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Above-below surface interactions mediate effects of seagrass disturbance on meiobenthic diversity, nematode and polychaete trophic structure

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Abstract

Ecological interactions between aquatic plants and sediment communities can shape the structure and function of natural systems. Currently, we do not fully understand how seagrass habitat degradation impacts the biodiversity of belowground sediment communities. Here, we evaluated indirect effects of disturbance of seagrass meadows on meiobenthic community composition, with a five-month *in situ* experiment in a tropical seagrass meadow. Disturbance was created by reducing light availability (two levels of shading), and by mimicking grazing events (two levels) to assess impacts on meiobenthic diversity using high-throughput sequencing of 18S rRNA amplicons. Both shading and simulated grazing had an effect on meiobenthic community structure, mediated by seagrass-associated biotic drivers and sediment abiotic variables. Additionally, shading substantially altered the trophic structure of the nematode community. Our findings show that degradation of seagrass meadows can alter benthic community structure in coastal areas with potential impacts to ecosystem functions mediated by meiobenthos in marine sediments.

Introduction

Feedback between above- and below-surface components of soil and sediment ecosystems are a vital mechanism controlling biodiversity and ecosystem processes [1]. Anthropogenic pressure can directly affect above-surface communities, by changing community composition, resource distribution patterns, or habitat structure, which in turn can have strong effects on below-surface biota [2,3]. On the other hand, below surface communities have an important function in organic matter mineralization and can create feedbacks that benefit above surface communities [1,4]. Although linkages between above and below-surface habitats in driving ecosystem structure and function in terrestrial ecosystems has received

considerable attention [1,2], much remains unknown about such interrelationships in marine coastal systems.

Similar to terrestrial ecosystems, plants in marine habitats provide a highly complex spatial environment with several niches for different species [5]. Seagrasses are an example of such plant communities that encompass some of the most productive habitats in marine ecosystems [6], providing a number of high-value ecosystem services [7]. Marine plant species are recognized to be autogenic ecosystem engineers shaping the shallow coastal environment through multiple and complex pathways [8]. The physical structures of seagrasses can modify local hydrodynamics and sedimentary habitats, thereby having a large controlling effect on subsurface environments by altering sediment granulometry, stabilizing sediments, storing atmospheric CO_2, trapping detritus and providing a wide range of food sources that support a high diversity of consumers [9].

The abundance and diversity of below-surface metazoan consumers in marine sediments is dominated by meiobenthos (microscopic benthic invertebrates between 0.04 and 1 mm in size) [10]. Meiobenthic communities play an important role in benthic ecosystem processes [11–13]. In seagrass beds, meiobenthos are often characterized by high densities and biomass, possessing short life cycles and high turnover rates [14] that often translate into high secondary production [15]. Although the importance of seagrasses for epiphytic invertebrate biodiversity (invertebrates associated with seagrass blades and leaves) has been well documented [16], their effects on the meiobenthos in the sediment are not as well understood [17–19], in part due to the practical difficulties in large scale studies focusing on a taxonomically hyperdiverse groups such as meiobenthos [20]. The application of high-throughput sequencing (HTS) approaches to the study of meiobenthos can considerably improve our understanding of the ecological patterns and environmental drivers of biodiversity in marine sediments [21,22],

65 including in seagrass beds, by allowing biodiversity assessments of microscopic metazoans at
66 a scale and with coverage previously unfeasible [20]. Nevertheless, to our knowledge no study
67 has looked at meiobenthic diversity in seagrass beds using HTS.
68 Seagrass habitats and their productive below-ground communities are highly
69 vulnerable to anthropogenic stress as they are often located in areas contiguous to intense
70 human activities [23]. As a result, seagrass habitats have been declining worldwide due to
71 anthropogenic activity [24]. Increased eutrophication, and sedimentation, resulting in light
72 reduction and decreased photosynthesis, are among the principal anthropogenic disturbances
73 to seagrass ecosystems [25]. Light reduction has multiple negative effects on seagrass plants,
74 spanning from reduced growth and loss of biomass [26] to lower carbohydrate storage in plant
75 rhizomes [27,28]. An additional important source of disturbance in seagrass beds comes from
76 increased fishing pressure. The removal of predatory fishes such as wrasses (Labridae),
77 snappers (Lutjanidae) and emperors (Lethrinidae) [29] can disturb the balance between
78 herbivory and seagrass production and potentially induce cascading effects [30] in these
79 ecosystems. Although, grazing is a vital process for controlling fast-growing epiphytic algae
80 in eutrophic systems [31], release of grazers like sea urchins from predation can provoke intense
81 grazing events that consume considerable amounts of seagrass above-surface biomass [32,33].
82 High densities of sea urchins and consequent overgrazing of seagrasses have been more
83 frequently reported in the last few decades [32,33] and can have enduring impacts on above-
84 ground seagrass biomass [32], with potential important knock-on effects for sediment properties
85 [34] and the structure and function of benthic fauna communities. Studies on the impacts of
86 human-induced disturbances on above surface communities and linkages to below-surface
87 diversity in marine systems are scarce. As meiobenthos mediate important benthic ecosystem
88 processes, it is crucial to understand how indirect effects of eutrophication and overfishing-

induced changes on plant above and below ground biomass affect meiobenthic communities. Such an understanding is crucial to predict future impacts on marine ecosystem structure and function [35].

Here, we address this important knowledge gap with a 5-month field experiment where we manipulated seagrass plots in a *Thalassia hemprichii* meadow and used HTS to assess impacts of shading and simulated grazing on meiofauna species richness and evenness metrics (alpha-diversity); variations in meiofauna community composition (beta-diversity) following the framework described by Anderson et al [36], and lastly nematode and polychaete trophic structure. The seagrass plot manipulations included two independent variables (shading and clipping) each with two levels (high and low). We used shading to mimic the effects of reduced light availability to seagrasses due to eutrophication and/or sedimentation, and simulated a high intensity grazing event due to herbivores being released from predation. Herbivory was simulated by clipping of shoots to mimic two different levels of grazing pressure. The design was used to test the following two hypotheses: shading causes a reduced seagrass root- and rhizome biomass with potential feedback effects on meiobenthic diversity, community, and trophic structure; and secondly, continued grazing causes a decrease of seagrass above-ground biomass that leads to a reduction in sediment stability and intensified erosion of the sediment surface, also with indirect effects on meiobenthic diversity, community and trophic structure. Our findings indicates that disturbance of *T. hemprichii* meadows can substantially change meiobenthic community composition and trophic structure of nematodes and polychaetes in coastal ecosystems.

Results

HTS data output

The Illumina Miseq dataset of eukaryotic 18S rRNA amplicons generated a total of 10,320,000 raw paired-end reads from 24 samples, resulting in a total of 6,180,945 quality-filtered reads after read merging and primer trimming, which led to an average of 257,539 sequences per sample (minimum- 83,262; maximum- 360,378). Clustering at 96% OTU similarity produced 14106 different OTUs (minimum cluster size >2 reads), of which 9034 OTUs were from metazoan taxonomic groups. Accumulation plots of number of OTUs vs number of reads for each sample are presented in Supplementary Information (Supplementary Figure 1).

Taxonomic composition

The percentage of OTUs belonging to metazoan groups was high for all seagrass treatments (on average 86%, 80%, 87%,87% and 86% in Control (CTRL), High clipping (HC), High shading (HS), Low clipping (LC), Low shading (LS), respectively), and highest in the unvegetated treatment with 96% (Supplementary Figure 2), confirming that sieving and density extraction is an effective way to isolate metazoan organisms as found in previous works [37]. The OTUs assigned to non-Metazoan Eukaryotes were excluded from the remaining analysis. Nematodes and copepods were the most abundant metazoan taxa in all treatments comprising approximately 40-70% of all relative abundance, followed by polychaetes, gastrotrichs and platyhelminths (Fig. 1-A). Supplementary Data 1 presents a list of all OTUs, its taxonomic classifications and sequence counts.

At a meiobenthos group level there was an effect of treatment in the relative abundances of OTUs belonging to Nematoda (PERMANOVA, pseudo- $F_{5,18}$ =13.9, p=0.001) and Copepoda (PERMANOVA, pseudo- $F_{5,18}$ =4.9, p=0.004). Relative abundance of nematode OTUs where significantly higher in the Unvegetated treatment than in the CTRL

(PERMANOVA, pseudo- $F_{5,18}$ =13.9, p= 0.028), while the opposite was found for copepods (PERMANOVA, pseudo- $F_{5,18}$ =4.9, p= 0.03). Within the nematodes, there were differences among treatments in relative abundances of its taxa (Fig. 1-B and 1-C). CTRL presented a significantly higher relative abundance of nematodes belonging to the order Monhysterida than in unvegetated plots (PERMANOVA, pseudo- $F_{5,18}$ =8.6, p= 0.029) and Chromadorida (PERMANOVA, pseudo- $F_{5,18}$ =4.8, p= 0.027). On the other hand relative abundances of Desmodorida nematodes were significantly lower in the CTRL when compared to the unvegetated treatment (PERMANOVA, pseudo- $F_{5,18}$ =31, p= 0.029- Fig. 1-B). At the nematode genus level there was a conspicuous difference in dominance between the seagrass plots (CTRL, HS, LS, HC and LC) and the Unvegetated treatments. While the former were dominated by *Molgolaimus* and Monhysterids nematodes (PERMANOVA, pseudo- $F_{5,18}$ =6.1, p= 0.002, pseudo- $F_{5,18}$ =29, p= 0.001, respectively) the latter treatment was dominated by nematodes of the genus *Catanema* (PERMANOVA, pseudo- $F_{5,18}$ =64.3, p< 0.001- Fig. 1-C)

Differences among treatments in alpha diversity

Alpha diversity metrics showed the same general trend for all three metrics we analysed: Observed number of unique OTUs and the ACE and Shannon index (Fig. 2). There was a significant effect of treatment on observed unique OTUs (PERMANOVA, pseudo- $F_{5,18}$ =3.9 ; p=0.01) which was lower in Unvegetated than in any other treatments, except HC. No additional significant differences in observed unique OTUs were found between the manipulated seagrass treatments (HC, HS, LC and LS) and the CTRL. The same pattern and effect of treatment was seen for ACE (PERMANOVA, pseudo- $F_{5,18}$ =4.8; p=0.003) and Shannon indexes (PERMANOVA, pseudo- $F_{5,18}$ =4.6; p=0.01). Again, both these metrics were significantly lower in the Unvegetated treatment but not in any of the pairwise

comparisons between CTRL, HC, HS, LC and LS.

Meiofauna beta-diversity differences among treatments

Figure 3 shows an NMDS ordination of samples based on meiobenthic community structure across all treatments. The PERMANOVA (*adonis*, pseudo-$F_{5,18}$= 2.0, p=0.001) analysis revealed a significant effect of treatment in meiobenthic community composition. A pairwise comparison performed with the *pairwise.perm.manova* showed significant differences in meiobenthic community composition between CTRL and all other treatments (PERMANOVA, p =0.02 for CTRL vs HS, p=0.05 for CTRL vs LS, p =0.04 for CTRL vs LC) except HC (PERMANOVA, p =0.09 for CTRL vs HC). A Principal Coordinates Analysis (PCoA) with UniFrac distance was also performed showing a similar pattern (*adonis*, Pseudo-$F_{5,18}$= 4.6, p=0.001, Supplementary Figure 3). Differences in community composition between the CTRL and all other treatments (HC, HS, LC, LS and Unvegetated) were mostly driven by turnover, and this pattern was constant for all comparisons (Supplementary Figure 4). There was also a difference among treatments in community beta-diversity as measured by average distance to centroid using the altGower distance (*betadisp,* PERMDISP, pseudo-$F_{5,18}$= 2.4, p=0.039) (Fig.4). Average distance to centroid in the CTRL treatment was significantly higher from all other treatments with the exception of HC indicating that the disturbances simulated in our experiment had a demonstrable effect on meiobenthic community beta-diversity (*betadisp*, PERMDISP, p<0.02 for all pairwise comparisons between CTRL and HS, LS and LC). A significant difference in average distance to centroid was also observed when comparing the LS and LC treatments (Fig.4, *betadisp*, PERMDISP, p=0.0007).

Regarding the relationship between meiofaunal community structure and

environmental variables, the BIOENV analysis showed that the environmental variables that best explained differences in meiobenthic community composition included both abiotic sediment variables (sediment C:N ratio, sediment %C content) and seagrass related biotic variables (rhizome biomass, community metabolism and N in plants) (Table 1, Fig. 5). How each of these variables varied among treatments is presented in supplementary information (Supplementary Figure 5). The CCA analysis showed that 43% of the total constrained inertia of the final selected model was explained, with the three retained environmental variables, sediment C:N ratio, N content in plant and C in rhizome, showing significant associations with community composition in the seagrass treatments (R^2=0.79 p=0.001, R^2=0.67 p=0.004 and R^2=0.76 p=0.001, respectively).

Trophic composition of nematodes and polychaetes

The abundance of trophic groups of nematodes and polychaetes were different among treatments. With regards to the nematodes, there was a significant effect of shading on the abundance of OTUs with taxonomic assignments corresponding to selective deposit feeder nematodes. The abundances of these OTUs were significantly higher in both the HS and in the LS treatment than in the CTRL (Fig. 6-B and 6-D, $p_{(DESeq2)}$ = 0.0008 and $p_{(DESeq2)}$ = 0.001, for LS vs CTR and HS vs CTRL, respectively). Conversely, the abundance of OTUs of epistrate feeder nematodes were lower in the two shading treatments than in the controls, but this difference was only significant for the HS treatment (Fig. 6-B and 6-D, $p_{(DESeq2)}$ = 0.055 and $p_{(DESeq2)}$ = 0.001 LS vs CTR and HS vs CTRL, respectively). Significant effects of clipping on nematode trophic structure were also observed. The abundance of epistrate feeder nematode OTUs were significantly less abundant in both clipping treatments (LC and HC) than in the CTRL (Fig. 6-A and 6-C, $p_{(DESeq2)}$ = 0.021 and $p_{(DESeq2)}$ = 0.044 for LC vs CTR

and HC vs CTRL, respectively). In addition, the abundance of non-selective deposit feeders was on average higher in the LC and HC treatments than in the CTRL, but this difference was only significant for LC (Fig. 6-A and 6-C , $p_{(DESeq2)}$=0.06 and $p_{(DESeq2)}$=0.02 for HC vs CTRL and LC vs CTRL, respectively). All trophic groups were significantly different between the Unvegetated treatment and the CTRL, with the predator/omnivore and nonselective nematode feeders showing an increase in abundance of OTUs in the Unvegetated treatment, whereas epistrate and selective feeding nematodes showed a decreased number of OTUs compared to the Unvegetated treatment (Fig. 6-E, all $p_{(DESeq2)} < 0.0001$)

Significant differences among treatments were also seen in the assessment of the polychaete feeding guilds. As found for nematode feeding groups, shading significantly increased the abundance of OTUs of deposit feeders polychaete when compared to the CTRL, but this difference was only significant for LS, (Fig. 7-A, $p_{(DESeq2)}$= 0.038). In addition, significantly fewer OTUs of carnivore polychaetes were found in LS compared to the CTRL (Fig. 7-A, $p_{(DESeq2)}$ =0.028). No other significant differences were found between the CTRL and the remaining manipulated seagrass treatments (HS, HC and LC). Conversely, all polychaete feeding guilds analyzed here, with the exception of suspension feeders were significantly different in the unvegetated treatment when compared to the CTRL (Fig. 7-B). The Unvegetated plots had less OTUs of subsurface deposit feeders ($p_{(DESeq2)}$= 0.007) and higher abundances of OTUs in the carnivore ($p_{(DESeq2)}$= 0.014) and omnivorous $p_{(DESeq2)}$= 0.043) feeding guilds when compared to the CTRL.

Discussion

While shading and corresponding reduced light availability did not affect meiobenthic community alpha diversity in our study, it had a significant effect on meiobenthic community structure. Reduced light availability to seagrasses is often coupled to eutrophication and/or increased sedimentation in seagrass beds [25]. Decreased light availability as a result of increased phytoplankton and epiphytic algae production is one of the principal mechanisms through which eutrophication impacts seagrass meadows [24,28]. Seagrasses can acclimate to reduced light regimes by decreasing above and below-ground biomass and photosynthetic activity[28,34,38], which in turn potentially shape sediment abiotic conditions for meiobenthic communities [17]. In particular, *T. hemprichii* has a comparatively well-developed root and rhizome network [39] that can confer stability to the sediment and increase its microscale complexity that favors microbial growth and diversity [40]. As such, a decrease in below-ground biomass of *T. hemprichii* could potentially impact such microscale habitat complexity and sediment characteristics for the meiobenthos.

Lower biomass and photosynthetic activity as a result of reduced light availability will cause a lower transport of oxygen from the shoots to the roots, decreasing "radial oxygen loss (ROL)" from the root-tips and thereby reduce the oxygenation of the sediment [41]. Reduction in photosynthetic rates can also lead to higher H_2S levels in the sediments of disturbed seagrass meadows [41,42]. Both lower oxygen conditions and increased H_2S concentrations in sediments have the potential to change meiofauna diversity and community composition [43,44]. In addition, photosynthetically derived dissolved organic carbon (DOC) has been shown to greatly stimulate the activity of microorganisms around *T. hemprichii* roots when it is transported to belowground tissue and excreted from the root system [45]. Both disturbances here tested shading and clipping probably reduced the amount of DOC extruded from the roots to the sediment. As bacteria and some nematodes can utilize DOC as an energy source

257 these direct and indirect changes in resource availability are likely to have effects of
258 meiobenthic community structure. Similar *in situ* studies have shown that shading resulted in
259 a significant decrease in root biomass and photosynthetic activity in the HS treatments [28], and
260 the BIOENV analysis in our study identified rhizome biomass as one of the variables that
261 correlated with meiobenthic beta-diversity. These results suggest that a reduced microhabitat
262 complexity could be related to the changes in meiobenthic community beta-diversity in the
263 shading treatments.
264 In addition to an effect on meiobenthic community beta-diversity, we found that
265 the relative abundances of OTUs assigned to nematodes of different feeding types differed
266 significantly between the control and the shading treatments, the latter showing a lower
267 proportion of epistrate feeders that seemed to be replaced by selective deposit feeders.
268 Nematodes are generally one of the most abundant metazoans in seagrass systems and
269 associated trophic structures are determined by abiotic factors such as grain size, sediment
270 porosity, temperature, salinity and food availability [10]. In our study, both temperature and
271 salinity varied in the same way among treatments and differences in sediment porosity
272 and compactness did not explain changes in the trophic structure of nematodes (BIOENV,
273 Table 1). As such, our results suggest that the reduction in OTUs of epistrate feeder and
274 increase in OTUs of selective deposit feeders is related to changes in the food resources of
275 these two feeding types of nematodes. Changes in food quantity and quality have been
276 coupled to nematode trophic structure in seagrass *Posidonia oceanica* meadows [15,46] and
277 in other coastal ecosystems [47]. We propose that shading reduced important phytoplankton
278 food sources to epistrate feeder nematodes as well as sedimentation in these plots, thereby
279 decreasing the relative abundance of epistrate feeders. We expected such effects would be
280 noticeable in the sediment Chl*a* content and net community production (NCP). A study that

281 used the same experimental system as ours found community metabolism to be significantly
282 lower in the HS treatment than in the CTRL plots[34]. While Chl*a* sediment content was on
283 average higher in the controls than in the shading treatments, this difference was not
284 statistically significant [34]. On the other hand, nematodes classified as selective deposit
285 feeders are generally considered to depend on different food sources than epistrate feeders,
286 as they preferentially feed on bacteria, small particulate food or dissolved organic matter.
287 As such, selective deposit feeders would therefore not be affected by the changes
288 microphytobenthic production and phytoplankton sedimentation. The reduced competition
289 with other nematodes could explain the increase in selective deposit feeders. Changes in
290 nematode trophic structure should be interpreted cautiously as recent work suggests that
291 most nematodes in their natural environment might exhibit a certain level of generalist
292 and opportunistic feeding behavior [48]. Nevertheless, the classification of Wieser (1953)
293 still provides valuable information about the feeding guilds of nematode community.
294 The increase in deposit feeders in the shading treatments observed in the
295 nematode community was also seen in polychaetes (Fig.7). Unlike what was seen with
296 nematode feeding types, the abundance of predator polychaetes was reduced in one our
297 shading treatments. This is in accordance with previous studies that have found an
298 increase in dominance of polychaete deposit feeders and a decrease proportion of
299 carnivores as an observed response to anthropogenic disturbance in benthic ecosystems
300 [49,50]. Taken together our results clearly show an indirect effect of shading on meiobenthic
301 community composition and trophic structure that is mediated by seagrass response to
302 eutrophication/and or increased sedimentation. Our results suggest that the impacts of
303 eutrophication on seagrass meiofauna community and nematode and polychaete trophic
304 structure can at least in part be due to indirect effects mediated by the response of seagrasses

305 to reduced light availability, and that above-below ground interactions can play an
306 important role in mediating sediment community structure in marine ecosystems.
307 Clipping also produced seagrass mediated effects on meiobenthic beta-diversity,
308 but these were less clear than what could be observed in the shading treatments. The largest
309 impact of these manipulations on the seagrass was the continuous removal above-ground
310 photosynthetic shoot from the replicate plots, an effect that simulates the impact of intense
311 grazing events [51]. This loss of biomass is known to disrupt the carbon sequestration and the
312 trapping of allochthonous organic matter, an important component of organic carbon in
313 seagrass beds [52]. Therefore, it was expected that a loss of above□ground biomass would
314 result in a lower accumulation of allochthonous organic matter in the clipping treatments.
315 Indeed, Dahl et. al [34] found a lower organic carbon content in the first 2.5 cm layer of
316 sediment of the clipping treatments in the same experimental system here reported. Organic
317 carbon content has been shown to be one of the most important factors structuring
318 meiobenthic communities [46] and it is likely that seagrass mediated effects on sediment carbon
319 dynamics affected the meiofauna community structure in the clipping treatments. Indeed,
320 BIOENV analysis found both sediment carbon content and sediment C:N ratio correlated with
321 changes in meiofauna community structure. An additional notable consequence of continuous
322 shoot biomass removal is an increased sediment erosion due to reduced capacity of shoots to
323 decrease wave action [34]. A decreased root and rhizome biomass (significant only in the HC
324 treatment) would also reduce sediment stability and allow for a higher degree of erosion [53],
325 which is particularly relevant in our experimental area characterized by large tides and strong
326 wave action [54]. This increase in tidal disturbance and sediment erosion as a result of seagrass
327 biomass removal has been seen as a response to large grazing effects by sea urchins [55]. As
328 such, both reduction of allochthonous organic matter trapping and increased erosion are

329 expected to impact sediment abiotic conditions important for the structuring of meiobenthic
330 communities. Additionally, loss of canopy can also reduce protection from predation.
331 Macrophytes provide shelter from predation for both macro- [5] and meiobenthos [17]. It is
332 possible that increased predation pressure contributed to the differences in meiobenthic
333 community structure. However, we did not measure predation pressure in our experiment and
334 are unable to confirm the connection with the data available. We expected the effects on
335 meiobenthic community should have been more pronounced in the HC than the LC treatment.
336 However, we found that community beta-diversity to be significantly different from the
337 CTRL in the LC but not in the HC treatment. It is possible that the high erosion and tidal
338 action in HC increased the variability within replicates, thereby decreasing our power to
339 detect statistical differences. An additional explanation is that, although simulated grazing
340 treatments can reduce the biomass of rhizomes, the root and rhizome network is still present
341 and minimizes potential negative effects of above-ground disturbances on meiobenthic
342 communities. It would therefore be interesting to test the effects of high clipping with higher
343 amounts of replication.
344 We also anticipated changes in sediment condition in the HC to affect the
345 trophic structure of nematodes; in particular the abundance of OTUs of epistrate feeders as
346 Dahl et al [34] found significantly higher Chl*a* content in HC sediments. This higher Chl*a*
347 content found in that study would suggest a higher microphytobenthos production as a result
348 of a greater light availability due to the removal of seagrass above ground biomass. However
349 we did not detect a higher OTU abundance of epistrate feeder nematodes in the HC treatment
350 when compared to the CTRL but rather the opposite. It is likely that the sediment erosion and
351 high hydrodynamics of our experimental system, would increase with lower seagrass canopy
352 and induce the observed patterns in nematode trophic structure. Although an effect of clipping

353 was detected on meiobenthic beta-diversity, community composition and nematode trophic
354 structure, our results indicate that disturbance related to clipping has less pronounced effects
355 when compared to shading.
356
357 There were clear differences between unvegetated areas and CTRL in most
358 response metrics here studied including meiobenthic alpha diversity, meiobenthic community
359 beta-diversity, nematode and polychaete trophic structure. The CTRLs had higher alpha
360 diversity, abundance of epigrowth feeder nematodes and carnivore polychaetes than the
361 unvegetated plots. Positive effects of seagrasses on macrofauna diversity and abundance of
362 macrofauna are well known [56,57] but regarding the less studied meiobenthos, the available
363 literature shows contrasting results [17 and references therein]. For example, Arrivillaga & Baltz [58]
364 found no significant differences in meiobenthic abundance, species richness or diversity
365 between sediments in tropical *T. testudinum* meadows and unvegetated sediments.
366 Furthermore, a number of studies have shown meiobenthos abundance to be negatively
367 correlated to seagrass cover as a result of increased predation pressure by macrofauna on
368 vegetated sediments [59,60]. Nevertheless, the positive effects of *T. hemprichii* for meiobenthic
369 alpha and beta-diversity, and trophic structure were clear in our study. Seagrass cover
370 increases the stabilization of sediments, habitat complexity and sediment organic matter
371 content, all of which could have positive effects on meiobenthos [17,18,61]. Our results suggest
372 that this habitat modulation by seagrasses influenced nematode community composition.
373 Unvegetated sediments where dominated by Desmodorida, particularly of the genus
374 *Catanema* that seem to find unstable fluid sediments in unvegetated areas advantageous [14,18].
375 However, other studies have found *Catanema* to be common in seagrass areas at sediment
376 depths deeper than the ones sampled in our experiment [18,19]. *Catanema* was replaced by

377 *Molgolaimus* in our seagrass plots, a common nematode genus in sediments of *T. hemprichii*
378 meadows, particularly in its top layer [18]. These seagrass plots were clearly dominated by
379 Monhysterida, which are likely positively impacted by increased amounts of fine particles and
380 detritus normally found in sediments in seagrass meadows [62]. Effects of seagrass on
381 nematodes and other meiobenthos may, nevertheless, be dependent on seagrass species'
382 composition and density and on other abiotic factors not examined here.
383 In summary, our results indicate that disturbance of seagrass meadows have
384 propagating effects on meiobenthic communities that are mediated by above-below ground
385 interactions. Shading altered meiobenthic community composition and nematode and
386 polychaete trophic structure to a larger dominance of deposit feeders. Such responses to
387 shading by the meiobenthos seem to be related to reduced seagrass root and rhizome biomass
388 reported in previous studies [28,34]. The continued grazing in the clipping treatments also
389 resulted in significant changes in meiobenthic community and trophic structure, although
390 these were not as clear as the shading treatments. Our study suggests that such changes are
391 connected to a decrease in above-ground biomass and intensified erosion of the sediment
392 surface reported in previous work [34]. Since human-induced disturbances are increasing the
393 rate of seagrass bed habitat degradation [63] it is crucial to improve our understanding of what
394 such losses mean for the structure and functioning of benthic ecosystems. Our results
395 highlight the complex role of above-below ground interactions in marine systems. Seagrasses
396 function as ecosystem engineers for benthic faunal communities, and how they respond to
397 disturbances can have significant indirect effects of meiobenthic community diversity and
398 trophic structure. Considering that meiobenthos can have important roles in benthic foodwebs
399 [10,35] and mediate vital benthic ecosystem function [11,13], prolonged disturbances of seagrass

habitats as presently seen in many coastal waters, are likely to have important cascading effects for benthic ecosystem structure and function.

Methods

Study area and experimental setup

We performed an *in situ* experiment for 5 months (November to March 2015) in a seagrass meadow in Chwaka Bay on Zanzibar Island (Unguja), Tanzania. Chwaka Bay is a large (approximately 50 km^2) semi-enclosed bay on the east coast of Zanzibar Island with a maximum (spring tide) tidal fluctuation of 3.2 m [54]. The bay is composed by seagrass meadows (with as many as 11 seagrass species) and unvegetated bare sediment habitats [64]. Within the bay, an experimental site (06°09'S 39°26'E) was selected in the middle of a one kilometer-wide seagrass meadow dominated by *Thalassia hemprichii*; a common species in the region as well as in tropical areas elsewhere [65]. The experimental site was located in the intertidal zone with a water depth of approximately 10 cm during low spring tide. Salinity was 34 in the experimental area and was measured with a multimeter Multi 340i, CellOx 325 (WTW).

The experimental design comprised of six treatments; low- and high clipping intensity treatments (LC and HC, respectively), low- and high shading treatments (LS and HS, respectively) as well as controls of non-manipulated seagrass plots (CTRL). Unvegetated bare sediments plots were selected in an area adjacent to the manipulated plots. Four replicate plots for each treatment were placed within a 40 x 40 m experimental site using a random block design, with each plot covering 10 m^2 [28,34] (Fig. 8). The LS and HS plots were covered with plastic semi-transparent shading nets, mounted approximately 40 cm above the sediment surface; the LS treatment was covered with one shading net and the HS treatment with double shading nets. This procedure reduced the light irradiance from 470 µmol quanta m^{-2} s^{-1} in the

seagrass control plots, to 356 µmol quanta m^{-2} s^{-1} in the LS treatment (a mean light reduction over day of 64% in relation to CTRL) and 307 µmol quanta m^{-2} s^{-1} in the HS treatment(a mean light reduction over day of 75% in relation to CTRL). A Photosynthetic Active Radiation (PAR) Logger (Odyssey, New Zealand) was used to measure light intensity levels in LS, HS, and control plots. Each day the shading nets were cleaned of debris and fouling organisms, and the nets were replaced two times during the experiment due to natural wear. For LC and HC treatments, 50% and 100% of the original shoot biomass was removed, respectively. In the LC treatment, the shoot height was reduced by approximately half the natural shoot length (~10 cm) and in the HC treatment, the shoots were cut just above the meristematic region. The clipping was performed at a 3 to 5 day interval until three weeks before terminating the experiment after which no additional clipping was done.

Sediment sampling, sample preparation and sequencing

After 5 months at the termination of the experiment the sediment of each of the 24 replicate plots was sampled with six handheld Perspex sediment cores taken from the exact same location within each of the plots. The handheld sampling units were 45 mm diameter with a surface area of 17 cm^2, a size suitable for sampling of microbial benthic metazoans such as meiofauna [66,67]. The top 3 cm of each core were sliced and sieved through 500 µm and 40 µm stacked sieves, pooled and preserved in 20% DESS before storage at 4°C. After two weeks the sediment and animals were again placed in a 40 µm sieve and rinsed thoroughly in filtered artificial saltwater (salinity 34) close to *in situ* salinity to remove the DESS. The meiofauna individuals were isolated and separated from the sediment particles using density extraction by washing the content of the 40 µm sieve into a 500-mL E-flask with LevasilH 200A 40% colloidal silica solution (H.C. Starck SilicaSol GmbH) with a density of 1.3 and

448 shaken vigorously as described previously in Nascimento et al. [11]. After aeration, the solution
449 was left to settle for 5 min. The top 100 mL of the LevasilH solution was sieved through a
450 sterilized 40 μm sieve and rinsed thoroughly in seawater. The 40 μm sieves were then washed
451 with 70% ethanol and autoclaved between each replicate. The density extraction procedure
452 was repeated twice (5-min and then 30-min settling time). The extracted meiofaunal animals
453 were then washed carefully from the sieve into a 50ml falcon tube with a volume of Milli-Q
454 ultrapure water that did not exceed 10 ml and frozen at -20 ^{O}C until DNA extraction.
455 DNA extraction
456 DNA from the meiofauna community was extracted with the PowerMax® Soil
457 DNA Isolation Kit (MOBIO, Cat#12988), in conformity with the protocol instructions. After
458 DNA extraction, samples were frozen at -20 °C in 3 mL of C6 solution (10mM Tris). After
459 this, 100 μL of each DNA extract was purified with PowerClean® Pro DNA Clean-Up Kit
460 (MOBIO, Cat# 12997-50) and stored in 100 μL of C5 (10mM Tris) solution at -20 °C. Before
461 PCR amplification, all DNA extracts were standardized to a concentration of 10 ng/μL. The
462 conservative metabarcoding primers TAReuk454FWD1 (5′-
463 CCAGCA(G/C)C(C/T)GCGGTAATTCC-3′) and TAReukREV3 (5′-
464 ACTTTCGTTCTTGAT(C/T)(A/G)A-3′) and Pfu DNA polymerase (Promega, Southampton,
465 UK) were used to amplify the 18S nSSU gene region with PCR, creating fragments between
466 365-410 bp excluding adaptors or barcodes. Each sample from the 24 replicate plots were
467 amplified in triplicates which were then pooled, dual-barcoded with Nextera XT index
468 primers following a modified version of Bista et al.[68] (2017) and visualized by gel
469 electrophoresis. The barcoded amplicons were then purified with the Agencourt AMPure XP
470 PCR Purification kit (Beckman Coulter), quantified with Qubit (Invitrogen, USA) and pooled
471 in equimolar quantities. The purified amplicons were sequenced in both directions on an

Illumina MiSeq platform at the National Genomics Institute (NGI -Stockholm, Sweden) as a single pool comprised of the 24 different samples with 24 unique index primer combinations (i.e., an index primer combination for each of the 4 replicates plots of our 6 experimental treatments).

Bioinformatics

Amplicon reads were demultiplexed by the sequencing facility, followed by initial data processing and quality-filtering in the QIIME 1.9.1 pipeline [69]. Paired-end Illumina reads were overlapped and merged using the join_paired_ends.py script in QIIME, followed by quality-filtering of raw reads using the multiple_split_libraries_fastq.py script with a minimum Phred quality score of 19. Unmerged (orphan) Illumina read pairs were discarded, and excluded from all downstream data analysis steps. PCR primer sequences were subsequently trimmed from merged reads using Trimmomatic version 0.32 [70](parameters used were ILLUMINACLIP:2:30:10, with all other parameters as default). Trimmed, merged reads which passed all quality-filtering steps were next subjected to open-reference OTU picking using a 96% pairwise identity cutoff, using the pick_open_reference_otus.py script in QIIME 1.9.1 (using the uclust algorithm with 10% subsampling, no prefiltering, and reverse strand match enabled). All resulting singleton OTUs were excluded from the resulting OTU table outputs. Taxonomy was assigned to representative OTU sequences with the RDP Classifier [71] in QIIME (assign_taxonomy.py with a confidence threshold of 0.7), using the SILVA 119 release as a reference database [72]. OTU representative sequences were aligned with PYNAST [73] using the align_seqs.py script.

Statistics and Reproducibility

The resulting OTU table and correspondent metadata set was imported into R v 3.4.3 and analysed using the phyloseq [74] and vegan [75] packages. The effect of both shading and clipping on alpha diversity metrics (observed OTUs, ACE index and Shannon index) and relative abundance of meiofauna taxonomic groups were tested with one-way PERMANOVA with the PAST 3.24[76]. Statistical significance was defined at $\alpha=0.05$ to cover all analyses.

Community composition was examined by first selecting and filtering metazoan OTUs and sub-sampling the OTUs counts to the lowest sample size (66 754 counts) with the *rarefy_even_depth* function in pyloseq. After Hellinger transformation, the dissimilarity between faunal assemblages in the different treatments was analysed by non-metric multidimensional scaling (NMDS), using the altGower distance [77], and by Principal Coordinates Analysis (PCoA) with UniFrac distance. To statistically test for the effects of treatment on community composition, we conducted a permutational multivariate analysis of variance (PERMANOVA) with the *adonis* function of the *vegan* package. The function *pairwise.perm.manova* of the RVAideMemoire package [78] was used to perform pairwise comparisons between CTRL and the remaining treatments in terms of differences in community composition. To examine differences in beta-diversity among treatments we used the community beta-diversity index [36] that is based on community OTU dissimilarity metrics and measured as average distance of each observation to the group centroid, using the *betadisper* function in the *vegan* package [75]. Pairwise differences between treatments in average distance to the group centroid were checked with the *permutest.betadisper* of the betadisp object that permutes model residuals and generates a permutation distribution of F with the null hypothesis that there is no difference in dispersion between groups. Furthermore, metrics to partition beta-diversity were utilized to calculate the relative importance of turnover and nestedness in the different treatments [79]. Beta-diversity can be divided into

519 dissimilarity as a result of turnover, i.e species replacement between sites or samples, and
520 dissimilarity as a result of nestedness, species loss from sample to sample. We used the R
521 package betapart [79] for this analysis. Additionally, a BIOENV ('biota-environment') analysis
522 [80] was performed to explore relationships between environmental variables and meiobenthic
523 community composition using Spearman's rank correlations. Concisely, BIOENV identifies
524 the combination of environmental variables, that best correlated with the changes in
525 community structure. For the analysis we included 21 variables measured and reported in
526 Dahl et *al.*[34] and Deyanova et *al* [28], studies based on the same experimental system.
527 Specifically we used two classes of environmental variables for the BIOENV analysis: firstly
528 we used seagrass traits namely: net community production (NCP); leaf biomass, C, N content
529 and C:N ratio; rhizome biomass C, N content and C:N ratio; root biomass, C, N content and
530 C:N ratio; and secondly sediment variables, specifically: density, porosity, sediment %C,
531 sediment %N, C:N ratio, sediment inorganic C and content in total hydrolysable amino acids
532 (THAA) and Chl*a*. The methodology used to derive these variables is described in detail in
533 Dhal et *al* [34] and Deyanova et *al.* [28]. Furthermore, and in order to complement the BIOENV
534 analysis and visualize the relationships between the environmental variables and community
535 composition, a canonical correspondence analysis (CCA) was performed with the best
536 combination of variables identified by BIOENV as a starting point. After exclusion of the
537 variables that had a correlation coefficient higher than 0.7 from the analysis, we used the
538 *envfit* function of the *vegan* package to test which environmental variables were significantly
539 correlated with meiobenthic community composition.
540 To investigate potential changes in nematode trophic structure we subset our
541 dataset to include only nematode OTUs that could be taxonomically classified to genus, in a
542 procedure similarly applied to terrestrial nematodes [81,82]. These 644 OTUs were categorized

into functional feeding groups as previously defined by Wieser [83], using nematode buccal cavity morphology to define four trophic groups: selective deposit-feeders (1A), nonselective deposit-feeders (1B), epistrate feeders (2A) and omnivorous-carnivorous (2B). A full list of nematode feeding type classifications for the genera used in this is available in Supplementary Data 2.

Furthermore, we investigated treatment related changes in the trophic structure of polychaetes, by subsetting the polychaete OTUs taxonomically assigned to Family (total 870 OTUs) and classifying them to relevant trophic guilds (eg deposit feeders, omnivore, herbivore or predators) following Jumars [84].

To assess differential OTU abundance between the CTRL and the other treatments in nematode and polychaete trophic structure, we used the DESeq2 statistical package [85]. DESeq2 accounts for the variance heterogeneity often observed in sequence data by using a negative binomial distribution as an error distribution to compare abundance of each OTU between groups of samples [85]. All statistical tests were performed on R v 3.4.3. All statistical analysis outputs can be found in Supplementary Data 3.

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Authors' contributions

F.J.A.N., M.D., D.D., L.D.L. M.B, S.C. and M.G designed the study. M.D., D.D and L.D.L. conducted the experiment and sampled in the field. F.J.A.N. conducted the laboratory work and analysed the data; T.S. and T.J.P. provided with bioinformatics and, with help from H.M.B. F.J.A.N. wrote the manuscript with contributions from M.D., H.M.B. M.B, S.C. and M.G and comments by D.D., L.D.L.

Competing interests

The authors declare that there are no financial or non-financial competing interests.

Data Accessibility

The raw sequence data have been uploaded and are available on the NCBI database with the following BioProject number: PRJNA540961

Fig. 1- Stacked bars of the average relative abundances of 18S rRNA gene for meiobenthos in the different treatments, n=4 biologically independent samples. The y-axis shows the treatments, and x-axis shows relative abundance (%) of Metazoa phyla (A); order in the Nematoda (B); and genus in the Nematoda (C).

Fig. 2- Alpha-diversity metrics for meiobenthos in the different treatments. Figure panels show: number of observed unique OTUs (A), ACE index (B) and Shannon index (C). Central bars represent the mean of each treatment. Different letters indicate statistically significant differences (PERMANOVA, $p<0.05$) based on n=4 biologically independent samples.

Fig. 3- Plot of the non-metric multidimensional scaling (NMDS) analysis based on normalized OTU matrix for meiobenthos using altGower dissimilarities . Different colours represent the groupings of the different treatments.

Fig.4- Meiobenthic community β-diversity index showing the average distance from group centroid to each observation, n=4 biologically independent samples. Different letter codes indicate statistically significant differences (PERMDISP, $p<0.05$).

Fig. 5- Canonical correspondence analysis (CCA) biplot showing the co-variant relationship between significant non-correlated environmental factors (See methods) and meiobenthic community structure. Arrows are vectors representing the correlation between environmental variables and the axes.

Fig. 6- OTU abundance of nematode feeding types in the different treatments in relation to the Controls: High Clip (A), High Shade (B), Low Clip (C) and Low Shade (D), Unvegetated (E). The x axis shows the $\log_2$ fold changes of the four different nematode feeding types (y axis) calculated by the DESeq2 adjusted base mean (see Methods). A $\log_2$ fold change of >0 (green) indicate that abundance was higher in the Control than in the respective manipulated treatment, while a $\log_2$ fold change of <0 (red) indicates that abundance was lower in the Control than in the respective manipulated treatment. White asterisks show cases when differences were statistically significant ($p_{(DESeq2)} < 0.05$) and error bars represent SE, n=4 biologically independent samples.

Fig. 7- OTU abundance of polychaete feeding guilds in the different treatments in relation to the Controls: Low Shade (A) and Unvegetated (B). The x axis shows the $\log_2$ fold changes of the four different nematode feeding types (y axis) calculated by the DESeq2 adjusted base mean (see Methods). A $\log_2$ fold change of >0 (green) indicates that abundance was higher in the Control than in the respective manipulated treatment, while a $\log_2$ fold change of <0 (red) indicates that abundance was lower in the Control than in the respective manipulated treatment. White asterisks denote cases when differences were statistically significant ($p_{(DESeq2)} < 0.05$) and error bars represent SE, n=4 biologically independent samples. No other differences were detected between CTRL and the remaining manipulated seagrass treatments

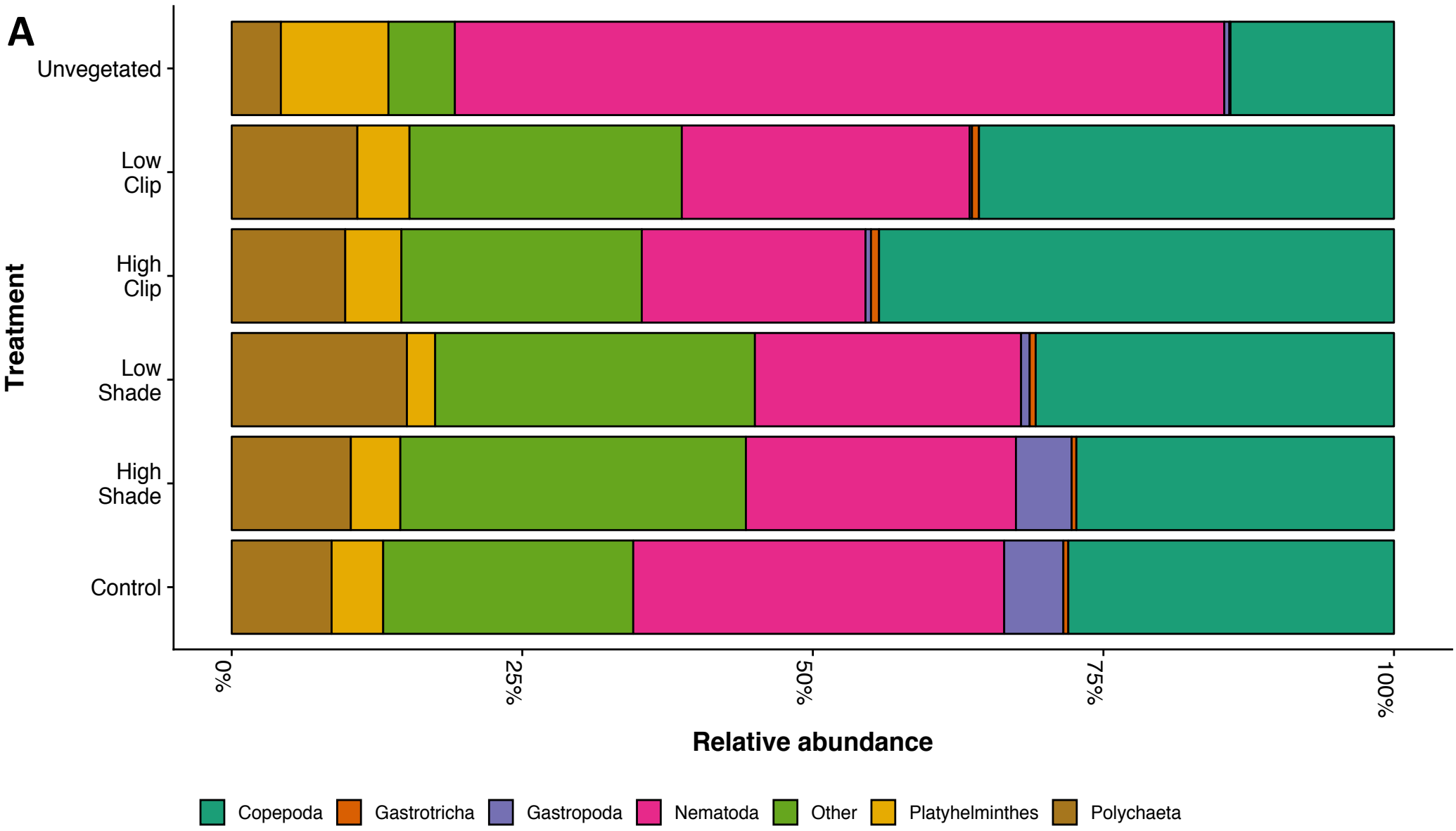
Fig. 8- Experimental approach. (a) Experimental approach displaying the randomized complete block design in our study. Different patterns correspond to the different experimental treatments (four biologically independent replicates per treatment). Letters

represent replicate blocks. (b) High Shading treatment, (c) High Clipping treatment. Photos by Martin Gullström

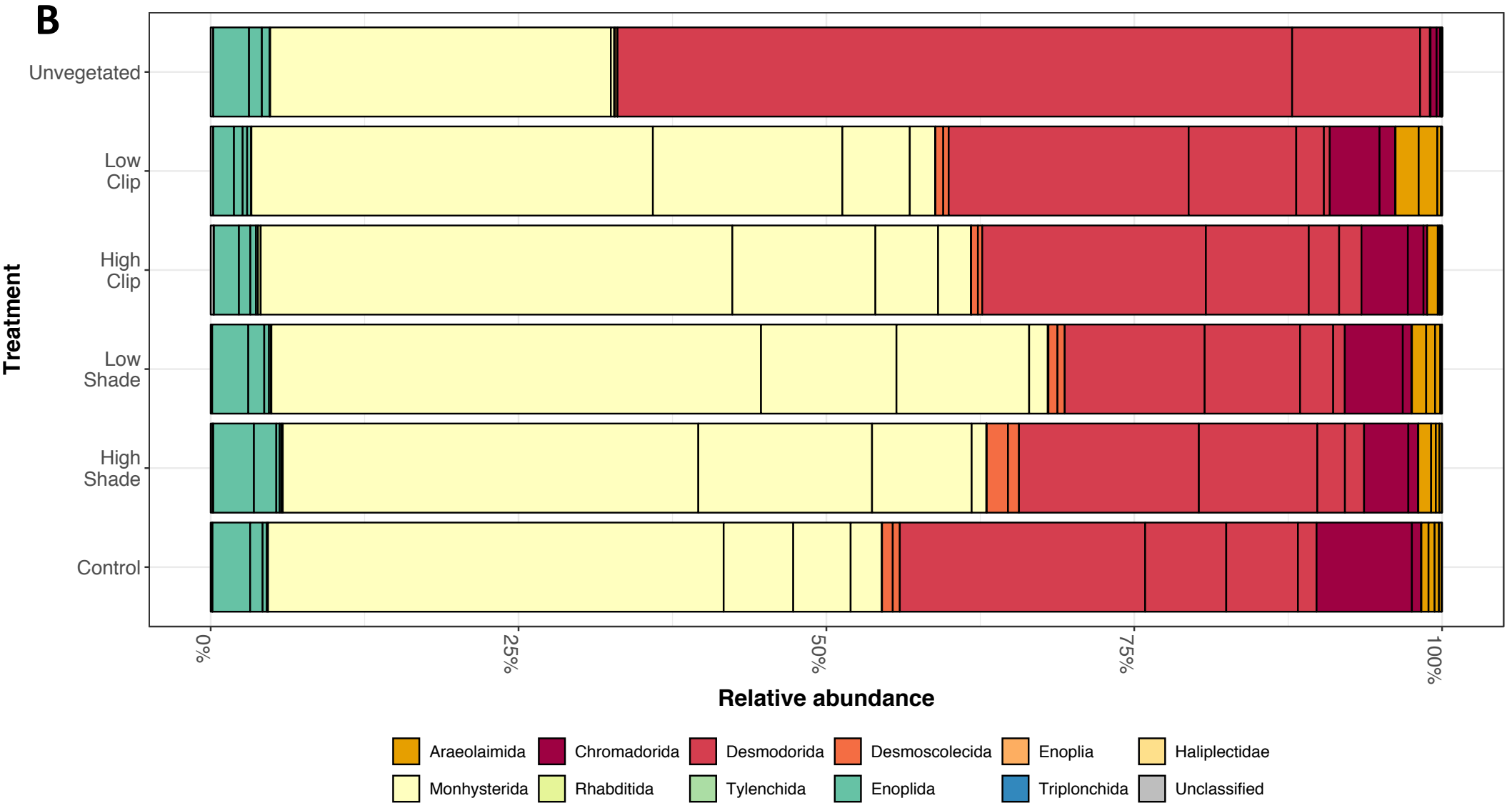
No of variables	Correlation	Environmental variables
7	0.6	Sed C:N ratio; Bulk C in core; Sed C inorg; Rhizome biomass; NCP; N in Plant; C in rhizomes
7	0.598	Sed C:N ratio; Bulk C in core; Sed C inorg; Leaf biomass; Rhizome biomass; NCP; C in rhizomes
6	0.597	Sed C:N ratio; Bulk C in core;Sed C inorg; Leaf biomass; Rhizome biomass; NCP;
8	0.594	Sed C:N ratio; Bulk C in core; Sed C inorg; Leaf biomass; Rhizome biomass; NCP; N in Plant; C in rhizomes
6	0.593	Sed C:N ratio; Bulk C in core; Sed C inorg; Rhizome biomass; NCP; N in Plant

Table 1- Biota-environment (BIOENV) analysis showing the 5 best combinations of variables linked with the highest correlation to the meiobenthos community composition. Correlation values represent Spearman's rank correlation coefficient. Environmental variables abbreviations: Sediment C:N ratio (Sed C:N ratio); Bulk carbon density (Bulk C in core); Sediment content in inorganic C (Sed C inorg); Rhizome biomass (Rhizome biomass); Community metabolism (NCP); Plant Nitrogen content (N in Plant); Rhizomes carbon content (C in rhizomes): Leaf biomass (Leaf biomass)

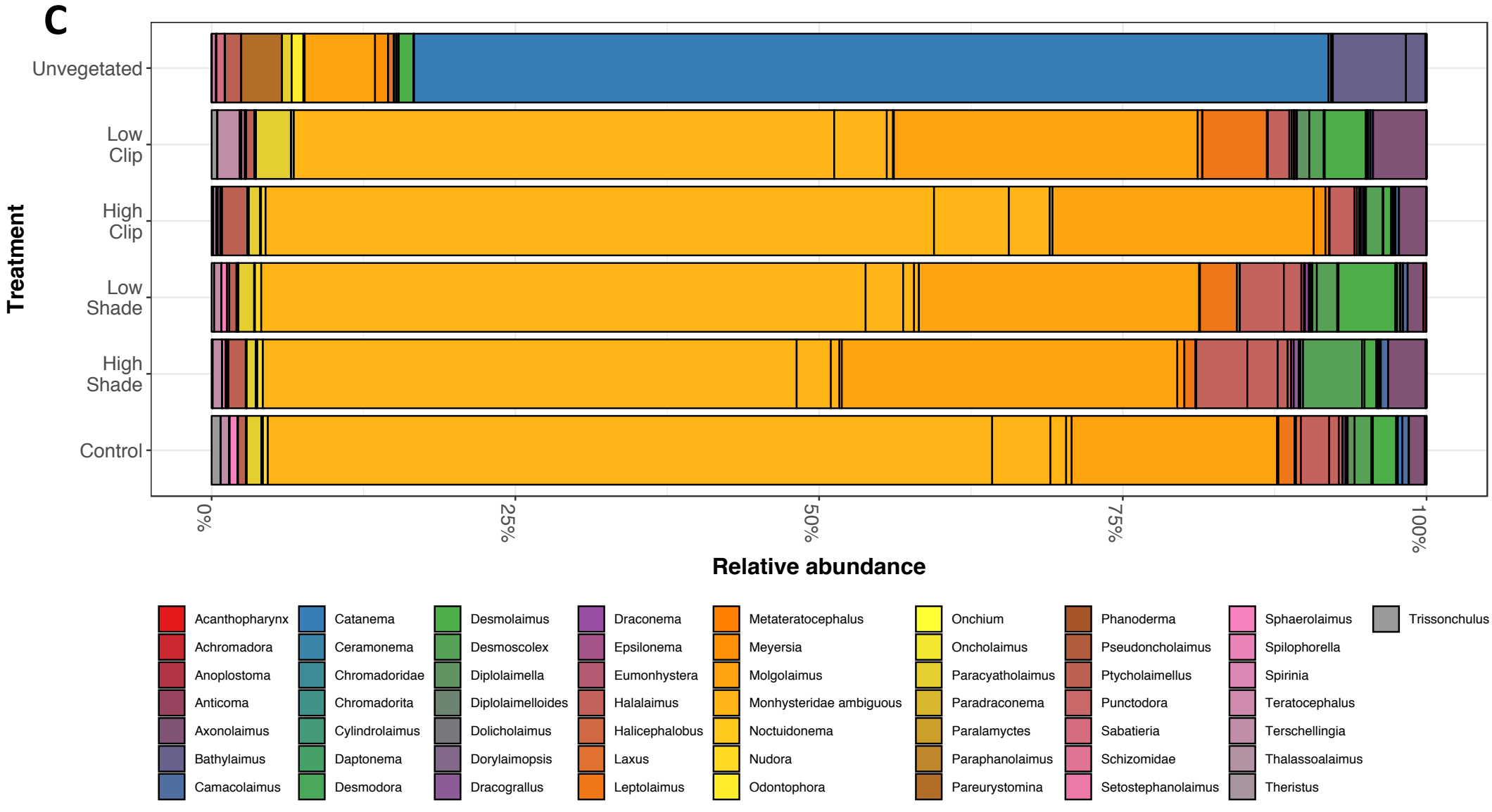
A

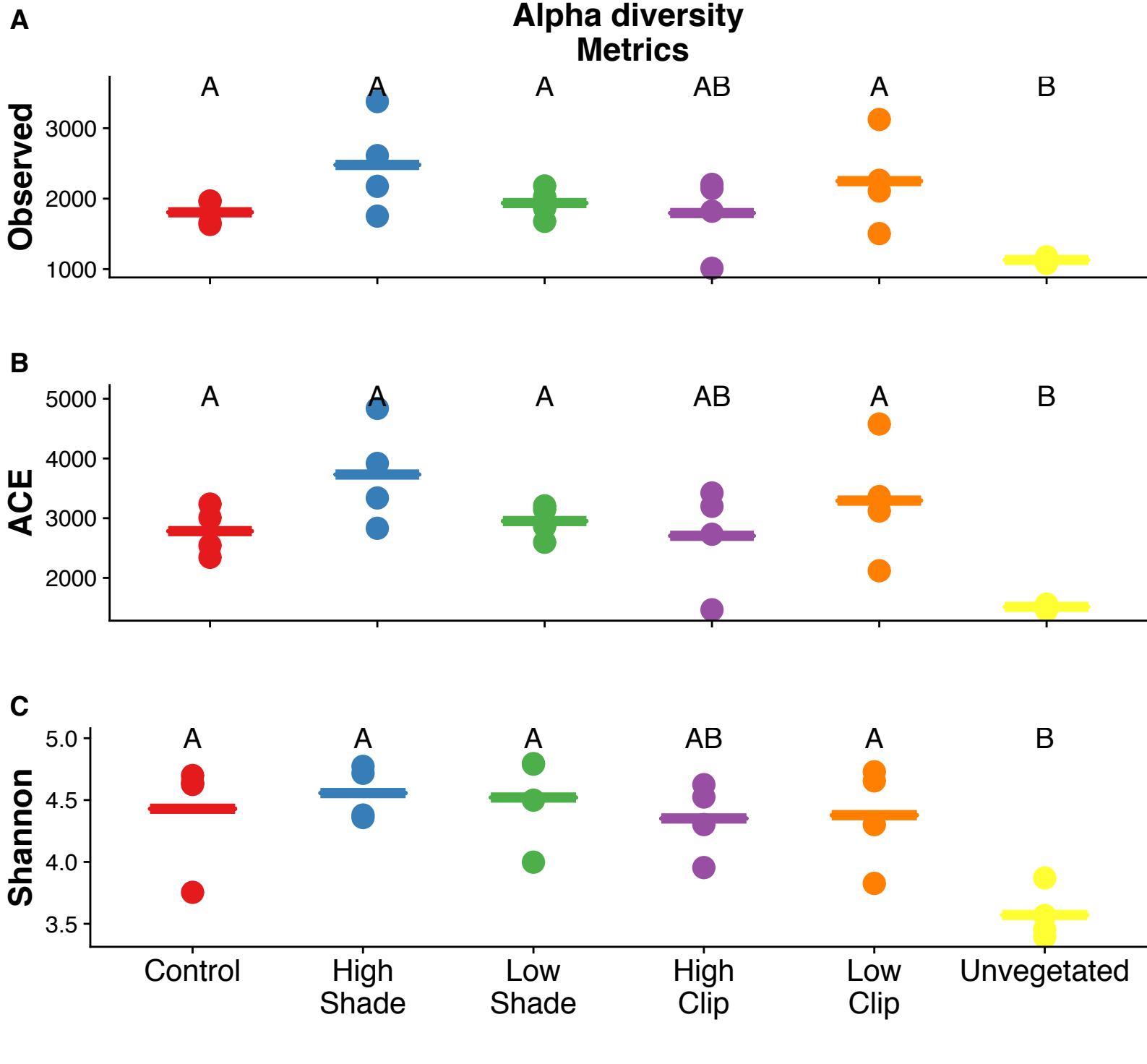

Unvegetated
Low Clip
High Clip
Low Shade
High Shade
Control
Treatment
0%
25%
50%
75%
100%
Relative abundance
Copepoda
Gastrotricha
Gastropoda
Nematoda
Other
Platyhelminthes
Polychaeta

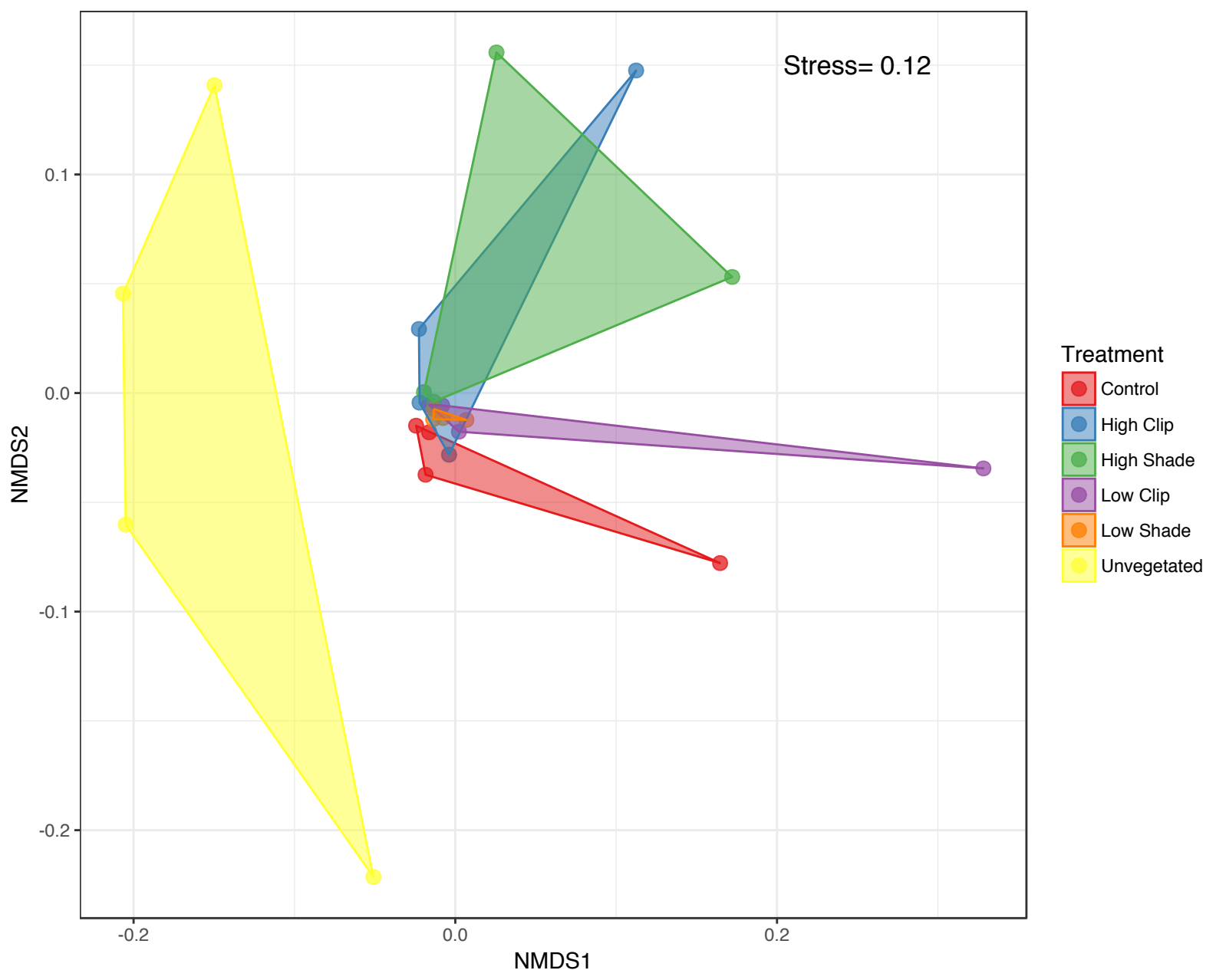
B

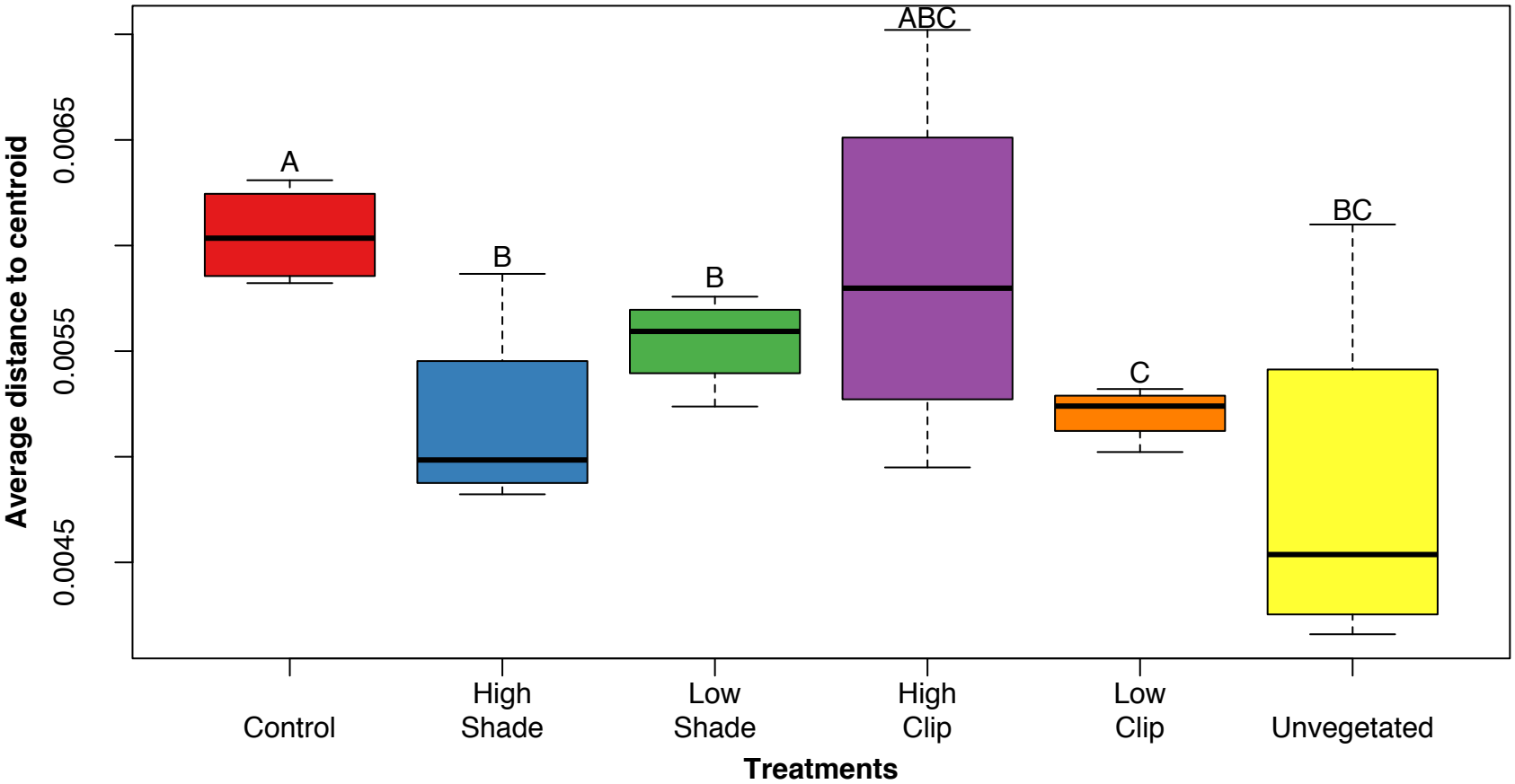

Unvegetated
Low Clip
High Clip
Low Shade
High Shade
Control
Treatment
0%
25%
50%
75%
100%
Relative abundance
Araeolaimida
Chromadorida
Desmodorida
Desmoscolecida
Enoplia
Haliplectidae
Monhysterida
Rhabditida
Tylenchida
Enoplida
Triplonchida
Unclassified

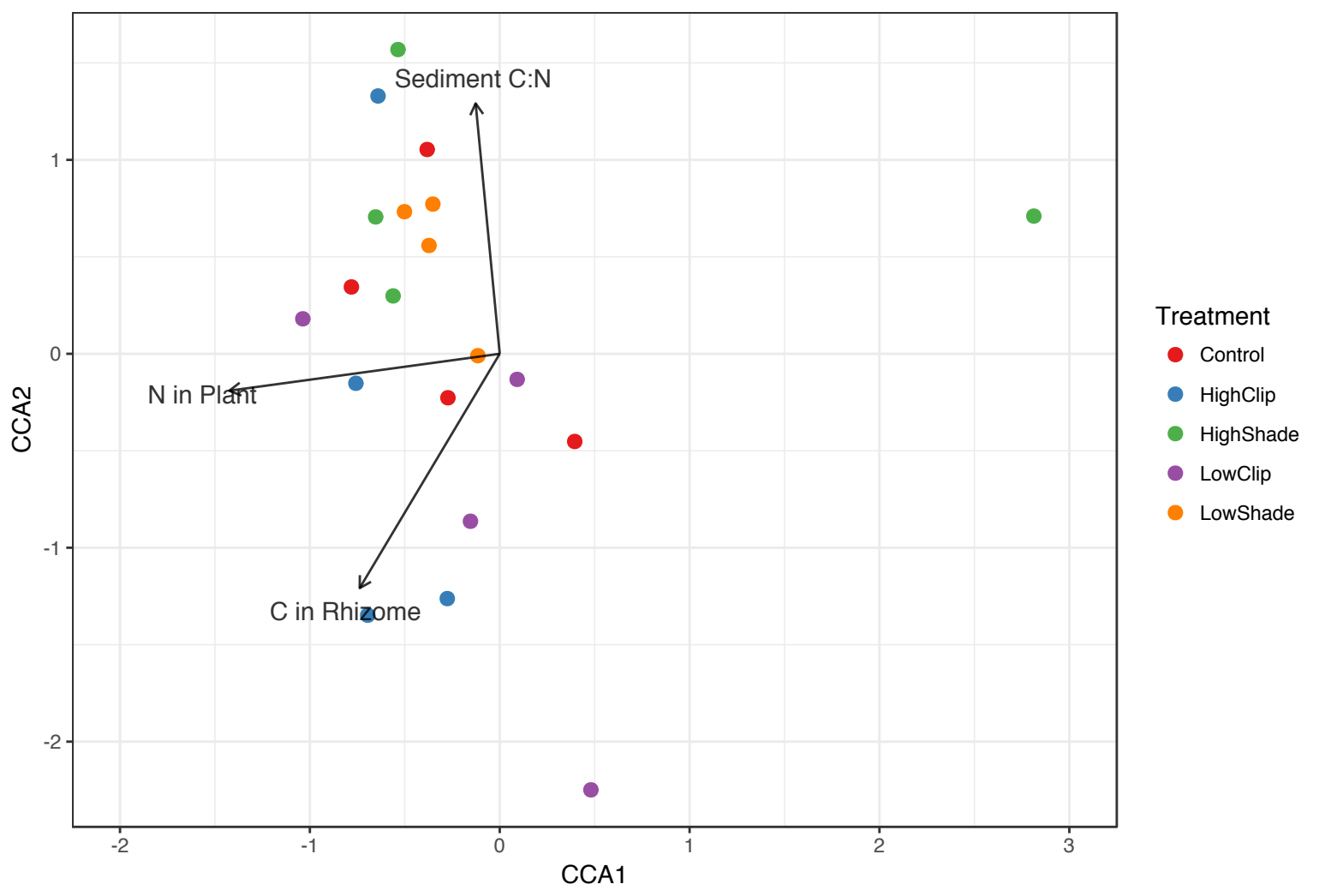
C

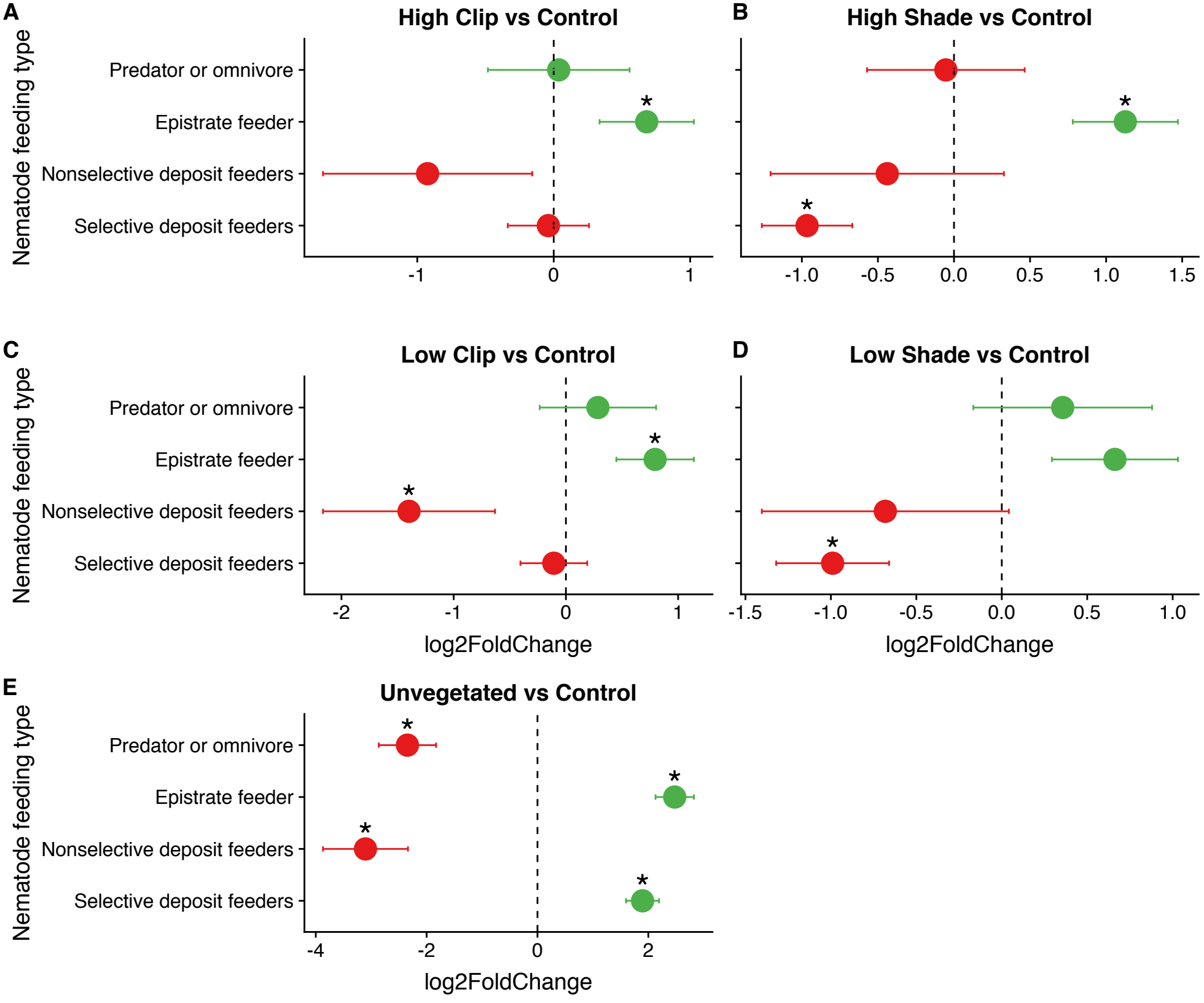

Unvegetated
Low Clip
High Clip
Low Shade
High Shade
Control
Treatment
0%
25%
50%
75%
100%
Relative abundance
Acanthopharynx
Achromadora
Anoplostoma
Anticoma
Axonolaimus
Bathylaimus
Camacolaimus
Catanema
Ceramonema
Chromadoridae
Chromadorita
Cylindrolaimus
Daptonema
Desmodora
Desmolaimus
Desmoscolex
Diplolaimella
Diplolaimelloides
Dolicholaimus
Dorylaimopsis
Dracograllus
Draconema
Epsilonema
Eumonhystera
Halalaimus
Halicephalobus
Laxus
Leptolaimus
Metateratocephalus
Meyersia
Molgolaimus
Monhysteridae ambiguous
Noctuidonema
Nudora
Odontophora
Onchium
Oncholaimus
Paracyatholaimus
Paradraconema
Paralamyctes
Paraphanolaimus
Pareurystomina
Phanoderma
Pseudoncholaimus
Ptycholaimellus
Punctodora
Sabatieria
Schizomidae
Setostephanolaimus
Sphaerolaimus
Spilophorella
Spirinia
Teratocephalus
Terschellingia
Thalassoalaimus
Theristus
Trissonchulus

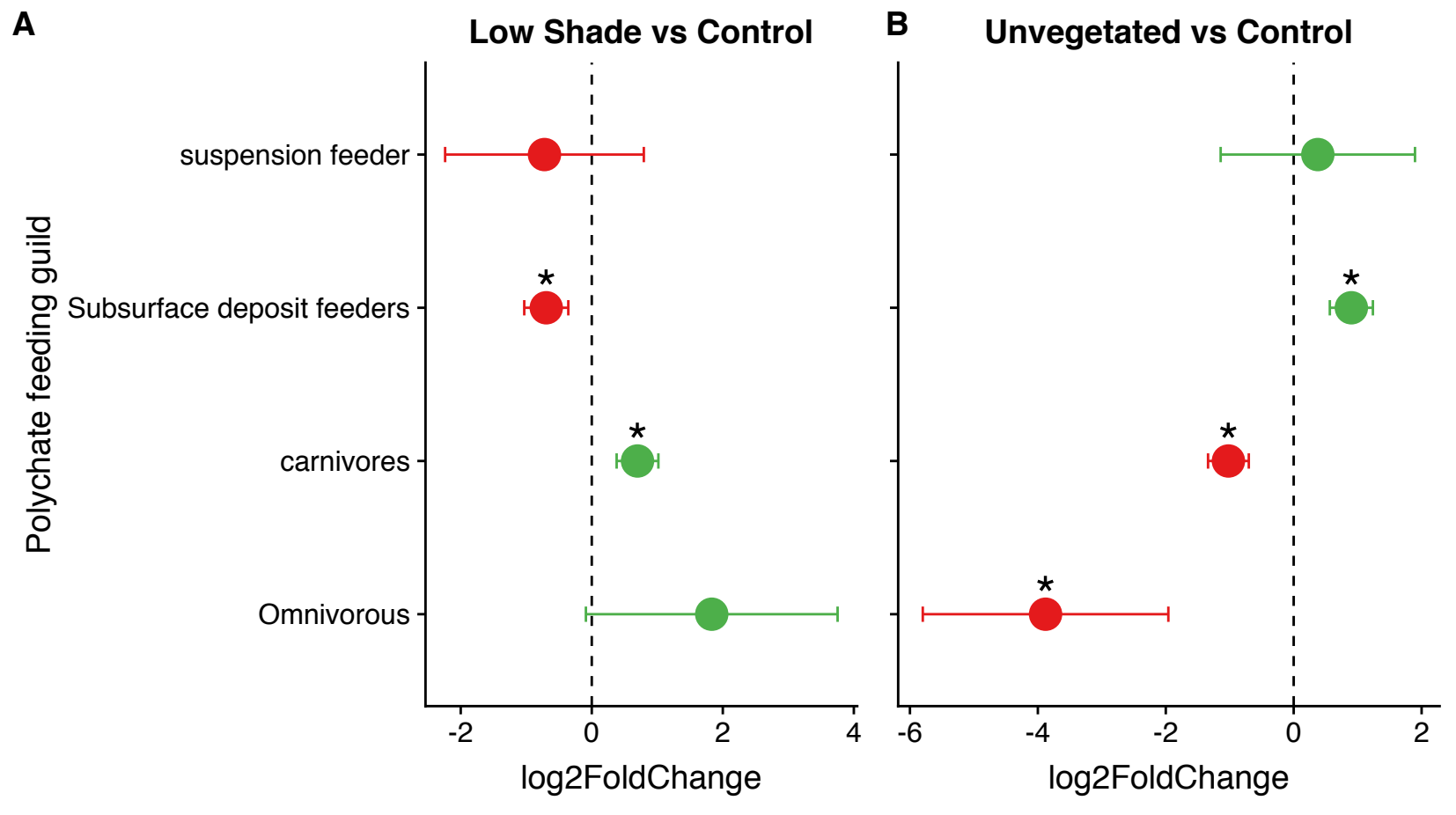

A
Alpha diversity Metrics
Observed
3000
2000
1000
A
A
A
AB
A
B
B
ACE
5000
4000
3000
2000
A
A
A
AB
A
B
C
Shannon
5.0
4.5
4.0
3.5
A
A
A
AB
A
B
Control
High Shade
Low Shade
High Clip
Low Clip
Unvegetated

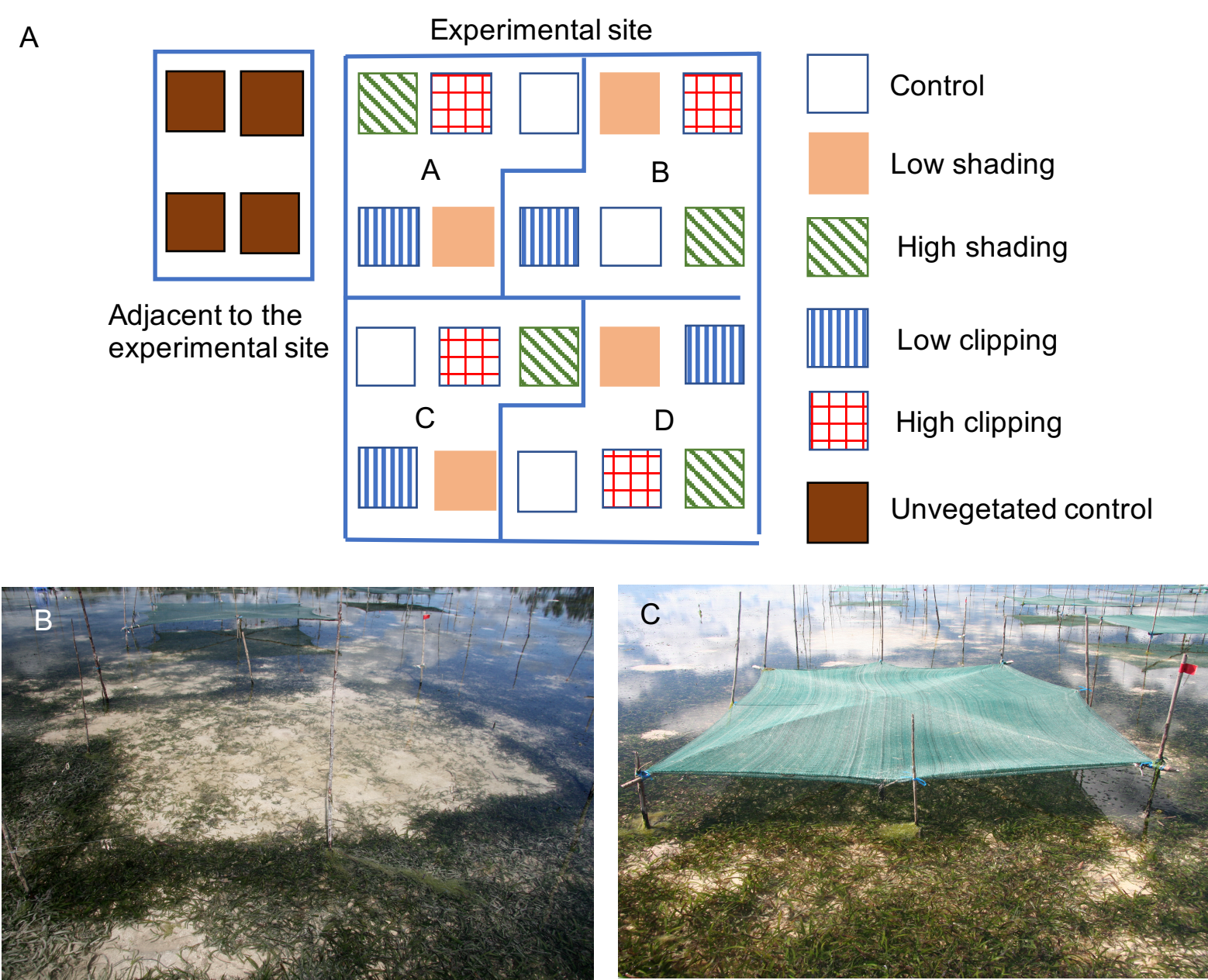

Stress= 0.12
NMDS2
NMDS1
0.1
0.0
-0.1
-0.2
-0.2
0.0
0.2
Treatment
Control
High Clip
High Shade
Low Clip
Low Shade
Unvegetated


Average distance to centroid
0.0045
0.0055
0.0065
A
B
B
ABC
C
BC
Control
High Shade
Low Shade
High Clip
Low Clip
Unvegetated
Treatments


Sediment C:N
N in Plant
C in Rhizome
CCA1
CCA2
-2
-1
0
1
2
3
Treatment
Control
HighClip
HighShade
LowClip
LowShade


A
High Clip vs Control
B
High Shade vs Control
C
Low Clip vs Control
D
Low Shade vs Control
E
Unvegetated vs Control
Nematode feeding type
Predator or omnivore
Epistrate feeder
Nonselective deposit feeders
Selective deposit feeders
log2FoldChange


A
Low Shade vs Control
B
Unvegetated vs Control
Polychate feeding guild
suspension feeder
Subsurface deposit feeders
carnivores
Omnivorous
*
-2
0
2
4
-6
-4
-2
0
2
log2FoldChange
log2FoldChange


A
Experimental site
Adjacent to the experimental site
A
B
C
D
Control
Low shading
High shading
Low clipping
High clipping
Unvegetated control
B
C