO_2 exposure have been reported (see Meadows et al., 2015, although Schade et al. (2016) reports no nematode community changes under high- CO_2 exposure). There are indications that different nematode species may respond differently to lower pH owing to different behaviour (activity, feeding behaviour), possession of different physiological needs and thresholds (Takeuchi et al., 1997), as well as in response to predatory release from other predator nematodes or turbellarians if those predators were less tolerant to changing conditions. However, our experiment revealed no nematode community differences as a result of different CO_2 exposures. The only community structure effects we observed were caused by the differences between day 0 and day 28 (for sand samples and sand and mud samples together; Table S2).

Aside from some CO_2 effects, we also observed a temperature effect on nematode genus richness in mud samples. Meadows et al. (2015) found a consistent negative effect of higher temperature (16 °C vs. 12 °C) across pH treatments on average nematode species richness, expected number of species (ES[50]), and Pielou evenness, whilst we found the opposite: nematode genus richness was lower under higher temperature (also 16 °C vs. 12 °C) across both CO_2 concentrations in mud sediments (Fig. 10). One potential explanation is that opportunistic species (r-strategists) may have outcompeted multiple K-strategy species, having benefited from the higher temperature. That being said, there are no other temperature effects observed in any of the tests on the nematode data (e.g. evenness, community structure) suggesting it may be a straightforward loss of some genera in the community.

As for the meiofauna discussed above, nematode communities vary substantially on a monthly and yearly basis, with sediments sampled in different seasons exhibiting different abundance, diversity and community structure (Schratzberger et al., 2008; Vanaverbeke et al., 2004). Changes in nematode communities are best explained through food quality, quantity (availability) and temperature in sublittoral systems since these environmental characteristics may influence different species differently (reflecting different life histories and feeding modes), resulting in dynamic communities from season to season (Heip et al., 1985; Moens and Vincx, 2000; Schratzberger et al., 2008; Yodnarasri et al., 2008). The expectation that monthly and seasonal differences are likely to affect the nematode community characteristics (Yodnarasri et al., 2008) in our experimental samples was confirmed through the significant Campaign effects on nematode diversity values (mud and sand samples together; no significant result for community structure, Table S4). To investigate further the nature of the Campaign effects, we performed two-way tests on nematode genus richness and Shannon-Wiener diversity, using Campaign and sediment type as main crossed factors, and saw that sediment type effects remained significant whilst Campaign effects were borderline (p=0.065-0.071; no significant interaction term). This implies that the sediment effects were indeed distinguishable from potential Campaign effects, hence validating the sediment effects discussed above, but also that there are differences between the sampling months which were unlikely to influence the other effects observed (CO_2, temperature, Time).

5.3. Conclusions and future work

Our experiment has shown that the meiofauna and nematode communities originating from intertidal muddy and sandy sediments are relatively tolerant to the direct effects of OAW in a relatively short (4-week) mesocosm experiment. With regard to our hypotheses, we have shown that: (H1) OAW affects meiofauna and nematode communities to a very limited extent; (H2) meiofauna and nematode community responses to OAW are very different in muddy versus sandy sediments, with effects mostly found in muddy sediments; and (H3) Ocean acidification and warming together to do not present additive or synergistic effects on meiofauna and nematode community characteristics.

Sediment type was by far the most discriminating factor for meiofauna and nematode community structure, diversity, and evenness. There were negative CO_2 effects on meiofauna abundance in mud samples, caused by a reduction in abundance of notably the nematodes, copepods and ostracods. High CO_2 exposure resulted in increased nematode genus richness, Shannon diversity and Pielou evenness. The only warming effect we observed, was a negative influence on nematode genus richness. In general, there were no significant interaction effects between CO_2 exposure and warming. These findings paint a complex picture whereby OAW influences meiofauna and nematode communities most likely through their food sources such as bacteria and microphytobenthos. Under OAW conditions, the ecological interactions may change: shifts in major taxa abundances and nematode genera trade-offs are likely consequences in benthic systems subjected to OAW.

Before meiofauna and in particular nematodes can be used as indicators for ocean acidification and warming a better understanding is needed of their spatial and temporal variation as well as improved knowledge on their physiology and life histories, and this in different environmental settings and marine habitats. Information on community responses do not necessarily provide a mechanistic view of the responses of the individuals and reasons behind different responses of species. As is often the case in meiofauna and nematode impact studies, information is often drawn from a multitude of studies in which particular meiofauna higher taxa or nematode genera or species have been observed to respond to a particular stressor or environmental change (Balsamo et al., 2012). In addition, our understanding of how different taxa and levels of biological organisation respond to ocean acidification and warming is limited, but it has been shown that different life stages will respond differently (Ingels et al., 2012; Kroeker et al., 2010). The ability to provide a comprehensive overview on meiofauna and nematode responses to ocean acidification and warming relies on studies which enable the separation of responses from higher taxa, species, and life stages to the different stressors. Moreover, and perhaps more importantly, insights into the ecological interactions between different meiofauna components as well as interactions between meiofauna and other biota are needed to achieve an understanding of true community responses to ocean acidification and warming. Our study has also shown that there is a need for integration between autecological and synecological studies and multi-stressor approaches to achieve an ecosystem view and to account for environmental interactions and revealing additive or synergistic effects of different types of stressors (Zeppilli et al., 2015). It has previously been asserted that more multi-stressor experiments are needed to reveal complex ecological and biological interactions in a changing marine environment (Zeppilli et al., 2015). Finally, identifying meiofauna and nematodes is time-intensive and costly. Metagenomic barcoding may provide a more cost-effective way of identifying impact responses of communities. However, DNA-based meiofauna and nematode identification cannot yet rely on a species- and life-stage-specific understanding of the mechanistics of responses as well as the interactions and variability of these responses. It is therefore important that studies are conducted to provide mechanistic lower-taxon insights as well as community understanding of responses to OAW.

6. Acknowledgements

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7. Figures and tables

	Muddy sediment				Sandy sediment			
Temperature	***12°C***	***16°C***	***12°C***	***16°C***	***12°C***	***16°C***	***12°C***	***16°C***
CO_2 level	***380 ppm***	***380 ppm***	***750 ppm***	***750 ppm***	***380 ppm***	***380 ppm***	***750 ppm***	***750 ppm***
NH_4 (µmol)	11.92 *±3.74*	7.57 *±2.61*	22.48 *±6.74*	5.41 *±1.93*	7.05 *±1.85*	4.58 *±1.33*	8.31 *±2.16*	3.99 *±1.38*
PO_4 (µmol)	0.11 *±0.02*	0.19 *±0.03*	0.08 *±0.01*	0.10 *±0.03*	0.13 *±0.03*	0.88 *±0.17*	0.65 *±0.16*	0.15 *±0.05*
Si (µmol)	0.77 *±0.43*	1.08 *±0.44*	0.58 *±0.16*	1.51 *±0.84*	1.12 *±0.38*	1.77 *±0.37*	1.66 *±0.39*	0.87 *±0.24*
NO_3 + NO_2 (µmol)	18.58 *±4.04*	40.11 *±6.54*	21.84 *±3.77*	27.70 *±7.33*	1.14 *±0.50*	10.21 *±3.84*	5.38 *±2.93*	0.70 *±0.18*
Dissolved Inorganic Carbon (mM)	2.63 *±0.04*	2.79 *±0.06*	2.71 *±0.04*	2.83 *±0.05*	2.33 *±0.02*	2.42 *±0.02*	2.42 *±0.02*	2.43 *±0.02*
Total Alkalinity (mM)	2.83 *±0.05*	2.97 *±0.05*	2.88 *±0.06*	2.88 *±0.07*	2.58 *±0.02*	2.67 *±0.03*	2.65 *±0.02*	2.65 *±0.03*

Table 1. Mean seawater chemistry values for the different treatments. Values in italics denote standard errors for means.

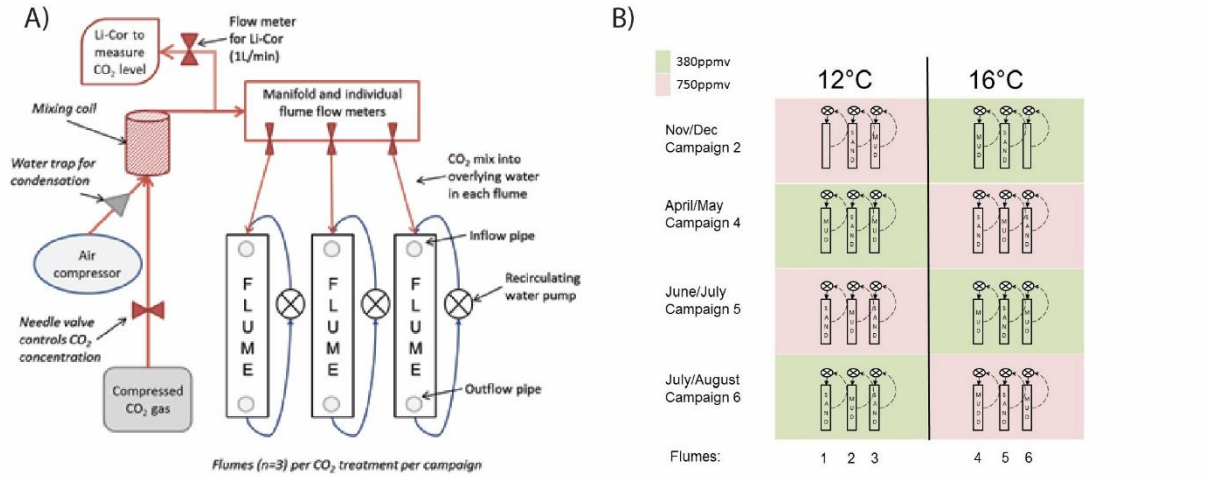


Fig. 1. (A) Schematic diagram of experimental setup and circulation system; (B) schematic of the experimental treatments in the flume tanks (empty flumes in Campaign 2 were not used for this study).

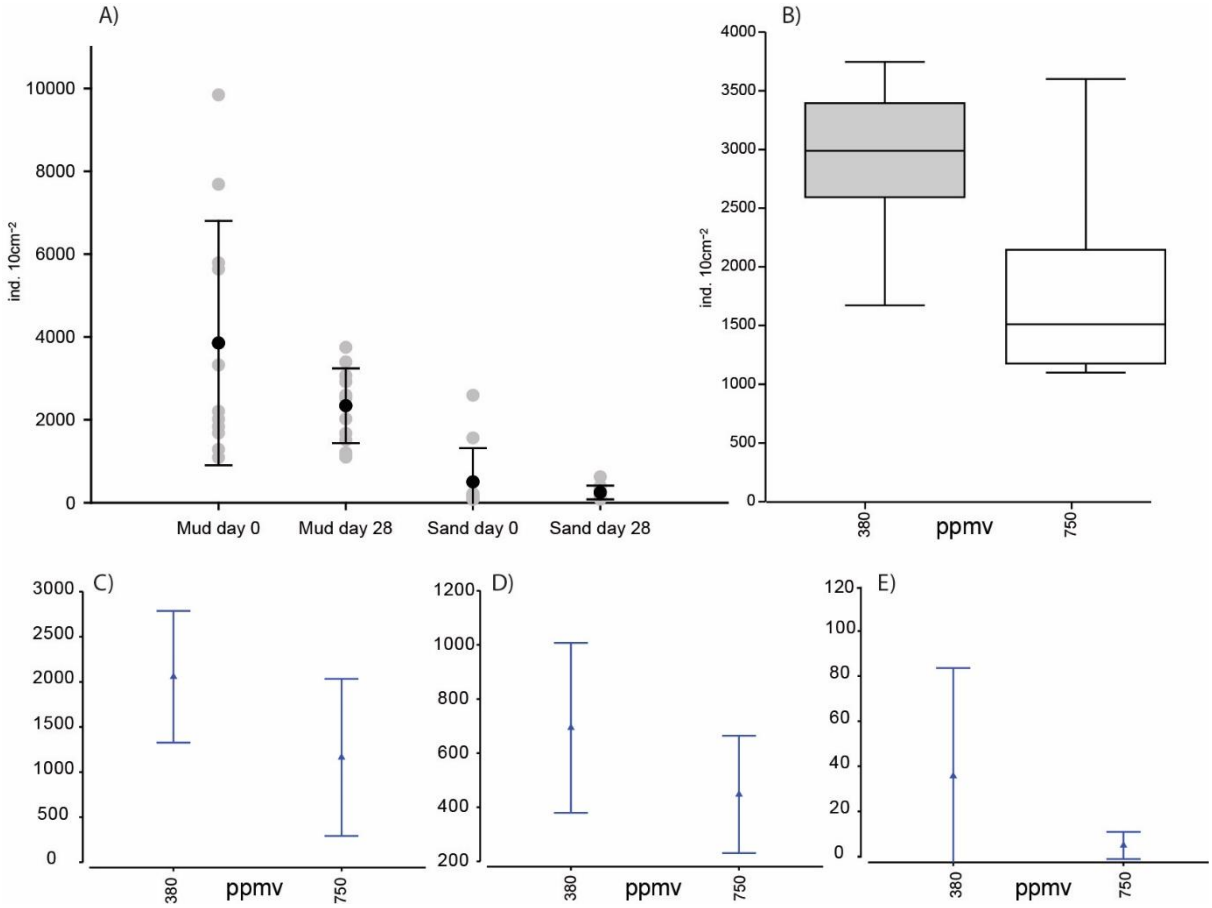


Fig. 2. (A) Meiofauna abundance for sediment-type-day groups of samples (average (±SD, n=11) in black and sample abundances in grey), (B) Box and whisker plot of meiofauna abundance (ind. 10 cm^{-2}) for mud samples at day 28 with 380 ppmv (n=6) and 750 ppmv treatment (n=5). Box boundaries represent 25-75 percentile, line within box represents median, error bars indicate 90th and 10th percentiles. C-E) abundance (average ind. 10cm^{-2}, error bars indicate 95 % confidence intervals) comparison between 380 (n=6) and 750 ppmv (n=5) at 28 days in mud samples, C) nematodes, D) copepods, E) ostracods.

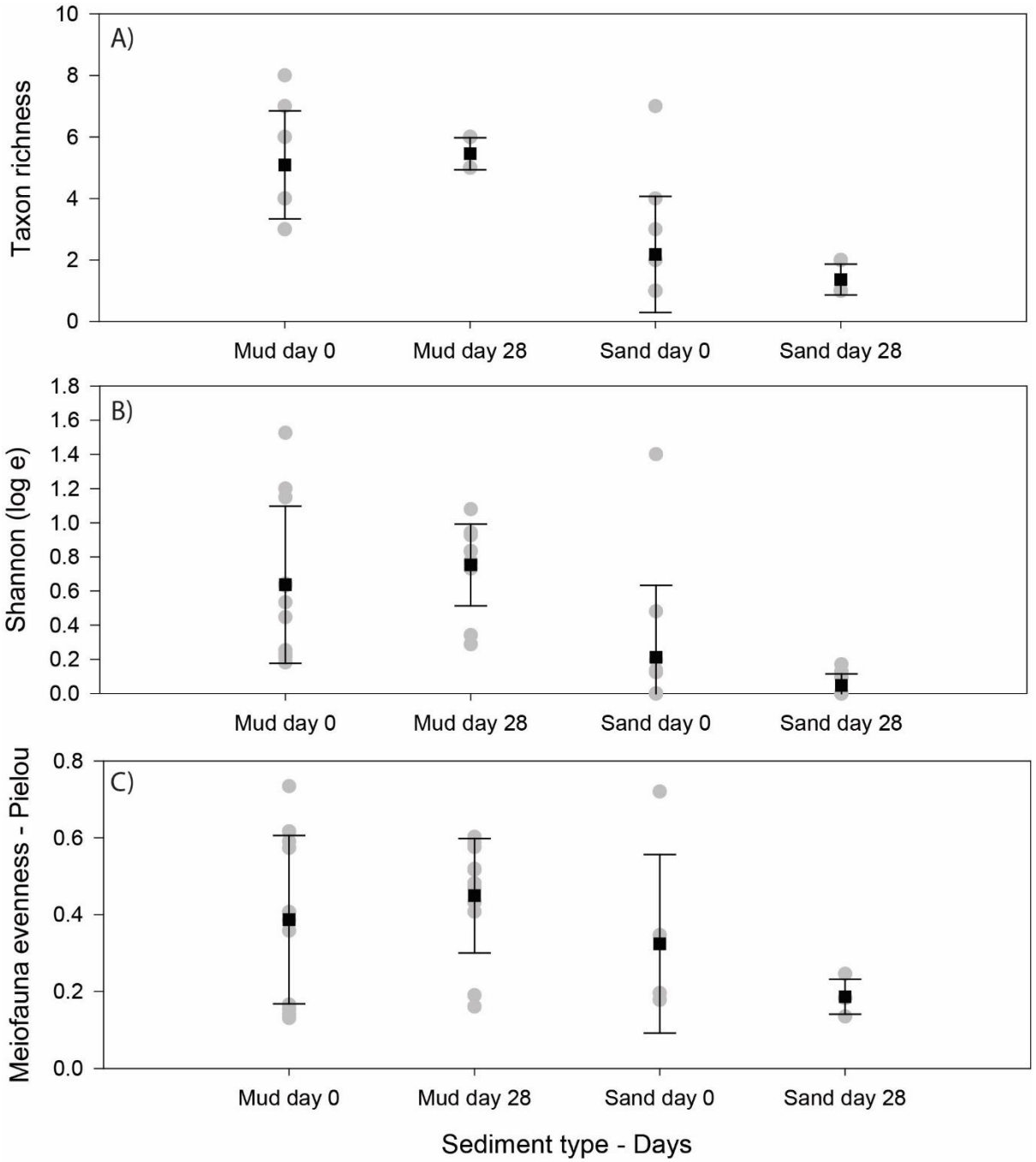


Fig. 3. Meiofauna diversity measures for sediment-type-day groups of samples (average (±SD, n=11) in black and sample abundances in grey). (A) Meiofauna taxon richness (S), (B) Meiofauna Shannon diversity (log e), C) Meiofauna Pielou evenness.

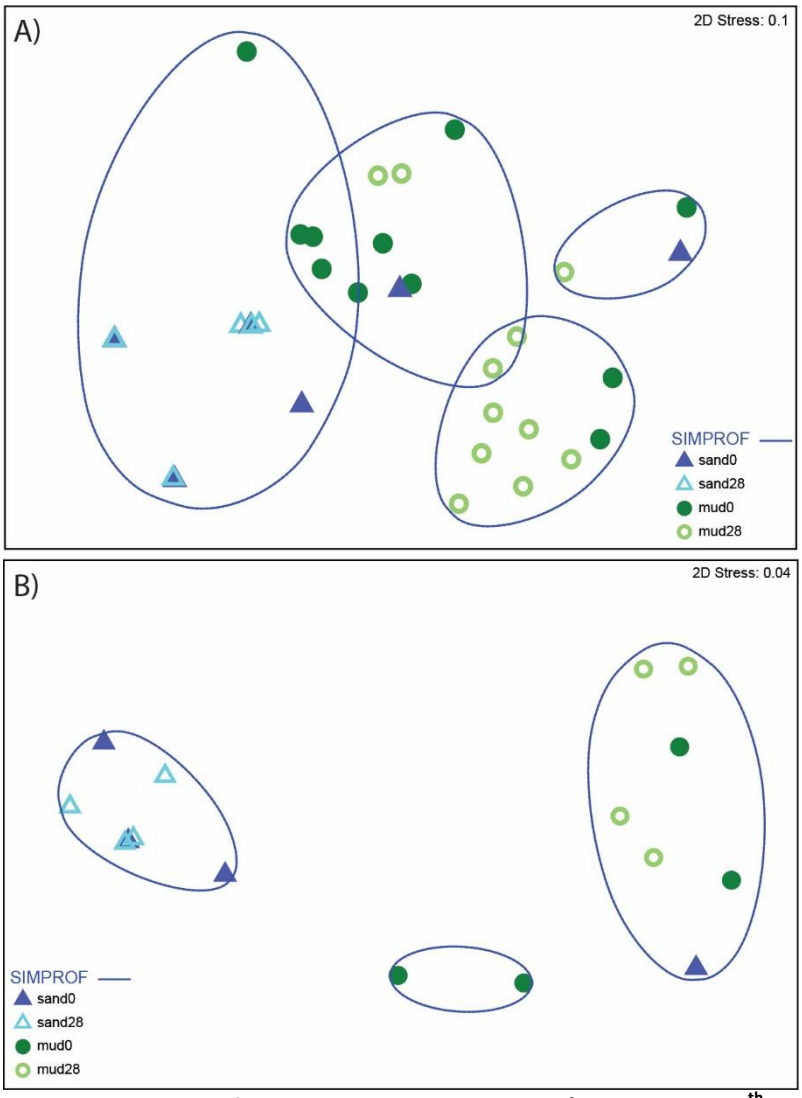


Fig. 4. Non-metric MDS plots on meiofauna major taxa abundance (standardised, 4th root transformation, Bray Curtis resemblance). Symbols represent samples belonging to two sediment type (sand, mud) and two time (0 days, 28 days) combinations. A) all samples, B) samples averaged over replicates. Blue lines indicate SIMPROF significance at the 5 % level.

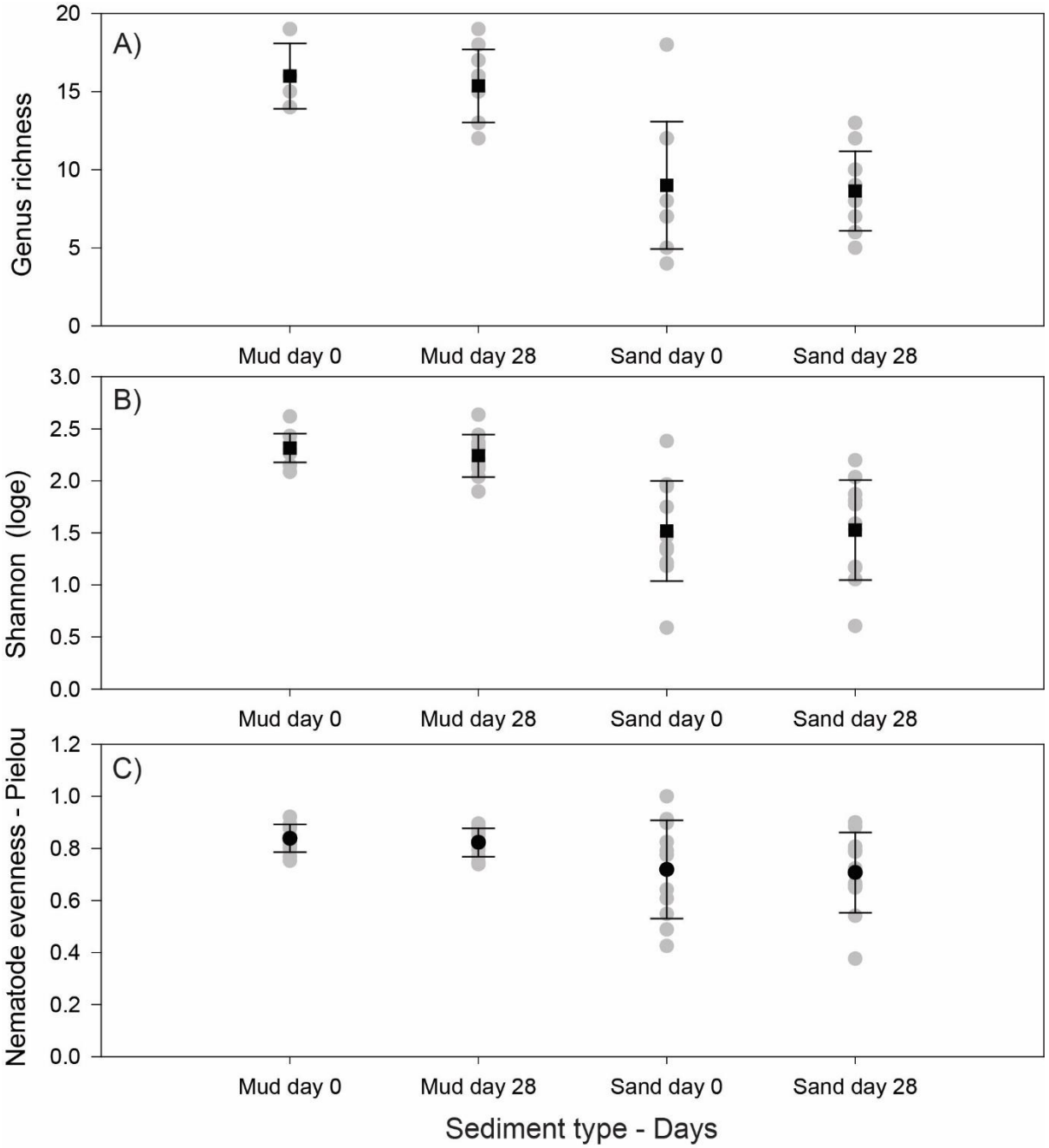
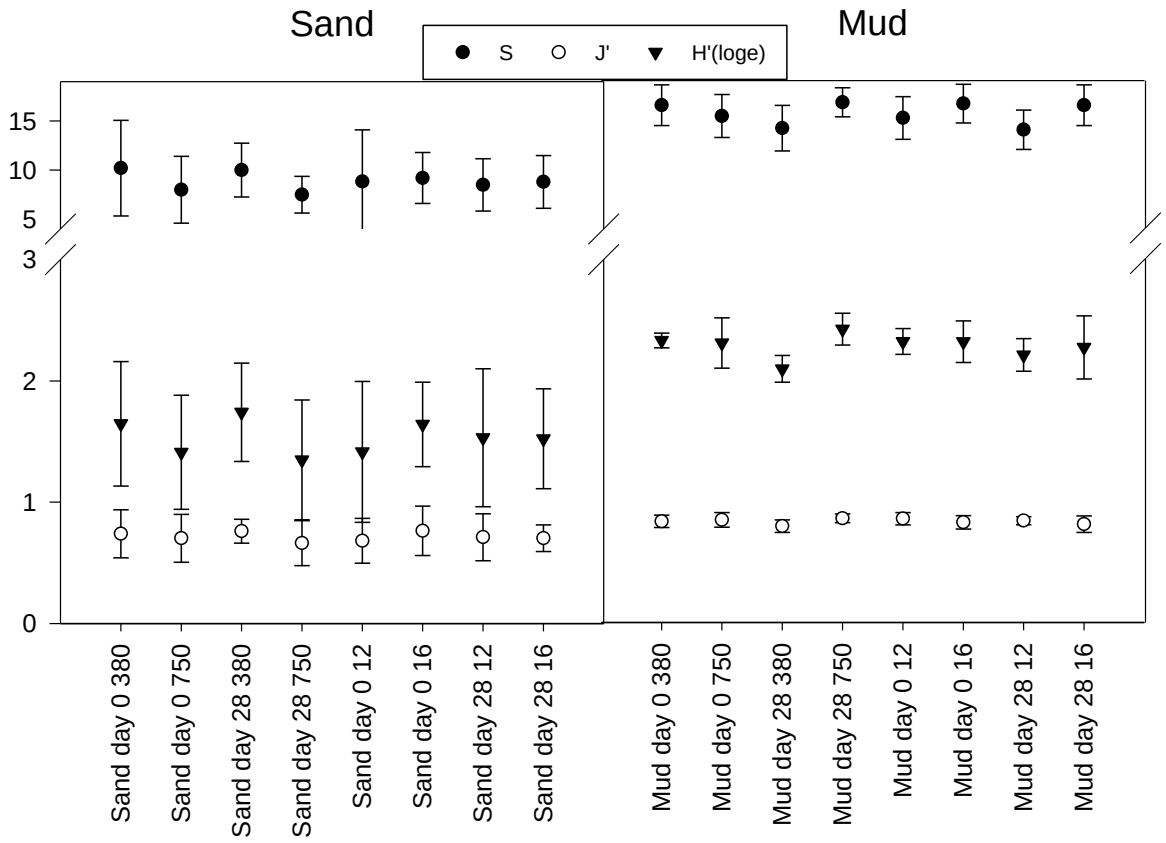


Fig. 5. Nematode diversity measures for sediment-type-day groups of samples (average (±SD, n=11) in black and sample abundances in grey). (A) Nematode genus richness (S), (B) Nematode Shannon diversity (log e), C) Nematode Pielou evenness.



928
929 **Fig. 6. Nematode diversity indices for each combination of sediment type, day and CO_2 exposure or**
930 **Temperature, indicating consistent differences in nematode genus richness and Shannon Wiener diversity**
931 **between sediment types, whilst Pielou evenness did not differ significantly and no differences were**
932 **observed between CO_2 and temperature treatments (n= 5 or n=6 depending on the treatment combination).**
933
934

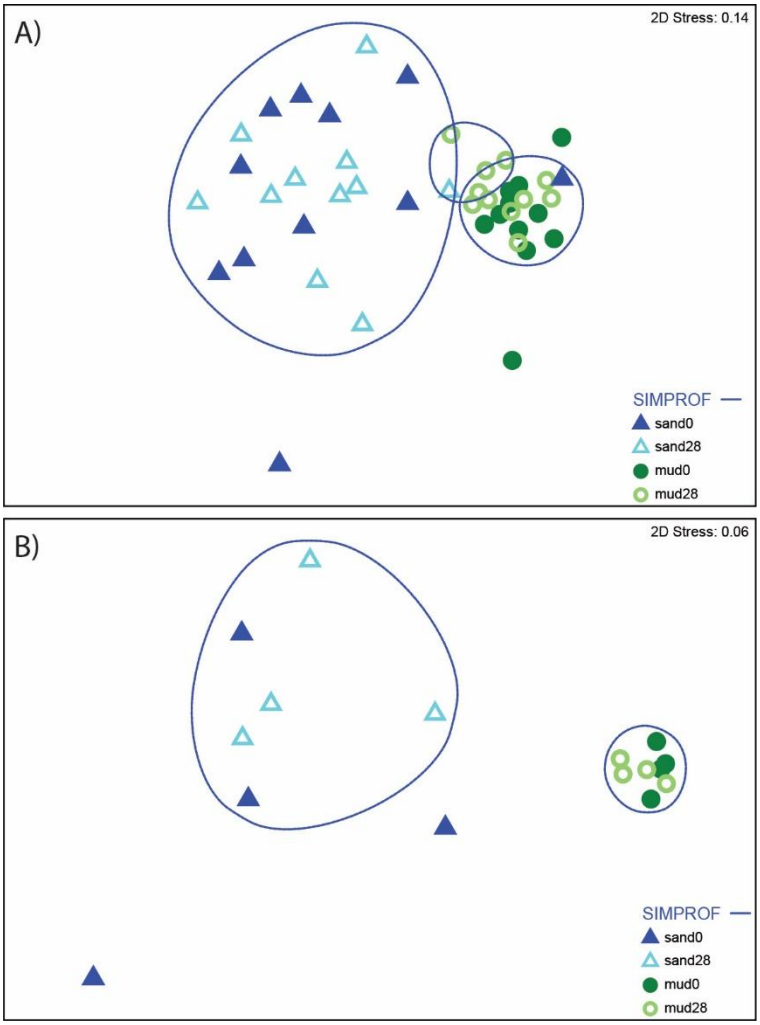


Fig. 7. Non-metric MDS on nematode genera abundance (standardised, sq. root transformation, Bray Curtis resemblance). Symbols represent samples belonging to two sediment type (sand, mud) and two time (0 days, 28 days) combinations. (A) all samples, (B) samples averaged over replicates. Blue lines indicate SIMPROF significance at the 5 % level.

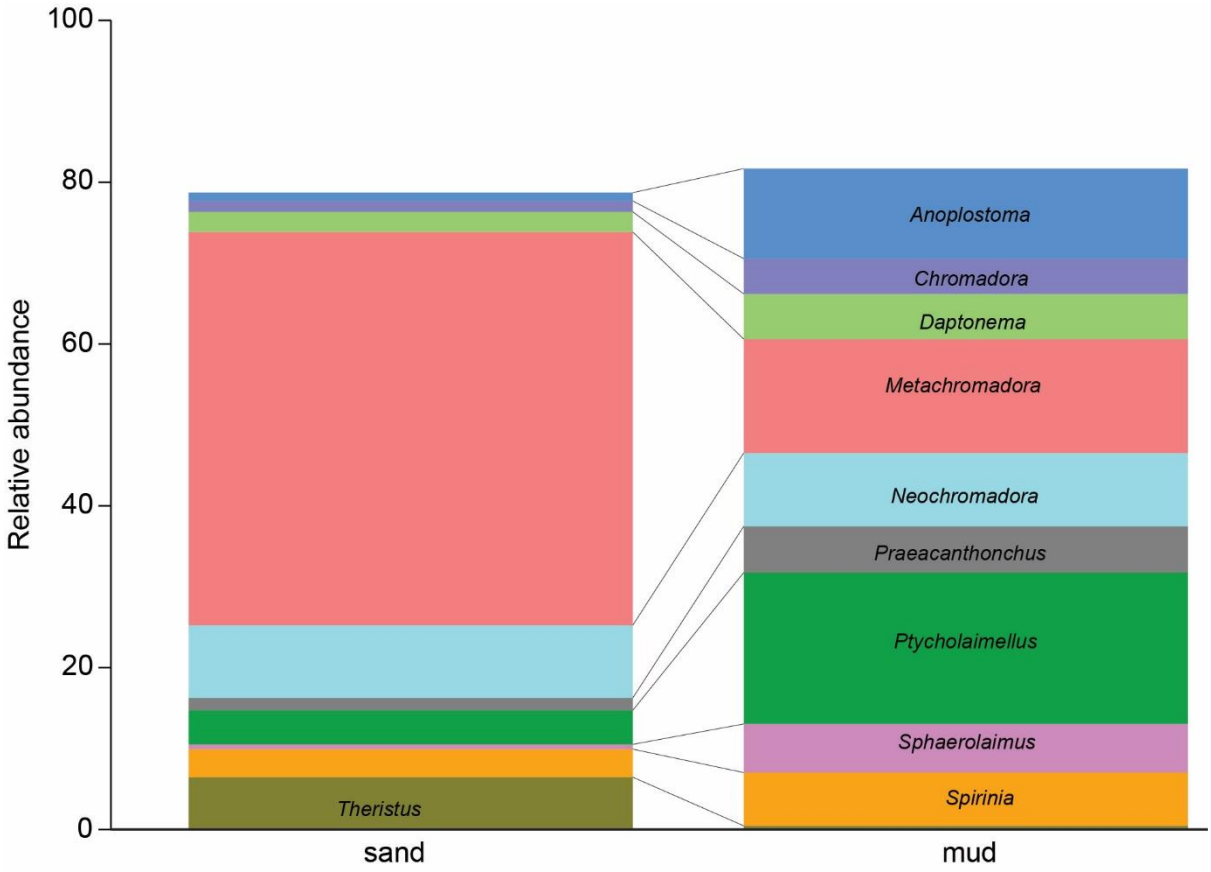


Fig. 8. Average relative abundance for the sand and mud samples representing only the 10 most important genera, indicating the dominance of *Metachromadora* in sand samples and the more evenly distributed community in mud samples.

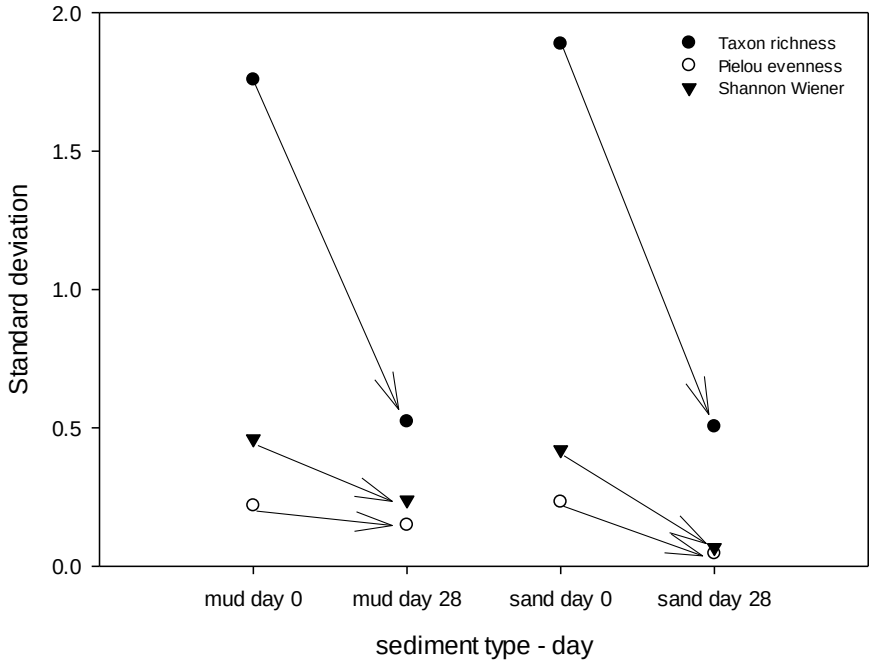


Fig. 9. Standard deviations of meiofauna diversity measures for the two sediment types at day 0 and day 28, indicating a consistent decline of variation over the duration of the experiment.

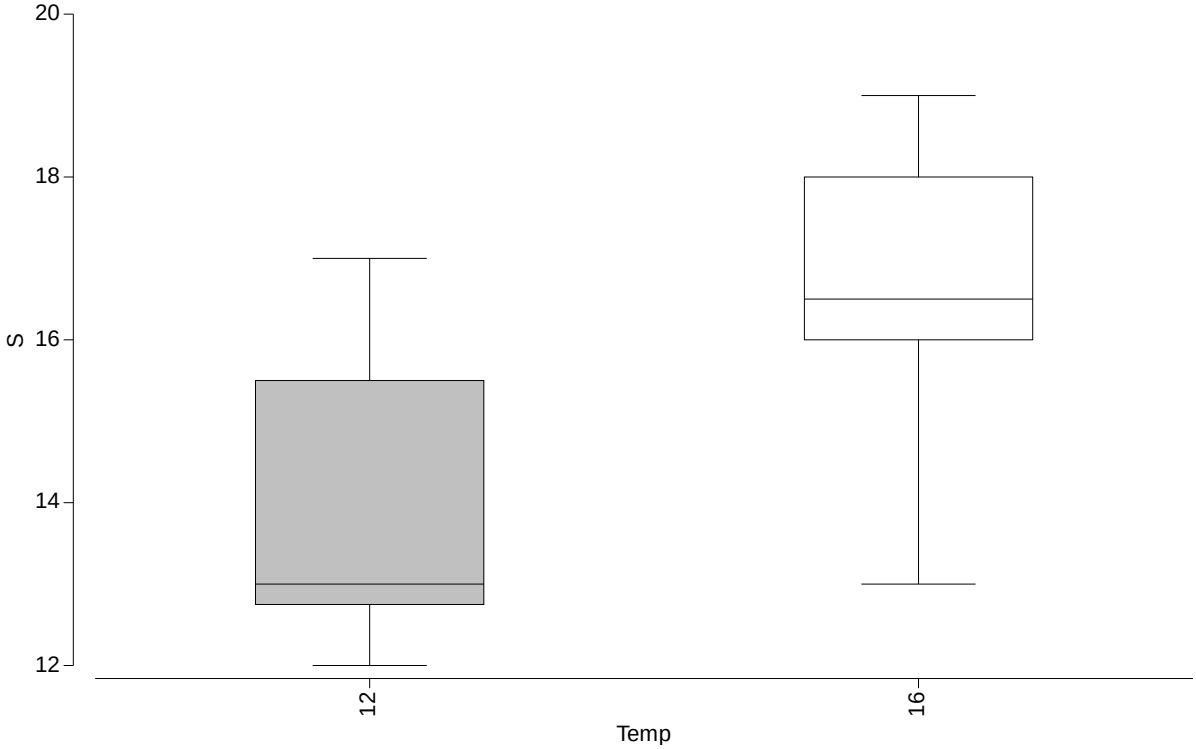


Fig. 10. Box and whisker plots for nematode genus richness (number of genera, S) for both temperature treatments in mud sediments after 28 days. Box boundaries represent 25-75 percentile, line within box represents median, error bars indicate 90th and 10th percentiles (n=22 for both treatments).

8. Supplementary Tables

Table S1. Meiofauna PERMANOVA results testing treatment effects. Time (Ti; 0, 28 days), Sediment (Se; sand, mud), CO_2 (380, 750 ppmv), temperature (Te; 12, 16°C)), MC: Monte Carlo, ECV: estimated component of variation.

Meiofauna	Test	Source	Pseudo-F	P(perm) [MC]	Unique perms	√ECV
Abundance	4way (Ti, Te, CO2, Se)	Ti	2.8285	0.109	998	521.54
		Se	24.885	**0.001**	993	1884.9
		CO2	1.0194	0.307	998	53.737
		Te	0.0094802	0.938	996	-383.86
		TixSe	1.3974	0.248	997	343.83
		TixCO2	0.0024605	0.959	994	-544.78
		TixTe	0.028894	0.868	995	-5.38E+02
		SexCO2	0.087656	0.777	999	-5.21E+02
		SexTe	0.017407	0.903	998	-5.41E+02
		CO2xTe	0.92238	0.336	998	-151.96
		TixSexCO2	0.5033	0.482	996	-5.44E+02
		TixSexTe	0.090993	0.762	998	-735.45
		TixCO2xTe	0.087656	0.784	997	-7.37E+02
		SexCO2xTe	0.8053	0.414	995	-3.40E+02
		TixSexCO2xTe	0.18126	0.671	998	-987.08
Abundance (sand)	3-way (Ti, Te, CO2)	Te	0.0050507	0.932	993	-187.14
		Ti	1.0553	0.363	999	4.41E+01
		CO2	2.1519	0.162	994	2.01E+02
		TexTi	0.073264	0.819	996	-2.55E+02
		TexCO2	0.016785	0.91	994	-2.63E+02
		TixCO2	1.8399	0.194	995	2.43E+02
		TexTixCO2	0.071071	0.805	998	-3.62E+02
Abundance (mud)	3-way (Ti, Te, CO2)	Te	0.013972	0.907	998	-742.97
		Ti	2.1794	0.155	997	812.57
		CO2	0.45303	0.494	997	-553.36
		TexTi	0.059106	0.806	995	-1026.4
		TexCO2	0.9171	0.371	999	-304.66
		TixCO2	0.15309	0.706	998	-973.78
		TexTixCO2	0.13845	0.721	994	-1389
Abundance (sand, d0)	2-way (Te, CO2)	CO2	2.08E+00	0.249	978	3.82E+02
		Te	1.04E-02	0.909	985	-365.05
		CO2xTe	0.0049088	0.906	987	-517.69
Abundance (sand, d28)	2-way (Te, CO2)	CO2	0.070032	0.792	984	-75.557
		Te	0.66967	0.428	986	-45.031
		CO2xTe	0.89987	0.37	985	-35.062
Abundance (mud, d0)	2-way (Te, CO2)	CO2	0.020522	0.892	986	-1456.7

		Te	0.0040323	0.938	989	-1468.9
		CO2xTe	0.45693	0.522	991	-1533.9
Abundance (mud, d28)	2-way (Te, CO2)	CO2	8.6977	**0.023**	985	749.18
		Te	1.0024	0.362	989	13.101
		CO2xTe	2.6327	0.13	985	487.95
S	4way (Ti, Te, CO2, Se)	Ti	1.47	0.236	998	0.15827
		Se	113.57	**0.001**	995	2.4493
		CO2	2.5451	0.117	997	0.28695
		Te	0.91623	0.327	998	-0.066815
		TixSe	4.4346	**0.038**	995	0.60504
		TixCO2	6.8453	**0.014**	998	0.78931
		TixTe	0.19953	0.673	994	-0.29209
		SexCO2	1.47	0.245	998	0.22383
		SexTe	10.592	**0.008**	998	1.0111
		CO2xTe	2.9686	*0.098*	998	0.45806
		TixSexCO2	1.47	0.216	993	0.31654
		TixSexTe	7.5294	**0.007**	997	1.1798
		TixCO2xTe	2.9686	*0.084*	993	0.6478
		SexCO2xTe	0.0040721	0.94	997	-0.46076
		TixSexCO2xTe	1.1768	0.298	997	0.27458
S (sand)	3-way (Ti, Te, CO2)	Te	2.4231	0.131	985	0.40642
		Ti	5.0556	*0.051*	998	0.6861
		CO2	3.6197	*0.081*	996	0.55142
		TexTi	2.4231	0.143	986	0.57477
		TexCO2	1.4658	0.244	998	0.32884
		TixCO2	6.7308	**0.031**	995	1.1534
		TexTixCO2	3.6197	*0.074*	998	1.1028
S (mud)	3-way (Ti, Te, CO2)	Te	9.7356	**0.008**	996	0.92099
		Ti	0.43806	0.538	998	-0.23359
		CO2	0.08046	0.813	995	-0.29881
		TexTi	5.5875	**0.042**	995	0.94386
		TexCO2	1.5109	0.219	993	0.31497
		TixCO2	1.0817	0.303	997	0.12599
		TexTixCO2	0.2235	0.675	998	-0.54917
S (sand, d0)	2-way (Te, CO2)	CO2	5.715	**0.041**	668	1.3916
		Te	2.7391	0.133	607	0.84515
		CO2xTe	2.7391	0.151	622	1.1952
S (sand, d28)	2-way (Te, CO2)	CO2	1.037	0.339	757	0.044544
		Te	2.79E-16	1	114	-0.23146
		CO2xTe	1.037	0.355	747	0.062994
S (mud, d0)	2-way (Te, CO2)	CO2	0.50815	0.5	937	-0.40581
		Te	8.7215	**0.036**	947	1.6079
		CO2xTe	0.84	0.44	634	-0.32733
S (mud, d28)	2-way (Te, CO2)	CO2	1.037	0.309	374	0.044544
		Te	1.037	0.336	369	0.044544

		CO2xTe	1.037	0.32	368	0.062994
Pielou	4way (Ti, Te, CO2, Se)	Ti	0.10894	0.754	997	-0.080648
		Se	2.691	0.107	994	0.10368
		CO2	0.1118	0.73	996	-0.079209
		Te	0.0015066	0.973	996	-7.97E-02
		TixSe	0.6279	0.449	997	-0.071286
		TixCO2	0.39126	0.551	996	-0.095778
		TixTe	0.0080253	0.925	998	-0.11639
		SexCO2	0.0087918	0.925	996	-0.11834
		SexTe	0.0011741	0.972	997	-1.13E-01
		CO2xTe	0.18895	0.664	996	-0.10704
		TixSexCO2	0.427	0.477	996	-0.13546
		TixSexTe	0.34001	0.546	998	-0.13882
		TixCO2xTe	0.048172	0.825	998	-0.12703
		SexCO2xTe	0.39052	0.562	997	-0.13123
		TixSexCO2xTe	No test			
Pielou (sand)	3-way (Ti, Te, CO2)	Te	0.0070428	0.8332	234	-0.16535
		Ti	0.19619	0.7006	613	-0.16069
		CO2	0.078668	0.764	235	-0.17204
		TexTi	0.024625	0.87	598	-0.25034
		TexCO2	0.028535	0.8496	220	-0.24983
		TixCO2	0.26412	0.6684	113	-0.23245
		TexTixCO2	No test			
Pielou (mud)	3-way (Ti, Te, CO2)	Te	0.0087954	0.923	998	-0.061858
		Ti	0.36002	0.546	995	-0.049704
		CO2	0.10593	0.773	996	-0.058749
		TexTi	0.84817	0.384	994	-0.034238
		TexCO2	2.0545	0.178	997	0.090229
		TixCO2	0.0045076	0.947	999	-0.087669
		TexTixCO2	0.052889	0.824	997	-0.12093
Pielou (sand, d0)	2-way (Te, CO2)	CO2	0.34436	[0.665]	15	-0.20524
		Te	0.027524	[0.907]	15	-0.24996
		CO2xTe	0.014267	[0.927]	15	-0.3559
Pielou (sand, d28)	2-way (Te, CO2)	CO2	No test			
		Te	No test			
		CO2xTe	No test			
Pielou (mud, d0)	2-way (Te, CO2)	CO2	0.022836	0.876	990	-0.10499
		Te	0.35237	0.586	985	-0.085474
		CO2xTe	0.49554	0.503	983	-0.10668
Pielou (mud, d28)	2-way (Te, CO2)	CO2	0.14302	0.704	987	-0.059711
		Te	0.63486	0.422	986	-0.038976
		CO2xTe	2.567	0.176	989	0.11419
Shannon	4way (Ti, Te, CO2, Se)	Ti	0.39091	0.542	998	-0.054547
		Se	29.531	**0.001**	999	0.37332
		CO2	1.9715	0.168	998	0.06889

		Te	0.0070287	0.932	999	-0.069646
		TixSe	2.5029	0.131	997	0.12117
		TixCO2	2.4052	0.134	997	0.11717
		TixTe	0.16837	0.698	996	-0.090138
		SexCO2	0.27302	0.638	997	-0.084276
		SexTe	2.345	0.141	997	0.11463
		CO2xTe	0.027734	0.842	997	-0.097462
		TixSexCO2	0.87151	0.371	996	-0.050106
		TixSexTe	4.1731	**0.046**	994	0.249
		TixCO2xTe	1.3446	0.246	995	0.082061
		SexCO2xTe	2.63	0.123	997	0.17847
		TixSexCO2xTe	0.35279	0.562	996	-0.15904
Shannon (sand)	3-way (Ti, Te, CO2)	Te	2.0261	0.21	998	0.080338
		Ti	3.7839	*0.074*	999	0.13233
		CO2	2.8829	0.133	999	0.10882
		TexTi	2.0698	0.177	999	0.11601
		TexCO2	1.6447	0.233	995	0.090052
		TixCO2	4.7937	**0.046**	998	0.21846
		TexTixCO2	2.3881	0.146	999	0.18688
Shannon (mud)	3-way (Ti, Te, CO2)	Te	0.77247	0.418	999	-0.054907
		Ti	0.33753	0.581	999	-0.09369
		CO2	0.28654	0.608	998	-0.097228
		TexTi	2.2187	0.151	999	0.17971
		TexCO2	1.179	0.315	997	0.068872
		TixCO2	0.14049	0.725	999	-0.15092
		TexTixCO2	0.11795	0.732	997	-0.21621
Shannon (sand, d0)	2-way (Te, CO2)	CO2	3.9283	*0.085*	944	0.26618
		Te	2.1295	0.198	927	0.16531
		CO2xTe	2.0787	0.201	935	0.22847
Shannon (sand, d28)	2-way (Te, CO2)	CO2	1.5774	0.238	862	0.023586
		Te	0.0015201	0.969	863	-0.031016
		CO2xTe	0.45128	0.494	859	-0.032516
Shannon (mud, d0)	2-way (Te, CO2)	CO2	0.26185	0.601	989	-0.1759
		Te	1.7733	0.217	985	0.18003
		CO2xTe	0.17422	0.673	983	-0.2631
Shannon (mud, d28)	2-way (Te, CO2)	CO2	0.03078	0.846	985	-0.10366
		Te	0.44565	0.538	989	-0.078392
		CO2xTe	2.4416	0.179	986	0.17878
Community	4way (Ti, Te, CO2, Se)	Ti	1.9693	0.123	999	3.964
		Se	32.96	**0.001**	998	22.762
		CO2	2.2966	*0.084*	998	4.5847
		Te	Negative			-4.084
		TixSe	3.6671	**0.018**	999	9.2991
		TixCO2	2.6707	**0.046**	999	7.3599

			TixTe	Negative			-5.8064
			SexCO2	1.0512	0.377	999	1.2883
			SexTe	2.981	**0.041**	999	8.0144
			CO2xTe	2.2261	*0.085*	998	6.3049
			TixSexCO2	0.37641	0.77	999	-6.359
			TixSexTe	3.1858	**0.03**	999	11.905
			TixCO2xTe	2.4876	*0.077*	999	9.8215
			SexCO2xTe	1.2673	0.284	999	4.1635
			TixSexCO2xTe	1.6569	0.184	999	9.2302
	Community (sand)	3-way (Ti, Te, CO2)	Te	1.4215	0.263	999	2.9934
			Ti	3.1856	*0.055*	999	6.8161
			CO2	3.3893	*0.07*	997	7.1266
			TexTi	1.8832	0.156	998	6.1278
			TexCO2	2.2216	0.135	997	7.2067
			TixCO2	4.9062	**0.03**	999	12.887
			TexTixCO2	2.9172	*0.089*	999	12.768
	Community (mud)	3-way (Ti, Te, CO2)	Te	1.5027	0.231	999	4.681
			Ti	2.639	*0.071*	999	8.4523
			CO2	0.83735	0.489	998	-2.6626
			TexTi	1.4217	0.256	999	6.0629
			TexCO2	1.5151	0.211	999	6.7009
			TixCO2	Negative			-9.908
			TexTixCO2	1.6602	0.192	999	10.729
	Community (sand, d0)	2-way (Te, CO2)	CO2	5.6563	**0.034**	925	16.89
			Te	2.0977	0.152	937	8.2008
			CO2xTe	3.5044	*0.078*	924	17.518
	Community (sand, d28)	2-way (Te, CO2)	CO2	0.258	0.799	926	-4.199
			Te	0.50418	0.594	919	-3.4325
			CO2xTe	0.1588	0.876	905	-6.3228
	Community (mud, d0)	2-way (Te, CO2)	CO2	0.48201	0.74	992	-7.5694
			Te	2.016	0.142	991	10.601
			CO2xTe	0.44341	0.739	989	-11.097
	Community (mud, d28)	2-way (Te, CO2)	CO2	0.13629	0.9	989	-7.4196
			Te	0.50108	0.632	988	-5.6392
			CO2xTe	3.5734	**0.045**	983	18.112

Table S2. Nematode PERMANOVA results testing treatment effects [Time (Ti; 0, 28 days), Sediment (Se; sand, mud), CO_2 (380, 750 ppmv), temperature (Te; 12, 16°C)), MC: Monte Carlo, ECV: estimated component of variation].

Nematoda	Test	Source	Pseudo-F	P(perm) [MC]	Unique perms	√ECV
S	4way (Ti, Te, CO2, Se)	Ti	0.12061	0.727	998	-0.59668
		Se	55.233	**0.001**	998	4.6858
		CO2	0.99112	0.33	995	-0.059969
		Te	1.8659	0.174	993	0.59209
		TixSe	0.013401	0.924	998	-0.89379
		TixCO2	0.8153	0.4	998	-0.38672
		TixTe	0.043418	0.847	999	-0.88009
		SexCO2	2.7021	0.118	997	1.174
		SexTe	0.58374	0.463	996	-0.58056
		CO2xTe	0.013401	0.913	999	-0.89379
		TixSexCO2	1.5057	0.215	996	0.90496
		TixSexTe	0.0048243	0.933	995	-1.2695
		TixCO2xTe	1.287	0.269	998	0.68175
		SexCO2xTe	0.043418	0.846	994	-1.2446
		TixSexCO2xTe	0.090589	0.767	995	-1.7162
S (sand)	3-way (Ti, Te, CO2)	Te	0.11581	0.744	998	-1.0583
		Ti	0.017132	0.893	998	-1.1158
		CO2	2.2264	0.165	995	1.2464
		TexTi	0.0061674	0.952	993	-1.5868
		TexCO2	0.033578	0.855	995	-1.5647
		TixCO2	0.033578	0.857	997	-1.5647
		TexTixCO2	0.65854	0.439	998	-1.3154
S (mud)	3-way (Ti, Te, CO2)	Te	5.2082	**0.037**	995	1.2182
		Ti	0.24613	0.626	998	-0.51563
		CO2	0.48242	0.477	997	-0.42725
		TexTi	0.088608	0.764	996	-0.80178
		TexCO2	0.0098453	0.929	996	-0.83571
		TixCO2	5.2082	*0.05*	996	1.7229
		TexTixCO2	0.79747	0.368	994	-0.53452
S (sand, d0)	2-way (Te, CO2)	CO2	0.55811	0.491	942	-1.3108
		Te	0.022324	0.872	971	-1.9498
		CO2xTe	0.32236	0.597	974	-2.2956
S (sand, d28)	2-way (Te, CO2)	CO2	3.0168	0.115	980	1.5417
		Te	0.18855	0.664	977	-0.97793
		CO2xTe	0.42424	0.535	959	-1.165
S (mud, d0)	2-way (Te, CO2)	CO2	1.0159	0.368	877	0.11785
		Te	1.5873	0.261	960	0.71686
		CO2xTe	0.25397	0.617	951	-1.1426
S (mud, d28)	2-way (Te,	CO2	5.8333	**0.041**	893	1.6091

	CO2)					
		Te	4.3815	*0.068*	974	1.3459
		CO2xTe	0.64815	0.467	979	-0.61399
Pielou	4way (Ti, Te, CO2, Se)	Ti	0.061963	0.792	996	-0.029443
		Se	7.0391	**0.018**	996	0.074706
		CO2	0.14893	0.728	996	-0.028045
		Te	0.024952	0.881	999	-0.030018
		TixSe	0.010636	0.916	998	-0.042762
		TixCO2	0.011355	0.919	996	-0.042747
		TixTe	0.16883	0.707	998	-0.039195
		SexCO2	1.5947	0.228	993	0.033154
		SexTe	0.72059	0.404	996	-0.022725
		CO2xTe	0.22314	0.637	998	-0.037892
		TixSexCO2	0.45872	0.467	997	-0.044731
		TixSexTe	0.16828	0.659	995	-0.055448
		TixCO2xTe	0.27461	0.607	996	-0.051783
		SexCO2xTe	0.11193	0.757	998	-0.057295
		TixSexCO2xTe	0.91523	0.332	997	-0.025034
Pielou (sand)	3-way (Ti, Te, CO2)	Te	0.26901	0.605	998	-0.050454
		Ti	0.0056404	0.931	997	-0.058845
		CO2	0.72136	0.402	997	-0.03115
		TexTi	0.17892	0.678	997	-0.075622
		TexCO2	0.0050408	0.955	999	-0.083245
		TixCO2	0.16305	0.65	998	-0.076349
		TexTixCO2	0.58182	0.464	994	-0.076322
Pielou (mud)	3-way (Ti, Te, CO2)	Te	2.0603	0.181	997	0.015067
		Ti	0.53494	0.469	998	-0.0099788
		CO2	3.3189	*0.094*	995	0.022283
		TexTi	1.93E-06	0.999	999	-2.07E-02
		TexCO2	2.8104	0.114	998	0.027843
		TixCO2	1.4059	0.259	998	0.013184
		TexTixCO2	0.80789	0.398	997	-0.012827
Pielou (sand, d0)	2-way (Te, CO2)	CO2	0.081089	0.772	981	-0.088506
		Te	0.36223	0.558	981	-0.073734
		CO2xTe	0.28399	0.589	982	-0.11049
Pielou (sand, d28)	2-way (Te, CO2)	CO2	1.0117	0.392	986	0.0079684
		Te	0.0058982	0.942	989	-0.073301
		CO2xTe	0.30833	0.601	984	-0.086469
Pielou (mud, d0)	2-way (Te, CO2)	CO2	0.13197	0.71	989	-0.023871
		Te	0.67072	0.459	984	-0.014702
		CO2xTe	0.19722	0.64	987	-0.032465
Pielou (mud, d28)	2-way (Te, CO2)	CO2	9.6823	**0.035**	977	0.041673
		Te	2.2097	0.176	983	0.015556

		CO2xTe	7.0992	**0.03**	985	0.049396
Shannon	4way (Ti, Te, CO2, Se)	Ti	0.014571	0.894	999	-0.078486
		Se	43.59	**0.001**	998	0.51598
		CO2	0.64839	0.432	998	-0.046882
		Te	0.51329	0.489	997	-0.055159
		TixSe	0.19221	0.677	999	-0.10049
		TixCO2	0.10953	0.741	996	-0.10551
		TixTe	0.084303	0.791	999	-0.107
		SexCO2	4.304	**0.046**	997	0.20324
		SexTe	0.24394	0.644	997	-0.097224
		CO2xTe	0.40775	0.517	995	-0.086049
		TixSexCO2	1.4533	0.22	996	0.10646
		TixSexTe	0.18452	0.664	994	-0.1428
		TixCO2xTe	1.3748	0.273	995	0.096802
		SexCO2xTe	0.021054	0.896	998	-0.15645
		TixSexCO2xTe	1.5654	0.24	998	0.16815
Shannon (sand)	3-way (Ti, Te, CO2)	Te	0.39555	0.539	997	-0.1183
		Ti	0.027255	0.867	997	-0.15007
		CO2	2.2393	0.149	996	0.16939
		TexTi	0.13994	0.732	997	-0.19956
		TexCO2	0.065747	0.79	995	-0.20799
		TixCO2	0.20653	0.667	995	-0.19168
		TexTixCO2	1.5861	0.199	997	0.23297
Shannon (mud)	3-way (Ti, Te, CO2)	Te	0.16704	0.676	997	-0.03929
		Ti	1.0545	0.313	998	0.010048
		CO2	5.4349	**0.039**	997	0.09066
		TexTi	0.065361	0.803	998	-0.058859
		TexCO2	2.0714	0.196	997	0.063017
		TixCO2	7.9628	**0.012**	999	0.16065
		TexTixCO2	0.020868	0.897	998	-0.085197
Shannon (sand, d0)	2-way (Te, CO2)	CO2	0.55	0.475	987	-0.14341
		Te	0.50962	0.466	986	-0.1497
		CO2xTe	1.1639	0.29	990	0.12241
Shannon (sand, d28)	2-way (Te, CO2)	CO2	1.8786	0.235	983	0.203
		Te	0.032057	0.869	992	-0.21307
		CO2xTe	0.49655	0.51	985	-0.21731
Shannon (mud, d0)	2-way (Te, CO2)	CO2	0.09535	0.763	982	-0.065048
		Te	0.0092821	0.916	990	-0.068072
		CO2xTe	0.66427	0.497	987	-0.056041
Shannon (mud, d28)	2-way (Te, CO2)	CO2	17.988	**0.005**	990	0.21559
		Te	0.29899	0.574	988	-0.043795
		CO2xTe	1.6989	0.219	983	0.061842
Community	4way (Ti, Te,	Ti	2.0103	**0.044**	999	7.0165

		CO2, Se)					
			Se	20.265	**0.001**	998	30.639
			CO2	0.91862	0.495	999	-1.9914
			Te	1.1103	0.352	999	2.3189
			TixSe	0.97277	0.449	999	-1.6289
			TixCO2	1.0127	0.438	999	1.1141
			TixTe	0.85108	0.582	999	-3.8096
			SexCO2	1.2649	0.26	998	5.0809
			SexTe	0.92792	0.524	998	-2.6504
			CO2xTe	0.94451	0.491	999	-2.3254
			TixSexCO2	0.77986	0.639	999	-6.5504
			TixSexTe	1.4564	0.171	999	9.4318
			TixCO2xTe	0.85272	0.568	998	-5.3579
			SexCO2xTe	0.5049	0.864	997	-9.8235
			TixSexCO2xTe	0.65144	0.752	998	-11.657
	Community (sand)	3-way (Ti, Te, CO2)	Te	0.8011	0.619	998	-3.3122
			Ti	2.2265	**0.022**	999	8.2247
			CO2	1.2266	0.292	999	3.5351
			TexTi	0.20914	0.986	999	-9.3403
			TexCO2	1.3825	0.207	999	6.4961
			TixCO2	1.0008	0.476	999	0.29005
			TexTixCO2	0.28183	0.969	999	-12.587
	Community (mud)	3-way (Ti, Te, CO2)	Te	1.1052	0.383	999	3.834
			Ti	1.2015	0.306	998	5.307
			CO2	1.0385	0.419	998	2.321
			TexTi	1.5265	0.147	998	12.132
			TexCO2	0.46508	0.861	997	-12.228
			TixCO2	0.85507	0.583	998	-6.3647
			TexTixCO2	0.93767	0.494	998	-5.903
	Community (sand, d0)	2-way (Te, CO2)	CO2	1.0375	0.409	987	3.5338
			Te	1.5638	0.182	989	13.695
			CO2xTe	0.56316	0.769	990	-17.048
	Community (sand, d28)	2-way (Te, CO2)	CO2	0.81347	0.612	983	-6.4979
			Te	0.95152	0.464	992	-3.3127
			CO2xTe	0.90449	0.483	981	-6.5756
	Community (mud, d0)	2-way (Te, CO2)	CO2	0.97491	0.461	996	-1.8239
			Te	0.36487	0.904	992	-9.1761
			CO2xTe	1.0786	0.401	992	4.5652
	Community (mud, d28)	2-way (Te, CO2)	CO2	1.3226	0.272	984	5.3296
			Te	0.71629	0.6	985	-4.998
			CO2xTe	0.46117	0.813	985	-9.741

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Table S3. Meiofauna PERMANOVA results testing Campaign (Ca) and Flume (Fl) effects; MC: Monte Carlo, ECV: estimated component of variation.

Meiofauna	Test	Source	Pseudo-F	P(perm) [MC]	Unique perms	√ECV
Abundance	2way (Ca, Fl)	Ca	0.5146	0.675	999	-234.81
		Fl	0.7293	0.634	999	-191.34
		CaxFl	1.4449	0.270	999	290.21
Abundance (sand)	2way (Ca, Fl)	Ca	0.5146	0.691	999	-234.81
		Fl	0.7293	0.624	999	-191.34
		CaxFl	1.4449	0.279	999	290.21
Abundance (mud)	2way (Ca, Fl)	Ca	2.4096	0.108	999	1451.40
		Fl	0.4122	0.822	998	-1022.50
		CaxFl	0.6044	0.574	999	-992.60
Abundance (sand, d0)	2way (Ca, Fl)	Ca	0.76411	0.628	998	-368.24
		Fl	0.6548	0.673	998	-486.05
		CaxFl	excluded			
Abundance (sand, d28)	2way (Ca, Fl)	Ca	12.265	*0.071*	996	185.91
		Fl	4.7232	0.177	997	116.62
		CaxFl	excluded			
Abundance (mud, d0)	2way (Ca, Fl)	Ca	3.5461	0.224	999	2729.80
		Fl	0.92261	0.619	999	-519.29
		CaxFl	excluded			
Abundance (mud, d28)	2way (Ca, Fl)	Ca	2.9502	0.286	998	712.92
		Fl	1.3681	0.474	997	337.94
		CaxFl	excluded			
S	2way (Ca, Fl)	Ca	0.80252	0.497	999	-0.19133
		Fl	1.2233	0.358	999	0.24762
		CaxFl	5.7622	**0.002**	996	2.1698
S (sand)	2way (Ca, Fl)	Ca	0.96855	0.419	992	-0.15076
		Fl	0.92704	0.492	999	-0.25054
		CaxFl	0.31132	0.749	975	-0.91079
S (mud)	2way (Ca, Fl)	Ca	0.66503	0.598	999	-0.39409
		Fl	1.5206	0.25	999	0.53604
		CaxFl	0.053922	0.946	998	-0.85502
S (sand, d0)	2way (Ca, Fl)	Ca	1.5873	0.389	979	1.1106
		Fl	1.1238	0.546	931	0.55635
		CaxFl	excluded			
S (sand, d28)	2way (Ca, Fl)	Ca	0.44444	0.728	722	-0.40825
		Fl	0.4	0.825	259	-0.46291
		CaxFl	excluded			
S (mud, d0)	2way (Ca, Fl)	Ca	2.4167	0.288	923	0.75277
		Fl	6.95	0.107	850	1.6833
		CaxFl	excluded			
S (mud, d28)	2way (Ca, Fl)	Ca	0.44444	0.761	694	-0.40825
		Fl	0.4	0.831	100	-0.46291
		CaxFl	excluded			

Pielou	2way (Ca, Fl)	Ca	1.6647	0.255	999	0.033498
		Fl	0.41956	0.716	999	-0.038101
		CaxFl	1.1732	0.413	999	0.039488
Pielou (sand)	2way (Ca, Fl)	Ca	4480.5	**0.001**	999	0.09994
		Fl	195.94	**0.005**	998	0.022748
		CaxFl	empty cells			
Pielou (mud)	2way (Ca, Fl)	Ca	1.6681	0.221	998	0.084955
		Fl	0.83609	0.561	996	-0.045911
		CaxFl	0.44114	0.642	999	-0.10031
Pielou (sand, d0)	2way (Ca, Fl)	Ca	no test			
		Fl	no test			
		CaxFl	no test			
Pielou (sand, d28)	2way (Ca, Fl)	Ca	no test			
		Fl	no test			
		CaxFl	no test			
Pielou (mud, d0)	2way (Ca, Fl)	Ca	4.5353	0.183	998	0.15526
		Fl	5.2012	0.178	997	0.18467
		CaxFl	excluded			
Pielou (mud, d28)	2way (Ca, Fl)	Ca	10.727	*0.088*	998	0.17437
		Fl	0.64888	0.718	997	-0.036148
		CaxFl	excluded			
Shannon	2way (Ca, Fl)	Ca	0.33553	0.811	999	-0.089517
		Fl	0.89379	0.503	999	-0.04356
		CaxFl	2.7993	**0.020**	998	0.34019
Shannon (sand)	2way (Ca, Fl)	Ca	1.6429	0.240	998	0.14207
		Fl	1.0397	0.488	999	0.038509
		CaxFl	0.13358	0.877	999	-0.21292
Shannon (mud)	2way (Ca, Fl)	Ca	0.90042	0.475	999	-0.067524
		Fl	0.91205	0.513	999	-0.06924
		CaxFl	0.5111	0.600	998	-0.19316
Shannon (sand, d0)	2way (Ca, Fl)	Ca	5.4871	0.176	993	0.43119
		Fl	2.9816	0.262	993	0.31265
		CaxFl	excluded			
Shannon (sand, d28)	2way (Ca, Fl)	Ca	0.22091	0.869	988	-0.072161
		Fl	0.29642	0.904	983	-0.074822
		CaxFl	excluded			
Shannon (mud, d0)	2way (Ca, Fl)	Ca	1.3031	0.487	997	0.13782
		Fl	2.7816	0.280	998	0.36457
		CaxFl	excluded			
Shannon (mud, d28)	2way (Ca, Fl)	Ca	15.669	**0.047**	998	0.28864
		Fl	1.0839	0.556	997	0.023813
		CaxFl	excluded			
Community	2way (Ca, Fl)	Ca	0.943	0.473	999	-1.5902
		Fl	1.0122	0.435	998	0.89404
		CaxFl	2.6141	**0.004**	999	19.542
Community (sand)	2way (Ca, Fl)	Ca	0.84842	0.523	999	-4.0046

			Fl	1.1012	0.389	998	3.5695
			CaxFl	0.20794	0.865	999	-11.818
Community (mud)	2way (Ca, Fl)		Ca	0.81893	0.598	999	-5.679
			Fl	0.82026	0.626	998	-6.1735
			CaxFl	0.37947	0.884	998	-13.572
Community (sand, d0)	2way (Ca, Fl)		Ca	1.2713	0.485	990	9.46
			Fl	0.96296	0.590	993	-3.8141
			CaxFl	excluded			
Community (sand, d28)	2way (Ca, Fl)		Ca	0.35924	0.783	990	-7.9264
			Fl	0.68379	0.729	982	-6.0754
			CaxFl	excluded			
Community (mud, d0)	2way (Ca, Fl)		Ca	1.4854	0.304	999	9.345
			Fl	2.6989	0.122	995	19.076
			CaxFl	excluded			
Community (mud, d28)	2way (Ca, Fl)		Ca	10.172	**0.027**	999	18.086
			Fl	3.0219	*0.087*	996	9.2652
			CaxFl	excluded			

Table S4. Nematode PERMANOVA results testing Campaign (Ca) and Flume (Fl) effects; MC: Monte Carlo, ECV: estimated component of variation.

Nematodes	Test	Source	Pseudo-F	P(perm) [MC]	Unique perms	√ECV
S	2way (Ca, Fl)	Ca	5.5659	**0.007**	999	1.2746
		Fl	8.778	**0.001**	998	2.0249
		CaxFl	11.041	**0.001**	997	4.3653
S (sand)	2way (Ca, Fl)	Ca	2.4766	0.104	998	1.2371
		Fl	7.6566	**0.001**	999	2.8658
		CaxFl	2.7018	0.112	996	1.7145
S (mud)	2way (Ca, Fl)	Ca	1.1819	0.322	999	0.47514
		Fl	1.1846	0.365	998	0.52223
		CaxFl	0.38278	0.713	998	-1.1298
S (sand, d0)	2way (Ca, Fl)	Ca	2.3827	0.328	995	1.9322
		Fl	4.3111	0.202	953	3.2623
		CaxFl	Excluded			
S (sand, d28)	2way (Ca, Fl)	Ca	0.31183	0.7978	982	-1.4606
		Fl	1.9161	0.355	995	1.8387
		CaxFl	Excluded			
S (mud, d0)	2way (Ca, Fl)	Ca	1.2807	0.4945	617	0.7303
		Fl	0.97895	0.572	968	-0.21822
		CaxFl	Excluded			
S (mud, d28)	2way (Ca, Fl)	Ca	0.20614	0.894	995	-2.4563
		Fl	0.31316	0.8799	994	-2.4928
		CaxFl	Excluded			
Pielou	2way (Ca, Fl)	Ca	5.1224	**0.013**	999	0.053886
		Fl	4.0279	**0.015**	998	0.056211
		CaxFl	3.3074	**0.012**	998	0.093102
Pielou (sand)	2way (Ca, Fl)	Ca	4.7809	**0.02**	998	0.12094
		Fl	2.5582	*0.088*	999	0.084715
		CaxFl	0.25875	0.798	999	-0.069134
Pielou (mud)	2way (Ca, Fl)	Ca	2.5209	0.132	999	0.03118
		Fl	2.168	0.147	997	0.029813
		CaxFl	0.56505	0.562	999	-0.021526
Pielou (sand, d0)	2way (Ca, Fl)	Ca	32.187	**0.028**	999	0.19308
		Fl	6.8105	0.142	998	0.090931
		CaxFl	Excluded			
Pielou (sand, d28)	2way (Ca, Fl)	Ca	0.97593	0.544	998	-0.014827
		Fl	1.6374	0.434	997	0.083254
		CaxFl	Excluded			
Pielou (mud, d0)	2way (Ca, Fl)	Ca	0.8772	0.572	999	-0.013148
		Fl	1.3279	0.496	998	0.02344
		CaxFl	Excluded			
Pielou (mud, d28)	2way (Ca, Fl)	Ca	4.5247	0.193	997	0.05674
		Fl	1.34	0.478	999	0.019227
		CaxFl	Excluded			

Shannon	2way (Ca, Fl)	Ca	4.5679	**0.012**	998	0.13554
		Fl	9.7202	**0.001**	999	0.25792
		CaxFl	9.4515	**0.001**	996	0.48177
Shannon (sand)	2way (Ca, Fl)	Ca	3.2666	*0.068*	999	0.22682
		Fl	6.8308	**0.007**	999	0.39694
		CaxFl	1.104	0.373	998	0.06271
Shannon (mud)	2way (Ca, Fl)	Ca	1.6941	0.23	998	0.084377
		Fl	0.57755	0.724	999	-0.071821
		CaxFl	0.53813	0.613	999	-0.088856
Shannon (sand, d0)	2way (Ca, Fl)	Ca	14.45	*0.065*	999	0.4349
		Fl	10.536	*0.081*	997	0.39956
		CaxFl	Excluded			
Shannon (sand, d28)	2way (Ca, Fl)	Ca	0.2306	0.87	999	-0.25966
		Fl	2.1207	0.365	996	0.34193
		CaxFl	Excluded			
Shannon (mud, d0)	2way (Ca, Fl)	Ca	0.37243	0.784	999	-0.12236
		Fl	0.47095	0.824	995	-0.12258
		CaxFl	Excluded			
Shannon (mud, d28)	2way (Ca, Fl)	Ca	0.72732	0.65	999	-0.12007
		Fl	0.16732	0.966	996	-0.22894
		CaxFl	Excluded			
Community	2way (Ca, Fl)	Ca	1.3737	0.102	998	5.8911
		Fl	1.8498	**0.009**	999	10.813
		CaxFl	2.334	**0.001**	998	25.706
Community (sand)	2way (Ca, Fl)	Ca	0.87717	0.609	999	-7.3115
		Fl	1.1544	0.289	999	8.9449
		CaxFl	0.63175	0.831	998	-16.343
Community (mud)	2way (Ca, Fl)	Ca	1.9706	**0.019**	998	12.431
		Fl	0.95922	0.569	998	-2.7801
		CaxFl	0.91416	0.524	999	-4.7725
Community (sand, d0)	2way (Ca, Fl)	Ca	1.9065	0.134	997	23.123
		Fl	1.7368	0.146	994	22.745
		CaxFl	Excluded			
Community (sand, d28)	2way (Ca, Fl)	Ca	0.63957	0.743	997	-17.348
		Fl	0.77671	0.756	996	-14.898
		CaxFl	Excluded			
Community (mud, d0)	2way (Ca, Fl)	Ca	4.2823	**0.025**	999	22.761
		Fl	2.7208	*0.067*	994	17.982
		CaxFl	Excluded			
Community (mud, d28)	2way (Ca, Fl)	Ca	0.89175	0.585	998	-6.3942
		Fl	0.30531	*0.98*	997	-17.674
		CaxFl	Excluded			

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