

# 1 **CO<sub>2</sub> and nutrient-driven changes across multiple levels** 2 **of organization in *Zostera noltii* ecosystems**

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## 8 9 **Abstract**

10 Increasing evidence emphasizes that the effects of human impacts on ecosystems must  
11 be investigated using designs that incorporate the responses across levels of biological  
12 organization as well as the effects of multiple stressors. Here we implemented a  
13 mesocosm experiment to investigate how the individual and interactive effects of CO<sub>2</sub>  
14 enrichment and eutrophication, scale-up from changes in primary producers at the  
15 individual- (biochemistry) or population-level (production, reproduction, and/or  
16 abundance) to higher levels of community (macroalgae abundance, herbivory, and  
17 global metabolism) and ecosystem organization (detritus release and carbon sink  
18 capacity). The responses of *Zostera noltii* seagrass meadows growing in low- and high-  
19 nutrient field conditions were compared. In both meadows, the expected CO<sub>2</sub> benefits  
20 on *Z. noltii* leaf production were suppressed by epiphyte overgrowth, with no direct CO<sub>2</sub>  
21 effect on plant biochemistry or population-level traits. Multi-level meadow response to  
22 nutrients was faster and stronger than to CO<sub>2</sub>. Nutrient enrichment promoted the  
23 nutritional quality of *Z. noltii* (high N, low C:N and phenolics), the growth of epiphytic  
24 pennate diatoms and purple bacteria, and shoot mortality. In the low-nutrient meadow,  
25 individual effects of CO<sub>2</sub> and nutrients separately resulted in reduced carbon storage in  
26 the sediment, probably due enhanced microbial degradation of more labile organic  
27 matter. These changes, however, had no effect on herbivory nor on community  
28 metabolism. Interestingly, individual effects of CO<sub>2</sub> or nutrient addition on epiphytes,  
29 shoot mortality, and carbon storage were attenuated when both nutrients and CO<sub>2</sub> acted  
30 simultaneously. This suggests CO<sub>2</sub>-induced benefits on eutrophic meadows. In the high-  
31 nutrient meadow, a striking shoot decline caused by amphipod overgrazing masked the

32 response to CO<sub>2</sub> and nutrient additions. Our results reveal that under future scenarios of  
33 CO<sub>2</sub>, the responses of seagrass ecosystems will be complex and context dependent,  
34 being mediated by epiphyte overgrowth rather than by direct effects on plant  
35 biochemistry. Overall, we found that the responses of seagrass meadows to individual  
36 and interactive effects of CO<sub>2</sub> and nutrient enrichment varied depending on interactions  
37 among species and connections between organization levels.

38

## 39 **1 Introduction**

40 Understanding community and ecosystem responses to human impacts is a challenge  
41 that requires integrating not only the organism-level responses across populations and  
42 entire systems (Russell et al., 2012), but also synergistic or antagonistic effects of  
43 multiple stressors (Woodward et al., 2010). A large number of articles has been  
44 published on the effects of ocean acidification, and reviewed among others by Doney et  
45 al. (2009) and Kroeker et al. (2010). This body of research has revealed that ocean  
46 acidification can be detrimental to most marine calcifying organisms, while increasing  
47 carbon dioxide (CO<sub>2</sub>) concentration can benefit primary productivity of phytoplankton,  
48 cyanobacteria, fleshy algae, and seagrasses. Our current understanding of these effects  
49 is largely based on the species-specific responses of individuals or populations.  
50 However, the broad variability in responses among organisms may influence species  
51 interactions and drive unforeseen impacts on marine communities and ecosystems  
52 (Hall-Spencer et al., 2008; Kroeker et al., 2013a).

53 The interactive effect of multiple stressors on ecological communities remains largely  
54 unknown (Crain et al., 2008). Atmospheric CO<sub>2</sub> concentration has increased from  
55 preindustrial levels of approximately 280 ppm to 397 ppm in 2013 (NOAA, Mauna Loa  
56 Observatory, Hawaii), leading to a rise in the CO<sub>2</sub> absorbed by the ocean with an  
57 associated pH decrease of 0.1 units. An additional pH decrease of 0.07-0.31 units is  
58 expected by the end of the 21<sup>th</sup> century based on the 'Intergovernmental Panel on  
59 Climate Change' predictions (IPCC, 2013). In the marine environment, ocean  
60 acidification can locally interact with excess nutrients from coastal eutrophication to  
61 accelerate changes in ecosystem structure and functioning (Russell et al., 2009).

62 Human impacts that alter the availability of environmental resources are shifting the  
63 nutritional quality of primary producers through changes at the biochemical or

64 individual levels of the biological organization (e.g. allocation of resources to growth,  
65 storage, and chemical defences). In addition, changes in environmental resources can  
66 favour different types of producers and alter the inter-species competitiveness and  
67 producers' abundances (Kroeker et al., 2013b). Overall, this may affect ecological  
68 interactions and fluxes, leading to shifts at community and ecosystem levels. Under  
69 elevated nutrient concentrations, aquatic and terrestrial ecosystems with higher producer  
70 nutritional quality often support higher rates of herbivory, more rapid decomposition  
71 rates and recycling of nutrients, and lower net accumulation of soil carbon (Wardle et  
72 al., 2004; Cebrian et al., 2009). The effects of nutrient enrichment have been widely  
73 described on terrestrial, freshwater, and marine ecosystems, whereas the scaling up of  
74 elevated CO<sub>2</sub> effects has been mostly studied in terrestrial plants. Under elevated CO<sub>2</sub>  
75 levels, and especially if nutrient availability is limiting to growth, terrestrial plants  
76 typically increase the accumulation of carbohydrates and/or carbon-based secondary  
77 compounds (mostly phenolics). This increases C:N ratios ("nitrogen dilution" effect)  
78 and sometimes leaf toughness through increasing indigestible polymers such as  
79 cellulose and lignin (Zvereva and Kozlov 2006; Lindroth, 2010; Robinson et al., 2012).  
80 Herbivores usually compensate for this lower food quality by eating more (Stiling and  
81 Cornelissen, 2007). In addition, CO<sub>2</sub> enrichment may shift the biomass and composition  
82 of soil microbial communities, directly through different responses of microbial groups  
83 to high CO<sub>2</sub> / low pH (Krause et al., 2012; Lidbury et al., 2012) or indirectly through  
84 reducing foliar and detritus quality (Drigo et al., 2007). Overall, high CO<sub>2</sub> levels may  
85 have both positive and negative consequences on the decomposition of soil organic  
86 matter and nutrient recycling (Lindroth, 2010).

87 In this study, we use seagrass meadows as model ecosystems to investigate the scaling  
88 up of the effects of elevated CO<sub>2</sub> and nutrient levels on marine coastal environments.  
89 Seagrass beds are widely distributed habitats that host high biodiversity and provide  
90 valuable ecosystem services (Orth et al., 2006). A rich epiphyte community usually  
91 colonizes seagrass leaves, thus providing a useful system for studying how changes in  
92 environmental resources can favour different types of producers (e.g. non-calcareous vs.  
93 calcareous). They are highly productive systems that sequester larger amounts of carbon  
94 per area than tropical forests, providing for a long-term removal of carbon dioxide from  
95 the atmosphere (Pidgeon, 2009; Fourqurean et al., 2012). The maintenance of the key

96 services provided by seagrass ecosystems under global change is thus of prime  
97 importance for human well-being.

98 A mesocosm experiment was conducted to assess: (1) how CO<sub>2</sub> and nutrient  
99 enrichments affect primary producers' at the individual- (plant biochemistry including  
100 CN and allocation of resources to carbohydrate reserves and carbon-based chemical  
101 defences) or population-level (plant allocation of resources to biomass and  
102 reproduction, and composition and abundance of seagrass epiphytes), and (2) whether  
103 these changes propagate to the community (macroalgae abundance, meso-herbivory,  
104 whole-community metabolism) and to the ecosystem (detritus production and organic  
105 carbon storage in sediment). The responses of meadows of the seagrass *Zostera noltii*  
106 Horneman developing in low- and high- nutrient conditions in the field were compared  
107 to assess if they react differently.

108

## 109 **2 Methods**

### 110 **2.1 Study meadows**

111 Samples of *Z. noltii* community were collected from two meadows separated 5.5 km  
112 from each other within the Ria Formosa lagoon (South Portugal). This shallow  
113 mesotidal lagoon is dominated by monospecific beds of the seagrass *Z. noltii* that  
114 occupy ca. 45% of the intertidal area. One meadow was developing under prior field  
115 conditions of low nutrient levels (36°59'40''N 7°58'00''W; hereafter low-nutrient  
116 meadow) and the other under high nutrient levels (37°01'15''N 8°00'56.50''W;  
117 hereafter high-nutrient meadow). Low-intertidal samples exposed to a small emersion  
118 period only during low spring tides were selected. Table 1 presents seawater nutrient  
119 concentrations and seagrass meadow traits that reveal the substantial initial differences  
120 between meadows.

### 121 **2.2 Mesocosm experiment**

122 The study was conducted in an outdoor mesocosm system at the Ramalhete field station  
123 of the Centre of Marine Sciences, which is located at the Ria Formosa lagoon. To assess  
124 the effects of CO<sub>2</sub> and nutrients on *Z. noltii* meadows an enrichment experiment was  
125 conducted for 6 weeks during August-September 2011, after 4 days of acclimation to

126 the experimental mesocosms. This time span is sufficient to detect any treatment-driven  
127 changes in physiological, morphological and population traits of this fast-growing  
128 species (e.g. Peralta et al., 2002). Core samples of *Z. noltii* community, including  
129 sediment and algal, faunal and microbial components, were randomly collected from  
130 each donor meadow and allocated to flowerpots of 20 cm of diameter and height. Three  
131 flowerpots were placed in each of the 16 experimental mesocosms (tanks of 110 L),  
132 which were exposed to combinations of two CO<sub>2</sub> and two nutrient levels in a crossed  
133 design with two replicates.

134 Experimental levels of CO<sub>2</sub> encompassed present (pH 8.00±0.02, equivalent to ca. 400  
135 ppm CO<sub>2</sub>) and future conditions (pH 7.83±0.01, equivalent to ca. 800 ppm CO<sub>2</sub>) in Ria  
136 Formosa lagoon. The mesocosms received sand-filtered seawater from two head tanks  
137 of 1000 L at a rate of 240 L h<sup>-1</sup>. In one of the head tanks, the water CO<sub>2</sub> was  
138 manipulated to ensure fixed pH differences between treatment means within the range  
139 predicted for 2100 by the IPCC (pH decline = -0.18 units) following a commonly used  
140 method (e.g. Alsterberg et al., 2013). The CO<sub>2</sub> injection was controlled by an auto-  
141 analyzer (Yokogawa, EXAxt 450, Tokyo, Japan), which continuously monitored the  
142 water pH and temperature. Total alkalinity, pH, temperature and salinity within the  
143 mesocosms, as well as the seawater DIC and carbon speciation are provided on  
144 Appendix B (Supplement).

145 Water nutrient levels encompassed the natural values found in the lagoon and the values  
146 of highly eutrophic conditions (N: 45x and P: 11x natural, see Appendix B,  
147 Supplement). The nutrient enrichment was obtained by adding a solubilised mixture of  
148 the fertilizers ammonium nitrate and monoammonium phosphate directly into the water  
149 column of each enriched mesocosm using a multi-channel dosing pump. Water samples  
150 were collected weekly to analyze nutrient concentrations using a loop-flow analyzer  
151 ( $\mu$ Mac-1000; Systea, Anagni, Italy).

152 The water within mesocosms was homogenized using a submersible circulation pump  
153 placed at leaf height. Pumps were stopped 2 h twice per day to simulate tidal currents.  
154 Twice a week, the epiphytes growing in the mesocosm walls were removed and the  
155 position of flowerpots within each mesocosm was reassigned to minimize potential  
156 spatial differences. Natural settlement and growth of leaf epiphytes and small animals  
157 were allowed throughout the experiment.

### 158 **2.3 Producers' traits at the individual- or population-level**

159 Changes on producers' at the individual or population levels were assessed by  
160 measuring: (1) plant biochemistry and allocation of resources to biomass and  
161 reproduction; and (2) the composition and abundance of seagrass epiphytes.

162 Allocation of plant resources to biomass and reproduction at the population-level was  
163 monitored almost every week. Shoot recruitment or mortality were quantified within  
164 each flowerpot excluding shoots growing around the border to avoid edge effects.  
165 Allocation to reproduction was quantified as density of flowering shoots. Five shoots at  
166 the beginning and at the end of the experiment and three shoots in between sampling  
167 events were randomly chosen within each flowerpot to quantify the number of leaves  
168 and the leaf area index (LAI) as indicators of aboveground productivity. To estimate  
169 LAI, leaf area was measured on these shoots, averaged, multiplied by the number of  
170 shoots within the pot, and scaled per surface area.

171 After four weeks of experiment, leaf epiphyte composition was determined in the oldest  
172 leaves of three randomly chosen shoots. The surface covered by each taxon was  
173 quantified under a microscope and standardized per 10 cm<sup>2</sup> of leaf area.

174 After six weeks, all shoots from each mesocosm were harvested and plant traits  
175 quantified in each flowerpot. Belowground productivity was estimated from the vertical  
176 or horizontal rhizome length and from the total root length (number of roots multiplied  
177 by the average root length) of five shoots per pot. The above- and below-ground  
178 biomass allocation was quantified after drying at 60 °C until constant weight. The  
179 pooled epiphyte load of three shoots was removed using a glass slide and quantified as  
180 relative to leaf area after drying at 60 °C until constant weight. Pooled material of five  
181 shoots was separated into leaves (without epiphytes) and rhizomes, freeze-dried,  
182 weighted, ground to fine powder and used in subsequent analyses of plant biochemistry.  
183 Carbon and nitrogen concentrations were analyzed using an elemental analyzer (Carlo-  
184 Erba, Milan, Italy). Total non-structural carbohydrates were measured in rhizomes using  
185 the phenol-sulfuric acid colorimetric method (Dubois et al., 1956) with glucose as  
186 standard, after sugar extraction in hot ethanol and enzymatic conversion of starch to  
187 glucose equivalents (Smith and Zeeman, 2006). Total phenolics were quantified as  
188 indicators of plant allocation of resources to chemical defences. Phenolics were  
189 extracted from leaf material with methanol 50% for 24 h under constant agitation at 4

190 °C and determined with a spectrophotometer using chlorogenic acid as standard  
191 following a modified Folin-Ciocalteu method (Bolser et al., 1998).

## 192 **2.4 Community- and ecosystem-level traits**

193 The response of the seagrass community to CO<sub>2</sub> and nutrient enrichments was  
194 quantified weekly by: (1) the percentage of flowerpot surface covered by *Ulva* spp.; (2)  
195 the feeding activity of mesograzers (percentage of leaves showing bite marks in the  
196 same shoots used to measure the morphological traits); and (3) the whole-community  
197 metabolism quantified from the oxygen evolution within benthic chambers of 17 cm  
198 diameter (4.8±0.01 L incubated) fitted to the flowerpots for 30-45 minutes at midday  
199 (12-14 h). A transparent acrylic chamber to estimate net production and a dark chamber  
200 to estimate respiration were simultaneously deployed within each mesocosm. Dissolved  
201 oxygen concentration was measured by spectrophotometry using the Winkler method  
202 (Labasque et al., 2004) in three water samples collected before and after incubations  
203 into 12 mL soda glass vials. Community metabolism was estimated from the net change  
204 in oxygen concentration during incubations integrated by the chamber volume and  
205 standardized by incubation time and bottom area. There were no effects of enclosure on  
206 the water temperature within chambers (measured with onset HOBO loggers, Southern  
207 MA, USA). All incubations were run under irradiances of photosynthetically active  
208 radiation (PAR) averaging 283±8.6 μmol quanta m<sup>-2</sup> s<sup>-1</sup> (measured with a Li-192SA  
209 underwater PAR quantum sensor, Li-Cor, USA), when *Z. noltii* photosynthesis is light  
210 saturated and not photoinhibited (Peralta et al., 2002).

211 At the ecosystem level, detritus production (fresh weight of all floating material  
212 collected during a 24h period) was quantified almost every week. Organic matter in the  
213 sediment (loss of dry weight after combustion at 450 °C, 4h) was measured at the end of  
214 the experiment as indicator of the carbon sink capacity.

## 215 **2.5 Statistical analyses**

216 The effects of CO<sub>2</sub> and nutrient treatments throughout the experiment were tested using  
217 three-way repeated-measures analyses of variance (RM ANOVA). The subject  
218 repeatedly sampled was the mesocosm, CO<sub>2</sub> and nutrients were the among-subject  
219 factors (two fixed crossed factors) and Time the within-subject factor. To avoid the  
220 masking effect of the strong initial differences between meadows on the responses to

221 experimental treatments, data for the low- and high-nutrient meadows were analyzed  
222 separately. Data were checked for parametric assumptions and transformed where  
223 needed. When sphericity was not met, corrected degrees of freedom from Greenhouse-  
224 Geisser adjustment were used (Quinn and Keough, 2002).

225 The effects of CO<sub>2</sub> and nutrient treatments on variables measured at the end of the  
226 experiment were tested using two-way ANOVAs (two fixed crossed factors) after  
227 testing parametric assumptions. A normal distribution with unequal variances was found  
228 for all variables, which is usual when the sample size is small. Following  
229 recommendation by Quinn and Keough (2002), we proceeded with the analyses but  
230 making significance level more restrictive to minimize the possibility of Type I error  
231 (mistakenly detection of differences). Welch *t* tests that are robust against unequal  
232 variances were used to interpret significant interactions. Again, data for the low- and  
233 high-nutrient meadows were analyzed separately.

234 To assess the ordination of treatments based on differences in the composition of leaf  
235 epiphyte assemblages, a non-metric Multi-Dimensional Scaling analysis (NMDS) with  
236 Bray-Curtis distances was carried out. Because NMDS axes are arbitrary, the final  
237 solution was rotated using a Principal Component Analysis (PCA) to align the largest  
238 variance in the first axis. The significance of the effect of CO<sub>2</sub> and nutrient treatments  
239 on assemblage composition was tested with a two-way permutational analysis of  
240 variance (PERMANOVA; two fixed crossed factors). To perform the test, Bray-Curtis  
241 distances were calculated from untransformed data and 999 permutations were used  
242 under a reduced model.

243 Finally, two PCAs, one for each meadow, were performed to assess links among the  
244 several traits and the trajectory of treatment responses through time. Traits showing the  
245 highest correlation with the components ( $r \geq 0.7$ ) were selected for interpretations.  
246 Since our variables were not dimensionally homogeneous, principal components were  
247 computed from the correlation matrix.

248



## 249 3 Results

### 250 3.1 Responses of meadow traits measured through time

251 The response of the low-nutrient meadow showed a threshold at the third week of the  
252 experiment, when most variables responded differently from the first two weeks (Fig. 1,  
253 left). Shoot recruitment occurred mostly in unfertilized but also in CO<sub>2</sub>-enriched  
254 conditions until the third week (Fig. 1a), after which shoot mortality progressively  
255 increased. Figure 1d suggests that the *Z. noltii* leaf area index (LAI) tended to increase  
256 with CO<sub>2</sub> enrichment until the third week of the experiment. A positive, significant  
257 effect of the CO<sub>2</sub> enrichment was observed on detritus production throughout the  
258 experiment (Fig. 1c). Nutrient addition increased shoot mortality (Fig. 1a), whereas it  
259 decreased leaf number and LAI (Fig. 1b and d). Shoot mortality induced by the nutrient  
260 enrichment was attenuated by the simultaneous addition of CO<sub>2</sub>, especially from the  
261 third week onwards (Fig. 1a). A treatment and time interaction was detected on the  
262 community production and respiration (Fig. 1e and f). These variables showed high  
263 variability with similar ranges of variation in unfertilized and enriched conditions. No  
264 treatment effects were detected throughout the experiment on shoot flowering or meso-  
265 herbivory (see Appendix C, Supplement).

266 The responses of the high-nutrient meadow to CO<sub>2</sub> enrichment included an increased  
267 shoot mortality during the second and third weeks and an increased detritus production  
268 at the end of the experiment (Fig. 1g and i). Nutrient addition decreased the number of  
269 leaves per shoot and increased detritus production throughout the experiment (Fig. 1h  
270 and i). CO<sub>2</sub> enrichment interacted with nutrients to alleviate the nutrient-induced  
271 reduction of the number of leaves (Fig. 1h). No effects of CO<sub>2</sub> or nutrient enrichment  
272 were observed through time on LAI, meso-herbivory, *Ulva* spp. cover, shoot flowering,  
273 and community production or respiration (Figs. 1j-l and C, Supplement). Independently  
274 of the experimental treatments, overgrazing by the herbivorous amphipod *Cymadusa*  
275 *filosa* Savigny severely affected the plants from the high-nutrient meadow causing  
276 massive shoot mortality (Fig. 1k and g). At the end of the experiment a mean ( $\pm$ se) of  
277 89 ( $\pm$ 3.7)% of shoots died, 81 ( $\pm$ 9.1)% of the seagrass leaves showed bite marks and  
278 leaf area was reduced from 5.0 ( $\pm$ 0.2) to 1.0 ( $\pm$ 0.4) cm<sup>2</sup> shoot<sup>-1</sup>. Similarly, *Ulva* spp.  
279 cover progressively decreased, being close to 0% in all treatments after six weeks (Fig.  
280 1l). At the end of the experiment, all *Z. noltii* shoots and *Ulva* spp. fronds disappeared

281 from three flowerpots (one unfertilized and two CO<sub>2</sub>-and-nutrient-enriched). However,  
282 net production and respiration in these pots were within the range observed in the other  
283 pots (Fig. Cd and e, Supplement), indicating that the metabolism of the sediment  
284 microbial community was similar to that of the *Z. noltii* community.

285 We did not detect any significant effect of CO<sub>2</sub> or nutrient enrichment on plant damage  
286 by meso-herbivory in the low- or high-nutrient meadows. However, at the end of the  
287 experiment plants from the high-nutrient meadow showed 81 (+9.1)% of leaves with  
288 bite marks, compared to only 6.9 (+3.2)% in the low-nutrient meadow (Figs. 1k and Cb,  
289 Supplement). These between meadow differences, as well as the link between shoot  
290 mortality and meso-herbivory in masking the enrichment effects in the high-nutrient  
291 meadow, were further confirmed by a PCA of responsive variables from the low- and  
292 high-nutrient meadows at the end of the experiment (see Appendix E, Supplement).

### 293 **3.2 Responses of meadow traits measured at the end of the experiment**

294 In plants from the low-nutrient meadow (Fig. 2, grey bars), nutrient enrichment  
295 enhanced the leaf nutritional quality (high leaf nitrogen and low leaf C:N ratio, Fig. 2a  
296 and c) and the accumulation of nitrogen in rhizomes (high rhizome nitrogen  
297 concentration and low rhizome C:N ratio, Fig. 2d and b), whereas it had a negative  
298 impact on the accumulation of leaf phenolics (Fig. 2f). A significant interaction of CO<sub>2</sub>  
299 and nutrient additions was detected for epiphyte load and sediment organic matter (Fig.  
300 2g and h). The leaf epiphyte load increased significantly under CO<sub>2</sub> addition, whereas  
301 nutrient enrichment and especially the interactive CO<sub>2</sub> and nutrient additions had a  
302 lower and not significant effect (*t* test comparisons in Fig. 2g). Similarly, CO<sub>2</sub> and  
303 nutrient interaction resulted in maintenance of the organic matter content in the  
304 sediment, which tended to decrease with separated CO<sub>2</sub> and nutrient additions (*t* test  
305 comparisons in Fig. 2h).

306 In plants from the high-nutrient meadow (Fig. 2, black bars), CO<sub>2</sub> enrichment decreased  
307 rhizome C:N (Fig. 2b) and increased epiphyte loads (Fig. 2g). The CO<sub>2</sub>-induced  
308 increase of the epiphyte load was maintained under the simultaneous addition of  
309 nutrients. Nutrient addition enhanced the leaf nutritional quality (high leaf nitrogen  
310 concentration, Fig. 2a). A reduction of leaf C:N ratio and phenolics was detected  
311 apparently in response to CO<sub>2</sub> and/or nutrient enrichments (Fig. 2c and f), but this was  
312 actually caused by an increase of these traits in the unfertilized plants at the end of the

313 experiment in relation to the initial field conditions (Table 1). A synergistic interaction  
314 between CO<sub>2</sub> and nutrient additions caused an increase of the rhizome length (Fig. 2e).  
315 Variables for which no significant effects of CO<sub>2</sub> or nutrient addition were detected are  
316 shown in Appendix D (Supplement).

### 317 **3.3 Responses of *Z. noltii* epiphytes**

318 Both, CO<sub>2</sub> and nutrient additions altered the relative abundance of epiphyte populations,  
319 whereas elevated nutrient levels also modified the epiphyte composition (Fig. 3a). In the  
320 unfertilized plants, the epiphyte cover was low and the most abundant leaf epiphytes  
321 were the fanlike diatoms *Licmophora* spp. The second-most-abundant epiphyte in plants  
322 from the low-nutrient meadow was the encrusting coralline algae *Melobesia*  
323 *membranacea*, whereas in plants from the high-nutrient meadow it was the  
324 cyanobacterium *Microcoleus* spp. The response to the CO<sub>2</sub> enrichment in both, low- and  
325 high-nutrient meadows was a great increase of epiphyte cover, mostly due to a bloom of  
326 *Microcoleus* spp. (73% of the total cover) that outcompeted the diatoms *Licmophora*  
327 spp. and the encrusting corallines. Under nutrient-enrichment pennate diatom  
328 populations dominated by *Navicula* spp. outcompeted the other taxa. In the nutrient- and  
329 CO<sub>2</sub>-and-nutrient- treatments the composition of epiphyte assemblages was similar, but  
330 with a reduced replacement of *Licmophora* spp. by pennate diatoms in the CO<sub>2</sub>-and-  
331 nutrient- treatment. Chlorophytes (mainly *Ulva prolifera*) and filamentous rhodophytes  
332 (mainly *Bangia* spp. and *Stylonema alsidii*) were also present in all treatments.  
333 Temporal changes in epiphyte abundances within the enriched mesocosms involved a  
334 shift from relatively low epiphyte loads until the second week to increasing epiphyte  
335 loads from the third week onwards, with the occurrence of purple bacteria in nutrient-  
336 and CO<sub>2</sub>-and-nutrient-enriched treatments during the fourth week.

337 NMDS ordination of treatments based on the epiphyte composition showed clear CO<sub>2</sub>  
338 effects (Fig. 3b). CO<sub>2</sub> treatments were separated along axis I (51% of variance  
339 explained), whereas the other treatments were ordered along axis II (49% of variance  
340 explained) from unfertilized to CO<sub>2</sub>-, CO<sub>2</sub>-and-nutrient-, and nutrient-enriched.  
341 Separation of CO<sub>2</sub> enrichments along axis I was due to a higher epiphyte cover (mean  
342  $\pm$ se: 22 $\pm$ 2.3 cm<sup>2</sup> per 10 cm<sup>2</sup> of leaf) than the unfertilized, nutrient-enriched and CO<sub>2</sub>-  
343 and-nutrient-enriched treatments (7.6 $\pm$ 1.4, 11 $\pm$ 1.3 and 8.4 $\pm$ 3.2 cm<sup>2</sup> per 10 cm<sup>2</sup> of leaf,  
344 respectively). Treatments of both, low- and high-nutrient meadows were nearby in the

345 ordination diagram, reflecting minor differences among meadows in the response of the  
346 epiphyte assemblage. NMDS pattern was further confirmed by the PERMANOVA  
347 results, which showed significant effects of CO<sub>2</sub>, nutrients and their interaction (Fig.  
348 3b).

349 The above-mentioned PCA of traits from the low- and high-nutrient meadows further  
350 confirmed the increased epiphyte load and the change in epiphyte composition as main  
351 drivers of the meadow responses to CO<sub>2</sub> and nutrient enrichments (see Appendix E,  
352 Supplement).

### 353 **3.4 Response trajectories through time**

354 The first two PCA components of the low-nutrient meadow traits measured through  
355 time, explained 41% (component I) and 20% (component II) of the variance. The *Z.*  
356 *noltii* traits that highly correlated with component I were the LAI and the number of  
357 leaves, which were negatively correlated with shoot mortality and herbivory (Fig. 4a,  
358 right graph). Flowering, community production and community respiration highly  
359 correlated with component II (variable loadings are presented in Table E2, Supplement).  
360 The variability of all treatment scores on the component I during the first week and of  
361 unfertilized and CO<sub>2</sub>-enriched treatments during the second and third weeks were within  
362 the initial range of natural variability (week 0, grey rectangle in Fig. 4a, left graph). At  
363 this time, *Z. noltii* plants showed higher LAI and higher number of leaves. The time  
364 series ordination of the rest of treatments along component I revealed that the effects of  
365 nutrient addition started during the second week, when the scores of nutrient- and CO<sub>2</sub>-  
366 and-nutrient-treatments suddenly shifted to higher values. These nutrient effects were  
367 dominated by high mortality of *Z. noltii* shoots and to a less extent by high meso-  
368 herbivory. The highest scores on component I were attained by the nutrient-treatment  
369 during the fourth to sixth weeks. The system response to the CO<sub>2</sub> treatment was slower  
370 (starting at week 4) and of lower magnitude than the response to nutrient- and CO<sub>2</sub>-and-  
371 nutrient-treatments. This analysis supported the previous indication of a temporal  
372 threshold for the meadow responses, which was the second week for elevated nutrients  
373 and the fourth week for elevated CO<sub>2</sub>. No clear ordination of treatments was detected  
374 along component II, indicating that traits highly correlated with this component were  
375 substantially influenced by natural variability.

376 The first two PCA components of the high-nutrient meadow traits measured through  
377 time explained 64% (component I) and 12% (component II) of the variance. The *Z.*  
378 *noltii* traits that highly correlated with component I were the LAI, the number of leaves,  
379 the community production and the abundance of *Ulva*, which were negatively correlated  
380 with mortality, herbivory and detritus production (Fig. 4b, right graph). Community  
381 respiration highly correlated with component II (see variable loadings in Table E2,  
382 Supplement). The range of initial natural variability of all treatment scores was  
383 narrower than for the low-nutrient meadow (Fig. 4b, left graph). The system was  
384 initially dominated by high seagrass LAI and number of leaves, cover of *Ulva* spp., and  
385 community production. This progressively shifted to a later stage (week 6) dominated  
386 by high *Z. noltii* mortality, herbivory and detritus production. Contrary to the response  
387 of the low-nutrient meadow, there were no relevant differences in the time course and in  
388 the final stage attained by PCA scores of both unfertilized and enriched treatments. The  
389 shoot mortality of *Z. noltii* was positively correlated with meso-herbivore activity and  
390 detritus production, and negatively correlated with LAI, number of leaves and *Ulva*  
391 cover. No clear ordination of treatments was detected along component II.

392

## 393 **4 Discussion**

### 394 **4.1 Effects of CO<sub>2</sub> enrichment in low-nutrient meadows**

395 The CO<sub>2</sub> enrichment had no direct effects on *Z. noltii* biochemistry (Fig. 5a). We found  
396 no evidence of increased nonstructural carbohydrates and subsequent nitrogen dilution  
397 effect (increased C:N ratio) as has been previously observed in the seagrass *Thalassia*  
398 *hemprichii* (Jiang et al., 2010) and *T. testudinum* (Campbell and Fourqurean, 2013). As  
399 well, there was no increase of phenolic contents as predicted by the carbon-nutrient  
400 balance hypothesis and no propagation to susceptibility to herbivory. Several studies in  
401 terrestrial plants reveal that this lack of response is not uncommon (reviewed by  
402 Peñuelas and Estiarte, 1998 and Bidart-Bouzat and Imeh-Nathaniel, 2008). The lack of  
403 accumulation of carbohydrates and phenolics that we observed could be explained by  
404 the trade-off between secondary metabolism and plant growth predicted by the growth-  
405 differentiation balance hypothesis under no light and nutrient limitation (review by  
406 Stamp, 2003). However, we found no significant increase of seagrass productivity under  
407 CO<sub>2</sub> enrichment to support this trade-off, probably due to light limitation induced by

408 epiphyte overgrowth from the third week of experiment onwards. Photosynthesis  
409 enhancements have been reported under CO<sub>2</sub> addition in *Z. noltii* (Alexandre et al.,  
410 2012) and *Z. marina* (Zimmerman et al. 1997), but they do not always translate into  
411 seagrass growth since other determinant factors such as light and nutrient availability  
412 are also at play (Palacios and Zimmerman, 2007; Alexandre et al., 2012; Campbell and  
413 Fourqurean, 2013). In accordance with the previously cited terrestrial studies, our  
414 results suggest that the seagrass responses to elevated CO<sub>2</sub> levels are highly context-  
415 and species-specific, and are not as readily consistent and predictable as resource  
416 availability hypotheses would suggest.

417 The most striking response of *Z. noltii* meadows to the CO<sub>2</sub> enrichment was the increase  
418 of the epiphyte load, with changes in the relative abundance but not in the identity of the  
419 main epiphyte taxa. Interestingly, epiphyte-induced shading did not cause seagrass  
420 mortality as occurred under nutrient enrichment (see below). This suggests attenuation  
421 of the negative effect of reduced light availability by increased CO<sub>2</sub> availability, which  
422 may reduce the energy cost of carbon uptake (Koch et al., 2013). The epiphyte bloom  
423 was mostly caused by the proliferation of the colonial and filament-forming  
424 cyanobacterium *Microcoleus* spp. at the expense of a reduction of coralline algae crusts  
425 of *Melobesia membranacea* and fanlike diatoms *Licmophora* spp. This is in accordance  
426 with previous studies that showed elevated CO<sub>2</sub> / low pH to stimulate cyanobacteria  
427 growth and photosynthesis (Liu et al., 2010) and to decrease abundance of coralline  
428 algae (Hall-Spencer et al., 2008; Martin et al., 2008; Kuffner et al., 2008, Campbell and  
429 Fourqurean, 2014). As well, Hervé et al. (2012) reported negative effects of low pH on  
430 diatom valve formation and porosity, which were alleviated by a simultaneous nutrient  
431 addition. We found that the activity of mesograzers was insufficient to regulate the  
432 epiphyte proliferation in response to increased CO<sub>2</sub> levels, despite their recognized  
433 capacity of controlling epiphyte biomass (Hughes et al., 2004) and particularly  
434 cyanobacteria blooms (Neckles et al., 1993). Likely explanations are that the feeding  
435 capacity of the most abundant mesograzers in the experiment, the amphipod *C. filosa*,  
436 was exceeded by the cyanobacterium overgrowth or that the amphipod was not targeting  
437 these particular epiphytes.

438 Epiphyte overgrowth resulted in increased detritus production and decreased organic  
439 matter accumulated in the sediment. This suggests that bacterial decomposition in the  
440 sediment was accelerated due to the highly labile organic matter of epiphytes, as

441 reported under nutrient enrichment (see below). The acceleration of bacterial  
442 degradation of organic matter polysaccharides at low pH reported by Piontek et al.  
443 (2010) would also support this explanation. Our findings may have relevant  
444 implications, since the organic carbon produced in seagrass meadows sustains important  
445 detritus-based food webs (Pergent et al., 1994; Moore and Fairweather, 2006) and  
446 provides a major global carbon sink (Pidgeon, 2009; Fourqurean et al., 2012).

#### 447 **4.2 Nutrient enrichment and interaction with CO<sub>2</sub> in low-nutrient** 448 **meadows**

449 We found that nutrient enrichment had a faster and greater effect than CO<sub>2</sub> addition on  
450 meadows developing in low-nutrient conditions. Nutrient enrichment enhanced leaf  
451 nutritional quality (high nitrogen and low C:N ratio) and reduced the accumulation of  
452 phenolic compounds (Fig. 5a). Both, the overall increase of plant nitrogen (e.g. Cabaço  
453 et al., 2008; Invers et al., 2004) and the decrease of phenolics (e.g. van Katwijk et al.,  
454 1997; Goecker et al., 2005) have been widely described in seagrasses as a result of  
455 nutrient additions. The nitrogen increase was higher in leaves than in rhizomes, as  
456 expected for this fast-growing species that acquires preferentially ammonium through  
457 the leaves and shows minimal translocation of nitrogen to belowground tissues  
458 (Alexandre et al., 2011).

459 A reduction of the number of *Z. noltii* leaves and of leaf area index, and an increase of  
460 shoot mortality were observed after the second week in response to nutrient addition.  
461 These effects can be linked to ammonium toxicity, which has previously been reported  
462 in *Z. noltii* (Brun et al., 2002) and other seagrass species (Santamaría et al., 1994, van  
463 Katwijk et al., 1997). In addition, nutrient-induced changes in the epiphyte assemblage  
464 may also contribute to shoot mortality by reducing light availability to seagrass leaves.  
465 This seems supported by the abrupt increase of shoot mortality after the third week,  
466 coinciding with the shift of the epiphyte assemblage from coralline algae and fanlike  
467 diatoms to a dense layer of pennate diatoms (mostly of the genus *Navicula*), with a  
468 purple bacteria biofilm developing as well during the fourth week. Towards the end of  
469 the experiment, excess organic matter was released within the system due to increased  
470 shoot mortality and epiphyte shifts. Coincidentally, the accumulation of organic carbon in  
471 the sediments decreased, suggesting that an accelerated microbial decomposition was  
472 promoted by the higher nutritional quality of producers as reported elsewhere for

473 terrestrial systems (Wardle et al., 2004) and seagrass beds (López et al., 1998; Holmer  
474 et al., 2004; Spivak et al., 2007).

475 The simultaneous addition of CO<sub>2</sub> and nutrients did not modify the individual effects of  
476 nutrient enrichment on plant biochemistry, but attenuated the proliferation of certain  
477 epiphyte taxa (also occurring under high CO<sub>2</sub>) and subsequent nutrient-induced *Z. noltii*  
478 mortality. The interactive attenuation of epiphyte overgrowth may result from an  
479 increase in the interspecific competition between the species that dominated the  
480 epiphyte community under elevated CO<sub>2</sub> (i.e. the cyanobacterium *Microcoleus* spp.) and  
481 under elevated nutrient levels (i.e. diatoms of the genus *Navicula*). Our findings are in  
482 agreement with the negative effects of interspecific competition on the involved species  
483 (i.e. symmetrical competition) that have long been reported in ecological studies  
484 (Connel, 1983). Together, attenuation by simultaneous CO<sub>2</sub> and nutrient additions of the  
485 overgrowth of certain epiphytes and of nutrient-induced *Z. noltii* mortality, reduced the  
486 amount of more labile organic matter reaching the sediments compared to the individual  
487 CO<sub>2</sub> or nutrient enrichment. This probably resulted in the maintenance at control levels  
488 of the bacterial decomposition rates and of the sediment capacity to store organic  
489 matter. To our knowledge, this is the first report of the interactive effect of CO<sub>2</sub> and  
490 nutrient enrichments on the meadow carbon sink capacity. Overall, we found that under  
491 simultaneous addition of CO<sub>2</sub> and nutrients, species interactions attenuated the direct  
492 effects of individual stressors on *Z. noltii* and on sensitive epiphyte species or  
493 taxonomic groups.

#### 494 **4.3 High- vs. low- nutrient meadows**

495 Our results revealed that the expected benefits of high CO<sub>2</sub> predicted for the end of the  
496 century on seagrass productivity might be restrained by epiphyte overgrowth and by the  
497 interaction with local eutrophication. In both low- and high-nutrient meadows, CO<sub>2</sub>  
498 effects were more important in epiphyte populations than in the seagrass *Z. noltii*. These  
499 findings strengthen the increasingly recognized importance of species interactions in  
500 modulating the direct effects of eutrophication or acidification in single species,  
501 populations, and ultimately in ecosystem functioning (Orth et al., 2006; Kroeker et al.,  
502 2013b). The effect of nutrient enrichment was greater in the low- than in the high-  
503 nutrient meadow (Fig. 5a vs. b), with nutrient-induced mortality of *Z. noltii* only  
504 appearing in the former. When CO<sub>2</sub> and nutrient enrichments interacted, an increase of



505 epiphyte load was observed in the high-nutrient meadow as opposed to the low-nutrient  
506 meadow. These results highlight the context-dependence of the effects of multiple  
507 stressors in agreement with the meta-analysis of Crain et al. (2008). We observed that  
508 the accumulation of phenolics and carbohydrates was higher under lower nutrient  
509 regimes in the initial field conditions, and also in the experimental conditions for  
510 phenolics. This suggests that nutrient deficiency rather than a direct effect of high CO<sub>2</sub>  
511 drives the accretion of carbon-based compounds in *Z. noltii*. These observations are in  
512 agreement with previous studies in both, terrestrial plants (Lambers, 1993; Peñuelas and  
513 Estiarte, 1998) and seagrasses (Campbell et al., 2012), and reinforce the idea of the  
514 context-dependence of seagrass responses to CO<sub>2</sub> enrichment.

515 Interestingly, we detected little evidence that CO<sub>2</sub> or nutrient addition affected seagrass  
516 herbivory by mesograzers. However, clear differences in herbivory between meadows  
517 were observed. Plants from the high-nutrient meadow experienced a 12-fold higher  
518 amphipod grazing than plants from the low-nutrient meadow, which resulted in a  
519 massive loss of shoots. Blooms of the amphipod *C. filosa* may occur in warmer months  
520 (Appadoo and Myers, 2004). This tube-building amphipod is widely distributed and  
521 uses macroalgae for feeding (Ceh et al., 2005) and shelter (Appadoo and Myers, 2003).  
522 To our knowledge, these observations constitute the first report of *C. filosa* using the  
523 seagrass *Z. noltii* for both feeding and shelter-construction. We found that the  
524 consequences of plant-specific vulnerability to grazing on seagrass meadows can be  
525 stronger than the effects of CO<sub>2</sub> and eutrophication. This result concurs with findings by  
526 Alsterberg et al. (2013), which showed that the presence of grazers masked the response  
527 of benthic microalgae to ocean acidification and warming. Further studies aiming to  
528 identify the factors underlying the plant-specific seagrass vulnerability to grazers are  
529 thus of vital importance.

530 Our results showed that separated CO<sub>2</sub> or nutrient enrichment individually result in a  
531 loss of the carbon sink capacity of the low-nutrient meadow, as opposed to the high-  
532 nutrient meadow. This loss contrasts with results of previous studies conducted in situ  
533 with other seagrass species, which found that the meadow carbon sequestration capacity  
534 was unaffected by nutrient addition (Antón et al., 2011) or increased due to CO<sub>2</sub>  
535 enrichment (Russell et al., 2013). In both studies, the whole-community metabolism  
536 was used as indicator of the carbon storage capacity of seagrass meadows. In our study,  
537 however, the loss of carbon storage occurred without a significant response of the

538 whole-community metabolism, indicating a metabolic compensation between the *Z.*  
539 *noltii* populations and the leaf epiphyte and sediment microbial communities. A similar  
540 dynamic global balance has also been reported for marine pelagic systems under CO<sub>2</sub>  
541 addition (Silyakova et al., 2013).

542 Overall, we found that shifts in the community dynamics of leaf epiphytes or sediment  
543 bacteria mediated the multi-level responses of *Z. noltii* meadows to independent CO<sub>2</sub> or  
544 nutrient addition. They also modulated the attenuation of individual effects under  
545 simultaneous CO<sub>2</sub> and nutrient enrichment. Overgrazing masked the response to CO<sub>2</sub>  
546 enrichment and eutrophication but only in the high-nutrient meadow. Our findings  
547 highlight the importance of integrative multi-level and ecosystem-based approaches  
548 considering not only species interactions and connections between organization levels,  
549 but also the effect of interactive stressors, to anticipate the evolution of seagrass  
550 meadows in the near future and to endorse conservation efforts.

551

## 552 **Appendices**

553 Appendix A. Full comparative description of the low- and high-nutrient meadows of  
554 *Zostera noltii* in June-August 2011, prior to the start of the experiment.

555 Appendix B. Seawater chemistry within the experimental mesocosms.

556 Appendix C. Full results of the response to CO<sub>2</sub> and nutrient additions of *Zostera noltii*  
557 plant-, community-, and ecosystem-level traits measured through time.

558 Appendix D. Full results of the response to CO<sub>2</sub> and nutrient additions of *Zostera noltii*  
559 plant-, community-, and ecosystem-level traits measured at the end of the experiment.

560 Appendix E. Results of principal component analyses.

561

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772 dioxide and temperature for plant-herbivore interactions: a metaanalysis, *Glob. Change*  
773 *Biol.*, 12, 27-41, 2006.
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775 Table 1. Environmental and *Zostera noltii* community traits that revealed significant  
 776 differences between the low- and high-nutrient donor meadows during June-August  
 777 2011, prior to the start of the experiment, tested using unpaired t-tests. <sup>a</sup>Sqrt-  
 778 transformed data to meet normality. <sup>b</sup>Mann-Whitney rank tests were conducted for  
 779 variables that did not meet normality even after transformation. All measured traits and  
 780 methods are shown in Appendix A (Supplement).

	Low-nutrient meadow	High-nutrient meadow
Leaf phenolics (mg (gDW) <sup>-1</sup> )	48 <sub>±</sub> 1.2	29 <sub>±</sub> 3.2
Leaf nitrogen (mg gDW <sup>-1</sup> )	21 <sub>±</sub> 0.8	25 <sub>±</sub> 0.6
Leaf C : N	19 <sub>±</sub> 0.7	16 <sub>±</sub> 0.4
Rhizome starch (mgGlu (gDW) <sup>-1</sup> )	473 <sub>±</sub> 14	355 <sub>±</sub> 27
Rhizome TNC (mg Glu g (DW) <sup>-1</sup> )	668 <sub>±</sub> 18	532 <sub>±</sub> 28
Shoot area (cm <sup>2</sup> shoot <sup>-1</sup> ) <sup>a</sup>	7.8 <sub>±</sub> 0.6	4.5 <sub>±</sub> 0.7
<i>Z. noltii</i> density (shoots m <sup>-2</sup> )	5517 <sub>±</sub> 755	2664 <sub>±</sub> 411
<i>Z. noltii</i> cover (% of sediment surface) <sup>b</sup>	96 <sub>±</sub> 2.2	18 <sub>±</sub> 9.8
<i>Ulva</i> spp. cover (% of sediment surface) <sup>b</sup>	absent	38 <sub>±</sub> 12
Seawater nitrate (μM) <sup>b</sup>	< 0.01	1.1 <sub>±</sub> 0.2
Seawater ammonium (μM)	0.7 <sub>±</sub> 0.2	3.0 <sub>±</sub> 0.4
Seawater phosphate (μM)	0.5 <sub>±</sub> 0.1	1.2 <sub>±</sub> 0.1

781

782 **Figure captions**

783 Fig. 1. Effects of CO<sub>2</sub> and nutrient additions on *Zostera noltii* plant-, community-, and  
784 ecosystem-level traits from the low-nutrient (left graphs) and high-nutrient meadow  
785 (right graphs) through time. Symbols are means ( $\pm$ se,  $n = 2$ ).  $F$  statistics and  $p$  levels  
786 from RM ANOVA tests are shown for among-subject factors (CO<sub>2</sub>; Nut: nutrients) and  
787 their interaction (CO<sub>2</sub> x Nut), and for within-subject factor (time) and interactions (Time  
788 x CO<sub>2</sub>, Time x Nut, Time x CO<sub>2</sub> x Nut). Only the significant effects ( $p < 0.05$ ) and  
789 useful traits for results interpretation are shown here; the non-significant effects are  
790 shown in Appendix C (Supplement). \*Variables that did not meet normality after  
791 transformation, for which the significance level was more restrictive ( $p < 0.03$ ) to  
792 minimize the possibility of Type I error. \*\*Variable sqrt-transformed to meet normality.

793 Fig. 2. Effects of CO<sub>2</sub> and nutrient additions on *Zostera noltii* plant-, community-, and  
794 ecosystem-level traits measured at the end of the experiment from the low-nutrient (grey  
795 bars) and high-nutrient meadow (black bars). Bars are means ( $\pm$ se,  $n = 2$ ). The  $F$   
796 statistics and  $p$  levels from two-way ANOVA tests are shown for each fixed crossed  
797 factor (CO<sub>2</sub>; Nut: nutrients) or interaction (CO<sub>2</sub> x Nut). Lowercase letters above bars  
798 show significant differences between treatments for significant interactions ( $t$  tests, see  
799 Methods). Only restrictive significant effects ( $p < 0.03$ ) selected to minimize the  
800 possibility of Type I error due to unequal variances are shown here; the non-significant  
801 effects are shown on Appendix D (Supplement).

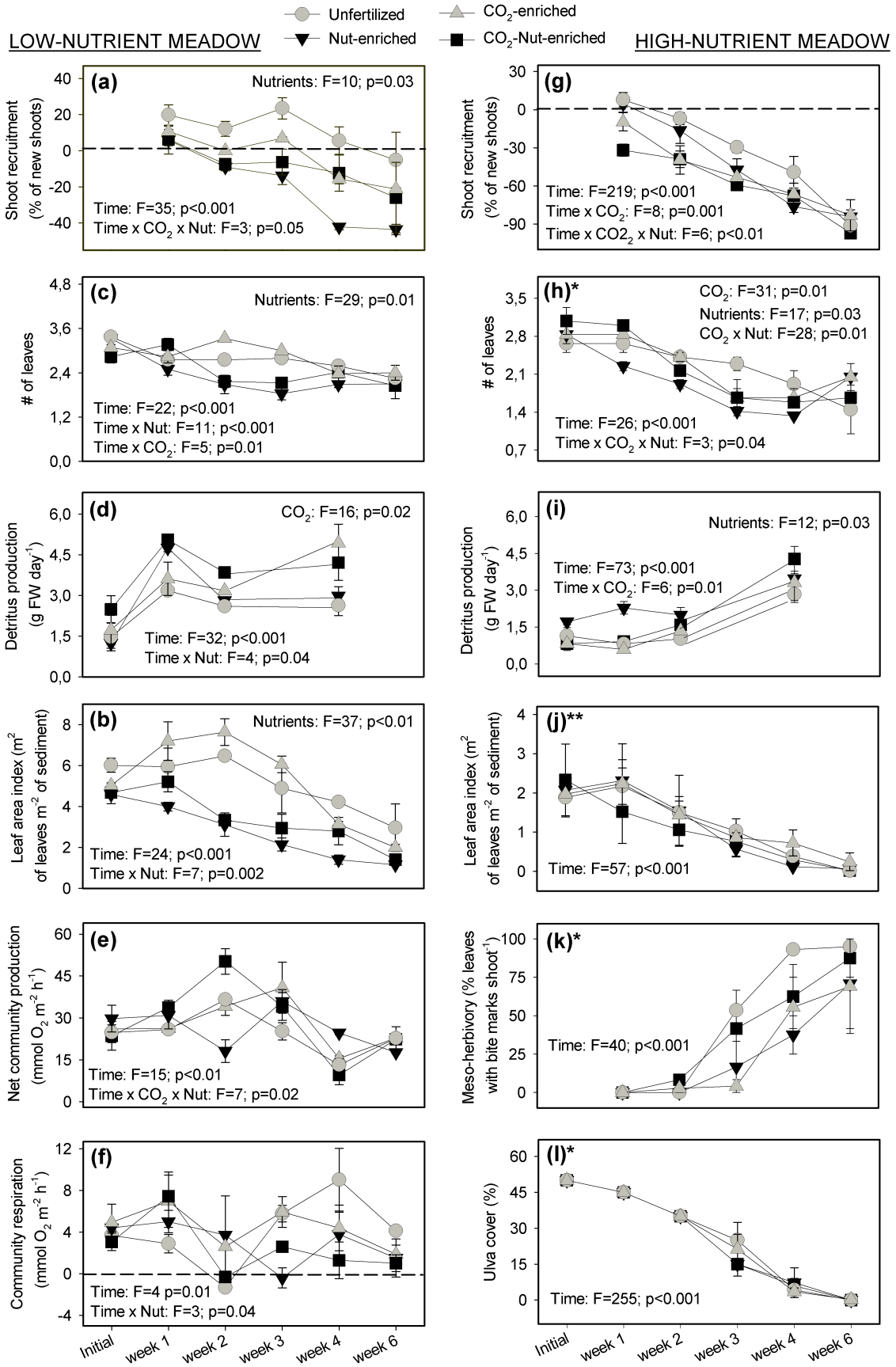
802 Fig. 3. Responses of *Zostera noltii* epiphyte populations to experimental addition of  
803 CO<sub>2</sub> and nutrients: (a) Relative abundance of the main epiphyte taxa of plants exposed  
804 to experimental treatments; and (b) NMDS ordination of experimental treatments (see  
805 symbol legend below treatment names in Fig. 3a) based on leaf epiphytes of plants from  
806 the low-nutrient (L; grey symbols) and high-nutrient meadow (H; black symbols).  
807 Pseudo- $F$  statistics and  $p$  levels from two-way PERMANOVA test are shown in the  
808 NMDS diagram for each fixed crossed factor (CO<sub>2</sub>; Nut: nutrients) and their interaction  
809 (CO<sub>2</sub> x Nut).

810 Fig. 4. Principal components analysis of *Zostera noltii* plant-, community, and  
811 ecosystem- level responses to treatments through time: (a) low-nutrient and (b) high-  
812 nutrient meadow. Numbers inside the symbols indicate sampling weeks from 0 to 6.  
813 The initial variability (week 0) along component I is incorporated within a grey

814 rectangle to highlight the range of initial natural variability. Variable loadings on the  
815 two principal components are depicted in right graphs. LAI refers to leaf area index,  
816 NCP to net community production and CR to community respiration.

817 Fig. 5. Summary of the effects of CO<sub>2</sub> (blue line) and nutrient (green line) additions, and  
818 when significant of their interaction (red line), on *Zostera noltii* plant- community and  
819 ecosystem- level traits of low-nutrient (a) and high-nutrient (b) meadows. Solid lines  
820 indicate significant effects on variables measured at the end of the experiment and  
821 dashed lines on variables measured through time. Dotted lines indicate no significant  
822 effects. Letters below the x-axis denote unfertilized (U) and enriched (E) treatment,  
823 which is the pooled mean response to the respective enrichment over the other when  
824 there was no significant interaction (no red line). For shoot mortality and net community  
825 production (NCP) in the low-nutrient meadow a significant Time x CO<sub>2</sub> x Nutrients  
826 interaction was detected and values were represented as for CO<sub>2</sub> x Nutrients significant  
827 interactions. \* Apparent effect due to a drastic increase in unfertilized plants in relation  
828 to the initial field conditions (see results).

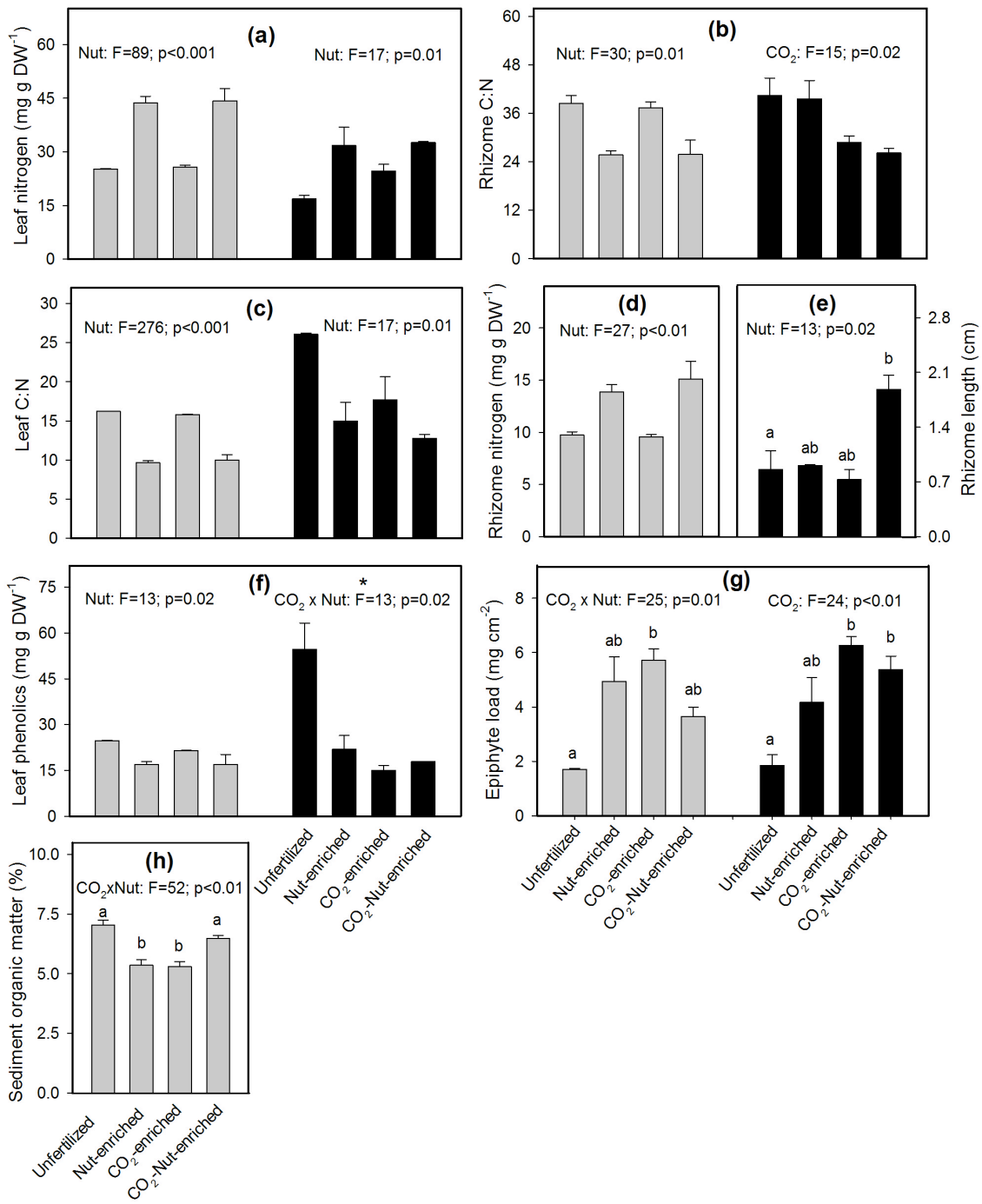
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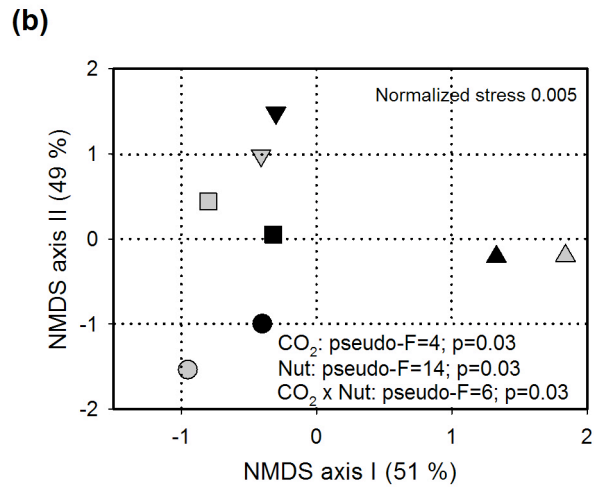
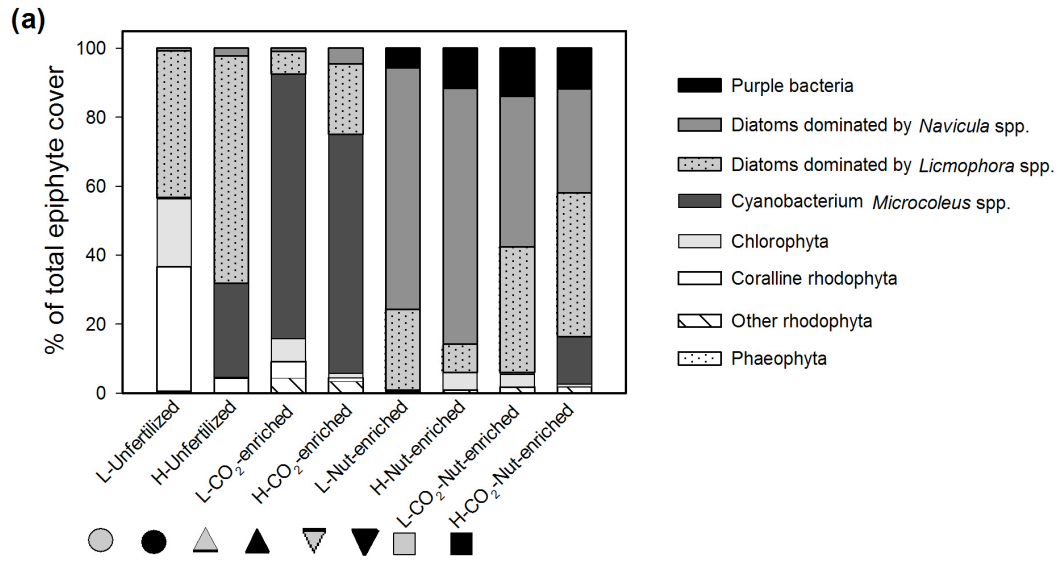


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831 Figure 1

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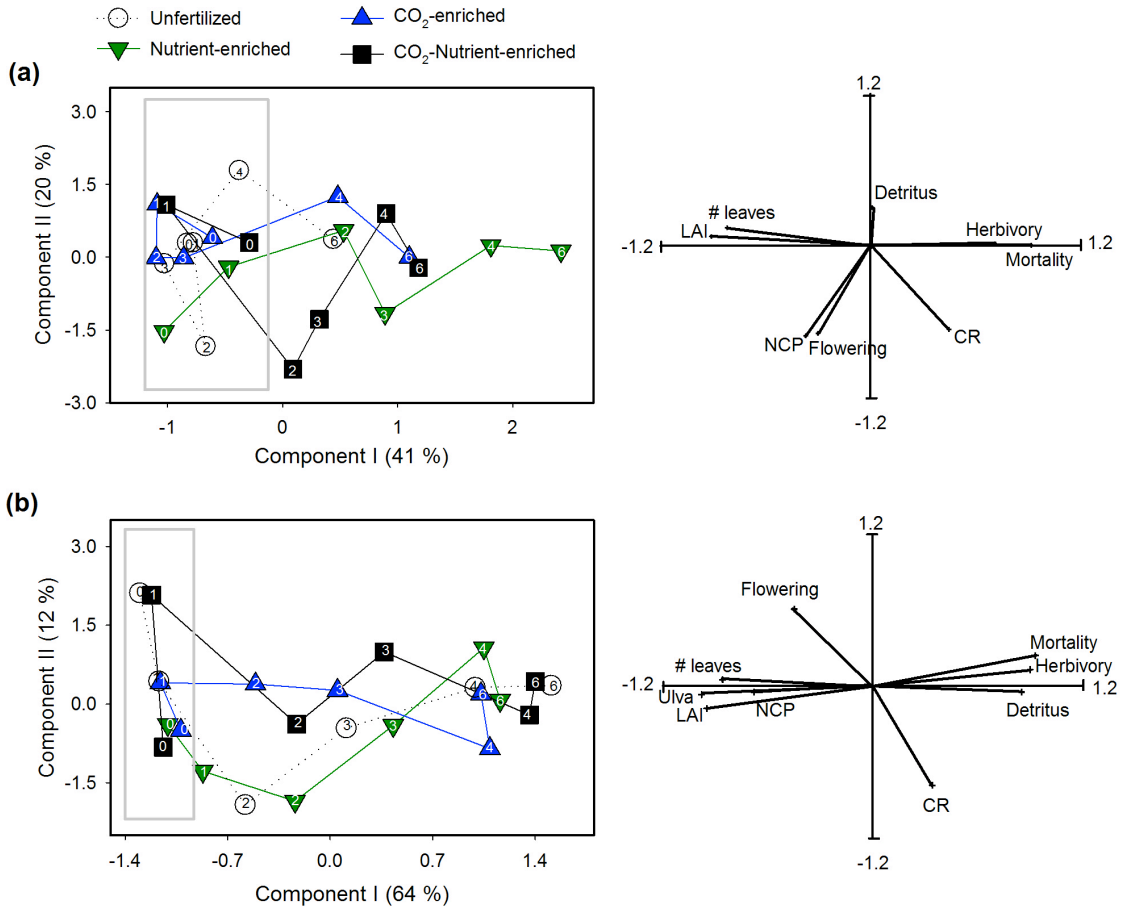


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837 Figure 3

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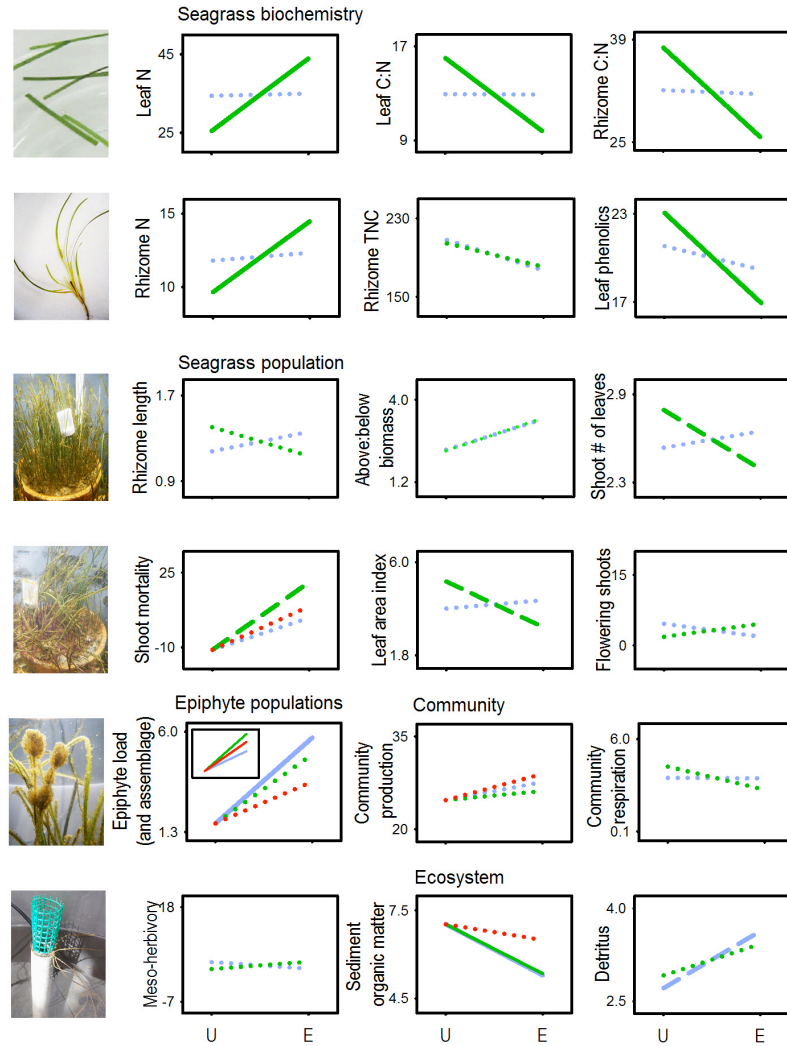




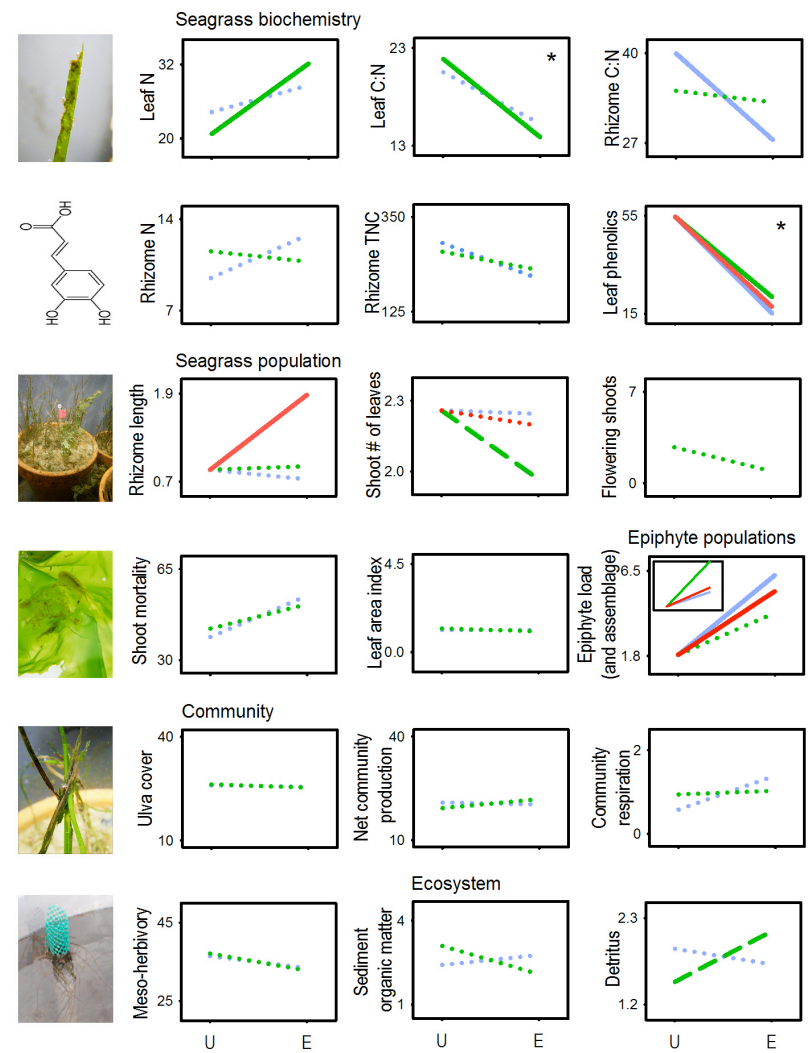
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840 Figure 4.

**(a) Low-nutrient meadow**



**(b) High-nutrient meadow**



841 Figure 5