

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

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

4

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16

17 **Abstract**

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

31

32 **Introduction**

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

41 considerable attention <sup>1,2</sup>, much remains unknown about such interrelationships in marine  
42 coastal systems.

43                 Similar to terrestrial ecosystems, plants in marine habitats provide a highly  
44 complex spatial environment with several niches for different species <sup>5</sup>. Seagrasses are an  
45 example of such plant communities that encompass some of the most productive habitats in  
46 marine ecosystems <sup>6</sup>, providing a number of high-value ecosystem services <sup>7</sup>. Marine plant  
47 species are recognized to be autogenic ecosystem engineers shaping the shallow coastal  
48 environment through multiple and complex pathways <sup>8</sup>. The physical structures of seagrasses  
49 can modify local hydrodynamics and sedimentary habitats, thereby having a large controlling  
50 effect on subsurface environments by altering sediment granulometry, stabilizing sediments,  
51 storing atmospheric CO<sub>2</sub>, trapping detritus and providing a wide range of food sources that  
52 support a high diversity of consumers <sup>9</sup>.

53                 The abundance and diversity of below-surface metazoan consumers in marine  
54 sediments is dominated by meiobenthos (microscopic benthic invertebrates between 0.04 and  
55 1 mm in size) <sup>10</sup>. Meiobenthic communities play an important role in benthic ecosystem  
56 processes <sup>11-13</sup>. In seagrass beds, meiobenthos are often characterized by high densities and  
57 biomass, possessing short life cycles and high turnover rates <sup>14</sup> that often translate into high  
58 secondary production <sup>15</sup>. Although the importance of seagrasses for epiphytic invertebrate  
59 biodiversity (invertebrates associated with seagrass blades and leaves) has been well  
60 documented <sup>16</sup>, their effects on the meiobenthos in the sediment are not as well understood <sup>17-</sup>  
61 <sup>19</sup>, in part due to the practical difficulties in large scale studies focusing on a taxonomically  
62 hyperdiverse groups such as meiobenthos <sup>20</sup>. The application of high-throughput sequencing  
63 (HTS) approaches to the study of meiobenthos can considerably improve our understanding  
64 of the ecological patterns and environmental drivers of biodiversity in marine sediments <sup>21,22</sup>,

65 including in seagrass beds, by allowing biodiversity assessments of microscopic metazoans at  
66 a scale and with coverage previously unfeasible<sup>20</sup>. 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<sup>23</sup>. As a result, seagrass habitats have been declining worldwide due to  
71 anthropogenic activity<sup>24</sup>. Increased eutrophication, and sedimentation, resulting in light  
72 reduction and decreased photosynthesis, are among the principal anthropogenic disturbances  
73 to seagrass ecosystems<sup>25</sup>. Light reduction has multiple negative effects on seagrass plants,  
74 spanning from reduced growth and loss of biomass<sup>26</sup> to lower carbohydrate storage in plant  
75 rhizomes<sup>27,28</sup>. 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)<sup>29</sup> can disturb the balance between  
78 herbivory and seagrass production and potentially induce cascading effects<sup>30</sup> in these  
79 ecosystems. Although, grazing is a vital process for controlling fast-growing epiphytic algae  
80 in eutrophic systems<sup>31</sup>, release of grazers like sea urchins from predation can provoke intense  
81 grazing events that consume considerable amounts of seagrass above-surface biomass<sup>32,33</sup>.  
82 High densities of sea urchins and consequent overgrazing of seagrasses have been more  
83 frequently reported in the last few decades<sup>32,33</sup> and can have enduring impacts on above-  
84 ground seagrass biomass<sup>32</sup>, with potential important knock-on effects for sediment properties  
85<sup>34</sup> 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-

89 induced changes on plant above and below ground biomass affect meiobenthic communities.  
90 Such an understanding is crucial to predict future impacts on marine ecosystem structure and  
91 function<sup>35</sup>.  
92 Here, we address this important knowledge gap with a 5-month field experiment where we  
93 manipulated seagrass plots in a *Thalassia hemprichii* meadow and used HTS to assess  
94 impacts of shading and simulated grazing on meiofauna species richness and evenness metrics  
95 (alpha-diversity); variations in meiofauna community composition (beta-diversity) following  
96 the framework described by Anderson et al<sup>36</sup>, and lastly nematode and polychaete trophic  
97 structure. The seagrass plot manipulations included two independent variables (shading and  
98 clipping) each with two levels (high and low). We used shading to mimic the effects of  
99 reduced light availability to seagrasses due to eutrophication and/or sedimentation, and  
100 simulated a high intensity grazing event due to herbivores being released from predation.  
101 Herbivory was simulated by clipping of shoots to mimic two different levels of grazing  
102 pressure. The design was used to test the following two hypotheses: shading causes a reduced  
103 seagrass root- and rhizome biomass with potential feedback effects on meiobenthic diversity,  
104 community, and trophic structure; and secondly, continued grazing causes a decrease of  
105 seagrass above-ground biomass that leads to a reduction in sediment stability and intensified  
106 erosion of the sediment surface, also with indirect effects on meiobenthic diversity,  
107 community and trophic structure. Our findings indicates that disturbance of *T. hemprichii*  
108 meadows can substantially change meiobenthic community composition and trophic structure  
109 of nematodes and polychaetes in coastal ecosystems.

110

111

112 **Results**

113

114 HTS data output

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

123 Taxonomic composition

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

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

138 (PERMANOVA, pseudo-  $F_{5,18} = 13.9$ ,  $p = 0.028$ ), while the opposite was found for copepods  
139 (PERMANOVA, pseudo-  $F_{5,18} = 4.9$ ,  $p = 0.03$ ). Within the nematodes, there were differences  
140 among treatments in relative abundances of its taxa (Fig. 1-B and 1-C). CTRL presented a  
141 significantly higher relative abundance of nematodes belonging to the order Monhysterida  
142 than in unvegetated plots (PERMANOVA, pseudo-  $F_{5,18} = 8.6$ ,  $p = 0.029$ ) and Chromadorida  
143 (PERMANOVA, pseudo-  $F_{5,18} = 4.8$ ,  $p = 0.027$ ). On the other hand relative abundances of  
144 Desmodorida nematodes were significantly lower in the CTRL when compared to the  
145 unvegetated treatment (PERMANOVA, pseudo-  $F_{5,18} = 31$ ,  $p = 0.029$ - Fig. 1-B). At the  
146 nematode genus level there was a conspicuous difference in dominance between the seagrass  
147 plots (CTRL, HS, LS, HC and LC) and the Unvegetated treatments. While the former were  
148 dominated by *Molgolaimus* and Monhysterids nematodes (PERMANOVA, pseudo-  $F_{5,18}$   
149  $= 6.1$ ,  $p = 0.002$ , pseudo-  $F_{5,18} = 29$ ,  $p = 0.001$ , respectively) the latter treatment was dominated  
150 by nematodes of the genus *Catanema* (PERMANOVA, pseudo-  $F_{5,18} = 64.3$ ,  $p < 0.001$ - Fig. 1-  
151 C)

#### 152 Differences among treatments in alpha diversity

153           Alpha diversity metrics showed the same general trend for all three metrics we  
154 analysed: Observed number of unique OTUs and the ACE and Shannon index (Fig. 2). There  
155 was a significant effect of treatment on observed unique OTUs (PERMANOVA, pseudo-  $F_{5,18}$   
156  $= 3.9$  ;  $p = 0.01$ ) which was lower in Unvegetated than in any other treatments, except HC. No  
157 additional significant differences in observed unique OTUs were found between the  
158 manipulated seagrass treatments (HC, HS, LC and LS) and the CTRL. The same pattern and  
159 effect of treatment was seen for ACE (PERMANOVA, pseudo-  $F_{5,18} = 4.8$ ;  $p = 0.003$ ) and  
160 Shannon indexes (PERMANOVA, pseudo-  $F_{5,18} = 4.6$ ;  $p = 0.01$ ). Again, both these metrics  
161 were significantly lower in the Unvegetated treatment but not in any of the pairwise

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

163

#### 164 Meiofauna beta-diversity differences among treatments

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

185 Regarding the relationship between meiofaunal community structure and

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

196

#### 197 Trophic composition of nematodes and polychaetes

198           The abundance of trophic groups of nematodes and polychaetes were different  
199 among treatments. With regards to the nematodes, there was a significant effect of shading on  
200 the abundance of OTUs with taxonomic assignments corresponding to selective deposit  
201 feeder nematodes. The abundances of these OTUs were significantly higher in both the HS  
202 and in the LS treatment than in the CTRL (Fig. 6-B and 6-D,  $p_{(DESeq2)} = 0.0008$  and  $p_{(DESeq2)} =$   
203  $0.001$ , for LS vs CTR and HS vs CTRL, respectively). Conversely, the abundance of OTUs  
204 of epistrate feeder nematodes were lower in the two shading treatments than in the controls,  
205 but this difference was only significant for the HS treatment (Fig. 6-B and 6-D,  $p_{(DESeq2)} =$   
206  $0.055$  and  $p_{(DESeq2)} = 0.001$  LS vs CTR and HS vs CTRL, respectively ). Significant effects of  
207 clipping on nematode trophic structure were also observed. The abundance of epistrate feeder  
208 nematode OTUs were significantly less abundant in both clipping treatments (LC and HC)  
209 than in the CTRL ( Fig. 6-A and 6-C,  $p_{(DESeq2)} = 0.021$  and  $p_{(DESeq2)} = 0.044$  for LC vs CTR

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

218           Significant differences among treatments were also seen in the assessment of the  
219 polychaete feeding guilds. As found for nematode feeding groups, shading significantly  
220 increased the abundance of OTUs of deposit feeders polychaete when compared to the CTRL,  
221 but this difference was only significant for LS, (Fig. 7-A,  $p_{(DESeq2)}= 0.038$ ). In addition,  
222 significantly fewer OTUs of carnivore polychaetes were found in LS compared to the CTRL  
223 (Fig. 7-A,  $p_{(DESeq2)} =0.028$ ). No other significant differences were found between the CTRL  
224 and the remaining manipulated seagrass treatments (HS, HC and LC). Conversely, all  
225 polychaete feeding guilds analyzed here, with the exception of suspension feeders were  
226 significantly different in the unvegetated treatment when compared to the CTRL (Fig. 7-B).  
227 The Unvegetated plots had less OTUs of subsurface deposit feeders ( $p_{(DESeq2)}= 0.007$ ) and  
228 higher abundances of OTUs in the carnivore ( $p_{(DESeq2)}= 0.014$ ) and omnivorous ( $p_{(DESeq2)}=$   
229 0.043) feeding guilds when compared to the CTRL.

230

## 231 **Discussion**

232

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

246 Lower biomass and photosynthetic activity as a result of reduced light availability will cause  
247 a lower transport of oxygen from the shoots to the roots, decreasing “radial oxygen loss  
248 (ROL)” from the root-tips and thereby reduce the oxygenation of the sediment<sup>41</sup>. Reduction  
249 in photosynthetic rates can also lead to higher H<sub>2</sub>S levels in the sediments of disturbed  
250 seagrass meadows<sup>41,42</sup>. Both lower oxygen conditions and increased H<sub>2</sub>S concentrations in  
251 sediments have the potential to change meiofauna diversity and community composition<sup>43,44</sup>.

252 In addition, photosynthetically derived dissolved organic carbon (DOC) has been shown to  
253 greatly stimulate the activity of microorganisms around *T. hemprichii* roots when it is  
254 transported to belowground tissue and excreted from the root system<sup>45</sup>. Both disturbances  
255 here tested shading and clipping probably reduced the amount of DOC extruded from the  
256 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<sup>28</sup>, 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<sup>10</sup>. 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<sup>15,46</sup> and  
277 in other coastal ecosystems<sup>47</sup>. 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 *Chla* 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<sup>34</sup>. 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<sup>34</sup>. 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<sup>48</sup>. 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<sup>49,50</sup>. 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<sup>51</sup>. 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<sup>52</sup>. 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<sup>34</sup> 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<sup>46</sup> 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<sup>34</sup>. 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<sup>53</sup>,  
325 which is particularly relevant in our experimental area characterized by large tides and strong  
326 wave action<sup>54</sup>. 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<sup>55</sup>. 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-<sup>5</sup> and meiobenthos<sup>17</sup>. 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<sup>34</sup> 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<sup>56,57</sup> but regarding the less studied meiobenthos, the available  
363 literature shows contrasting results<sup>17 and references therein</sup>. For example, Arrivillaga & Baltz<sup>58</sup>  
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<sup>59,60</sup>. 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<sup>17,18,61</sup>. Our results suggest  
372 that this habitat modulation by seagrasses influenced nematode community composition.  
373 Unvegetated sediments were dominated by Desmodorida, particularly of the genus  
374 *Catanema* that seem to find unstable fluid sediments in unvegetated areas advantageous<sup>14,18</sup>.  
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<sup>18,19</sup>. *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<sup>18</sup>. 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<sup>62</sup>. 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<sup>28,34</sup>. 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<sup>34</sup>. Since human-induced disturbances are increasing the  
393 rate of seagrass bed habitat degradation<sup>63</sup> 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<sup>10,35</sup> and mediate vital benthic ecosystem function<sup>11,13</sup>, prolonged disturbances of seagrass

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

## 402 **Methods**

### 403 Study area and experimental setup

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

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

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

435

#### 436 Sediment sampling, sample preparation and sequencing

437           After 5 months at the termination of the experiment the sediment of each of the  
438 24 replicate plots was sampled with six handheld Perspex sediment cores taken from the exact  
439 same location within each of the plots. The handheld sampling units were 45 mm diameter  
440 with a surface area of  $17 \text{ cm}^2$ , a size suitable for sampling of microbial benthic metazoans  
441 such as meiofauna<sup>66,67</sup>. The top 3 cm of each core were sliced and sieved through 500  $\mu\text{m}$  and  
442 40  $\mu\text{m}$  stacked sieves, pooled and preserved in 20% DESS before storage at 4°C. After two  
443 weeks the sediment and animals were again placed in a 40  $\mu\text{m}$  sieve and rinsed thoroughly in  
444 filtered artificial saltwater (salinity 34) close to *in situ* salinity to remove the DESS. The  
445 meiofauna individuals were isolated and separated from the sediment particles using density  
446 extraction by washing the content of the 40  $\mu\text{m}$  sieve into a 500-mL E-flask with LevasilH  
447 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.<sup>11</sup>. 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 °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.<sup>68</sup> (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

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

#### 476 Bioinformatics

477            Amplicon reads were demultiplexed by the sequencing facility, followed by  
478 initial data processing and quality-filtering in the QIIME 1.9.1 pipeline <sup>69</sup>. Paired-end  
479 Illumina reads were overlapped and merged using the join\_paired\_ends.py script in QIIME,  
480 followed by quality-filtering of raw reads using the multiple\_split\_libraries\_fastq.py script  
481 with a minimum Phred quality score of 19. Unmerged (orphan) Illumina read pairs were  
482 discarded, and excluded from all downstream data analysis steps. PCR primer sequences were  
483 subsequently trimmed from merged reads using Trimmomatic version 0.32 <sup>70</sup>(parameters used  
484 were ILLUMINACLIP:2:30:10, with all other parameters as default). Trimmed, merged reads  
485 which passed all quality-filtering steps were next subjected to open-reference OTU picking  
486 using a 96% pairwise identity cutoff, using the pick\_open\_reference\_otus.py script in QIIME  
487 1.9.1 (using the uclust algorithm with 10% subsampling, no prefiltering, and reverse strand  
488 match enabled). All resulting singleton OTUs were excluded from the resulting OTU table  
489 outputs. Taxonomy was assigned to representative OTU sequences with the RDP Classifier <sup>71</sup>  
490 in QIIME (assign\_taxonomy.py with a confidence threshold of 0.7), using the SILVA 119  
491 release as a reference database <sup>72</sup>. OTU representative sequences were aligned with PYNAST  
492 <sup>73</sup> using the align\_seqs.py script.

493

#### 494 Statistics and Reproducibility

495                   The resulting OTU table and correspondent metadata set was imported into R v  
496 3.4.3 and analysed using the *phyloseq*<sup>74</sup> and *vegan*<sup>75</sup> packages. The effect of both shading  
497 and clipping on alpha diversity metrics (observed OTUs, ACE index and Shannon index) and  
498 relative abundance of meiofauna taxonomic groups were tested with one-way PERMANOVA  
499 with the PAST 3.24<sup>76</sup>. Statistical significance was defined at  $\alpha=0.05$  to cover all analyses.

500                   Community composition was examined by first selecting and filtering metazoan  
501 OTUs and sub-sampling the OTUs counts to the lowest sample size (66 754 counts) with the  
502 *rarefy\_even\_depth* function in *pyloseq*. After Hellinger transformation, the dissimilarity  
503 between faunal assemblages in the different treatments was analysed by non-metric  
504 multidimensional scaling (NMDS), using the *altGower* distance<sup>77</sup>, and by Principal  
505 Coordinates Analysis (PCoA) with UniFrac distance. To statistically test for the effects of  
506 treatment on community composition, we conducted a permutational multivariate analysis of  
507 variance (PERMANOVA) with the *adonis* function of the *vegan* package. The function  
508 *pairwise.perm.manova* of the *RVAideMemoire* package<sup>78</sup> was used to perform pairwise  
509 comparisons between CTRL and the remaining treatments in terms of differences in  
510 community composition. To examine differences in beta-diversity among treatments we used  
511 the community beta-diversity index<sup>36</sup> that is based on community OTU dissimilarity metrics  
512 and measured as average distance of each observation to the group centroid, using the  
513 *betadisper* function in the *vegan* package<sup>75</sup>. Pairwise differences between treatments in  
514 average distance to the group centroid were checked with the *permutest.betadisper* of the  
515 *betadis* object that permutes model residuals and generates a permutation distribution of F  
516 with the null hypothesis that there is no difference in dispersion between groups. Furthermore,  
517 metrics to partition beta-diversity were utilized to calculate the relative importance of  
518 turnover and nestedness in the different treatments<sup>79</sup>. 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*<sup>79</sup> for this analysis. Additionally, a BIOENV ('biota-environment') analysis  
522<sup>80</sup> 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.<sup>34</sup> and Deyanova et al.<sup>28</sup>, 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 Dahl et al.<sup>34</sup> and Deyanova et al.<sup>28</sup>. 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<sup>81,82</sup>. These 644 OTUs were categorized

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

548 Furthermore, we investigated treatment related changes in the trophic structure  
549 of polychaetes, by subsetting the polychaete OTUs taxonomically assigned to Family (total  
550 870 OTUs) and classifying them to relevant trophic guilds (eg deposit feeders, omnivore,  
551 herbivore or predators) following Jumars<sup>84</sup>.

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

558

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768

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779 **Authors' contributions**

780 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.  
781 conducted the experiment and sampled in the field. F.J.A.N. conducted the laboratory work  
782 and analysed the data; T.S. and T.J.P. provided with bioinformatics and, with help from  
783 H.M.B. F.J.A.N. wrote the manuscript with contributions from M.D., H.M.B. M.B, S.C. and  
784 M.G and comments by D.D., L.D.L.

785 **Competing interests**

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

787 **Data Accessibility**

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

790

791 Fig. 1- Stacked bars of the average relative abundances of 18S rRNA gene for meiobenthos in  
792 the different treatments, n=4 biologically independent samples. The y-axis shows the  
793 treatments, and x-axis shows relative abundance (%) of Metazoa phyla (A); order in the  
794 Nematoda (B); and genus in the Nematoda (C).  
795  
796  
797 Fig. 2- Alpha-diversity metrics for meiobenthos in the different treatments. Figure panels  
798 show: number of observed unique OTUs (A), ACE index (B) and Shannon index (C). Central  
799 bars represent the mean of each treatment. Different letters indicate statistically significant  
800 differences (PERMANOVA,  $p < 0.05$ ) based on n=4 biologically independent samples.  
801  
802 Fig. 3- Plot of the non-metric multidimensional scaling (NMDS) analysis based on  
803 normalized OTU matrix for meiobenthos using altGower dissimilarities . Different  
804 colours represent the groupings of the different treatments.  
805  
806 Fig.4- Meiobenthic community  $\beta$ -diversity index showing the average distance from group  
807 centroid to each observation, n=4 biologically independent samples. Different letter codes  
808 indicate statistically significant differences (PERMDISP,  $p < 0.05$ ).  
809  
810 Fig. 5- Canonical correspondence analysis (CCA) biplot showing the co-variant relationship  
811 between significant non-correlated environmental factors (See methods) and meiobenthic  
812 community structure. Arrows are vectors representing the correlation between environmental  
813 variables and the axes.  
814  
815 Fig. 6- OTU abundance of nematode feeding types in the different treatments in relation to the  
816 Controls: High Clip (A), High Shade (B), Low Clip (C) and Low Shade (D), Unvegetated (E).  
817 The x axis shows the  $\log_2$  fold changes of the four different nematode feeding types (y axis)  
818 calculated by the DESeq2 adjusted base mean (see Methods). A  $\log_2$  fold change of  $>0$  (green)  
819 indicate that abundance was higher in the Control than in the respective manipulated  
820 treatment, while a  $\log_2$  fold change of  $<0$  (red) indicates that abundance was lower in the  
821 Control than in the respective manipulated treatment. White asterisks show cases when  
822 differences were statistically significant ( $p_{(DESeq2)} < 0.05$ ) and error bars represent SE, n=4  
823 biologically independent samples.  
824  
825 Fig. 7- OTU abundance of polychaete feeding guilds in the different treatments in relation to  
826 the Controls: Low Shade (A) and Unvegetated (B). The x axis shows the  $\log_2$  fold changes of  
827 the four different nematode feeding types (y axis) calculated by the DESeq2 adjusted base  
828 mean (see Methods). A  $\log_2$  fold change of  $>0$  (green) indicates that abundance was higher in  
829 the Control than in the respective manipulated treatment, while a  $\log_2$  fold change of  $<0$  (red)  
830 indicates that abundance was lower in the Control than in the respective manipulated  
831 treatment. White asterisks denote cases when differences were statistically significant  
832 ( $p_{(DESeq2)} < 0.05$ ) and error bars represent SE, n=4 biologically independent samples. No other  
833 differences were detected between CTRL and the remaining manipulated seagrass treatments  
834  
835 Fig. 8- Experimental approach. (a) Experimental approach displaying the randomized  
836 complete block design in our study. Different patterns correspond to the different  
837 experimental treatments (four biologically independent replicates per treatment). Letters

838 represent replicate blocks. (b) High Shading treatment, (c) High Clipping treatment. Photos  
839 by Martin Gullström  
840

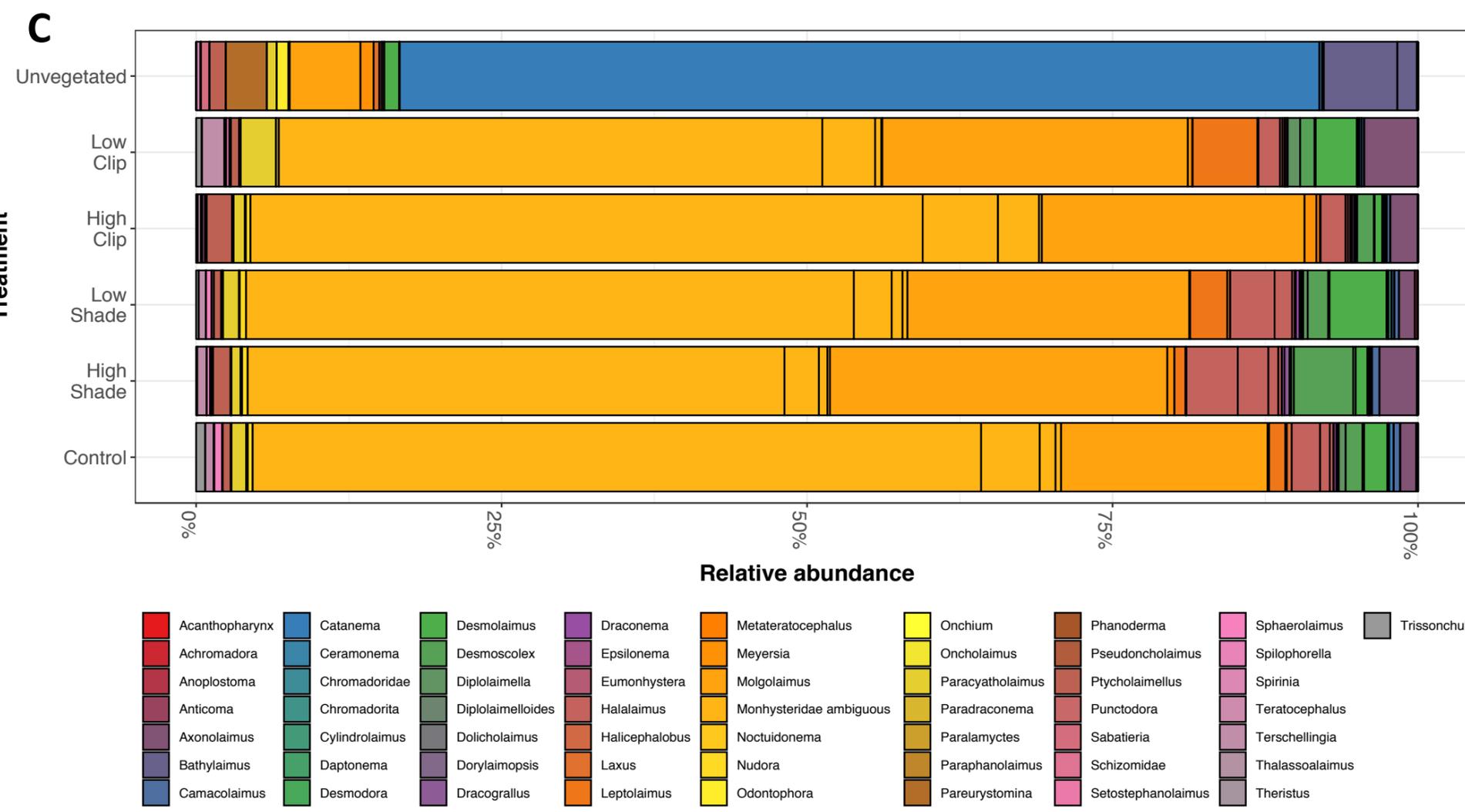
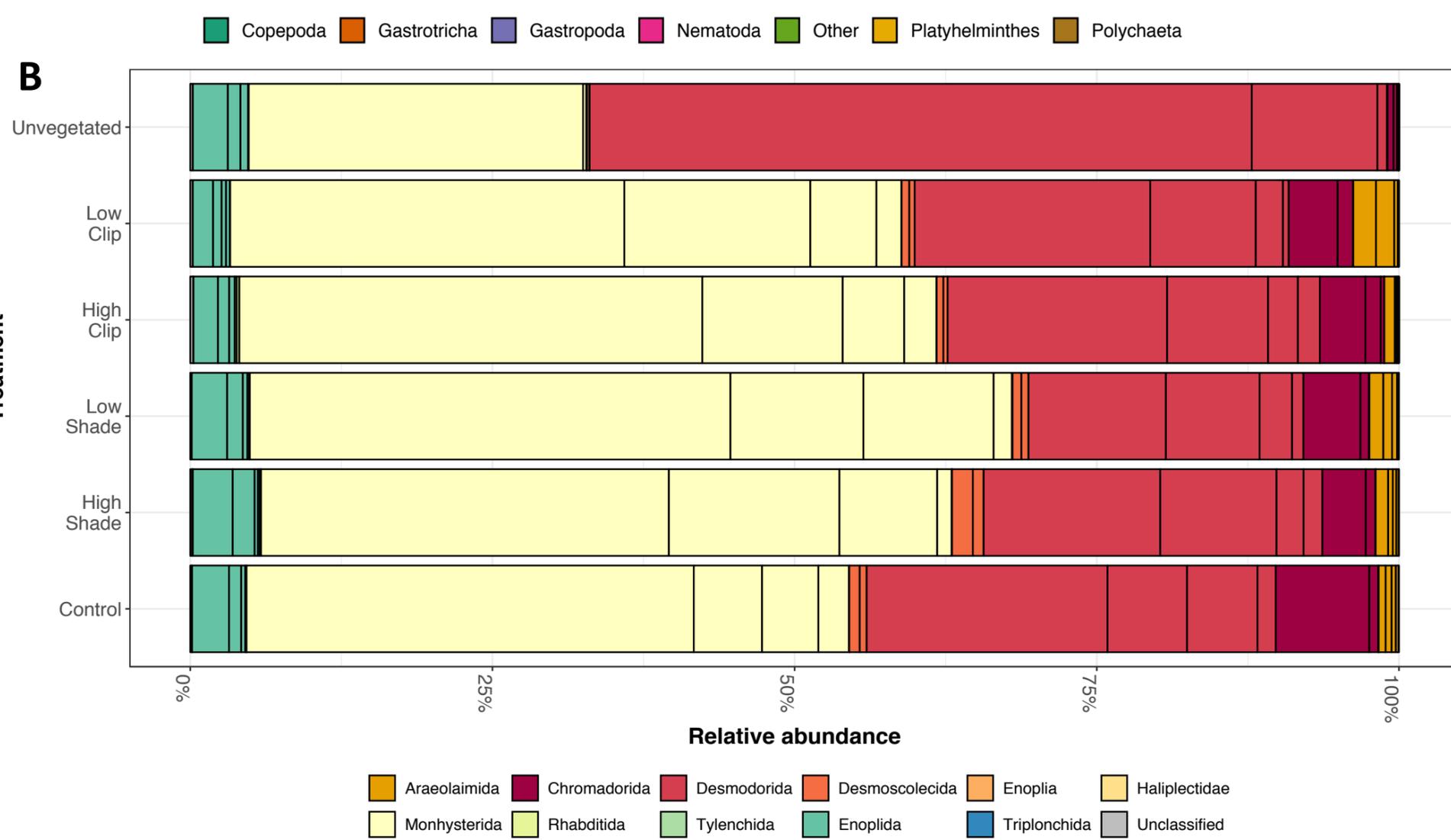
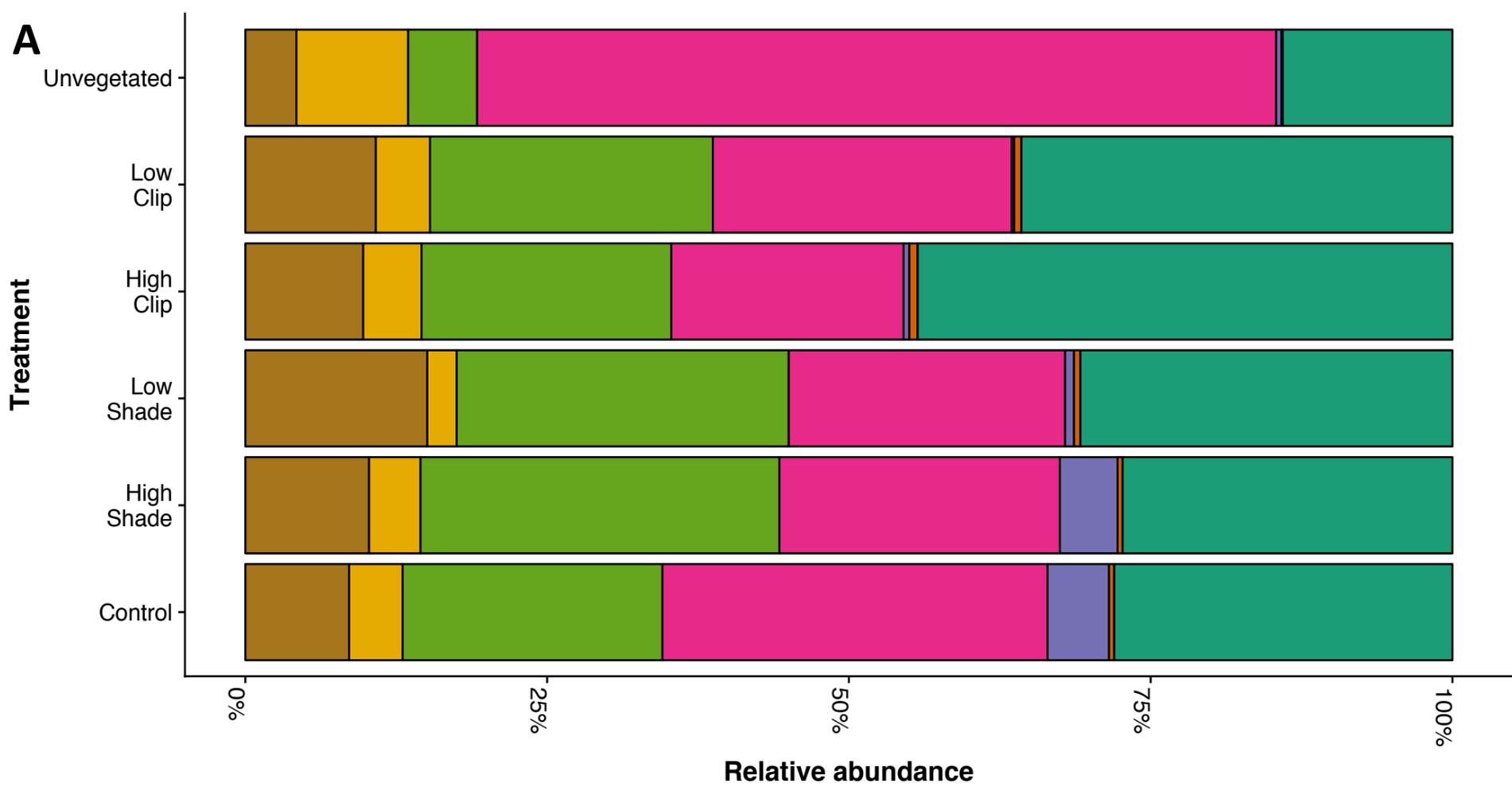
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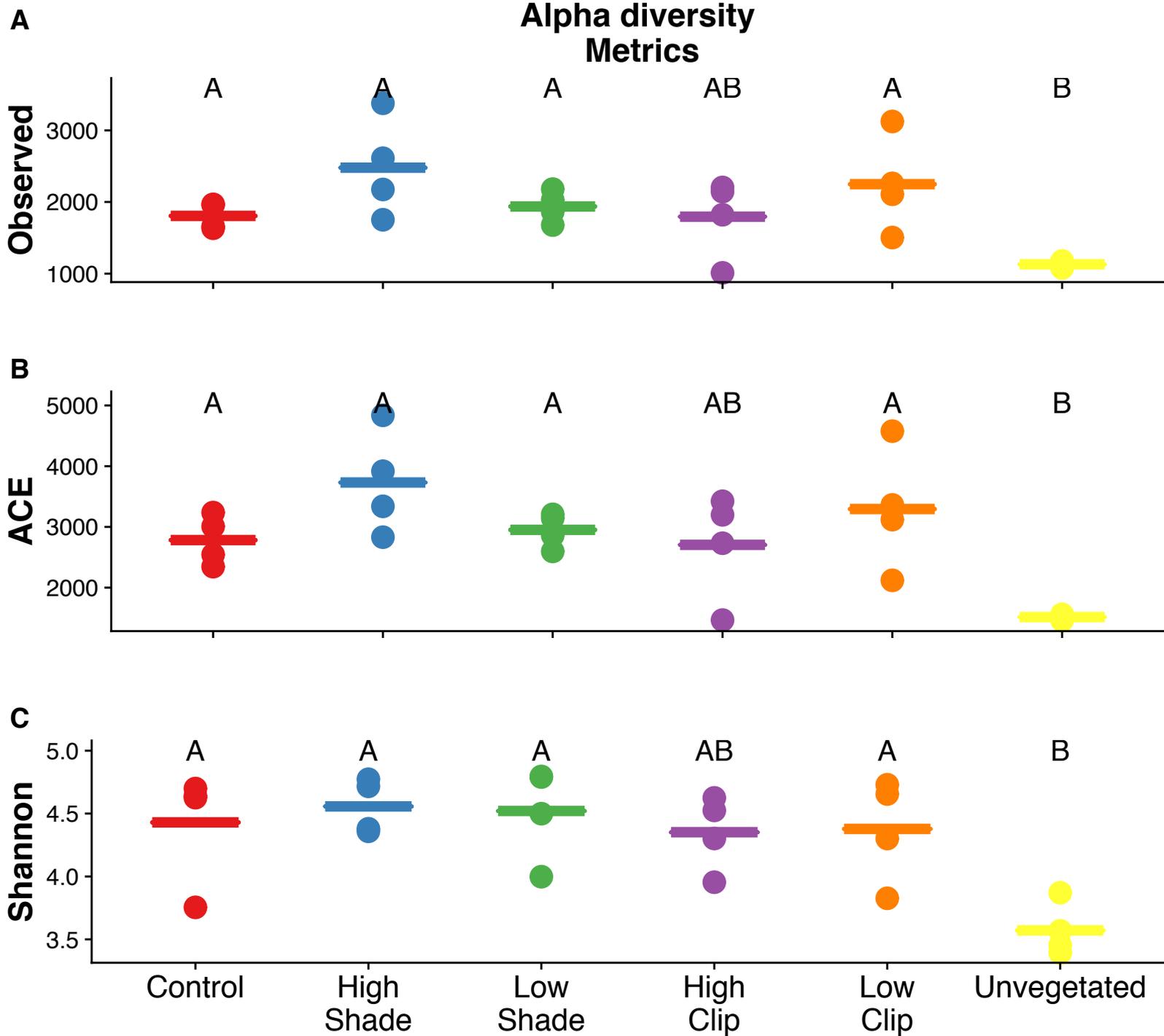
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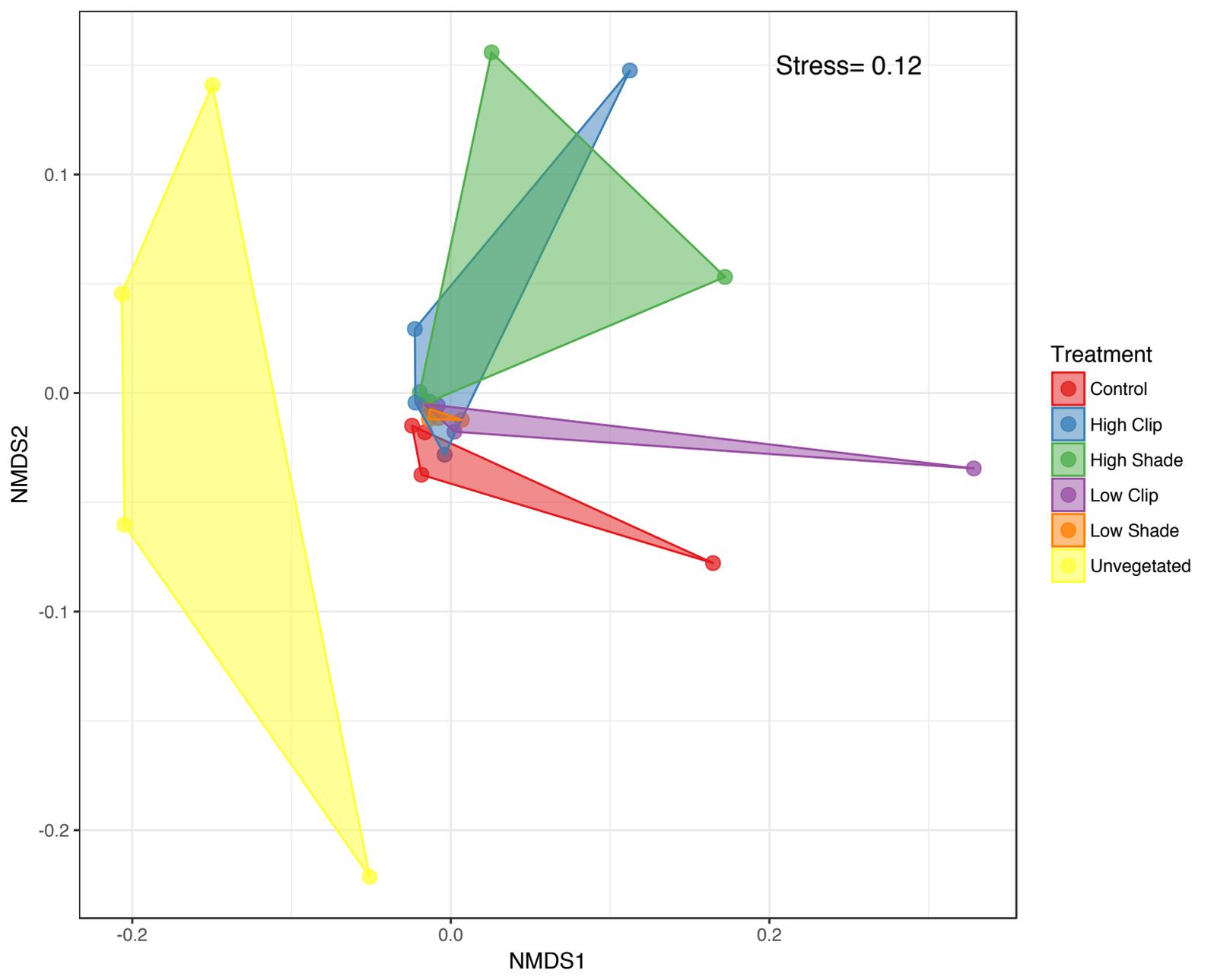
No of variables	Correlation	Environmental variables
7	0.6	Sed C:N ratio; Bulk C in core; Sed C inorg; Rhizome biomass; NCP; N in Plant; C in rhizomes
7	0.598	Sed C:N ratio; Bulk C in core; Sed C inorg; Leaf biomass; Rhizome biomass; NCP; C in rhizomes
6	0.597	Sed C:N ratio; Bulk C in core; Sed C inorg;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

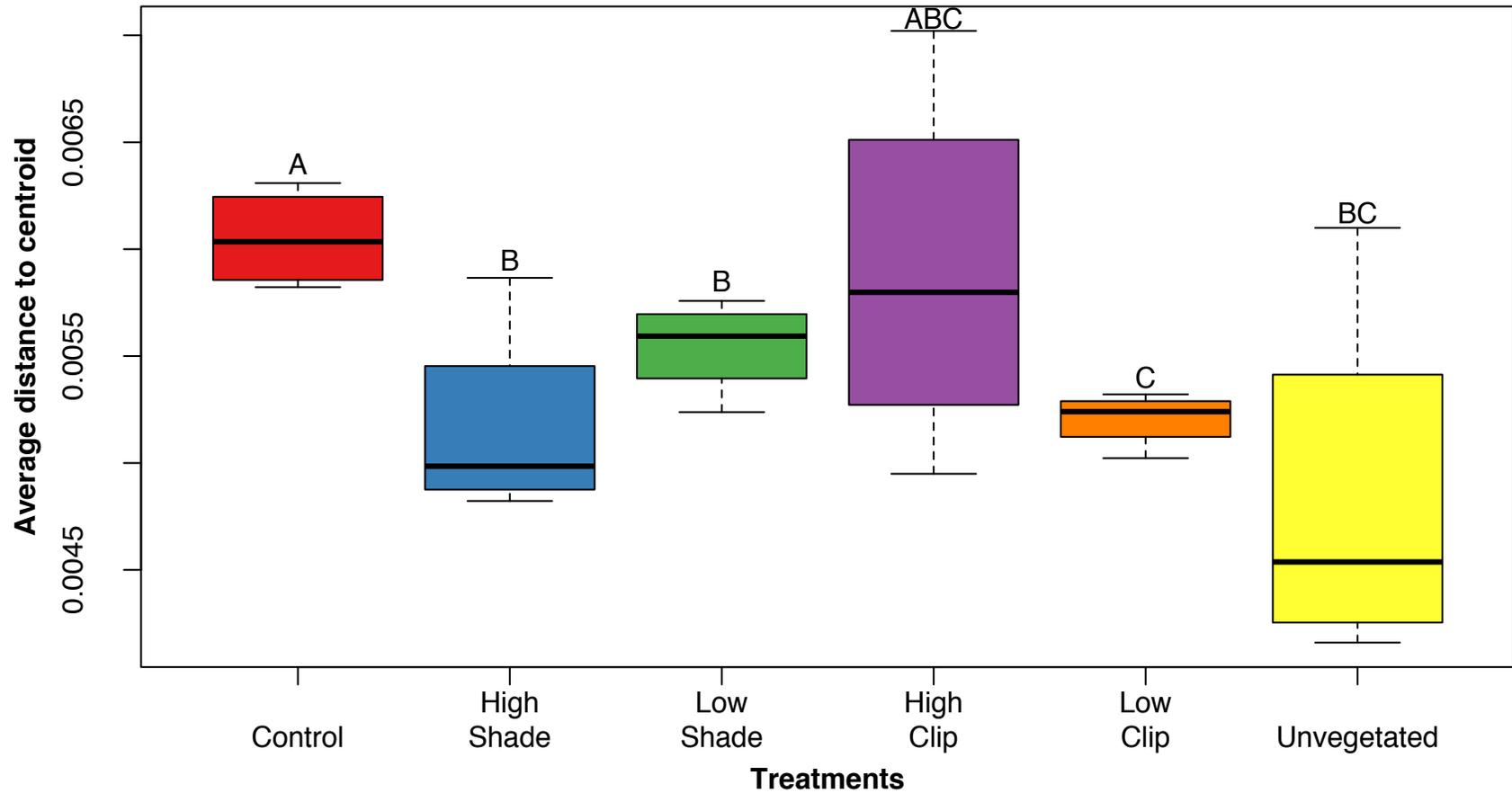
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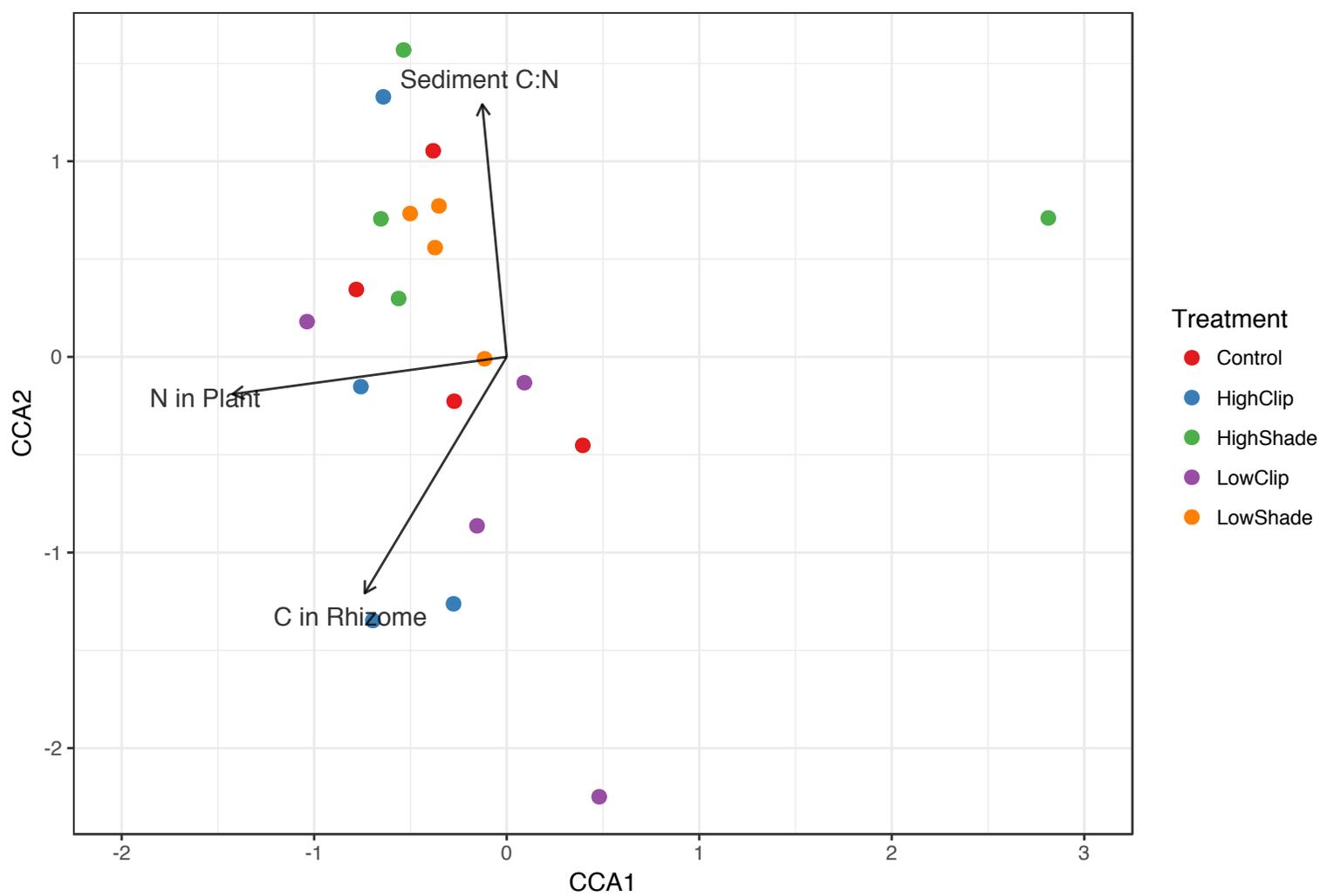
844 Table 1- Biota-environment (BIOENV) analysis showing the 5 best combinations of variables  
845 linked with the highest correlation to the meiobenthos community composition. Correlation  
846 values represent Spearman's rank correlation coefficient. Environmental variables  
847 abbreviations: Sediment C:N ratio (Sed C:N ratio); Bulk carbon density (Bulk C in core);  
848 Sediment content in inorganic C (Sed C inorg); Rhizome biomass (Rhizome biomass);  
849 Community metabolism (NCP); Plant Nitrogen content (N in Plant); Rhizomes carbon  
850 content (C in rhizomes); Leaf biomass (Leaf biomass)  
851

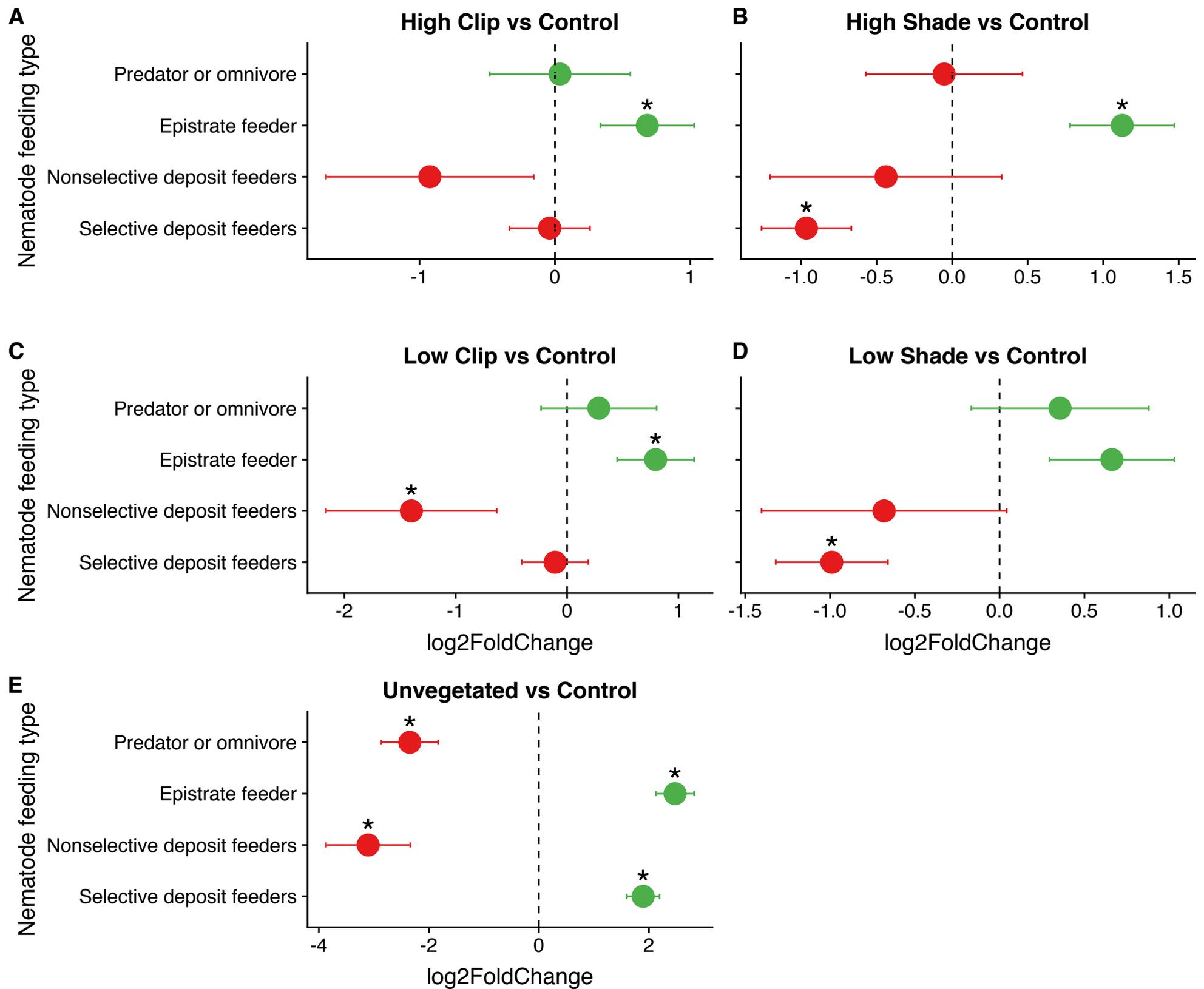






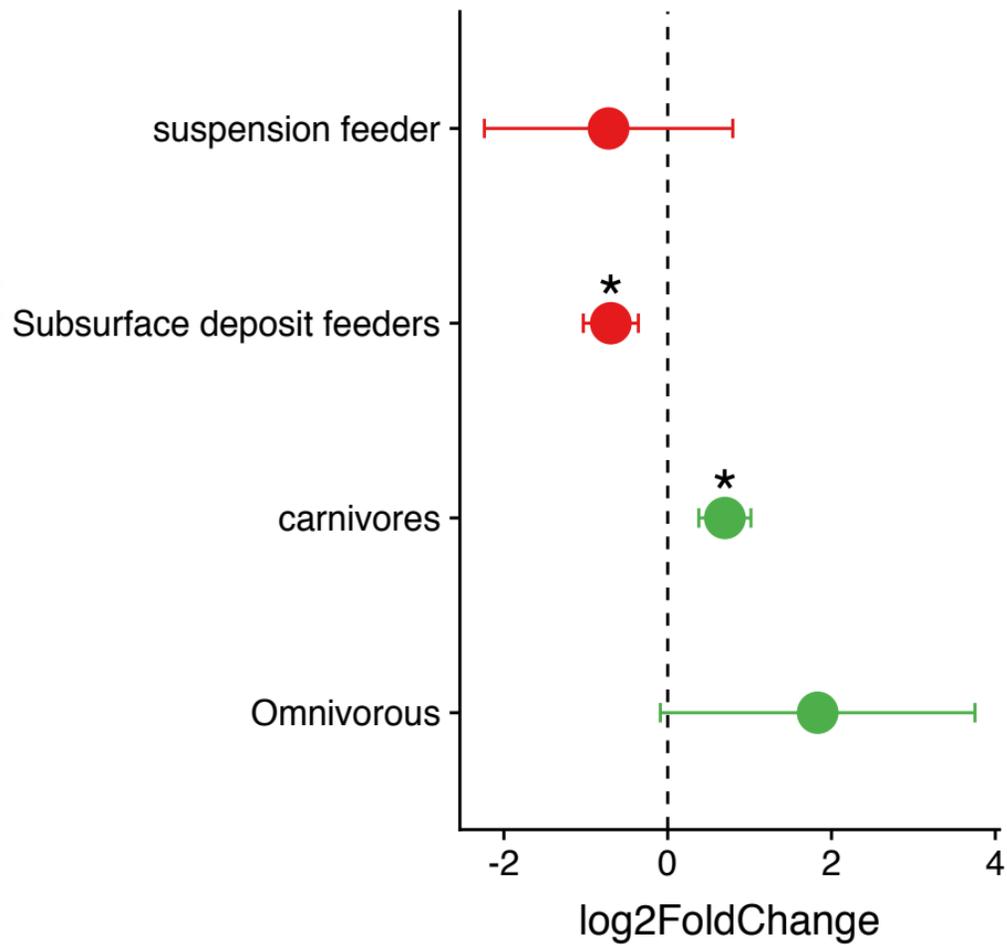
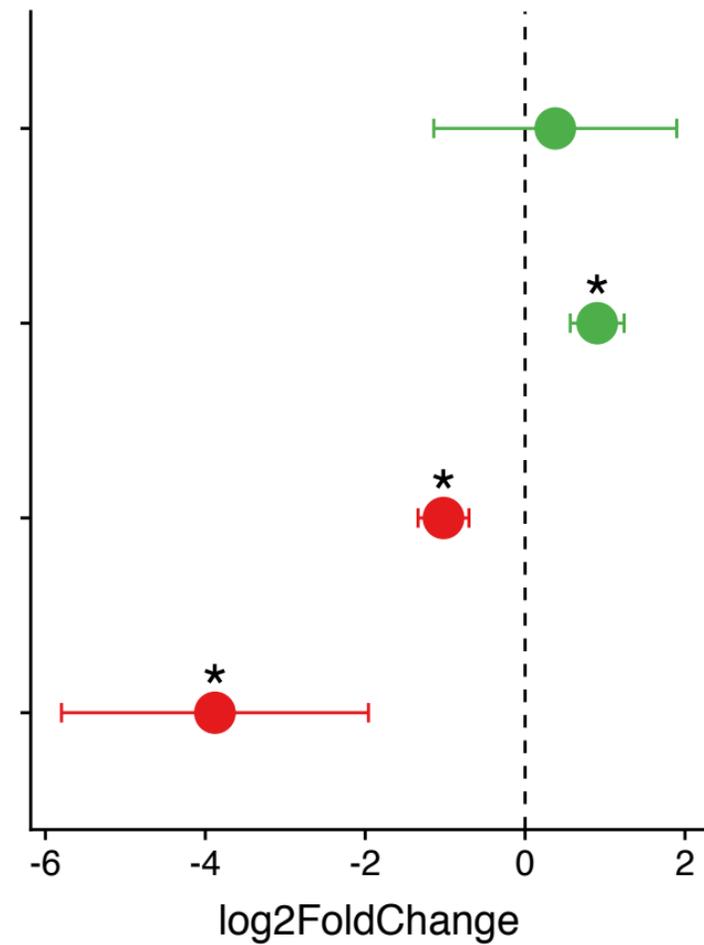






**A****Low Shade vs Control**

Polychaete feeding guild

**B****Unvegetated vs Control**

A

