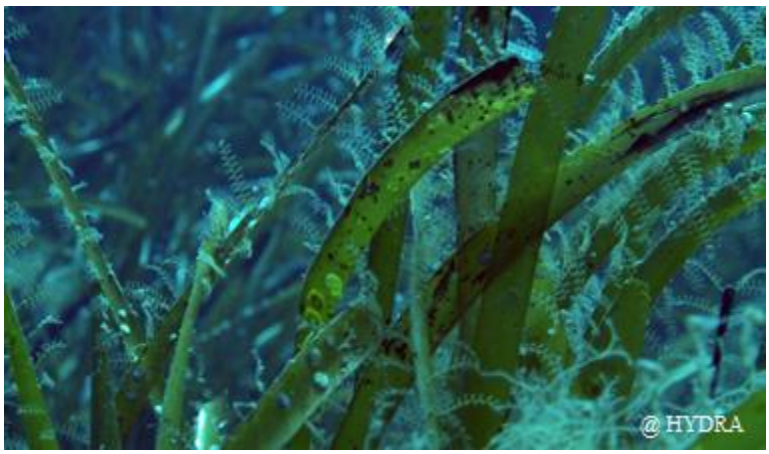
‘OCEANS AND LAKES’

INTERUNIVERSITY MASTER IN MARINE AND LACUSTRINE SCIENCE AND MANAGEMENT

Meiofauna associated with seagrasses at natural CO_2 seeps in the Mediterranean Sea



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ABSTRACT

Due to the elevated carbon-dioxide (CO_2) level in the atmosphere, safe and effective carbon capture and storage methods are gaining more interest. The risks associated with storage and potential leakages for the marine environment are, however, largely unknown. CO_2 release from sub-seabed reservoirs will have the greatest impact on the marine organisms living in or near the sea bottom. Areas where CO_2 of volcanic origin is leaking from the seabed since centuries provide natural laboratories to study the long-term effects of high CO_2 concentrations and subsequent seawater acidification. This study focused on the effects these environmental conditions have on meiofauna, and more particular on the community composition and diversity of the most two abundant taxa, i.e. nematodes and copepods. Samples were collected at natural CO_2 seeps in *Posidonia oceanica* seagrass meadows near Panarea Island. In conjunction, a colonisation experiment with seagrass mimics was also carried out to look into the short-term effects of CO_2 leakage.

Our observations indicated no significant differences in meiofauna densities between CO_2 impacted and non-impacted sites either on natural seagrass leaves, shoots or seagrass mimics. The only difference in meiofauna diversity was observed on natural seagrass shoots. On natural leaves, a shift in dominant harpacticoid species was found, however, community structure did not significantly differ. On the natural seagrass shoots, on the other hand, nematode communities showed a significant change in community structure and species dominance. The short-term colonisation experiment on seagrass mimics showed changes in the harpacticoid community structure at the seepage site; while the nematode community structure, showed no difference, only a change in species dominance was observed.

In general, a rather remarkable lack of strong meiofaunal response to the reduced pH may be depended on indirect consequences of CO_2 leakage, such as increased seagrass productivity, seasonal organic matter input and reduced macrofaunal predation, in addition to the species specific reactions to environmental disturbance. Since similar research has not been carried out in the shallow-water environment before, further studies are suggested to gain better knowledge of the adaption of meiofauna to the low pH/high CO_2 world.

Keywords: climate change, carbon capture and storage, CO_2 leakage, natural CO_2 seeps, *Posidonia oceanica*, meiofauna, nematodes, copepods, colonisation experiment

Introduction

Since the start of the Industrial Revolution, atmospheric carbon dioxide (CO_2) concentration has been increasing. The highest safe level of CO_2 (the level at which maximum 2°C warming can be achieved) was set at 350ppm (Hansen *et al*., 2008; Guivarch and Hallegatte, 2013). Nevertheless, in May 2013 the daily CO_2 level reading has reached 400ppm for the first time in modern human history and it continues to increase by ~ 2 ppm per year (Hansen *et al*., 2008; Keeling *et al*., 2013). Due to the increased CO_2 level, the atmospheric gas composition is changing and the global average temperature has increased by 0.8°C (Schellnhuber *et al*. 2012). Their combined effects are modifying the chemical composition of ocean water, causing acidification and disturbance of the marine ecosystem (Pachauri *et al*., 2007). Today the pH level of the surface ocean is 0.1 units lower than it was in preindustrial times and it is projected to decrease further by 0.4 units by the end of the century with atmospheric carbon level reaching 800ppm, at the current rate of emission (Caldiera *et al*., 2003).

The Kyoto Protocol, which was negotiated by the United Nations Framework Convention on Climate Change (UNFCCC), came into force in 2006 with the goal that the 37 industrialized nations stabilise greenhouse gas emission at the baseline level of 1990 within the period of 2008-2012. Methods of mitigation include carbon emission trading and taxing, development of green energy sources, and carbon capture and storage (CCS), which involves the removal of greenhouse gases from the exhaust of industrial plants and chemical factories, and transporting them to secure reservoir sites on land and/or deep seabed where it is injected in a liquefied form (Herzog *et al*., 2001).

The first CCS project was initialised at the Sleipner gas fields in the North Sea in 1996 (Statoil, 2009; Gurney *et al.*, 2012). Thus far, nineteen developed countries have been involved in CCS, and sixteen large-scale CCS projects have been established worldwide that are fully operating or are under construction (Gurney *et al*., 2012). Nevertheless, CCS holds several environmental risks. The potential of CO_2 leakage, for example while transporting via pipelines or due to ground displacement and seismicity, needs to be taken into account in order to carry out safe and effective CCS (Metz *et al*., 2005; Gurney *et al.*, 2012; Fig. 1).

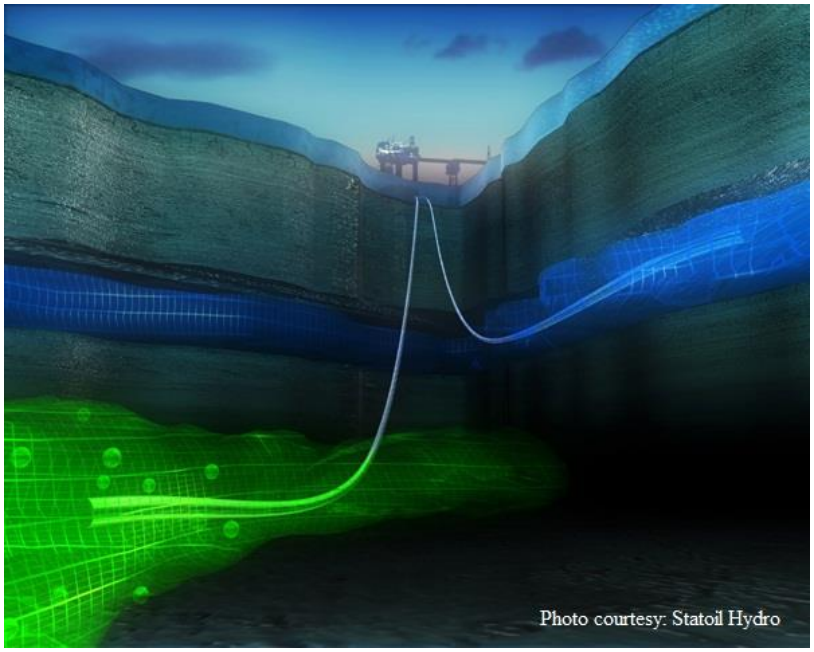

Figure 1: *Statoil's carbon storage at Sleipner (Image courtesy of Alligator film / BUG / Statoil)*

The European Union 7th Framework Programme initiated the ECO2 project – Sub-seabed CO_2 storage: Impact on Marine Ecosystem (2011-2015), to establish the best possible environmental practices for safe offshore CCS. The project merges together three different scientific issues: ocean acidification (OA), natural CO_2 leakage and CCS methods. The objectives of the project are to evaluate the likelihood of possible leakage from the storage sites and its ecological impact, as well as the economic and legal consequences (Wallman, 2012). The ECO2 project has seven main work packages (WP1-7) of which WP4 is designed to investigate the impact of leakage on marine ecosystems. The approach is threefold and includes short-, mid- and long-term monitoring at seepage sites, experimental work at natural volcanic CO_2 seepage areas, and the introduction of seawater acidification in laboratory experiments.

While the effects of OA are best studied on macrofauna that live near the water surface and have biogenic calcium carbonate ($CaCO_3$) skeletons or shells (e.g. corals, calcareous planktons, molluscs, and crustaceans), relatively little is known about the response of meiofauna communities to CO_2 level changes and subsequently pH variations (Kurihara *et al*., 2007; Hall-Spencer *et al*., 2008; Fabricius *et al*., 2011).

Meiofauna are regarded as an important measure of benthic health and energy flow (Giere, 2009). Limited mobility, fast growth and metabolic rate, and high abundance make them suitable to study long-term environmental effects (Kennedy *et al*. 1997; Kurihara *et al*., 2007). Up till now, experiments to study the effect of OA on meiofauna were mainly carried

out *in situ* in deep-sea environments and in laboratory conditions, each time on a short-term scale. Therefore, within the framework of the ECO2 Project, experiments were performed and samples were collected at natural volcanic CO_2 seeps at Panarea Island with the aim to investigate both the short- and long-term effects of elevated CO_2 concentrations on meiofauna that live associated with seagrass meadows.

The following hypotheses were tested:

1.) Long-term CO_2 seepage impact the meiofauna associated with seagrasses by causing changes in meiofauna densities and composition.

2.) Different meiofauna communities colonise seagrass mimics when influenced by CO_2 seepage.

3.) Copepods and nematodes respond differently to CO_2 seepage.

To test these hypotheses, a two-fold approach was used: 1.) sampling seagrass leaves and shoots in meadows at natural volcanic CO_2 seeps and 2.) performing a short-term colonization experiment with seagrass mimics (see De Troch *et al*., 2005). In both approaches the difference between the CO_2 impacted and the non-impacted reference site was compared in terms of meiofauna abundance, community structure and diversity with an emphasis on nematode and copepod species.

Material and methods

Sampling area

Sampling was carried out at natural analogue seepage sites at the Aeolian Islands in the Mediterranean Sea (Fig. 2). In this area, volcanic activity results in the escape of natural fluids and gases rich in CO_2 (> 90%) from open fractures and through the seabed sediment (Caramanna *et al*., 2011). This active and shallow (10 – 40 m) seepage site is the perfect "field laboratory" as it is easy to reach and sampling can be easily carried out, and the constant CO_2 outgassing provides excellent research possibilities for both short- and long-term monitoring of CO_2 leakage and its effects on the marine environment (Caramanna *et al*., 2011).

Figure 2: *Map of Aeolian Islands (Created by Norman Einstein)*

Sampling strategy

The sampling campaign at the island of Panarea (Fig. 2) took place from 2nd June to 12th June 2012. Underwater sampling with SCUBA (self-contained breathing apparatus) was carried out by the professional Elba diving team of HYDRA institute. Samples were collected east of the island of Basiluzzo at a depth of 14 – 16 m. Two sites were sampled: the acidic (down to pH 5.3) "RedPlus" site (38° 39.749'N, 15° 07.132'E; Fig. 3) where the sediments was red coloured, and evenly distributed gas seepage occurred, and the non-acidic (pH 8.2) "GreyMinus" site (38° 39.827'N, 15° 07.118'E; Fig. 4) where sediment was grey coloured and no gas seepage occurred (Meyer *et al*., 2012). ("RedPlus" and "GreyMinus" sites will also be referred to as impacted and non-impacted sites, respectively, in the text.) Water temperature at

the sampling depth was 19°C, and *Posidonia oceanica* meadows were present both at the impacted and the non-impacted site (Meyer *et al*., 2012).

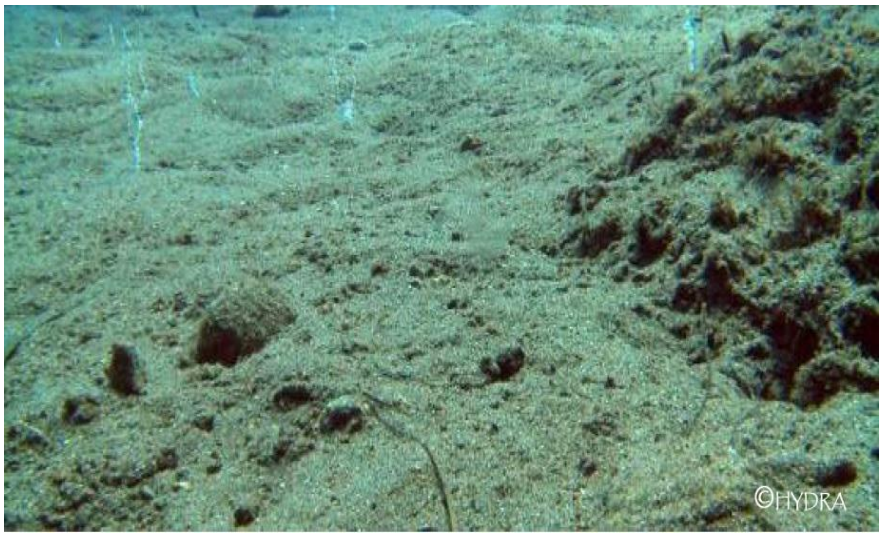

Figure 3: *RedPlus site (@HYDRA, Elba)*

Figure 4: *GreyMinus site (@HYDRA, Elba)*

Sampling method

At both “RedPlus” and “GreyMinus” sites three replicates of seagrass samples were collected. One replicate consisted of 12 to 18 leaves which were collected by placing plastic bags over the leaves and cutting off the leaves at their base, followed by closing the bag with an elastic band. The shoots were cut off from the rhizomes and also gently transferred into separate plastic bags. On land, the samples were poured on a 32µm mesh sieve to eliminate water and collect epiphytic meiofauna. The leaf and shoot samples were then stored in vials on a 4% formaldehyde-seawater solution.

Seagrass mimics and experimental set-up

Plastic seagrass mimics (Fig. 5) that resemble *Posidonia oceanica* (Bio Models CA, www.biomodelscompany.com) were planted in both “RedPlus” and “GreyMinus” meadow sites with the aim to study the colonisation by meiofauna under the different environmental conditions.

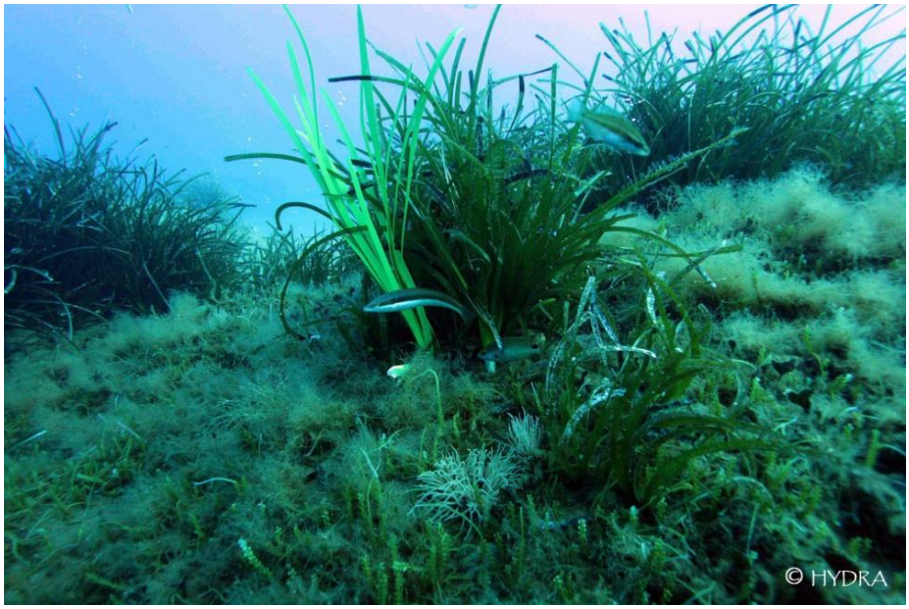

Figure 5: *Seagrass mimic at the RedPlus site (@HYDRA, Elba)*

The seagrass mimics were made up of 6 plastic blades that were 28 – 50 cm long and 7 – 11 mm wide, and were fixed with a 15 cm long pin. One replicate sample consisted of two seagrass mimics which had an average surface of 234 ± 13 cm^2 (n = 6). Six replicates were planted in the seagrass meadows at both the “RedPlus” and “GreyMinus” sites. After 13 days

the mimics were collected in plastic bags. The collected samples were poured over a 32 µm sieve and what remained on the sieve was stored in a 4% formaldehyde-seawater solution.

Sample processing

Under laboratory conditions at the University of Ghent, leaf and shoot samples were poured over stacked 1 mm and 32 µm sieves and rinsed thoroughly to retain macrofauna and meiofauna, respectively. Meiofauna were identified and counted under a stereo binocular microscope at the highest taxon level, while the first 120 copepods and 100 nematodes encountered were randomly handpicked with the use of a fine needle before further identification. Copepods were stored in 70% ethanol before being transferred into glycerine. The rotation of individuals was facilitated by placing small glass fragments under the cover-glass. Nematodes were transferred into glycerine (De Grisse I, II and III; Seinhorst, 1959) before being mounted on glass slides for identification.

In order to standardise meiofauna densities Image J software was used to measure seagrass leaf surfaces (expressed in individuals per 100 cm^2), while the volume of shoots was determined with the submersion method (expressed in individuals per 10 cm^3).

Species identification

The identification of both copepods and nematodes to species level was carried out with a Leica DMR compound microscope and Leica LAS 3.3 imaging software which allowed body measurements. Copepod species were identified to the lowest taxonomic level with the use of identification keys, reference books by Lang (1948), Huys *et al*., (1996), Boxshall and Halsey (2004) and original species descriptions. The identification of nematodes to species level was carried out with the online free-living nematodes identification key NeMys (http://nemys.ugent.be/) (NeMysKey©) and the pictorial key by Warwick *et al.* (1998).

Statistical analysis

Statistical analyses were carried out on meiofauna abundance and diversity, copepod and nematode community structure, and abundance and species diversity. Normality (Shapiro – Wilk's test) and homogeneity (Levene's test) were checked before a parametric analysis of variance (ANOVA) was used to detect significant differences between sample sites. Transformation of data was carried out, when necessary, to achieve the assumptions. Bray-Curtis analysis was performed as resemblance measure on square root transformed density data. Non-metric multidimensional scaling (MDS) plots were constructed to visualise

resemblance of community structure of sample sites. To analyse (dis)similarity of sample sites and habitats, one-way and two-way crossed analyses of similarities (ANOSIM) were used, and the variability within and between sample groups were revealed with similarity percentage analysis (SIMPER) on standardised and square root transformed density data. Margalef's (D) diversity index, Pielou's evenness index (J') and Hill's indices (N_0, N_1, N_2, N_{inf}) were also calculated on meiofauna higher taxa, and on copepod and nematode species (Hill, 1973; Pielou, 1975). All statistical analyses were carried out using Microsoft EXCEL (MS – EXCEL 2010), statistical software R (vers. 097.336) and PRIMER v6.0 software (Plymouth Marine Laboratory; Clark and Gorley, 2006).

Results

Natural seagrasses

Meiofauna

Average meiofauna densities on natural seagrass leaves at the "RedPlus" site were 93 ± 25 ind. 100 cm^{-2}, at the "GreyMinus" site 67 ± 14 ind. 100 cm^{-2}. The average meiofauna density on natural seagrass shoots was 68 ± 37 ind. 10 cm^{-3} at the "RedPlus" site and 26 ± 6 ind. 10 cm^{-3} at the "GreyMinus" site. Despite the fact that densities were on average higher at the impacted site than at the non-impacted site, no significant difference of meiofauna densities were detected between the "RedPlus" and the "GreyMinus" site either on the natural leaves (*one-way ANOVA, F = 3.6, p = 0.2, df = 1 and 4*) or on the natural shoots (*one-way ANOVA, F = 2.2, p = 0.1, df = 1 and 4*) (Fig. 6).

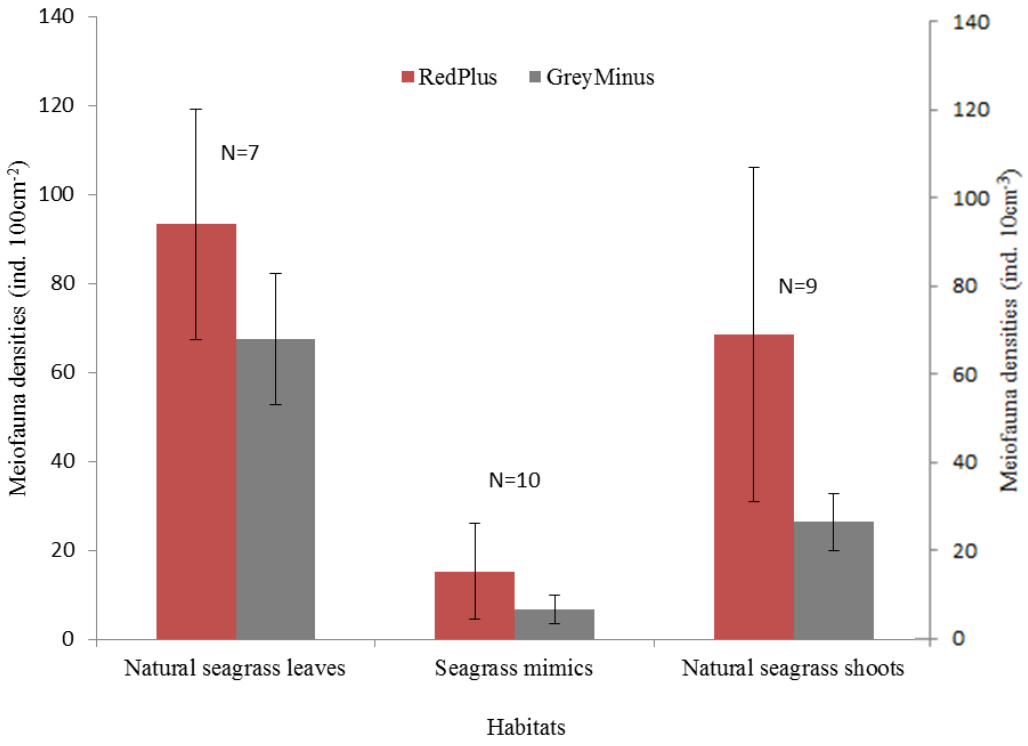

Figure 6: *Meiofauna densities (mean ± SD, N = number of taxa)*

On natural seagrass leaves seven different meiofauna taxa occurred. At both the "RedPlus" and the "GreyMinus" site, copepods (harpacticoids, calanoids, cyclopoids, siphonostomatoida and copepodites) dominated with an average relative abundance of 52% and 44% respectively, followed by nematodes (18.1%, 18.3%), polychaetes (11%, 13%), amphipods (9%, 13.9%),

isopods (3.8%, 1%), ostracods (2.7%, 2%) and cnidarians (1.6%, 5.9%). While no significant differences in relative abundance was observed between sites for any of the taxa, the density of cnidarians was significantly higher (one-way ANOVA, *F = 8.5, p = 0.04, df = 1 and 4*) at the "GreyMinus" compared to the "RedPlus" site (Fig. 7).

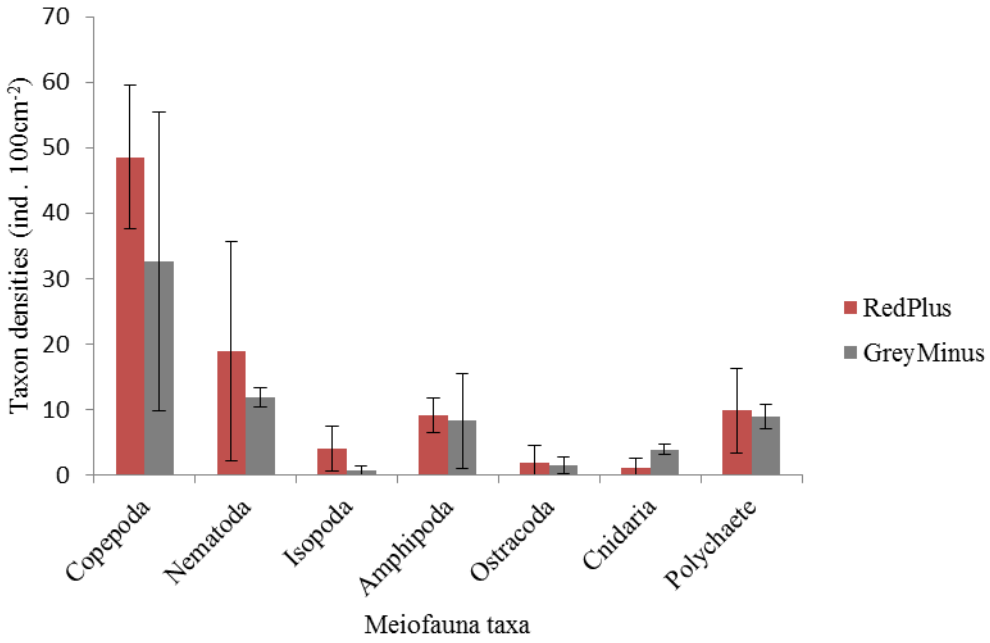

Figure 7: *Meiofauna taxon densities on the natural leaves (mean ± SD)*

The total number of taxa identified on natural seagrass shoots was different among sites (i. e. 8 at the "RedPlus" site and 10 at the "GreyMinus" site). At the "RedPlus" and the "GreyMinus" site, nematodes dominated with an average relative abundance of 37% and 55%, followed by copepods with 33% and 23%, respectively. The two most abundant taxa were followed by polychaetes (20%, 14%), isopods (3%, 0.5%), amphipods (2.3%, 1.4%), halacarids (1.3%, 0.5%), cnidarians (0.7%, 2.4%) and ostracods (0.8%, 0.2%). Additional taxa found at the "GreyMinus" site were tanaidaceans (0.8%) and cumaceans (0.3%). The relative abundance of nematodes (one-way ANOVA, *F = 16, p = 0.01, df = 1 and 4*) was significantly higher at the "GreyMinus" site, while the relative abundance of isopods (one-way ANOVA, *F = 11, p = 0.02, df = 1 and 4*) was higher at the "RedPlus" site. The density of isopods (one-way ANOVA, *F = 21, p = 0.009, df = 1 and 4*) and polychaetes (one-way ANOVA, *F = 20, p = 0.01, df = 1 and 4*) was significantly higher at the "RedPlus" than at the "GreyMinus" site (Fig. 8).

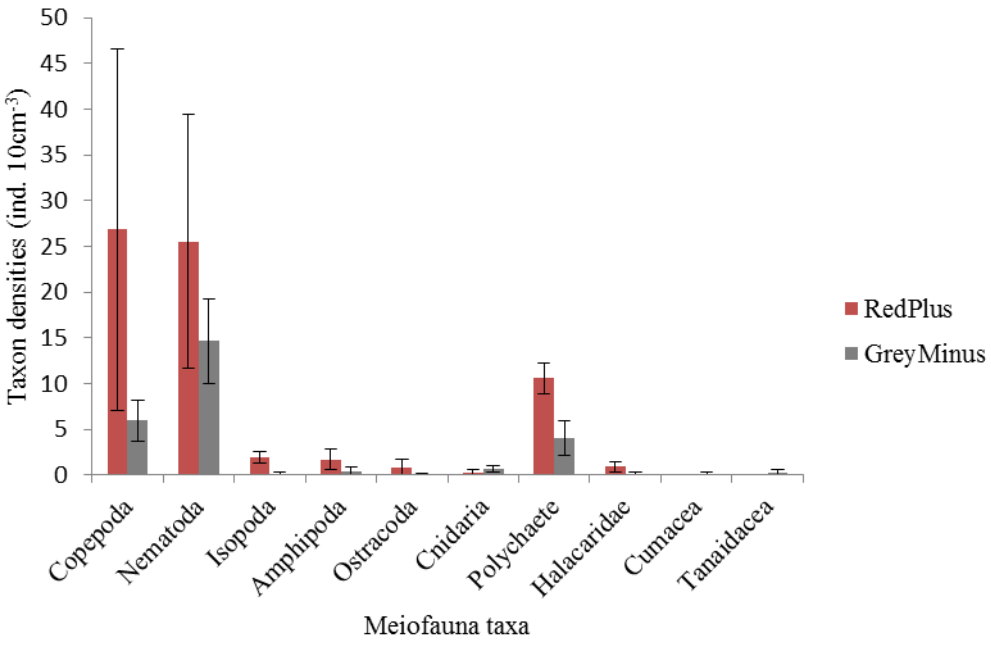

Figure 8: *Meiofauna taxon densities on the natural shoots (mean ± SD)*

Diversity of higher meiofauna taxa on natural leaves showed no significant differences between the two sampled sites. Significant differences were, however, detected for most Hill's indices (except N_0) between sites on natural seagrass shoots, with higher diversity at the "RedPlus" site (Table 1 and 2).

	Natural leaves		**Natural shoots**	
	RedPlus	GreyMinus	RedPlus	GreyMinus
D	0.9 ± 0.2	1.06 ± 0.09	1.04 ± 0.13	1.32 ± 0.44
J'	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.04	0.6 ± 0.09
N_0	6.3 ± 1.1	6.3 ± 0.5	7.3 ± 0.5	7.3 ± 2
N_1	3.7 ± 0.3	4.1 ± 1	*3.7 ± 0.1**	*3.3 ± 0.09**
N_2	2.82 ± 0.1	3.3 ± 1.2	*3.09 ± 0.2**	*2.56 ± 0.2**
N_{inf}	1.9 ± 0.1	2.31 ± 0.9	*2.4 ± 0.1**	*1.8 ± 0.2**

Table 1: *Diversity indices of meiofauna (mean ± SD) (* indicates significant difference)*

One-way ANOVA	N_1	N_2	N_{inf}
Natural shoots	*F = 13, p = 0.02, df = 1 and 4*	*F = 8, p = 0.04, df = 1 and 4*	*F = 11, p = 0.02, df = 1 and 4*

Table 2: *One-way ANOVA table of meiofauna diversity indices*

Since copepods and nematodes were the two most abundant taxa, their community composition, abundance and diversity were further investigated.

Copepod community

A total number of 36 copepod species belonging to 20 genera, 13 families and 4 orders were identified. The dominant order at both leaves and shoots was harpacticoids with an average relative abundance of 65% at natural seagrass leaves, and 69% at natural seagrass shoots. No significant difference of harpacticoid relative abundance was detected at any of the habitats between the sites. The orders Calanoida, Cyclopoida and Siphonostomatoida were also found but since they are planktonic, and therefore not strictly associated with the seagrasses, their identification was carried out only at order level.

The one-way ANOSIM analysis of natural seagrass leaves and shoots, respectively, showed no significant community structure differences at family, genus, and species level between the "RedPlus" and the "GreyMinus" site (Table 3 and Apppendix A).

	Natural leaves	Natural shoots
Family	R: 0.2, p = 10%	R: 0.3, p = 10%
Genus	R: 0.2, p = 20%	R: 0.3, p = 10%
Species	R: 0.2, p = 20%	R: 0.2, p = 10%

Table 3: *One-way ANOSIM of harpacticoids on natural leaves and shoots comparing the impacted and the non-impacted site*

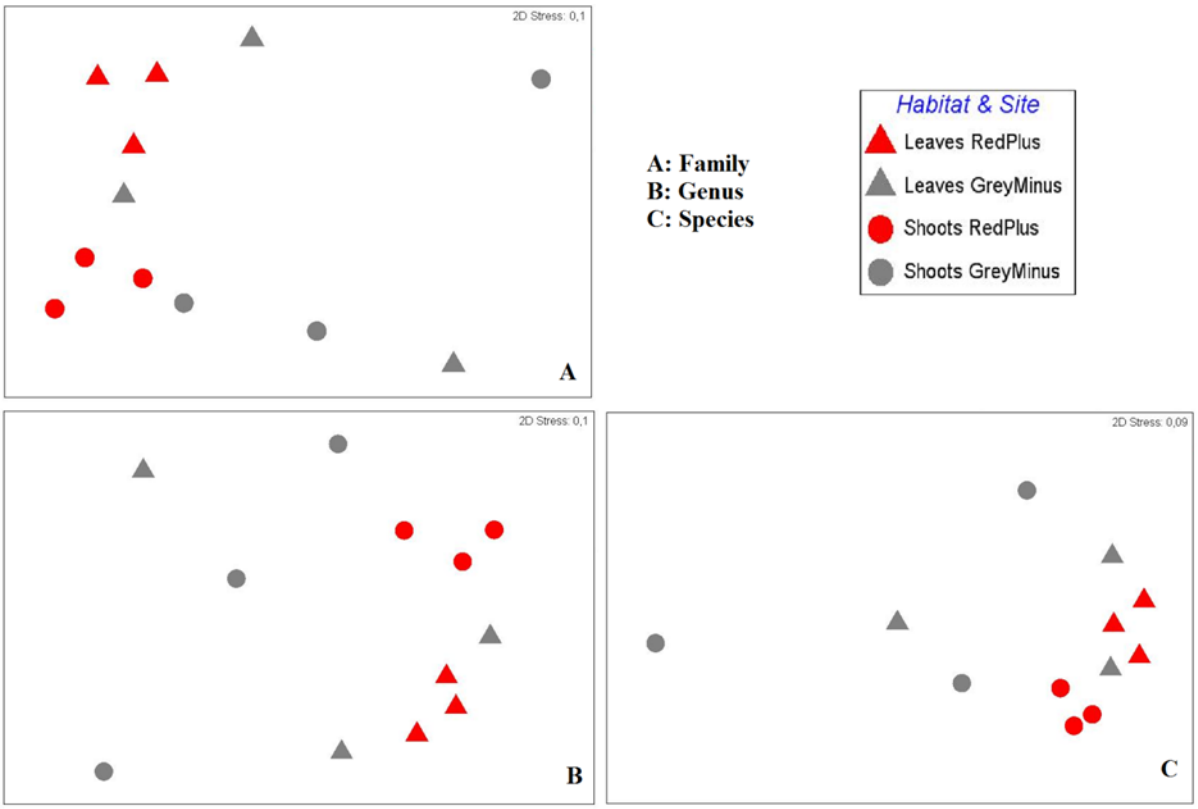

Figure 9*: MDS plot of harpacticoid communities at different taxonomic levels (A: family, B: genus, C: species) (Bray-Curtis analysis on standardised, square root transformed data)*

The two-way ANOSIM analysis showed no significant differences of harpacticoid communities across habitats and sites either (Table 4, Fig. 9).

	Habitat	**Site**
Family	R: 0.4, p = 8%	R: 0.2, p = 1%
Genus	R: 0.3, p = 9%	R: 0.2, p = 2%
Species	R: 0.4, p = 7%	R: 0.2, p = 2%

Table 4: *Two-way ANOSIM of harpacticoids comparing habitats and sites*

The one-way SIMPER analysis of harpacticoids on natural leaves revealed an average dissimilarity of 47% between the "RedPlus" and the "GreyMinus" site. *Tisbe ensifer* and *Amphiascus minutus* were the species contributing most to dissimilarity with 8% and 7%, respectively. Despite the fact that the one-way ANOSIM showed no significant difference of community structures on natural shoots at the sampled sites, the average dissimilarity of the

"RedPlus" and the "GreyMinus" site was 65%, with main contributing species *Amphiascus congener* and *Ameira minuta* (10%, 9%, respectively).

The diversity of harpacticoid species on natural leaves showed no significant differences between sites, while significantly higher number of species (*F = 15, p = 0.01, df = 1 and 4*) could be detected on natural shoots at the "RedPlus" site (Table 5).

	Natural leaves		**Natural shoots**	
	RedPlus	GreyMinus	RedPlus	GreyMinus
D	3.5 ± 0.2	0.2 ± 0.9	2.8 ± 1.9	0.3 ± 0.5
J'	0.8 ± 0.03	0.9 ± 0.05	0.8 ± 0.05	0.9 ± 0.05
N_0	16 ± 1	10.3 ± 6	*12.3 ± 0.5**	*5.3 ± 3**
N_1	11.3 ± 0.8	7.5 ± 4.8	8.3 ± 1	4.5 ± 2.2
N_2	8.8 ± 1.5	6.4 ± 3.4	6.2 ± 1.4	3.58 ± 1.6
N_{inf}	4.5 ± 1.2	3.6 ± 1.4	3.3 ± 0.8	2.5 ± 0.5

Table 5: *Harpacticoid species diversity (mean ± SD) (* indicates significant difference)*

On natural leaves, the "RedPlus" site was dominated by *Tisbe ensifer* (20%), while at the "GreyMinus" site *Ectinosoma dentatum* (24%) was dominant (see Appendix B). On the shoots, *Ameira longipes* dominated at both sites (32%, 33%, respectively) (Table 7). (see full species list in Appendix C)

Natural leaves				Natural shoots			
RedPlus (N=23)		GreyMinus (N=19)		RedPlus (N=16)		GreyMinus (N=10)	
Tisbe ensifer	20%	*Ectinosoma dentatum*	24%	*Ameira longipes*	32%	*Ameira longipes*	33%
Amphiascus minutus	11%	*Ameira minuta*	11%	*Amphiascus congener*	12%	*Ameira minuta*	13%
Ectinosoma dentatum	10%	*Tisbe ensifer*	11%	*Ectinosoma dentatum*	10%	*Ectinosoma dentatum*	10%
		Ameira longipes	9%	*Amphiascus catherinae*	10%	*Laophonte cornuta*	10%
		Amphiascus congener	8%	*Amphiascus minutus*	9%		
rest	59%	rest	37%	rest	27%	rest	34%

Table 6: *Relative abundance of abundant (> 7%) harpacticoids (N = number of species)*

To test higher taxon-surrogacy, relative abundance was calculated at family, genus and species level at both habitats. At family and genus level, the natural leaves at the "RedPlus" site were dominated by Tisbidae (12%) and *Tisbe* (23%), respectively, and at the "GreyMinus" site they were dominated by Ectinosomatidae (12%) and *Ectinosoma* (24%). At both sites the family Ameiridae (18%, 36%), and the genus *Ameira* (50%, 50%) dominated on natural shoots.
Adult/copepodite ratio showed significant difference (one-way ANOVA, $F = 20$, $p = 0.01$, $df = 1$ *and* 4) on natural seagrass leaves, with larger proportion of adults present at the "RedPlus" site.

Nematode community

The dominant taxon found on natural seagrass shoots, independent of the sample sites, were nematodes. A total number of 67 species belonging to 61 genera, 26 families and 8 orders were identified.
The one-way ANOSIM analysis of natural seagrass leaves showed no significant community structure differences at family, genus, and species level between the sites (Table 7). However, significant difference could be detected on natural shoots at family, genus and species level (Table 7 and Apppendix D).

	Natural leaves	Natural shoots
Family	R: -0.1, p = 70%	R: 0.5, p = 2%
Genus	R: -0.03, p = 80%	R: 0.5, p =10%
Species	R: -0.03, p = 90%	R: 0.5, p = 10%

Table 7: *One-way ANOSIM of nematodes on natural leaves and shoots comparing the impacted and the non-impacted site*

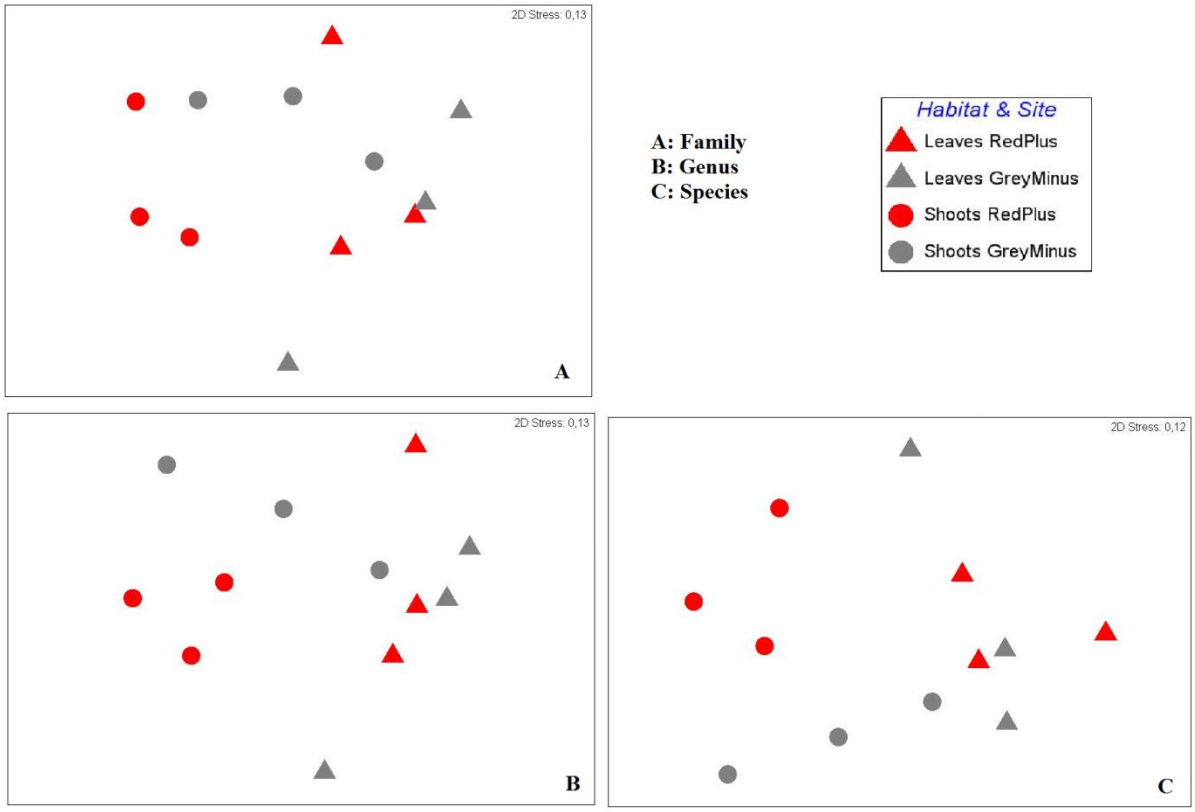

Figure 10: *MDS plot of nematode communities at difference taxonomic levels (A: family, B: genus, C: species)*
(Bray-Curtis analysis on standardised, square root transformed data)

The two-way ANOSIM analysis showed significant differences of nematode communities across habitats (Table 8, Fig 10).

	Habitat	Site
Family	R: 0.6, p = 1%	R: 0.2, p = 16%
Genus	R: 0.5, p = 2%	R: 0.2, p = 8%
Species	R: 0.6, p = 2%	R: 0.2, p = 9%

Table 8: *Two-way ANOSIM of nematodes comparing habitats and sites*

The one-way SIMPER analysis of nematode species on natural leaves revealed an average dissimilarity of 61% between the "RedPlus" and the "GreyMinus" site. *Prochromadorella dittlevseni* (9%) and *Desmodora nani* (8%) were the species contributing most to dissimilarity. The average dissimilarity of species at the "RedPlus" and the "GreyMinus" site on natural shoots was 69%, with main contributing species of *Chromadora nudicapitata* (5%) and *Desmodora nani* (4%).

The diversity of nematode species on natural leaves showed no significant differences between sites. However, the number of species on natural shoots was significantly higher (*F = 9, p = 0.03, df = 1 and 4*) on the "RedPlus" site (Table 9).

	Natural leaves		Natural shoots	
	RedPlus	GreyMinus	RedPlus	GreyMinus
D	2.39 ± 1.2	2.3 ± 0.2	5.91 ± 0.9	3.9 ± 1.2
J'	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.01	0.8 ± 0.1
N_0	9.6 ± 6.6	6.6 ± 0.5	*26 ± 3.6**	*16 ± 4.3**
N_1	4.7 ± 2.5	5.2 ± 1.01	18.7 ± 3.5	7.9 ± 8
N_2	3.08 ± 1.2	4.1 ± 1.4	13.4 ± 3.7	7.5 ± 4.9
N_{inf}	1.9 ± 0.3	2.4 ± 0.8	6.1 ± 3.09	3.9 ± 2.4

Table 9: *Nematode species diversity (mean ± SD) (* indicates significant difference)*

No difference in dominant species was observed on natural leaves since both at the "RedPlus" and the "GreyMinus" site *Chromadora nudicapitata* was dominant. On natural shoots on the other hand, a change in species dominance could be observed. At the "RedPlus" site *Desmodora nani* (12%) and *Acantholaimus setosus* (7%) were dominant, while at the "GreyMinus" site *Chromadora nudicapitata* (28%) and *Prochromadorella dittlevseni* (14%) dominated (Table 10) (see Appendix E and species list in Appendix F).

Natural leaves				Natural shoots			
RedPlus (N=23)		GreyMinus (N=15)		RedPlus (N=50)		GreyMinus (N=32)	
Chromadora nudicapitata	48%	*Chromadora nudicapitata*	43%	*Desmodora nani*	12%	*Chromadora nudicapitata*	28%
Loveninema unicornis	15%	*Prochromadorella dittlevseni*	9%	*Acantholaimus setosus*	7%	*Prochromadorella dittlevseni*	14%
rest	37%	rest	48%	rest	81%	rest	58%

Table 10: *Relative abundance of abundant (> 7%) nematode species (N = number of species)*

Similar to harpacticoids, higher-taxon surrogacy was also tested. On natural leaves the "RedPlus" and the "GreyMinus" site were dominated by Chromadoridae family (61%, 60%) and *Chromadora* genus (48%, 43%), respectively. On natural shoots, however, the dominance pattern differed. The "RedPlus" and the "GreyMinus" site were dominated by Chromadoridae family (29%, 67%), but on genus level the "RedPlus" site was dominated by *Desmodora* (20%) and the "GreyMinus" by *Chromadora* (28%).

No significant difference of adult/juvenile ratio of nematodes could be detected between the "RedPlus" and the "GreyMinus" site at either habitat.

Colonisation experiment

Meiofauna

Average meiofauna density on seagrass mimics was 15 ± 10 ind. 100 cm^{-2} at the "RedPlus" site, and 6 ± 3 ind. 100 cm^{-2} at the "GreyMinus" site. No significant difference (one-way ANOVA, *F = 1.2, p = 0.2, df = 1 and 4*) in meiofauna density was detected between the "RedPlus" and the "GreyMinus" site (Fig. 6). However, a significant difference (two-way ANOVA*, habitat: $p \leq 0.001$, site: p = 0.03, interaction: p = 0.4, df = 1, 1 and 14*) was detected between the meiofauna densities of natural seagrass leaves and the seagrass mimics (Fig. 6), since the mimics only contained 21% of the natural community densities.

The total number of taxa found on seagrass mimics differed only by one between the sampled sites, as at the "RedPlus" and the "GreyMinus" site the number of taxa was 10 and 9, respectively. At the "RedPlus" site, copepods dominated with 55%, followed by nematodes 12%. At the "GreyMinus" site, also copepods dominated with 47%, followed by ostracods 16% and then nematodes 13%. The dominant taxa were followed by polychaetes (13%, 9%),

halacarids (2.5%, 2.1%), amphipods (1.9%, 2.8%), isopods (2.6%, 3.2%), cnidarians (1%, 4.3%) and chaetognatha (1.2%, 0.7%). The relative abundance of ostracods was 8.4% at the "RedPlus" site. At the "RedPlus" site oligochaetas were also found with an average relative abundance of 1.2%, while they were not found at the "GreyMinus" site (Fig. 11).

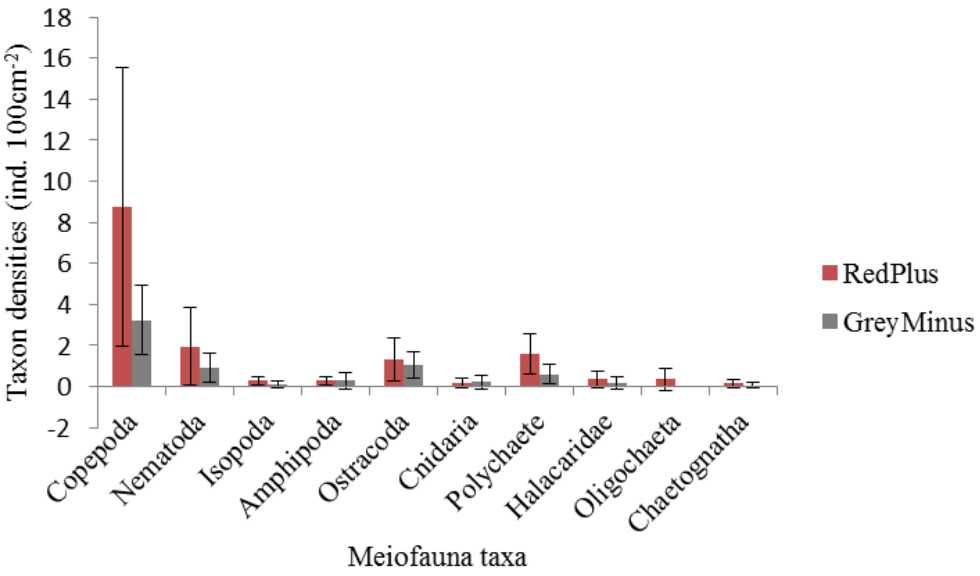

Figure 11: Meiofauna taxon densities on the seagrass mimics (mean ± SD)

The number of taxa found on seagrass mimics was higher than on natural leaves (Fig 7). A significant difference of copepod densities was detected between both of the two habitats and sites (two-way ANOVA, *habitat: $p \leq 0.001$, site: $p = 0.02$, interaction: $p = 0.6$, $df = 1, 1,$ and 14*). In the case of nematodes, isopods, amphipods, cnidarians and polychaetes densities, a significant difference ($p < 0.05$) could be detected in terms of habitats, but not in terms of the two sites. No significant difference was detected in the case of ostracods. The three extra taxa counted on mimics at the "RedPlus" site were Halacarida, Oligochaeta and Chaetognatha, and at the "GreyMinus" site Halacarida and Chaetognatha.

No significant differences were found in diversity of the colonised mimic communities between sample sites (Table 11).

	Natural leaves		Seagrass mimics	
	RedPlus	GreyMinus	RedPlus	GreyMinus
D	0.9 ± 0.2	1.06 ± 0.09	1.5 ± 0.5	1.4 ± 0.2
J'	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.06
N_0	6.3 ± 1.1	6.3 ± 0.5	7.5 ± 2.5	5.8 ± 0.7
N_1	3.7 ± 0.3	4.1 ± 1	3.9 ± 0.9	4.1 ± 0.5
N_2	2.82 ± 0.1	3.3 ± 1.2	2.82 ± 0.6	3.3 ± 0.6
N_{inf}	2.14 ± 0.1	2.3 ± 0.9	1.8 ± 0.2	2.1 ± 0.3

Table 11: Meiofauna taxon diversity (mean ± SD)

Copepod community

The one-way ANOSIM analysis of harpacticoid communities on seagrass mimics showed significant difference at genus and species level, contrary to the natural leaves (Table 12 and Appendix G).

	Natural leaves	Seagrass mimics
Family	R: 0.2, p = 10%	R: 0.1, p = 8%
Genus	R: 0.2, p = 20%	R: 0.3, p = 0.6%
Species	R: 0.2, p = 20%	R: 0.4, p = 0.4%

Table 12: *One-way ANOSIM analysis of harpacticoids on seagrasss mimics comparing the impacted and the non-impacted site*

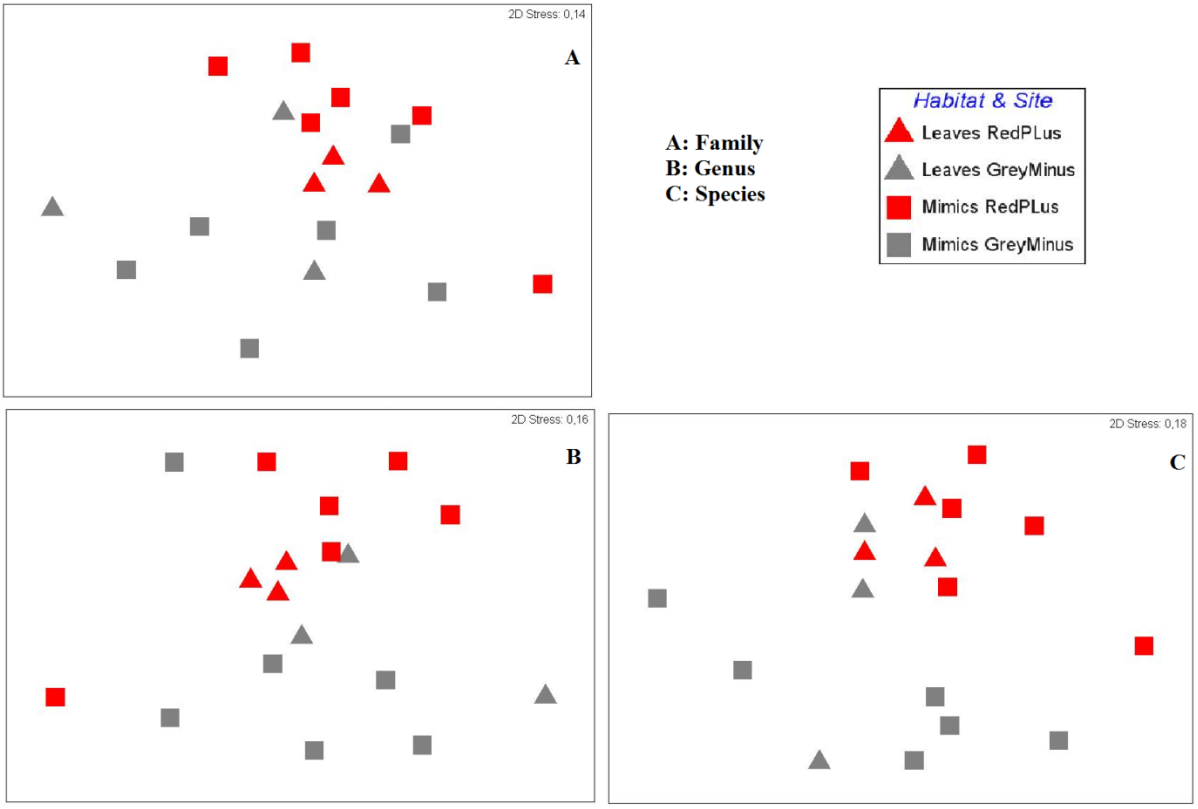

Figure 12: *MDS plot of harpacticoid communities at difference taxonomic levels (A: family, B: genus, C: species) (Bray-Curtis analysis on standardised, square root transformed data)*

The two-way ANOSIM analysis across habitats and sites showed significant differences at genus and species level between the "RedPlus" and the "GreyMinus" site (Table 13, Fig. 12).

	Habitat	**Site**
Family	R: -0.028, p = 55%	R: 0.1, p = 4.2%
Genus	R: 0.074, p = 30%	R: 0.3, p = 0.3%
Species	R: 0.2, p = 7.7%	R: 0.4, p = 0.1%

Table 13: *Two-way ANOSIM analysis of harpacticoids comparing habitats and sites*

The one-way SIMPER analysis of seagrass mimics showed an average dissimilarity of 69%, with *Tisbe ensifer* (12%) contributing most to dissimilarity. The two-way crossed SIMPER analysis showed an average 58% dissimilarity of habitats and an average dissimilarity of 64% between sites. *Ameira minuta* (10%) and *Tisbe ensifer* (11%) were the most representative species contributing to dissimilarity of habitats and sites, respectively.

On seagrass mimics, no significant difference in diversity of harpacticoids was found between the sites. However, the number of species and the N_1 diversity index was significantly higher ($p < 0.05$) on the natural leaves (Table 14).

	Natural leaves		**Seagrass mimics**	
	RedPlus	GreyMinus	RedPlus	GreyMinus
D	3.5 ± 0.2	0.2 ± 0.9	2.5 ± 0.5	1.9 ± 0.6
J'	0.8 ± 0.03	0.9 ± 0.05	0.9 ± 0.04	0.9 ± 0.07
N_0	*16 ± 1*	*10.3 ± 6*	*8.3 ± 3.3*	*4.6 ± 1.3***
N_1	*11.3 ± 0.8*	*7.5 ± 4.8*	*7 ± 2.4*	*4.2 ± 1.4***
N_2	8.8 ± 1.5	6.4 ± 3.4	6.1 ± 1.8	3.9 ± 1.6
N_{inf}	4.5 ± 1.2	3.6 ± 1.4	4.03 ± 0.9	3.4 ± 1.9

Table 14: Harpacticoids diversity on leaves and mimics (mean ± SD)
(** indicates significant difference)

Relative abundance of harpacticoids showed that the dominant species on mimics at both sites was *Ameira longipes*, dissimilar to natural leaves (Table 15) (see Appendix B).

Natural leaves				**Seagrass mimics**			
RedPlus (N=23)		GreyMinus (N=19)		RedPlus (N=20)		GreyMinus (N=13)	
Tisbe ensifer	20%	*Ectinosoma dentatum*	24%	*Ameira longipes*	21%	*Ameira longipes*	31%
Amphiascus minutus	11%	*Ameira minuta*	11%	*Tisbe ensifer*	12%	*Ameira minuta*	13%
Ectinosoma dentatum	10%	*Tisbe ensifer*	11%	*Porcellidium viride*	9%	*Amphiascus brevis*	9%
		Ameira longipes	9%	*Tisbe furcata*	8%	*Amphiascus minutus*	9%
		Amphiascus congener	8%			*Tisbe furcata*	9%
rest	59%	rest	37%	rest	50%	rest	29%

Table 15: Relative abundance of abundant (> 7%) harpacticoids (N = number of species)

Higher-taxon surrogacy was also tested and the relative abundance results showed dissimilar dominance patterns at family, genus and species level on seagrass mimics. The "RedPlus" site on family level was dominated by Tisbidae (28%) and at genus level *Ameira* (24%) dominated. At the "GreyMinus" site on both family and genus level Ameiridae and *Ameira* (17%, 34%, respectively) dominated.

Contrary to leaves, no significant difference (one-way ANOVA, *F = 0.08, p = 0.7, df = 1 and 4*) of adult/copepodite ratio was observed between sites on the seagrass mimics.

Nematode community

The one-way ANOSIM analysis of nematode communities on seagrass mimics showed no significant difference between the sampled sites, similar to natural leaves (Table 16 and Appendix G).

	Natural leaves	**Seagrass mimics**
Family	R: -0.1, p = 70%	R: 0.02, p = 39%
Genus	R: -0.03, p = 80%	R: 0.09, p = 20%
Species	R: -0.03, p = 90%	R: 0.09, p = 20%

Table 16: One-way ANOSIM *analysis of nematodes on seagrasss mimics comparing the impacted and the non-impacted site*

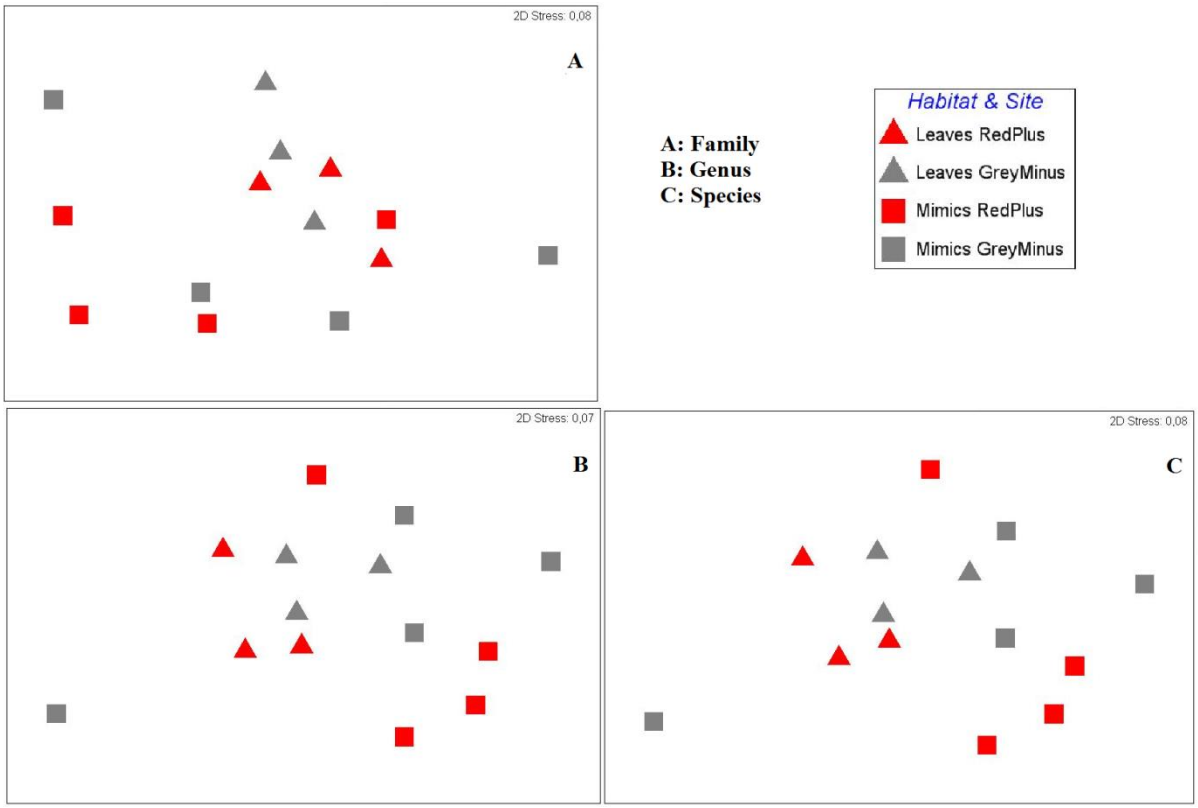

Figure 13: *MDS plot of nematode communities at difference taxonomic levels (A: family, B: genus, C: species) (Bray-Curtis analysis on standardised, square root transformed data)*

The two-way ANOSIM analysis across habitats showed significant differences at genus and species level between the "RedPlus" and the "GreyMinus" site (Table 17 and Fig. 13).

	Habitat	**Site**
Family	R: 0.2, p = 9%	R: -0.02, p = 53%
Genus	R: 0.3, p = 2.4%	R: 0.08, p = 21%
Species	R: 0.3, p = 2.2%	R: 0.08, p = 22%

Table 17: *Two-way ANOSIM analysis of nematodes comparing habitats and sites*

The one-way SIMPER analysis showed 83% dissimilarity between the sampled sites with *Microlaimus dimorphus* (28%), and *Camacolaimus monhystera* (15%) contributing most to dissimilarity. The two-way crossed SIMPER analysis of nematodes on seagrass mimics revealed an average dissimilarity of 93% of habitats and 76% of sites. The dominant species were *Chromadora nudicapitata* (19%), followed by *Microlaimus dimorphus* (13%) at habitats,

and *Microlaimus dimorphus* (21%) and *Prochromadorella dittlevseni* (13%) at sites. The dissimilarity of sites on mimics was higher than on natural leaves and the dominant species also differed.

Nematode diversity of seagrass mimics showed no significant differences between sites (Table 18).

	Natural leaves		**Seagrass mimics**	
	RedPlus	GreyMinus	RedPlus	GreyMinus
D	2.39 ± 1.2	2.3 ± 0.2	0.6 ± 1	0.9 ± 0.8
J'	0.7 ± 0.1	2.3 ± 0.2	1 ± 1	0.7 ± 0.8
N_0	9.6 ± 6.6	7.3 ± 0.5	2.2 ± 1.3	1.5 ± 0.5
N_1	4.7 ± 2.5	4.1 ± 1	2.2 ± 1.3	1.5 ± 0.5
N_2	3 ± 1.2	4.1 ± 1.4	2.2 ± 1.3	1.5 ± 0.5
N_{inf}	1.9 ± 0.3	2.4 ± 0.8	2.2 ± 1.3	1.5 ± 0.5

Table 18: Nematode diversity at leaves and mimics (mean ± SD)

On the seagrass mimics at the "RedPlus" site *Microlaimus dimorphus*, while at the "GreyMinus" site *Prochromadorella dittlevseni* was dominating (Table 19) (see Appendix E).

Natural leaves				Seagrass mimics			
RedPlus (N=23)		GreyMinus (N=15)		RedPlus (N=8)		GreyMinus (N=6)	
Chromadora nudicapitata	48%	*Chromadora nudicapitata*	43%	*Microlaimus dimorphus*	48%	*Prochromadorella dittlevseni*	28%
Loveninema unicornis	15%	*Prochromadorella dittlevseni*	10%	*Anticoma acuminata*	8%	*Acanthopharynx similis*	14%
						Camacolaimus monhystera	14%
						Leptolaimus ampullaceus	14%
						Microlaimus dimorphus	14%
						Pontonema ocellatum	14%
rest	37%	rest	47%	rest	44%	rest	2%

Table 19: Relative abundance of dominant (> 7%) nematode species

Higher-taxon surrogacy results showed similar dominance pattern at family, genus and species level. The "RedPlus" site was dominated by Microlaimidae (46%) and *Microlaimus* (46%) family and genus, respectively. The "GreyMinus" site was dominated at both family and genus level by Chromadoridae (28%) and *Prochromadorella* (28%), respectively.

Similar to natural leaves, no significant difference of adult/juvenile ratio was observed (one-way ANOVA, $F = 1$, $p = 0.3$, $df = 1$ and 4).

Discussion

Natural seagrasses

The present study reveals that meiofauna densities, both on natural leaves and shoots, are not affected by long-term CO_2 seepage. On natural leaves, no difference in composition and diversity is observed for the associated meiofauna. On natural shoots however, the diversity is significantly higher at the CO_2 impacted site. Here the abundance of copepods and nematodes are both high, while the polychaete and isopod density are also increased, resulting in higher evenness.

Natural seagrass shoots at natural CO_2 seepage sites can be considered a dynamic environment, as the escaping CO_2 bubbles can change the water movement and create a constant upward flow (Haugan and Drange, 1992; Caramanna *et al*., 2011). Due to the bubbling, which can be very intense locally, pH can fluctuate strongly compared to the non-impacted area. (Caramanna *et al*., 2011) Possibly, the higher meiofauna taxon diversity on natural shoots at the CO_2 impacted site can be explained by the intermediate disturbance theory. This would suggest that at gas seepage sites, where disturbance is higher than at non-impacted sites, the competitive exclusion is reduced, resulting in increased diversity levels (Huston, 1979). Similarly to the elevated levels of the meiofauna diversity at the impacted site, the higher nematode and copepod diversity on natural shoots at the CO_2 impacted site could also be explained by this theory. However, although meiofauna diversity is significantly higher for N_1, N_2 and N_{inf} at the impacted sites, it is only N_0 that is higher at nematode and copepod species levels, indicating that while evenness does not change, more nematode and copepod species are present at the impacted site on natural shoots. The exact process for increased biodiversity levels at the impacted sites is difficult to comprehend based on an occasional sampling event and further research is needed on species interactions under stressed and unstressed conditions.

The fact that densities of the dominant taxa are not lower at the impacted site is rather unexpected, especially since the average densities are even higher, although not statistically significant. Initial measurements during the sampling campaign at Panarea showed that dissolution of CO_2 occurred within the first 1-2 m from the seepage source (Meyer et *al.*, 2012). Since seagrass meadows are inorganic carbon (C_i) limited and their growth is not directly affected by the decreased pH, they are able to benefit from the increased CO_2 (Beer *et al*., 1996; Alexandre *et al*., 2012). Previous research at a similar environment close to Panarea (Ischia

Island) had shown that seagrass productivity increased due to the high CO_2, with significantly higher (30%) shoot density (Hall-Spencer *et al*., 2008). This increased seagrass activity in response to the elevated CO_2 also reflected in depth, based on increased below ground biomass (Beer *et al*., 1996; Invers *et al*., 2002). Consequently, the increased above ground productivity can result in excess organic matter input from phytodetritus, which stays trapped within the dense seagrass shoots. Organic matter can also become available from below the seafloor as the rising gas bubbles can bring detritus to the surface of sediments (Lopez *et al*., 1995; Dando *et al*., 1995). It is also important to take into account that sampling was carried out in June, following the spring bloom when a large number of phytodetritus input can occur, benefiting diatom grazing copepod species. Furthermore, during the summer period, seagrass production increases, which has a positive effect on both bacterial activity and abundance (Danvaro, 1996). While phytodetritus is mainly colonised by epiphytic and phyto-dwelling harpacticoids, the increased bacterial activity can benefit the epistrate feeder nematode species as well (Danvaro, 1996; Giere, 2009).

Earlier studies performed at shallow-water natural CO_2 seeps indicated that calcifying macrofauna species such as molluscs, gastropods and echinoderms are severely impacted by the reduced pH (Hall-Spencer *et al*., 2008). With decreased pH, non-calcifying animals can suffer from hypercapnia which occurs within the tissues of the organisms due to high H^+ levels and leads to imbalance of the acid-base level. When blood pH reduces to 7.35, acidosis occurs (Raven *et al*., 2005). Seagrass meadows act as nursery sites for juvenile fish, however, reduced pH can severely affect them as well, causing hypercapnia, acidosis, reduced growth and reproduction rate (Ishimatsu *et al*., 2004; Raven *et al*., 2005). These physiological changes induced by the elevated CO_2 level may lead to absence or reduced predation of macrofauna on meiofauna communities. Therefore we hypothesise that the reduced predation by macrofauna in combination with the increased seagrass production may stimulate the meiofauna communities, or at least compensate for negative impact of the pH stress, and explain the lack of density loss due to acidification.

Harpacticoid communities are largely unaffected by the CO_2 leakage. Only a slight shift in species dominance is observed. The ability of copepods to withstand CO_2 exposure is species-specific and their survival depends on the individual energy reserves that allow withstanding physiological changes (Vopel *et al*., 1998; Thistle *et al*., 2006). At this stage we lack precise quantitative information on how epibiont coverage was affected by the escaping CO_2 at Panarea. Based on the initial observation during the sampling, calcareous epibiont coverage was reduced at the CO_2 impacted site, while presence of hydrozoan and bryozoans

was high (Dr. Miriam Weber, personal communication). Since the relative abundance of the phyto-dwelling harpacticoid *Tisbe ensifer* is high on the natural leaves at the "RedPlus" site, this species perhaps benefits from both the increased seagrass and non-calcaerous epibiont production, while it is also able to tolerate the increased CO_2 level (Hicks and Coull, 1983; Hall-Spencer *et al*., 2008).

On natural shoots the average relative abundance of nematodes is significantly higher at the non-impacted site, since harpacticoids are more increased in abundance than the nematodes at the CO_2 impacted site (although statistically not significant at the 5% level). Nematode community structure shows a difference between the impacted and the non-impacted site and a shift in dominant species is also observed. Previous short-term (up to 7 weeks) laboratory studies have shown that the variation of pH values between 7.0 and 8.2 did not affect nematode relative abundance, however, a pH of 6.0 or less can significantly decrease relative abundance (Dashfield *et al*., 2008, and Takeuchi *et al*., 1997, respectively). The measured pH at the CO_2 impacted site was down to 5.3 which makes it likely that nematodes and also other organisms could suffer from acidosis. However, communities that live at natural CO_2 seeps may be adapted to low pH values due to their long time exposure, and the nematode dominance on natural shoots may also be influenced by the "gardening" effect (mucus extraction influences microbial presence that meiofaunal species feed upon) (Jensen, 1987; Giere, 2009). Nevertheless, precise details of nematode metabolic and physiological adaptions are missing so far.

Due to the lack of research done at shallow-water seepage areas, our results on nematode community composition and diversity can only be compared to other extreme systems such as cold seeps and hydrothermal vents. In the deep-sea, both at cold seeps and hydrothermal vents high dominance of a single species is typical (reviewed in Vanreusel *et al*., 2009). Our results show that the epistrate feeder *Desmodora* genus has become more dominant at the CO_2 impacted site on natural shoots, although the diversity has not decreased as observed at cold seeps and vents. The higher tolerance of this genus to disturbed environments was already recorded i.e. at deep-sea hydrothermal vents and cold-seeps (Van Gaever *et al*., 2009; reviewed in Vanreusel *et al*., 2010).

Higher-taxon surrogacy can only be applied to the harpacitoid data set on natural leaves (De Troch *et al*., 2008).

Adult/copepodite ratios show a significantly higher proportion of adults on natural leaves at the CO_2 impacted site which can indicate low copepodite recruitment. Kurihara *et al*., (2008) showed that high concentration of CO_2 had no effect on the development and survival rate of

copepods, however, egg production and hatching rate can decrease. While copepods may express larger sensitivity to decreased pH levels, the thick cuticle of nematodes can increase their tolerance and act as a buffer for short-term pH changes to maintain their internal acid-base balance (Barry *et al*., 2005; Kurihara *et al*., 2007; Widdicombe *et al*., 2009). This can explain the lack of difference in nematode adult/juvenile ratio between sites at any of the habitats.

In addition to the natural seagrass discussion, a rather unexpected observation was made. A very recently described species named *Loveninema unicornis* from a different habitat (depth of 30 – 60m at Skagerrak fjord, Sweden; Holovachov and Bostrom, 2012) is also found with high relative abundance (15 ± 10%) on natural seagrass leaves at the CO_2 impacted site. Since nothing is known on the ecology of this new species, we can only speculate on its exclusive association with leaves from the CO_2 impacted site at Panarea, while at Skaggerak no evidence of CO_2 seepage was mentioned by the authors of the genus description (see Appendix H).

Colonisation experiment

Similar to the long-term observation of natural seagrass leaves, the short-term (13 days) colonisation experiment with seagrass mimics shows that meiofauna density and diversity do not differ between the CO_2 impacted and non-impacted site. Harpacticoid community composition and diversity show a significant difference on mimics between the impacted and non-impacted site, although the dominant species are the same. This is very similar however, to the natural shoot harpacticoid results where the same species, *Ameira longipes*, could be observed at both sites as dominant species. Ameridiae is a sediment dwelling family and similarly to the results of the colonisation experiment by Daudi *et al*., (2013), they can be found in high abundance on the seagrass mimics. Adult/juvenile ratio on the mimics shows no difference, whereas on the natural leaves a higher proportion of adults are observed at the CO_2 impacted site. This can suggest that with time the number of copepodites on the natural leaves decreases due to the CO_2 leakage, as copepodite recruitment is affected by the lower pH.

The colonisation experiment also shows that nematode community composition does not differ significantly, while the dominant species is observed to be different. While on the natural leaves and mimics at the non-impacted site *Chromadora* is dominant, on the seagrass mimics at the CO_2 impacted site *Microlaimus* dominates (both genera are epistrate feeders, Wieser, 1953). Comparing the CO_2 impacted and non-impacted site, the change in dominant species suggests that with time more tolerant species appear. The *Microlaimus* genus is known to be

an early coloniser in disturbed environments and may be adapted to extreme environment has also been observed at other deep-sea seepage areas, where they occurred with high relative abundance (reviewed by Vanreusel *et al*., 2009).

Higher taxon-surrogacy on the harpacticoids data set cannot be applied, as at the different taxonomic levels dissimilar patterns are observed. However, the nematode data set at the different taxonomic levels shows a similar pattern (De Troch *et al*., 2008). This can be related to the colonisation experiment by De Troch *et al*., (2005) in which case the first few days of colonisation were dominated by nematodes and then copepods took over. Harpacticoid species are more mobile, which can suggest that at the beginning of colonisation large numbers of species try to settle, and with time species that are intolerant to the decreased pH level move away. Contrary to this, nematodes mobility is reduced and it may take longer to observe change in community structure and species composition.

Since seagrass mimics results are similar to natural seagrasses, the colonisation method is evaluated as highly suitable to test the research questions put forward. Even though epiphyte growth on live seagrass leaves is significantly higher than on seagrass mimics, the colonisation experiment by De Troch *et al*., (2005) showed increasing meiofauna colonisation with time (Pinckney *et al*., 1996). Since the density found on mimics after 13 days is significantly lower, repeating the colonisation experiment over a longer time-frame and a periodic recovery of mimics may help to gain a better insight into how changes in abundance and species composition occur, comparing the CO_2 impacted and non-impacted sites. Also, colonisation over longer time may allow the development of biofilm on the mimics since the available epiphyte and harpacticod abundance is expected to be positively correlated (De Troch *et al*., 2005).

Conclusion and future perspectives

In summary, our results showed that meiofaunal densities did not decrease while diversity increased at the CO_2 impacted site compared to the non-impacted. It is therefore suggested that the stress effect due to the high CO_2, low pH conditions is compensated by positive effects such as increased seagrass productivity, seasonal organic matter input and reduced macrofaunal predation at the CO_2 impacted site. On natural seagrass leaves harpacticoid community structure did not differ, however, there was a shift in dominant species at the CO_2 impacted site, indicating different tolerance level of the different species. On the other hand, observation on the natural shoots suggested that nematode community was impacted by the CO_2 leakage and a shift in species dominance also occurred. The colonisation experiment also showed that over short-term, different copepod and nematode species appeared on the seagrass mimics, compared to the long-term observation on natural leaves.

Thus far, studies carried out within natural environments (i.e. CO_2 seepage sites) only consider the projected pH (-0.4) reduction by 2100 (Caldiera *et al*., 2003). At the same time, research carried out in deep-sea and laboratory environments are only reflecting on short-term and sudden pH decrease (7.6 – 6.5) and are not considering other environmental variables such as detritus input, and trophic or other species interactions. As no previous research has been carried out to see how shallow-water meiofauna reacts to the elevated CO_2, further research is recommended. Future suggestions may include carrying out sampling during different seasons to investigate whether the seagrass production affects meiofauna densities at CO_2 seepage sites. They could also include laboratory experiments to look into trophic interactions and to see whether reduced predation of macrofauna occurs, which may benefit meiofauna survival. Lastly, the comparison of meiofauna communities in dynamic (shallow-water seepage) and more stable environment (deep-sea cold-seeps) may help to gain insight into (dis)similarities.

Acknowledgements

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References

Alexandre, A., Silva, J., Buapet, P., Bjork, M., Santos, R. (2012) Effects of CO_2 enrichment on photosynthesis, growth, and nitrogen metabolism of the seagrass *Zostera noltii*, *Ecology and Evolution*, pp 2620–2630

Barry, J. P., Buck, K., Lovera, C., Kuhnz, L., Whaling, P. J. (2005) Utility of deep sea CO_2 release experiments in understanding the biology of a high-CO_2 ocean: effects of hypercapnia on deep sea fauna, *Journal of Geophysical Research*, Vol. 110, pp 1-18

Beer, S., Koch, E. (1996) Photosynthesis of marine macroalgae and seagrasses in globally changing *CO_2* environments, *Marine Ecology Progress series*, Vol. 141: 199-204

Boxshall, G. A. and Hasley, S. H. (2004) An introduction to copepods diversity, London: The Ray Society.

Caldeira, K., Wickett, E. W. (2003) Anthropogenic carbon and ocean pH, *Nature*, Vol. 425, p 365

Caramanna, G., Voltattorini, N., Mercedes Maroto-Valer, M. (2011) Is Panarea Island (Italy) a valid and cost-effective natural laboratory for the development of detection and monitoring techniques for submarine CO_2 seepage?, *Greenhouse Gas Science Technology* 1, pp 200–210

Clarke, K.R. and Gorley, R.N. (2006) PRIMER v6. User Manual/Tutorial. Plymouth: PRIMER-E.

Dando, P. R., Hughes, I. A., Thiermann, F. (1995) Preliminary observations on biological communities at shallow hydrothermal vents in the Aegean Sea, *Geological Society*, London, Special Publications, pp 303-317

Danovaro, R. (1996) Detritus–bacteria–meiofauna interactions in a seagrass bed (Posidonia oceanica) of the NW Mediterranean, *Marine Biology* 127, pp 1–13.

Dashfield, S. L., Somerfield, P. J., Widdicombe, S., Austen, M. C., Nimmo, M. (2008) Impacts of ocean acidification and burrowing urchins on within-sediment pH profiles and subtidal nematode communities, *J. Exp. Mar. Biol. Ecol*. 365, pp 46–52

Daudi, L. N., Uku, J. N., De Troch, M. (2013) Role of the source community for recovery of seagrass associated meiofauna: a field colonisation experiment with seagrass mimics in Diani Beach, Kenya, *African Journal of Marine Science* 35(1), pp 1-8

De Troch, M., Vandepitte, L., Raes, M., Suarez-Morales, E., Vincx, M. (2005) A field colonization experiment with meiofauna and seagrass mimics: effect of time, distance and leaf surface area, *Marine Biology* 148, pp 73–86

De Troch, M., Raes, M., Muthumbi, A., Gheerardyn, H., Vanreusel, A. (2008) Spatial diversity of nematode and copepod genera of the coral degradation zone along the Kenyan coast, including a test for the use of higher-taxon surrogacy *Afr. J. Mar. Sci. 30(1)*: pp 25-33

Fabricius, K., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., De'ath, G., Okazaki, R., Muehllehner, N., Glas, M., and Loug, J. (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations, *Nature Climate Change* Vol.1 pp 163-168

Giere, O. (2008) *Meiobenthology: The Microscopic Motile Fauna of Aquatic Sediments*, Springer; 2nd ed.

Guivarch, C., Hallegatte, S., (2013) 2°C or not 2°C?, *Global Environmental Change* 23, pp 179–192

Gurney, E. (2012) *The Global Status of CCS: 2012* – Report, Global CCS Institute

Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, M. T., Rowley, S. J., Tedesco D., Buia, M. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification, *Nature*, Vol. 454, pp 96-99

Hansen, J., Sato, M., Kharecha, P., Beerling, D., Berner, R. (2008) Target Atmospheric CO_2: Where Should Humanity Aim? *The Open Atmospheric Science Journal*, pp 217-231

Haugan, P.M., & Drange, H. (1992) Sequestration of CO_2 in the deep ocean by shallow injection. *Nature* 357, pp 318-320

Herzog, H. J. (2001) What future for carbon capture and sequestion? *Environmental Science and Technology*, Vol. 35, Issue 7, pp 148-153

Hill, M. (1973) Diversity and Evenness: a Unifying Notation and its Consequences. *Ecology* 54, pp 427-32

Hicks, G. R. F., Coull, B.C. (1983) The ecology of marine meiobenthic harpacticoid copepods *Oceanography Mar Biol: Ann Rev* 21 pp 67–175

Holovachov, O., and Bostrom, S. (2012) Swedish Plectida (Nematoda). Part 3. The genus Loveninema gen. n. *Zootaxa* 3505 pp 26–38

Huston, M. (1979) A general hypothesis of species diversity, *American naturalist* 113 pp 81-101

Huys, R., Gee, J. M., Moore, C. G., Hamond, R. (1996) Marine and brackish water harpacticoid copepods, *Part I. In Synopsys of the British fauna (New Series) 5*, Cambridge University Press, Cambridge: 1-352

Invers, O., Tomàs, F., Pérez, M., Romero, J. (2002) Potential effect of increased global CO_2 availability on the depth distribution of the seagrass *Posidonia oceanica* (L.) Delile: A tentative assessment using a carbon balance mode, *Bulletin of Marine Science*, 71(3): pp 1191–1198

Ishimatsu, A., Kikkawa, T., Hayashi, M., Lee, K-S., Kita, K. (2004) Effects of CO_2 on marine fish: larvae and adults, *Journal of Oceanography*, Vol. 60, pp 731-741

Jensen, P. (1987) Feeding ecology of free-living aquatic nematodes, review, Marine Ecology Progress Series, Vol. 35, pp 187-196

Keeling, R. F., Piper S. C., Bollenbacher A. F., Walker S. J. (2013) Scripps CO_2 Program, Retrieved: 25th May 2013 http://scrippsco2.ucsd.edu/data/in_situ_co2/monthly_mlo.csv

Kennedy, A., Jacoby, C. (1997) Biological indicators of marine environmental health: meiofauna - a neglected benthic component? *Environmental Monitoring and Assessment*, Vol. 54, Issue 1, pp 47-68

Kurihara, H., Ishimatsu, A. (2007) Effects of elevated seawater CO_2 concentration on the meiofauna, *Journal of Marine Science and Technology*, Special Issue, pp 17-22

Kurihara, H., Ishimatsu, A. (2008) Effects of high CO2 seawater on the copepod. (Acartia tsuensis) through all life stages and subsequent generations, *Mar. Pollut. Bull.* 56, pp 1086–1090

Lang, K. (1948) *Monographie der Harpacticiden*. Lund: Haˆkan Ohlssons boktryckeri.

Lopez, N., Duarte, C., Vallespinos, F., Romero, J., Alcoverro, T. (1995) Bacterial activity in NW Mediterranean seagrass *(Posidonia oceanica)* sediments, *Journal of Experimental Marine Biology and Ecology* pp 39-49

Metz, B., Davidson, O., de Coninck, H., Loos, M., Meyer, L. (2005) IPCC Special report on carbon dioxide capture and storage

Meyer S., Weber M., Guilini K., Bigalke N., Vielstädte L., Wenzhöfer F., de Beer D., Schutting S., Ramette A., Lott C., Vanreusel A., Boetius A. (2012) CRUISE REPORT ECO2-3 (Panarea Island, Italy)

Pachauri, R. K., Reisinger, A. (Eds.) Climate Change (2007) Synthesis Report, *IPCC*, Geneva, Switzerland

Pielou, E.C. (ed) (1975) *Ecological Diversity.* Wiley, New York

Pinckney, J. L., Micheli, F. (1998) Microalgae on seagrass mimics: Does epiphyte community structure differ from live seagrasses? *Journal of Experimental Marine Biology and Ecology*, 221, pp 59–70

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebsell, U., Shepherd, J., Turley, C., Watson, A., Heap, R., Banes, R., Quinn, R. (2005) Ocean acidification due to increasing atmospheric carbon dioxide, *The Royal Society, Policy document 12/05*

Takeuchi, K., Fujioka, Y., Kawasaki, Y., Shirayama, Y. (1997) Impacts of High Concentration of CO_2 on Marine Organisms: a Modification of CO_2 Ocean Sequestration, *Energy Conversion and Management* Vol 38, Number unknown, pp 337-341(5)

Thistle, D., Sedlacek, L., Carman, K. R., Fleeger, J. W., Brewer, P. G., Barry, J. P. (2006) Simulated sequestration of industrial carbon dioxide at deep-sea site: effects on species of harpacticoid copepods, *Journal of Experimental Marine Biology and Ecology* 330, pp 151–158

Schellnhuber, H. J., Hare, H., Serdeczny, O., Adams, S., Coumou, D., Frieler, K., Martin, M., M. Otto, I., Perrette, M., Robinson, A., Rocha, M., Schaeffer, M., Schewe, J., Wang, X., Warszawski, L. (2012) Turn Down the Heat: Why a 4°C Warmer World Must be Avoided, *Potsdam Institute for Climate Impact Research and Climate Analytics*

Seinhorst, J. W. (1959) A rapid method for the transfer of nematodes from fixative to unhydrous glycerine, Nematologica 4: pp 67–69

Van Gaever S, Galeron J, Sibuet M, Vanreusel A. (2009) Deep-sea habitat heterogeneity influence on meiofaunal communities in the Gulf of Guinea, *Deep Sea Research Part II: Topical Studies in Oceanography*, Vol. 56 , N. 23 , pp 2259-2269

Vanreusel, A., Fonseca, G., Danovaro, R., da Silva, M. C., Esteves, A. M., Ferrero, T., Gad, G., Galtsova, V., Gambi, C., da Fonsêca G. V., Ingels, J., Ingole, B.,Lampadariou, N., Merckx, B., Miljutin, D., Miljutina, M., Muthumbi, A., Netto, S., Portnova, D., Radziejewska, T., Raes, M., Tchesunov, A., Vanaverbeke, J., Van Gaever, S., Venekey, V., Nara Bezerra, T., Flint, H., Copley, J., Pape, E., Zeppilli, D., Arbizu M. P. and Galeron, J. (2009) The contribution of deep-sea macrohabitat heterogeneity to global nematode diversity. *Marine Ecology*, 31, (1), pp 6-20

Vanreusel, A., De Groote, A., Gollner, S., Bright, M. (2010) Ecology and Biogeography of Free-Living Nematodes Associated with Chemosynthetic Environments in the Deep Sea: A Review, *PLoS ONE 5(8): e12449. doi:10.1371/journal.pone.0012449*

Vopel, K., Dehmlow, J., Arit, G., (1998) Effects of anoxia and sulphide on populations of *Cletocamptus confluens* (Copepoda, Harpacticoida), *Marine Ecology Progress Series*, Vol. 175, pp 121-128

Wallman, K. (2012) ECO2 briefing paper

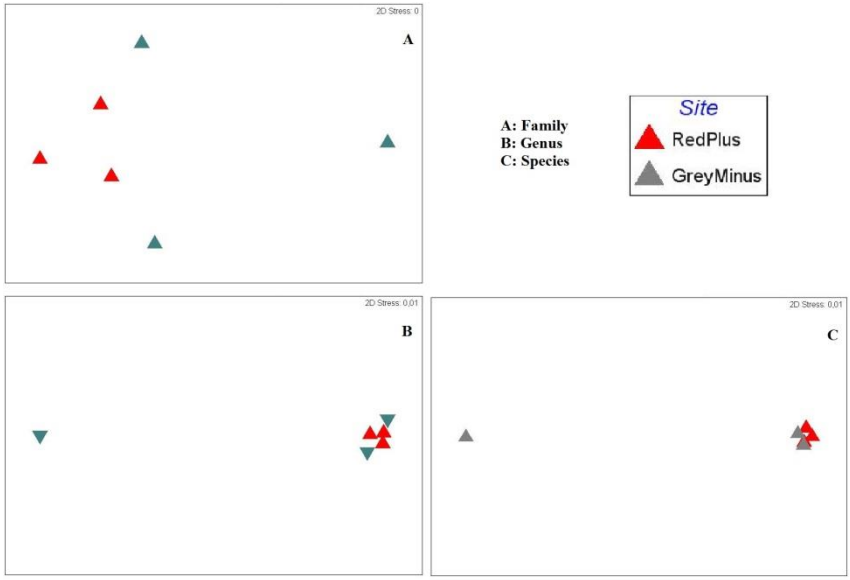
Warwick, R. M., Platt, H. M., and Somerfield J. P. (1998) Free-living marine nematodes. Part. III. Monhysterids, *Synopses of the British fauna (new series)*, No. 53. E J Brill/Dr W Backhuys, Leiden

Wieser, W. (1953) Die Beziehung zwischen Mundhohlenge-stalt, Ernahrungsweise und Vorkommen bei freilebenden marmen Nernatoden. *Ark. Zool*. 4: pp 439-484

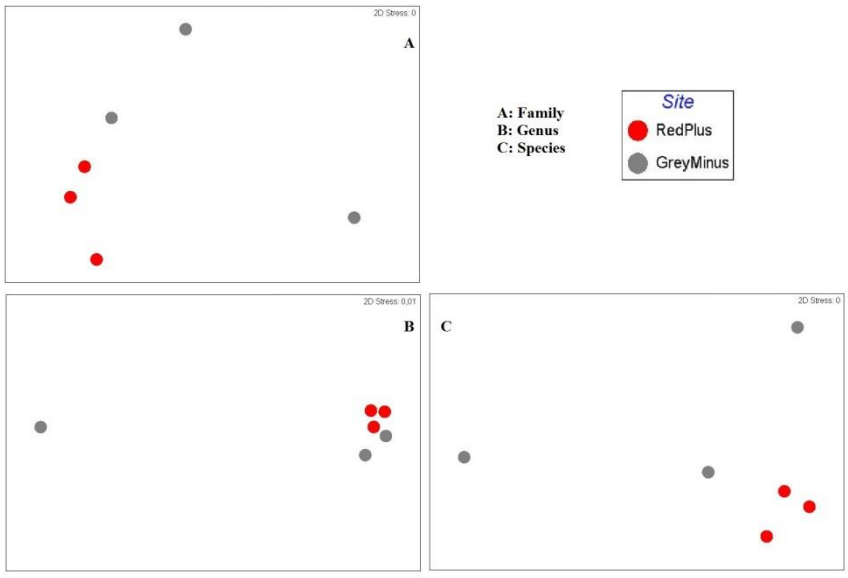
Widdicombe, S., Spicer, J. I. (2009) Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us?, *Journal of Experimental Marine Biology and Ecology* 366, pp 187–197

Appendices

Appendix A



MDS plot of harpacticoid communities on natural seagrass leaves at difference taxonomic levels (A: family, B: genus, C: species) (Bray-Curtis analysis on standardised, square root transformed data)



MDS plot of harpacticoid communities on natural seagrass shoots at difference taxonomic levels (A: family, B: genus, C: species) (Bray-Curtis analysis on standardised, square root transformed data)

Appendix B

Ameira longipes

Ectinosoma dentatum

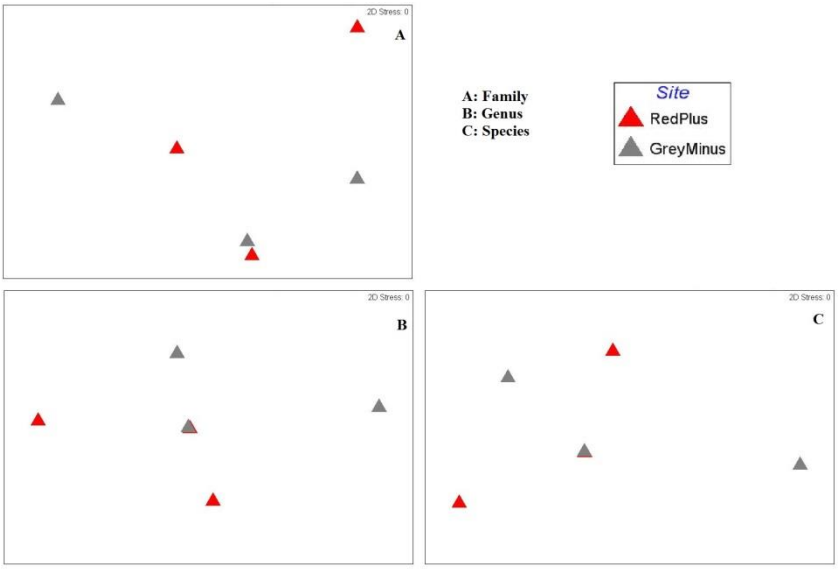
Tisbe ensifer

Appendix C

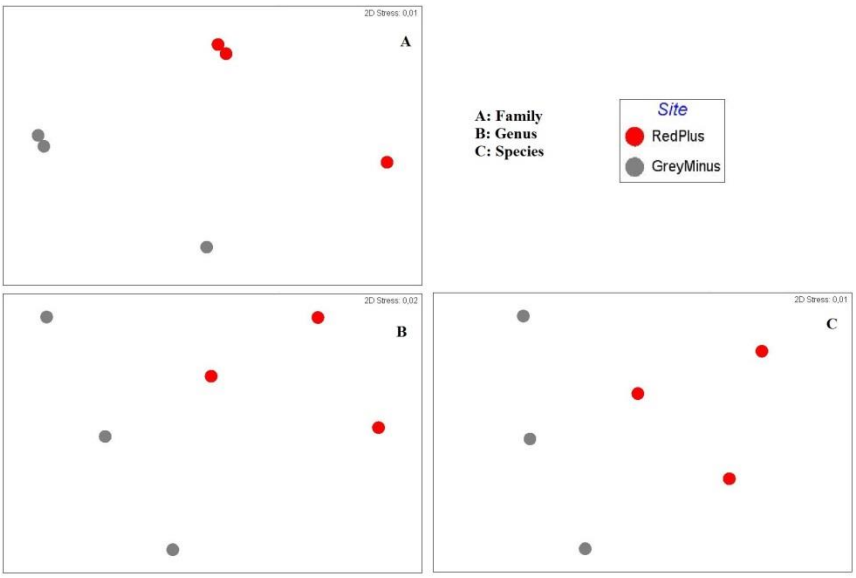
Harpacticoid species list:

Ameira longipes
Ameira minuta
Ameira gracilis
Ameira parvula
Amphiascus brevis
Amphiascus congener
Amphiascus catharinae
Amphiascus minutus
Dactylopusia tisboides
Diarthrodes nobilis
Ectinosoma dentatum
Ectinosoma sp1
Harpacticus littoralis
Harpacticus chelifer
Idyella tenuis
Idomene forficata
Laophonte cornuta
Lourinia armata
Microsetella sp1
Paralaophonte congenera
Paralaophonte brevistoris
Paradactylopodai latipes
Porcellidium sp1
Porcellidium ovatum
Porcellidium viride
Porcellidium tenuicauda
Phyllopodopsyllus bradyi
Sacodiscus littoralis
Tegastes satyrus
Thalestris rufoviolascens
Thalestris longimana
Tisbe ensifer
Tisbe furcata
Tisbe longicornis
Xouthous coronatus
Xouthous laticaudatus

Appendix D



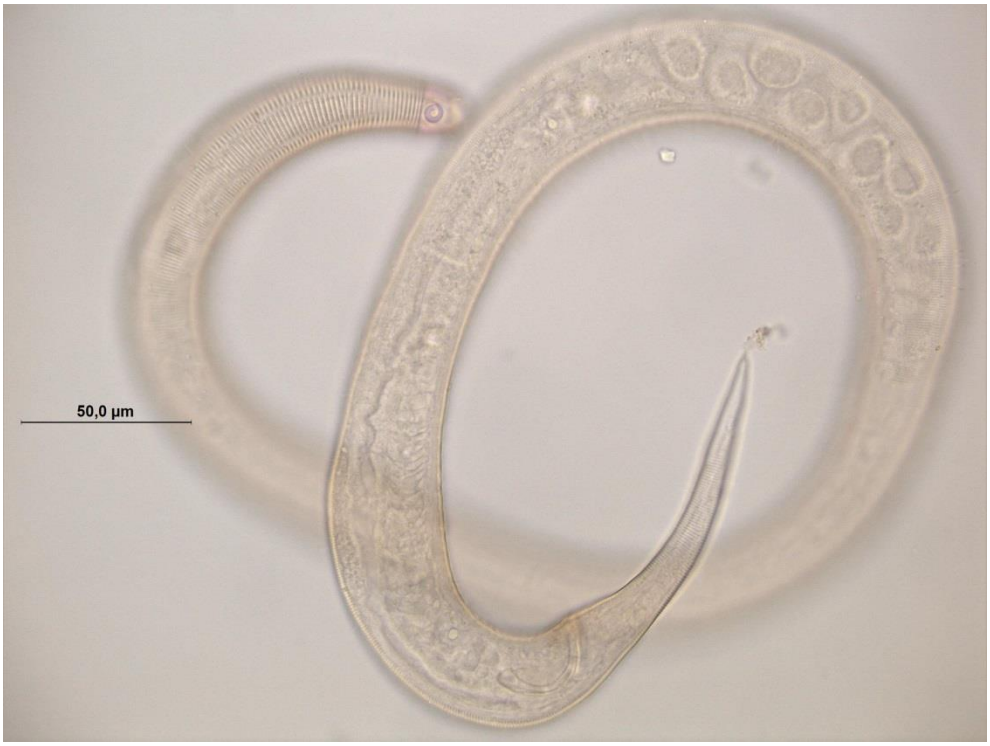
MDS plot of nematode communities on natural seagrass leaves at difference taxonomic levels (A: family, B: genus, C: species) (Bray-Curtis analysis on standardised, square root transformed data)



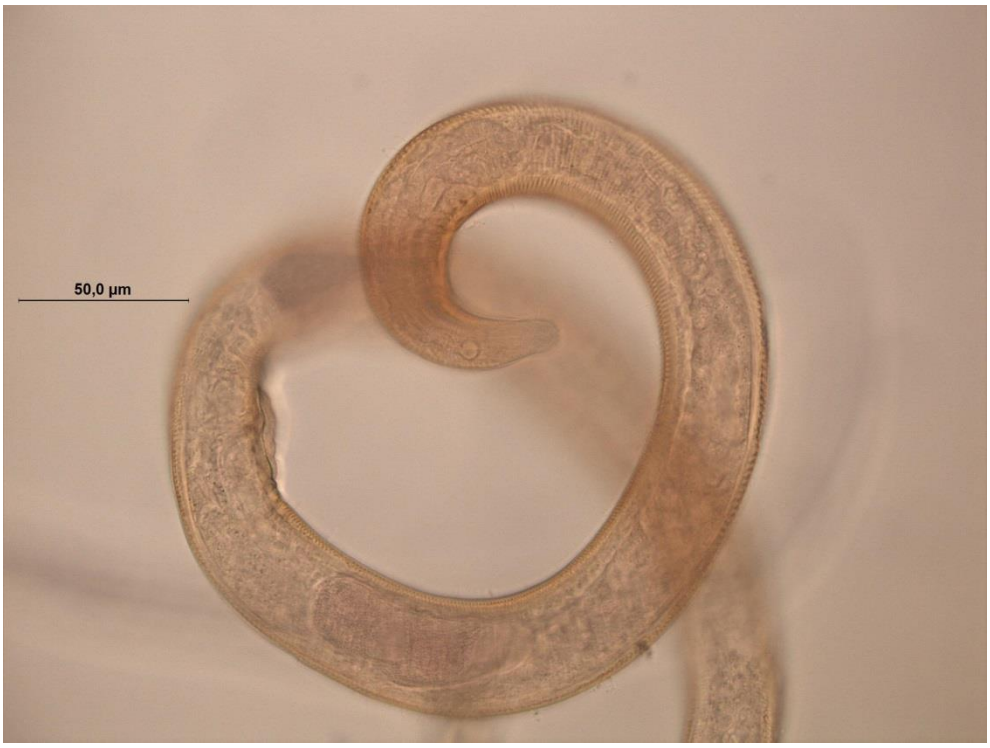
MDS plot of nematode communities on natural seagrass shoots at difference taxonomic levels (A: family, B: genus, C: species) (Bray-Curtis analysis on standardised, square root transformed data)

Appendix E

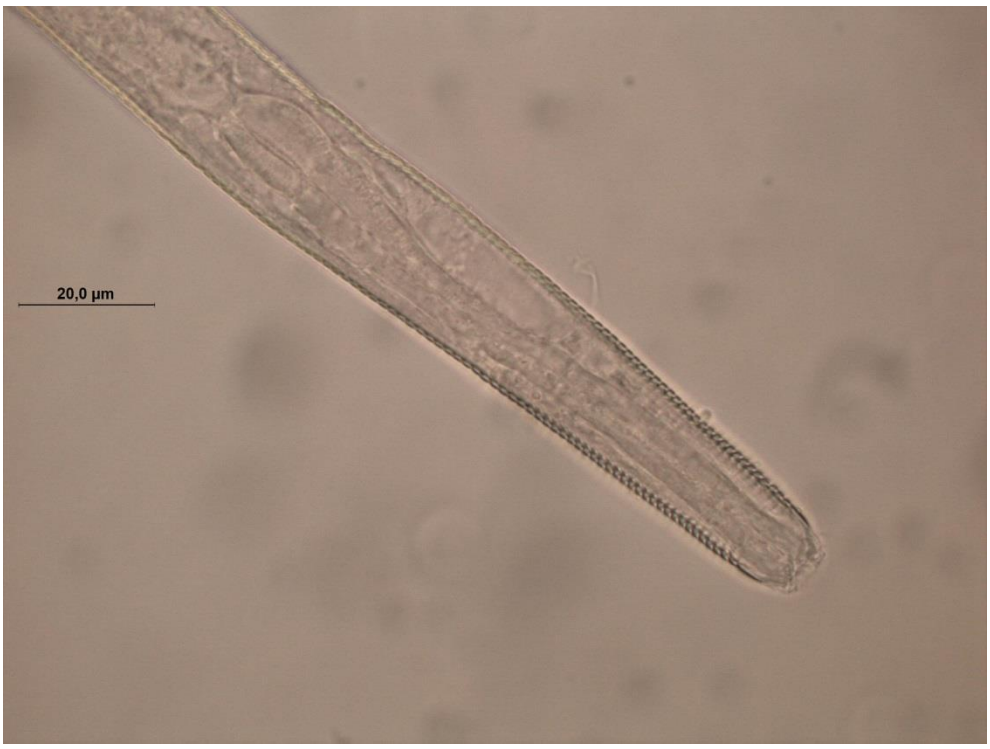
Chromadora nudicapitata



Desmodora nani



Microlaimus dimorphus



Prochromadorella dittlevseni

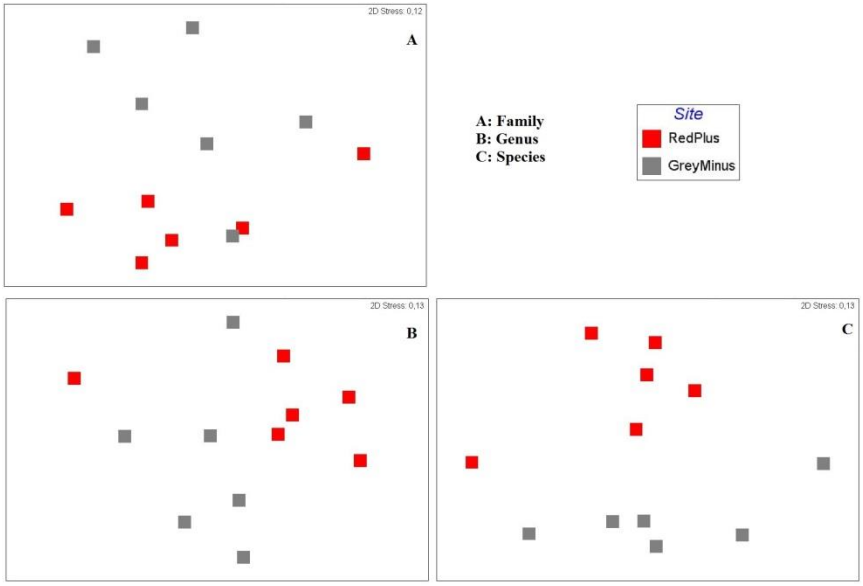
Appendix F

Nematode species list

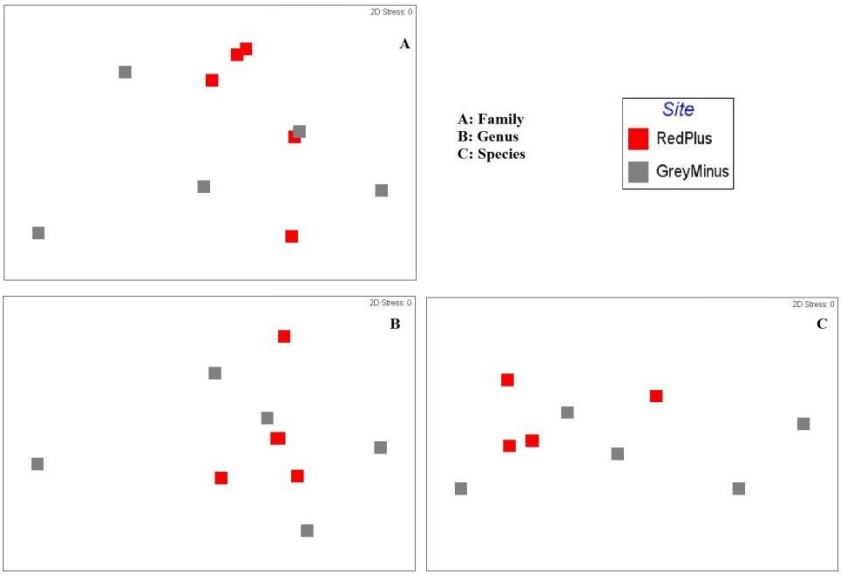
Acantholaimus setosus
Acanthopharynx similis
Actinonemafidatum
Ammotheristus helgolandicus
Anticoma acuminata
Araeolaimus sp1
Calyptonema acuminatum
Camacolaimus monhystera
Chromadora nudicapitata
Chromadora kreisi
Chromadorella parapoecilosoma
Chromadorina hiromii
Chromadorita sp1
Crenopharynx metagracilis
Daptonema deconincki
Desmodora nani
Desmodora campbelli
Desmodora sp3
Dichromadora arcospiculum
Diplopeltis cirrhatus
Draconema cephalatum
Endeolophos minutus
Enoplus benhami
Epacanthion brevispiculum
Euchromadora sp1
Eurystomina chilensis
Gammanema mediterraneum
Halalaimus sp1
Halalaimus sp2
Hypodontolaimus dimorphus
Leptolaimus ampullaceus
Leptolaimus ccupulatus
Leptosomatum revision
Loveninema unicornis
Mesacanthion sp1
Metadesmolaimus coronatus
Metalinhomoeus filiformis
Metepsilonema striatulum
Microlaimus dimorphus
Nannolaimoides sp1

Onchium hawaiinese
Paracanthonchus caecus
Paramonhystera levis
Paranticoma bandaensis
Phanoderma parafilipjevi
Polygastrophora hexabulba
Pontonema ocellatum
Prochromadorella dittlevseni
Pseudochromadora quadripapillata
Rynchonema sp1
Sabateria cobbi
Spilophorella paradoxa
Spirini sp1
Symplocostoma tenuicolle
Synonchiella micramphis
Synonchu hirsutus
Syringolaimus filicaudatus
Terschellingia parva
Thalassironus jungi
Thalassomonhystera sp1
Thalassomonhystera sp2
Thoracostoma microfenestratum
Trefusialaimus sp1
Tripyloides sp1
Viscosi viscosula
Wieseri sp1
Xenella sp1

Appendix G

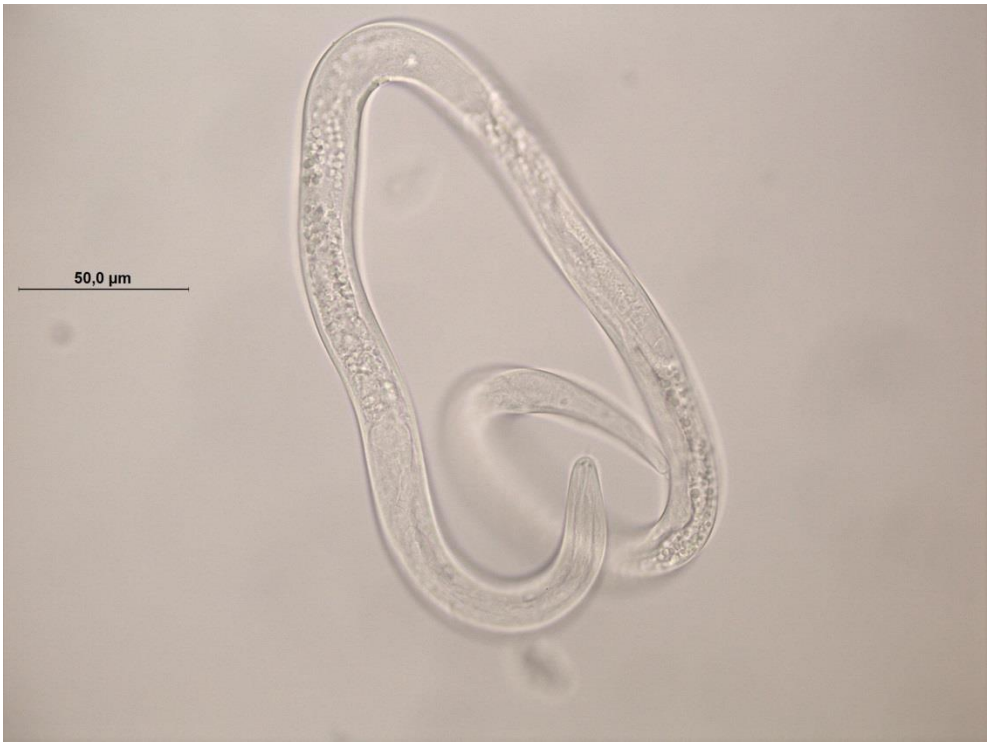


MDS plot of harpacticoid communities on seagrass mimics
at difference taxonomic levels
(A: family, B: genus, C: species)
(Bray-Curtis analysis on standardised, square root transformed data)

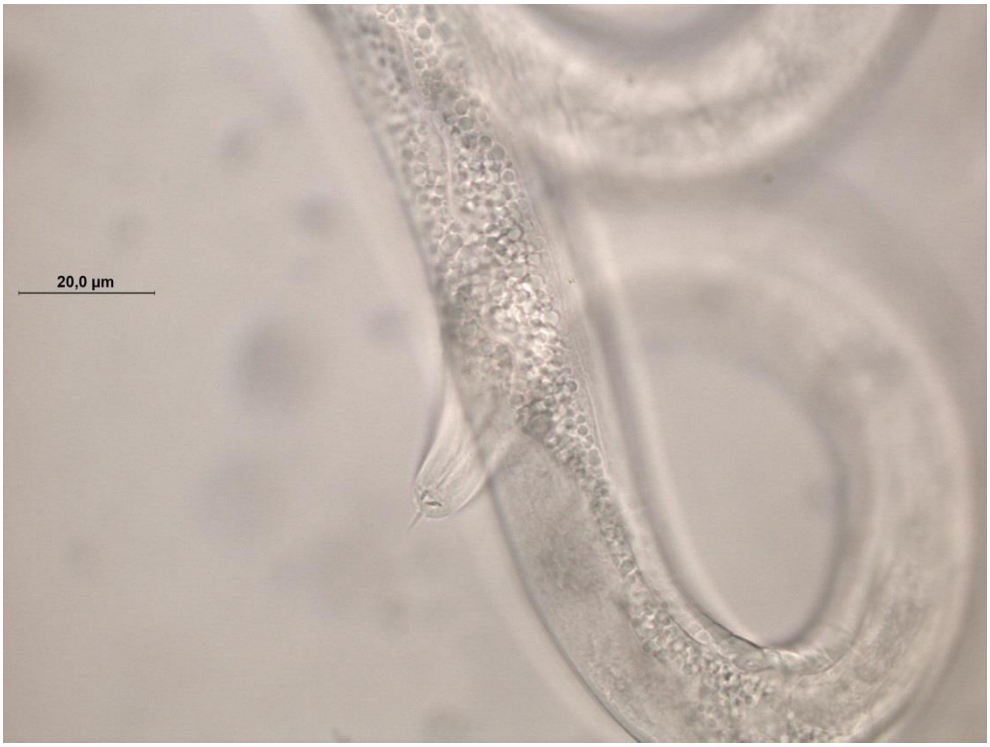


MDS plot of nematode communities on seagrass mimics
at difference taxonomic levels
(A: family, B: genus, C: species)
(Bray-Curtis analysis on standardised, square root transformed data)

Appendix H



Loveninema unicornis



Loveninema unicornis