Biogeosciences Discuss., 11, 5239–5274, 2014
www.biogeosciences-discuss.net/11/5239/2014/
doi:10.5194/bgd-11-5239-2014

Biogeosciences
Discussions
Open Access

CO_2 and nutrient-driven changes across multiple levels of organization in *Zostera noltii* ecosystems

B. Martínez-Crego, I. Olivé, and R. Santos

Centre of Marine Sciences (CCMAR), Universidade do Algarve – Campus de Gambelas,
8005-139 Faro, Portugal

Received: 19 February 2014 – Accepted: 20 March 2014 – Published: 3 April 2014

Correspondence to: B. Martínez-Crego (bmcrego@ualg.pt)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Increasing evidence emphasizes that the effects of human impacts on ecosystems must be investigated using designs that incorporate the responses across levels of biological organization as well as the effects of multiple stressors. Here we implemented a mesocosm experiment to investigate how the effects of CO_2 enrichment and its interaction with eutrophication, scale-up from changes in primary producers at the individual- (biochemistry) or population-level (production, reproduction, and/or abundance) to higher levels of community (macroalgae abundance, herbivory, and global metabolism) and ecosystem organization (detritus release and carbon sink capacity). The responses of *Zostera noltii* seagrass meadows growing in low- and high- nutrient field conditions were compared. In both meadows, the effect of elevated CO_2 levels was mediated by epiphyte proliferation (mostly the cyanobacterium *Microcoleus* spp.), but not through changes in plant biochemistry or population-level traits. In the low-nutrient meadow, epiphyte proliferation suppressed the CO_2 benefits on *Z. noltii* leaf production and led to increased detritus and decreased organic matter in sediment. Faster and stronger responses to nutrients than to CO_2 were observed. Nutrient addition enhanced the nutritional quality of *Z. noltii* (high N, low C : N and phenolics) and the loss of leaves and shoots, while promoted the proliferation of pennate diatoms and purple bacteria. These changes led to a reduced sediment organic matter, but had no significant effects on herbivory nor on community metabolism. Interestingly, the interaction with CO_2 attenuated eutrophication effects. In the high-nutrient meadow, a striking shoot decline caused by amphipod overgrazing was observed, with no response to CO_2 and nutrient additions. Our results reveal that under future scenarios of CO_2, the responses of seagrass ecosystems will be complex, being mediated by epiphyte proliferation rather than by effects on plant biochemistry. The multi-level responses of the system to nutrients overwhelmed those to CO_2 enrichment, but the interaction between stressors reduced the effects of eutrophication. Both, CO_2 and nutrient enrichments can reduce the carbon sink capacity of seagrass meadows.

1 Introduction

Understanding community and ecosystem responses to human impacts is a challenge that requires integrating not only the organism-level responses across populations and entire systems (Russell et al., 2012), but also synergistic effects of multiple stressors (Woodward et al., 2010). A large number of articles has been published on the effects of ocean acidification, and reviewed among others by Doney et al. (2009) and Kroeker et al. (2010). This body of research has revealed that ocean acidification can be detrimental to most marine calcifying organisms, while increasing carbon dioxide (CO_2) concentration can benefit primary productivity of phytoplankton, cyanobacteria, fleshy algae, and seagrasses. Our current understanding of these effects is largely based on the species-specific responses of individuals or populations. However, the broad variability in responses among organisms may influence species interactions and drive unforeseen impacts on marine communities and ecosystems (Hall-Spencer et al., 2008; Kroeker et al., 2013).

The interactive effect of multiple stressors on ecological communities remains largely unknown (Crain et al., 2008). Atmospheric CO_2 concentration has increased from preindustrial levels of approximately 280 ppm to 397 ppm in 2013 (NOAA, Mauna Loa Observatory, Hawaii), leading to a rise in the CO_2 absorbed by the ocean with an associated pH decrease of 0.1 units. An additional pH decrease of 0.07–0.31 units is expected by the end of the 21th century based on the "Intergovernmental Panel on Climate Change" predictions (IPCC, 2013). In the marine environment, ocean acidification can locally interact with excess nutrients from coastal eutrophication to accelerate changes in ecosystem structure and functioning (Russell et al., 2009).

Human impacts that alter the availability of environmental resources are shifting the nutritional quality of primary producers through changes at the biochemical or individual levels of the biological organization (e.g. allocation of resources to growth, storage, and chemical defences). In addition, changes in environmental resources can favour different types of producers and alter the inter-species competitiveness and producers' abundances. Overall, this may affect ecological interactions and fluxes, leading to shifts at community and ecosystem levels. Under elevated nutrient concentrations, aquatic and terrestrial ecosystems with higher producer nutritional quality often support higher rates of herbivory, more rapid decomposition rates and recycling of nutrients, and lower net accumulation of soil carbon (Wardle et al., 2004; Cebrian et al., 2009). The effects of nutrient enrichment have been widely described on terrestrial, freshwater, and marine ecosystems, whereas the scaling up of elevated CO_2 effects has been mostly studied in terrestrial plants. Under elevated CO_2 levels, and especially if nutrient availability is limiting to growth, terrestrial plants typically increase the accumulation of carbohydrates and/or carbon-based secondary compounds (mostly phenolics). This increases C : N ratios ("nitrogen dilution" effect) and sometimes leaf toughness through increasing indigestible polymers such as cellulose and lignin (Zvereva and Kozlov, 2006; Lindroth, 2010; Robinson et al., 2012). Herbivores usually compensate for this lower food quality by eating more (Stiling and Cornelissen, 2007). In addition, CO_2 enrichment may shift the biomass and composition of soil microbial communities, directly through different responses of microbial groups to high CO_2/low pH (Krause et al., 2012; Lidbury et al., 2012) or indirectly through reducing foliar and detritus quality (Drigo et al., 2007). Overall, high CO_2 levels may have both positive and negative consequences on the decomposition of soil organic matter and nutrient recycling (Lindroth, 2010).

In this study, we use seagrass meadows as model ecosystems to investigate the scaling up of the effects of elevated CO_2 and nutrient concentrations on marine coastal environments. A rich epiphyte community usually colonizes seagrass leaves, thus providing a useful system for studying how changes in environmental resources can favour different types of producers (e.g. non-calcareous vs. calcareous). Seagrasses beds are widely distributed habitats that host high biodiversity and provide valuable ecosystem services (Waycott et al., 2009). They are highly productive systems that sequester larger amounts of carbon per area than tropical forests, providing for a long-term removal of carbon dioxide from the atmosphere (Pidgeon, 2009; Fourqurean et al., 2012).

The maintenance of the key services provided by seagrass ecosystems under global change is thus of prime importance for human well-being.

A mesocosm experiment was conducted to assess: (1) how CO_2 and nutrient enrichments affect primary producers' at the individual- (plant biochemistry including CN and allocation of resources to carbohydrate reserves and carbon-based chemical defences) or population-level (plant allocation of resources to biomass and reproduction, and composition and abundance of seagrass epiphytes), and (2) whether these changes propagate to the community (macroalgae abundance, meso-herbivory, whole-community metabolism) and to the ecosystem (detritus production and organic carbon storage in sediment). The responses of *Zostera noltii* seagrass meadows developing in low- and high- nutrient conditions in the field were compared to assess if they differently react.

2 Methods

2.1 Study meadows

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

2.2 Mesocosm experiment

The study was conducted in an outdoor mesocosm system at the Ramalhete field station of the Centre of Marine Sciences, which is located at the Ria Formosa lagoon. To assess the effects of CO_2 and nutrients on *Z. noltii* meadows an enrichment experiment was conducted for 6 weeks during August–September 2011, after 4 days of acclimation to the experimental mesocosms. This time span is enough to detect any treatment-driven changes in physiological, morphological and population traits of this fast-growing species (e.g. Peralta et al., 2002). Core samples of *Z. noltii* community, including sediment and algal, faunal and microbial components, were randomly collected from each donor meadow and allocated to flowerpots of 20 cm of diameter and height. Three flowerpots were placed in each of the 16 experimental mesocosms (tanks of 110 L), which were exposed to combinations of two CO_2 and two nutrient levels in a crossed design with two replicates.

Experimental levels of CO_2 encompassed present (pH 8.00 ± 0.02, equivalent to ca. 400 ppm CO_2) and future conditions (pH 7.83 ± 0.01, equivalent to ca. 800 ppm CO_2) in Ria Formosa lagoon. The mesocosms received sand-filtered seawater from two head tanks of 1000 L at a rate of 240 $\mathrm{L\,h^{-1}}$. In one of the head tanks, the water CO_2 was manipulated to ensure fixed pH differences between treatment means within the range predicted for 2100 by the IPCC (pH decline = −0.18 units) following a commonly used method (e.g. Alsterberg et al., 2013). The CO_2 injection was controlled by an auto-analyzer (Yokogawa, EXAxt 450, Tokyo, Japan), which continuously monitored the water pH and temperature. Total alkalinity, pH, temperature and salinity within the mesocosms, as well as the seawater DIC and carbon speciation are provided on Appendix B (Supplement).

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

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

2.3 Producers' traits at the individual- or population-level

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

Allocation of plant resources to biomass and reproduction at the population-level was monitored almost every week. Shoot recruitment or mortality were quantified within each flowerpot excluding shoots growing around the border to avoid edge effects. Allocation to reproduction was quantified as density of flowering shoots. The number of leaves of three-five randomly chosen shoots within each flowerpot and the leaf area index (LAI) were quantified as indicators of aboveground productivity. To estimate LAI, leaf area was measured on these three-five shoots, averaged, multiplied by the pot number of shoots and scaled per surface area.

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

After six weeks, all shoots from each mesocosm were harvested and plant traits quantified in each flowerpot. Belowground productivity was estimated from the vertical or horizontal rhizome length and from the total root length (number of roots multiplied by the average root length) of five shoot per pot. The above- and below-ground biomass allocation was quantified after drying at 60 °C until constant weight. The pooled epiphyte load of three shoots relative to leaf area was quantified after drying at 60 °C until constant weight (two replicates per mesocosm). Pooled material of five shoots was separated into leaves (without epiphytes) and rhizomes, freeze-dried, weighted, ground to fine powder and used in subsequent analyses of plant biochemistry. Carbon and nitrogen concentrations were analyzed using an elemental analyzer (Carlo-Erba, Milan, Italy). Total non-structural carbohydrates were measured in rhizomes using the phenol-sulfuric acid colorimetric method (Dubois et al., 1956) with glucose as standard, after sugar extraction in hot ethanol and enzymatic conversion of starch to glucose equivalents (Smith and Zeeman, 2006). Total phenolics were quantified as indicators of plant allocation of resources to chemical defences. Phenolics were extracted from leaf material with methanol 50 % for 24 h under constant agitation at 4 °C and determined with a spectrophotometer using chlorogenic acid as standard following a modified Folin–Ciocalteu method (Bolser et al., 1998).

2.4 Community- and ecosystem-level traits

The response of the seagrass community to CO_2 and nutrient enrichments was quantified weekly by: (1) the percentage of flowerpot surface covered by *Ulva* spp.; (2) the feeding activity of mesograzers (percentage of leaves showing bite marks in the same shoots used to measure the morphological traits); and (3) the whole-community metabolism quantified from the oxygen evolution within benthic chambers of 17 cm diameter (4.8 ± 0.01 L incubated) fitted to the flowerpots for 30–45 min at midday (12–14 h). A transparent acrylic chamber to estimate net production and a dark chamber to estimate respiration were simultaneously deployed within each mesocosm. Dissolved oxygen concentration was measured by spectrophotometry using the Winkler method (Labasque et al., 2004) in three water samples collected before and after incubations into 12 mL soda glass vials. Community metabolism was estimated from the net change in oxygen concentration during incubations integrated by the chamber volume and standardized by incubation time and bottom area. There were no effects of enclosure on the water temperature within chambers (measured with onset HOBO loggers, Southern MA, USA). All incubations were run under irradiances of photosynthetically active radiation (PAR) averaging 283 ± 8.6 µmol quanta $m^{-2}\,s^{-1}$ (measured with a Li-192SA underwater PAR quantum sensor, Li-Cor, USA), when photosynthesis is light saturated and not photoinhibited (Peralta et al., 2002).

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

2.5 Statistical analyses

The effects of CO_2 and nutrient treatments throughout the experiment were tested using three-way repeated-measures analyses of variance (RM ANOVA). The subject repeatedly sampled was the mesocosm, CO_2 and nutrients were the among-subject factors (two fixed crossed factors) and time the within-subject factor. To avoid the masking effect of the strong initial differences between meadows on the responses to experimental treatments, data for the low- and high-nutrient meadows were analyzed separately. Data were checked for parametric assumptions and transformed where needed. When sphericity was not met, corrected significance levels from Greenhouse–Geisser adjustment were used (Quinn and Keough, 2002).

The effects of CO_2 and nutrient treatments on variables measured at the end of the experiment were tested using two-way ANOVAs (two fixed crossed factors) after checking parametric assumptions. *t* tests (student for equal or Welch for unequal variances) were used to interpret significant interactions and to confirm ANOVA results due to unequal variances. When no significant interaction was detected, the main effect of a given factor was pooled over the levels of the other factor in the *t* tests ($n = 4$). When *t* tests did not confirm ANOVA results the significance level was more restrictive to minimize the possibility of Type I error (mistakenly detection of differences). Again, data for the low- and high-nutrient meadows were analyzed separately.

To assess the ordination of treatments based on differences in the composition of leaf epiphyte populations, a non-metric Multi-Dimensional Scaling analysis (NMDS) with Bray–Curtis distances was carried out. Because NMDS axes are arbitrary, the final solution was rotated using a Principal Component Analysis (PCA) to align the largest variance in the first axis. The significance of the effect of CO_2 and nutrient treatments on assemblage composition was tested with a two-way permutational analysis of variance (PERMANOVA; two fixed crossed factors). To perform the test, Bray–Curtis distances were calculated from untransformed data and 999 permutations were used under a reduced model.

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

3 Results

3.1 Responses of meadow traits measured through time

The response of the low-nutrient meadow showed a threshold at the third week of the experiment, when most variables responded differently from the first two weeks (Fig. 1, left). Shoot recruitment occurred mostly in unfertilized but also in CO_2-enriched conditions until the third week (Fig. 1a), after which shoot mortality progressively increased. Figure 1b suggests that the *Z. noltii* leaf area index (LAI) tended to increase with CO_2 enrichment until the third week of the experiment. A positive, significant effect of the CO_2 enrichment was observed on detritus production throughout the experiment (Fig. 1d). Nutrient addition increased shoot mortality (Fig. 1a), whereas it decreased LAI and leaf number (Fig. 1b and c). Shoot mortality induced by the nutrient enrichment was attenuated by the simultaneous addition of CO_2, especially from the third week onwards (Fig. 1a). A treatment and time interaction was detected on the community production and respiration (Fig. 1e and f). These variables showed high variability with similar ranges of variation in unfertilized and enriched conditions. No treatment effects were detected throughout the experiment on shoot flowering or meso-herbivory (see Appendix C, Supplement).

The responses of the high-nutrient meadow to CO_2 enrichment included an increased shoot mortality during the second and third weeks and an increased detritus production at the end of the experiment (Fig. 1g and i). Nutrient addition decreased the number of leaves per shoot and increased detritus production throughout the experiment (Fig. 1h and i). CO_2 enrichment interacted with nutrients to alleviate the nutrient-induced reduction of the number of leaves (Fig. 1h). No effects of CO_2 or nutrient enrichments were observed through time on LAI, meso-herbivory, *Ulva* spp. cover, shoot flowering, and community production or respiration (Figs. 1j–l and C, Supplement). Independently of the experimental treatments, overgrazing by the herbivorous amphipod *Cymadusa filosa* Savigny severely affected the plants from the high-nutrient meadow causing massive shoot mortality (Fig. 1k and g). At the end of the experiment a mean (±se) of 89 (±3.7) % of shoots died, 81 (±9.1) % of the seagrass leaves showed bite marks and leaf area was reduced from 5.0 (±0.2) to 1.0 (±0.4) $cm^2\,shoot^{-1}$. Similarly, *Ulva* spp. cover progressively decreased, being close to 0 % in all treatments after six weeks (Fig. 1l). At the end of the experiment, all *Z. noltii* shoots and *Ulva* spp. fronds disappeared from three flowerpots (one unfertilized and two CO_2-and-nutrient-enriched). However, net production and respiration in these pots were within the range observed in the other pots (Fig. Cd and e, Supplement), indicating that the metabolism of the sediment microbial community was similar to that of the *Z. noltii* community.

We did not detect any significant effect of CO_2 or nutrient enrichments on plant damage by mesoherbivory in the low- or high-nutrient meadows. However, at the end of the experiment plants from the high-nutrient meadow showed 81 (±9.1) % of leaves with bite marks, compared to only 6.9 (±3.2) % in the low-nutrient meadow (Figs. 1k and Cb, Supplement). These between meadow differences, as well as the link between shoot mortality and meso-herbivory in masking the enrichment effects in the high-nutrient meadow, were further confirmed by a PCA of responsive variables from the low- and high-nutrient meadows at the end of the experiment (see Appendix E, Supplement).

3.2 Responses of meadow traits measured at the end of the experiment

In plants from the low-nutrient meadow (Fig. 2, grey bars), nutrient enrichment enhanced the leaf nutritional quality (high leaf nitrogen and low leaf C : N ratio, Fig. 2a and c) and the accumulation of nitrogen in rhizomes (high rhizome nitrogen concentration and low rhizome C : N ratio, Fig. 2d and b), whereas it had a negative impact on the accumulation of leaf phenolics (Fig. 2f). A significant interaction of CO_2 and nutrient additions was detected for sediment organic matter and epiphyte load (Fig. 2g and i). The leaf epiphyte load increased significantly under CO_2 addition, whereas nutrient enrichment and especially the interactive CO_2 and nutrient additions had a lower and not significant effect (*t* test comparisons in Fig. 2i). Similarly, CO_2 and nutrient interaction resulted in maintenance of the organic matter content in the sediment, which tended to decrease with separated CO_2 and nutrient additions (*t* test comparisons in Fig. 2g).

In plants from the high-nutrient meadow (Fig. 2, black bars), CO_2 enrichment decreased rhizome C : N (Fig. 2b) and increased epiphyte loads (Fig. 2i). The CO_2-induced increase of the epiphyte load was maintained under the simultaneous addition of nutrients. Nutrient addition enhanced the leaf nutritional quality (high leaf nitrogen concentration, Fig. 2a). A reduction of leaf C : N ratio and phenolics was detected apparently in response to CO_2 and/or nutrient enrichments (Fig. 2c and f), but this was actually caused by an increase of these traits in the unfertilized plants at the end of the experiment in relation to initial conditions (Table 1). A synergistic interaction between CO_2 and nutrient additions caused an increase of the rhizome length (Fig. 2h).

Variables for which no significant effects of CO_2 or nutrient additions were detected are shown in Appendix D (Supplement).

3.3 Responses of *Z. noltii* epiphytes

Both, CO_2 and nutrient additions altered the relative abundance of epiphyte populations, whereas elevated nutrient levels also modified the epiphyte composition (Fig. 3a). In the unfertilized plants, the epiphyte cover was low and the most abundant leaf epiphytes were the fanlike diatoms *Licmophora* spp. The second-most-abundant epiphyte in plants from the low-nutrient meadow was the encrusting coralline algae *Melobesia membranacea*, whereas in plants from the high-nutrient meadow it was the cyanobacterium *Microcoleus* spp. The response to the CO_2 enrichment in both, low- and high-nutrient meadows was a great increase of epiphyte cover, mostly due to a bloom of *Microcoleus* spp. (73 % of the total cover) that outcompeted the diatoms *Licmophora* spp. and the encrusting corallines. Under nutrient-enrichment pennate diatom populations dominated by *Navicula* spp. outcompeted the other taxa. In the nutrient- and CO_2-and-nutrient- treatments the structure of epiphyte assemblages was similar, with purple bacteria only appearing in them. Chlorophytes (mainly *Ulva prolifera*) and filamentous rhodophytes (mainly *Bangia* spp. and *Stylonema alsidii*) were also present in all treatments. Temporal changes in epiphyte abundances within the enriched mesocosms shifted from relatively low epiphyte loads until the second week to increasing epiphyte loads from the third week onwards, with the proliferation of purple bacteria in nutrient- and CO_2-and-nutrient-enriched treatments during the fourth week.

NMDS ordination of treatments based on the epiphyte composition showed clear CO_2 effects (Fig. 3b). CO_2 treatments were separated along axis I (51 % of variance explained), whereas the other treatments were ordered along axis II (49 % of variance explained) from unfertilized to CO_2-, CO_2-and-nutrient-, and nutrient-enriched. Separation of CO_2 enrichments along axis I was due to a higher epiphyte cover (mean ± se: $22 \pm 2.3\,\mathrm{cm}^2$ per $10\,\mathrm{cm}^2$ of leaf) than the unfertilized, nutrient-enriched and CO_2-and-nutrient-enriched treatments (7.6 ± 1.4, 11 ± 1.3 and $8.4 \pm 3.2\,\mathrm{cm}^2$ per $10\,\mathrm{cm}^2$ of leaf, respectively). Treatments of both, low- and high-nutrient meadows were nearby in the ordination diagram, reflecting low differences among meadows in the response of the epiphyte assemblage. NMDS pattern was further confirmed by the PERMANOVA results, which showed significant effects of CO_2, nutrients and their interaction (Fig. 3b).

The above-mentioned PCA of traits from the low- and high-nutrient meadows further confirmed the increased epiphyte load and the change in epiphyte composition as main drivers of the meadow responses to CO_2 and nutrient enrichments (see Appendix E, Supplement).

3.4 Response trajectories through time

The first two PCA components of the low-nutrient meadow traits measured through time, explained 41 % (component I) and 20 % (component II) of the variance. The *Z. noltii* traits that highly correlated with component I were the LAI and the number of leaves, which were negatively correlated with shoot mortality and herbivory (Fig. 4a, right graph). Flowering, community production and community respiration highly correlated with component II (variable loadings are presented in Table E2, Supplement). The variability of all treatment scores on the component I during the first week and of unfertilized and CO_2-enriched treatments during the second and third weeks were within the initial range of natural variability (week 0, grey rectangle in Fig. 4a, left graph). At this time, *Z. noltii* plants showed higher LAI and higher number of leaves. The time series ordination of the rest of treatments along component I revealed that the effects of nutrient addition started during the second week, when the scores of nutrient- and CO_2-and-nutrient-treatments suddenly shifted to higher values. These nutrient effects were dominated by high mortality of *Z. noltii* shoots and to a less extent by high mesoherbivory. The highest scores on component I were attained by the nutrient-treatment during the fourth to sixth weeks. The system response to the CO_2 treatment was slower (starting at week 4) and of lower magnitude than the response to nutrient- and CO_2-and-nutrient-treatments. This analysis supported the previous indication of a temporal threshold for the meadow responses, which was the second week for elevated nutrients and the fourth week for elevated CO_2. No clear ordination of treatments was detected along component II, indicating that traits highly correlated with this component were substantially influenced by natural variability.

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

4 Discussion

The response of the *Z. noltii* meadows to nutrients was faster and of higher magnitude than the response to CO_2 (Fig. 5a and b). Eutrophication and CO_2 caused significant shifts in primary producers' quality, which were mainly mediated by increased loads of diatoms or cyanobacteria epiphyte populations. Increased *Z. noltii* quality (high leaf nitrogen) was also a response to nutrient addition but not to CO_2 enrichment. In the meadow from low-nutrient field conditions, a decrease in sediment organic matter storage due to independent CO_2 and nutrient additions was evidenced at the ecosystem-level. Interestingly, all effects on epiphytes and sediment were attenuated when both nutrient and CO_2 acted simultaneously, as also occurred with the nutrient-induced shoot mortality. The higher plant quality induced by nutrients did not cascade up to an increase in herbivory, suggesting that the decrease of organic matter in the sediment was due to enhanced microbial activity. In the meadow from high-nutrient field conditions overgrazing by amphipods unrelated to CO_2 or nutrient enrichments drove, however, a striking seagrass decline. The connections between different organization levels assessed here can help to better understand the consequences of ocean acidification and eutrophication on ecosystems, as has been previously pointed out by other authors (Woodward et al., 2010).

4.1 Effects of CO_2 enrichment in low-nutrient meadows

The CO_2 enrichment had no direct effects on *Z. noltii* biochemistry (Fig. 5a), with no significant changes on the carbon-rich phenolics and the total carbohydrate reserves. This result is in accordance with resource allocation hypotheses subsumed in the Growth-Differentiation Balance hypothesis (Stamp, 2003), which predicts that under no light and nutrient limitation carbon is allocated to growth instead to the production of secondary metabolites. In fact, we found that CO_2 addition tended to enhance *Z. noltii* leaf production (LAI) in the first three weeks of the experiment. However, the effect on leaf production disappeared after the third week when a massive proliferation of epiphytes reduced the light available for *Z. noltii* photosynthesis. Our results are in agreement with previous studies, which have reported that under no light limitation, nutrient deficiency rather than a direct effect of high CO_2 levels drives the accumulation of carbohydrates and phenolic compounds in both terrestrial plants (Lambers, 1993; Peñuelas and Estiarte, 1998) and seagrasses (Campbell et al., 2012).

The response of the *Z. noltii* meadow to elevated CO_2 was mediated by an epiphyte bloom, which was mostly due to the proliferation of the colonial and filament-forming cyanobacterium *Microcoleus* spp. at the expense of a reduction of coralline algae crusts of *Melobesia membranacea* and fanlike diatoms *Licmophora* spp. This is in accordance with previous studies that showed elevated CO_2/low pH to stimulate cyanobacteria growth and photosynthesis (Liu et al., 2010) and to decrease abundance of coralline algae (Hall-Spencer et al., 2008; Martin et al., 2008; Kuffner et al., 2008). Similarly, Hervé et al. (2012) reported negative effects of low pH on diatom valve formation and porosity, which were alleviated by a simultaneous nutrient addition. We found that the activity of mesograzers was insufficient to regulate the epiphyte proliferation in response to increased CO_2 levels, despite their known controlling capacity (Hughes et al., 2004). A likely explanation is that mesograzer activity was exceeded by epiphyte overgrowth (Hauxwell et al., 1998) or that the most abundant mesograzer in our experiment was not a specialist epiphyte grazer (Hughes et al., 2004).

Epiphyte proliferation led to an increase in detritus production and tended to decrease the organic matter accumulated in the sediment. This may have relevant implications, since the organic carbon produced in seagrass meadows sustains important detritus-based food webs (Fenchel, 1977) and provides a major global carbon sink (Pidgeon, 2009). The decreased storage suggests a fast bacterial decomposition of more labile organic matter in the sediment. The acceleration of bacterial degradation of organic matter polysaccharides at low pH reported by Piontek et al. (2010) would also support this explanation.

4.2 Nutrient enrichment and interaction with CO_2 in low-nutrient meadows

We found that nutrient enrichment had a faster and stronger effect than CO_2 addition on meadows developing in low-nutrient conditions. Nutrient enrichment enhanced leaf nutritional quality (high nitrogen and low C : N ratio) and reduced the accumulation of phenolic compounds (Fig. 5a). Both, the overall increase of plant nitrogen (e.g. Cabaço et al., 2008; Invers et al., 2004) and the decrease of phenolics (e.g. van Katwijk et al., 1997; Goecker et al., 2005) have been widely described in seagrasses as a result of nutrient additions. We found that nitrogen concentration was higher in leaves than in rhizomes, as expected for this fast-growing species that acquires preferentially ammonium through the leaves and shows a scarce translocation of nitrogen to belowground tissues (Alexandre et al., 2011). Ammonium toxicity has been reported in *Z. noltii* (Brun et al., 2002) and other seagrass species (Santamaría et al., 1994, van Katwijk et al., 1997). To avoid toxicity, ammonium must be rapidly assimilated into amino acids at the expense of energy and carbon skeletons usually diverted from carbohydrate metabolism (Brun et al., 2002; Invers et al., 2004). In our experiment, ammonium toxicity was not apparent since nutrient addition had no significant effects on carbohydrate concentrations or plant survival. This finding concurs with previous studies, which showed that the effect of ammonium toxicity on carbohydrate reserves can be alleviated by high irradiance (van Katwijk et al., 1997) and simultaneous phosphate supply (Brun et al., 2008).

A reduction of the number of *Z. noltii* leaves and of leaf area index, and an increase of shoot mortality were observed after the second week due to excess nutrients. Shoot mortality abruptly increased after the third week, coinciding with the shift of the epiphyte assemblage from coralline algae and fanlike diatoms to a dense layer of pennate diatoms (mostly of the genus *Navicula*). During the fourth week, a purple bacteria biofilm developed as well on seagrass leaves. Towards the end of the experiment, excess organic matter was released within the system due to increased shoot mortality and epiphyte shifts. Coincidently, the accumulation of organic carbon in the sediments decreased, suggesting that an accelerated microbial decomposition was promoted by the higher nutritional quality of producers as reported elsewhere for terrestrial systems (Wardle et al., 2004) and seagrass beds (López et al., 1998; Holmer et al., 2004; Spivak et al., 2007).

The simultaneous addition of CO_2 and nutrients did not modify the eutrophication effects on plant biochemistry, but reduced the nutrient-induced changes in the epiphyte assemblages and the CO_2-induced increase in epiphyte loads. This led to maintenance at control levels of the capacity of the sediments to store organic matter. Most studies of the interactive effect of CO_2 and nutrients in terrestrial systems are focused on nutrient levels that alleviate nutrient limitation and increase plant growth at the expense of carbon reserves as predicted by the Growth-Differentiation Balance hypothesis (Johnson and Lincoln, 1991; Coley et al., 2002). However, the potential alleviation by CO_2 of the plant mortality that may occur due to imbalances induced by excess nutrients has not, to our knowledge, been previously reported.

4.3 High- vs. low- nutrient meadows

Our findings revealed that the expected benefits of high CO_2 predicted for the end of the century on seagrass productivity can be restrained by epiphyte proliferation and by the interaction with local eutrophication. The most striking responses of *Z. noltii* meadows to the CO_2 enrichment were an increase in the total epiphyte load and changes in the relative abundance, but not in the identity, of the main epiphyte taxa. In both low- and high-nutrient meadows, CO_2 effects were more important in epiphyte populations than in the seagrass *Z. noltii*. The effect of nutrient enrichment was higher in the low-nutrient than in the high-nutrient meadow (Fig. 5a vs. b). In both meadows, elevated nutrient levels altered leaf nitrogen concentration and epiphyte composition in the same way, but contrary to the low-nutrient meadow, there was no significant increase of the shoot mortality in the high-nutrient meadow. When CO_2 and nutrient enrichments interacted, the increased epiphyte load induced by elevated CO_2 levels was maintained in the high-nutrient meadow. In addition, there was an increase of the *Z. noltii* belowground productivity (longer rhizomes) that decreased the rhizome C : N ratio. The investment in vegetative growth of belowground rhizomes for colonizing adjacent areas is coherent with the Growth-Differentiation Balance hypothesis in the absence of nutrient limitation. This response has been reported for this species in relation to sedimentary (Cabaço and Santos, 2007) and hydrodynamic disturbances (Peralta et al., 2005), and under a 1 year CO_2 enrichment for *Zostera marina* (Palacios and Zimmerman, 2007). In the high-nutrient meadow, we found a reduction of leaf C : N ratios and phenolics, apparently in response to CO_2 and/or nutrient enrichments. However, this was actually due to a drastic increase in the C : N ratio and phenolic concentration of the unfertilized plants (Fig. 2c and f) in relation to the original field conditions (Table 1). This, together with the low phenolics observed in the field for the high-nutrient meadow support that nutrient deficiency was driving phenolic accumulation as previously pointed out for the low-nutrient meadow.

Interestingly, we detected little evidence that CO_2 or nutrient additions affected seagrass herbivory by mesograzers. However, clear differences in herbivory between meadows were observed. Plants from the high-nutrient meadow experienced a 12-fold higher amphipod grazing than plants from the low-nutrient meadow, which resulted in a massive loss of shoots. Blooms of the amphipod *C. filosa* may occur in warmer months, when sexual maturity attained at smaller sizes overlap the continuous reproduction described for the whole year (Appadoo and Myers, 2004). This tube-building amphipod is widely distributed and uses macroalgae for feeding (Ceh et al., 2005) and shelter (Appadoo and Myers, 2003). To our knowledge, these observations constitute the first report of *C. filosa* using the seagrass *Z. noltii* for both feeding and shelter-construction. We found that the consequences of plant-specific vulnerability to grazing on seagrass meadows can be stronger than the effects of CO_2 and eutrophication. This result concurs with findings by Alsterberg et al. (2013) which showed that the presence of grazers masked the response of benthic microalgae to ocean acidification and warming. Further studies aiming to identify the factors underlying the plant-specific seagrass vulnerability to grazers are thus of vital importance.

Our study revealed that CO_2 or nutrient enrichments may lead to a loss of the carbon sink capacity of low-nutrient meadows. These results are contradictory with studies by Antón et al. (2011) and Russell et al. (2013), who found no effect and an increase in the meadow carbon sequestration capacity of other seagrass species due to in situ nutrient and CO_2 enrichments, respectively. They used the whole-community metabolism as indicator of the carbon storage capacity of seagrass meadows. However, in our study, the loss of carbon storage occurred with no response of the whole-community metabolism, which showed high variability. Our results suggest a dynamic balance of the community metabolism in response to high CO_2 and nutrients, in which the changes in the *Z. noltii* metabolism are compensated by the metabolism of fast-growing leaf epiphyte and sediment microbial communities. A similar dynamic global balance has also been reported for marine pelagic systems under CO_2 addition (Silyakova et al., 2013).

Overall, the role of herbivory in driving differences between low- and high-nutrient meadows, as well as the role of epiphyte and microbial communities in mediating meadow responses to CO_2 and nutrients, illustrated the importance of interactions among species and connections between organization levels in ecosystem-based studies. The alleviation of nutrient effects by high CO_2 highlighted the relevance of incorporating responses to multiple stressors. This integrative ecosystem-based approach is an important challenge to endorse future conservation efforts.

Supplementary material related to this article is available online at http://www.biogeosciences-discuss.net/11/5239/2014/bgd-11-5239-2014-supplement.pdf.

Acknowledgements. This research was partially supported by the European Regional Development Fund through the COMPETE and by Portuguese funds through the FCT project PEst-C/MAR/LA0015/2011. Mesocosm facilities were provided by ASSEMBLE within the EU-FP7 program. We thank P. Arteaga for help during field works and experiment onset; J. Reis, P. Frias and M. Viegas for support during the mesocosm experiment; M. Mendes and S. Albano for laboratory assistance; M. Machado (grazers), M. Tavares and B. Fragoso (epiphytes) for identifications; J. Silva, A. Cunha and S. Cabaço for useful site information. B.M-C. and I.O. were supported by FCT post-doctoral fellowships (SFRH/BPD/75307/2010 and SFRH/BPD/71129/2010, respectively).

References

Alexandre, A., Silva, J., Bouma, T. J., and Santos, R.: Inorganic nitrogen uptake kinetics and whole-plant nitrogen budget in the seagrass *Zostera noltii*, J. Exp. Mar. Biol. Ecol., 401, 7–12, 2011.

Alsterberg, C., Eklöf, J. S., Gamfeldt, L., Havenhand, J. N., and Sundbäck, K.: Consumers mediate the effects of experimental ocean acidification and warming on primary producers, P. Natl. Acad. Sci. USA, 110, 8603–8608, 2013.

Antón, A., Cebrian, J., Heck, K. L., Duarte, C. M., Sheehan, K. L., Miller, M. E., and Foster, C. D.: Decoupled effects (positive to negative) of nutrient enrichment on ecosystem services, Ecol. Appl., 21, 991–1009, 2011.

Appadoo, C. and Myers, A. A.: Observations on the tube-building behaviour of the marine amphipod *Cymadusa filosa* Savigny (Crustacea: Ampithoidae), J. Nat. Hist., 37, 2151–2164, 2003.

Appadoo, C. and Myers, A. A.: Reproductive bionomics and life history traits of three gammaridean amphipods, *Cymadusa filosa* Savigny, *Ampithoe laxipodus* Appadoo and Myers and *Mallacoota schellenbergi* Ledoyer from the tropical Indian Ocean (Mauritius), Acta Oecol., 26, 227–238, 2004.

Bolser, R. C., Hay, M. E., Lindquist, N., Fenical, W., and Wilson, D.: Chemical defenses of freshwater macrophytes against crayfish herbivory, J. Chem. Ecol., 24, 1639–1658, 1998.

Brun, F. G., Hernández, I., Vergara, J. J., Peralta, G., and Pérez-Lloréns, J. L.: Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii*, Mar. Ecol.-Prog. Ser., 225, 177–187, 2002.

Brun, F. G., Olivé, I., Malta, E., Vergara, J. J., Ignacio, H., and Pérez-Lloréns, J. L.: Increased vulnerability of *Zostera noltii* to stress caused by low light and elevated ammonium levels under phosphate deficiency, Mar. Ecol.-Prog. Ser., 365, 67–75, 2008.

Cabaço, S. and Santos, R.: Effects of burial and erosion on the seagrass *Zostera noltii*, J. Exp. Mar. Biol. Ecol., 340, 204–212, 2007.

Cabaço, S., Machás, R., Vieira, V., and Santos, R.: Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*), Estuar. Coast. Shelf S., 78, 1–13, 2008.

Campbell, J. E., Yarbro, L. A., and Fourqurean, J. W.: Negative relationships between the nutrient and carbohydrate content of the seagrass *Thalassia testudinum*, Aquat. Bot., 99, 56–60, 2012.

Cebrian, J., Shurin, J. B., Borer, E. T., Cardinale, B. J., Ngai, J. T., Smith, M. D., and Fagan, W. F.: Producer nutritional quality controls ecosystem trophic structure, PLoS ONE, 4, e4929, doi:10.1371/journal.pone.0004929, 2009.

Ceh, J., Molis, M., Dzeha, T. M., and Wahl, M.: Induction and reduction of anti-herbivore defenses in brown and red macroalgae off the Kenyan coast, J. Phycol., 41, 726–731, 2005.

Coley, P., Massa, M., Lovelock, C., and Winter, K.: Effects of elevated CO_2 on foliar chemistry of saplings of nine species of tropical tree, Oecologia, 133, 62–69, 2002.

Crain, C. M., Kroeker, K., and Halpern, B. S.: Interactive and cumulative effects of multiple human stressors in marine systems, Ecol. Lett., 11, 1304–1315, 2008.

Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A.: Ocean acidification: the other CO_2 problem, Ann. Rev. Mar. Sci., 1, 169–192, 2009.

Drigo, B., Kowalchuk, G. A., Yergeau, E., Bezemer, T. M., Boschker, H. T. S., and van Veen, J. A.: Impact of elevated carbon dioxide on the rhizosphere communities of *Carex arenaria* and *Festuca rubra*, Glob. Change Biol., 13, 2396–2410, 2007.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F.: Colorimetric method for determination of sugars and related substances, Anal. Chem., 28, 350–356, 1956.

Fenchel, T.: Aspects of decomposition of seagrasses, in: Seagrass Ecosystems: a Scientific Perspective, edited by: McRoy, C. P. and Helfferich, C., Marcel Dekker, New York, USA, 123–145, 1977.

Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marba, N., Holmer, M., Mateo, M. A., Apostolaki, E. T., Kendrick, G. A., Krause-Jensen, D., McGlathery, K. J., and Serrano, O.: Seagrass ecosystems as a globally significant carbon stock, Nat. Geosci., 5, 505–509, 2012.

Goecker, M. E., Heck Jr., K. L., and Valentine, J. F.: Effects of nitrogen concentrations in turtle-grass *Thalassia testudinum* on consumption by the bucktooth parrotfish *Sparisoma radians*, Mar. Ecol.-Prog. Ser., 286, 239–248, 2005.

Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S. M., Rowley, S. J., Tedesco, D., and Buia, M. C.: Volcanic carbon dioxide vents show ecosystem effects of ocean acidification, Nature, 454, 96–99, 2008.

Hauxwell, J., McClelland, J., Behr, P., and Valiela, I.: Relative importance of grazing and nutrient controls of macroalgal biomass in three temperate shallow estuaries, Estuaries, 21, 347–360, 1998.

Hervé, V., Derr, J., Douady, S., Quinet, M., Moisan, L., and Lopez, P. J.: Multiparametric analyses reveal the pH-dependence of silicon biomineralization in diatoms, PLoS ONE, 7, e46722, doi:10.1371/journal.pone.0046722, 2012.

Holmer, M., Duarte, C. M., Boschker, T. S., and Barrón, C.: Carbon cycling and bacterial carbon sources in pristine and impacted Mediterranean seagrass sediments, Aquat. Microb. Ecol., 36, 227–237, 2004.

Hughes, A. R., Bando, K. J., Laura, F. R., and Susan, L. W.: Relative effects of grazers and nutrients on seagrasses: a meta-analysis approach, Mar. Ecol.-Prog. Ser., 282, 87–99, 2004.

Invers, O., Kraemer, G. P., Pérez, M., and Romero, J.: Effects of nitrogen addition on nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica*, J. Exp. Mar. Biol. Ecol., 303, 97–114, 2004.

IPCC: Summary for policymakers, in: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, edited by: Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M., Cambridge University Press, Cambridge, UK and New York, NY, USA, 27 pp., 2013.

Johnson, R. H. and Lincoln, D. E.: Sagebrush carbon allocation patterns and grasshopper nutrition: the influence of CO_2 enrichment and soil mineral limitation, Oecologia, 87, 127–134, 1991.

Krause, E., Wichels, A., Giménez, L., Lunau, M., Schilhabel, M. B., and Gerdts, G.: Small changes in pH have direct effects on marine bacterial community composition: a microcosm approach, PLoS ONE, 7, e47035, doi:10.1371/journal.pone.0047035, 2012.

Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G.: Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms, Ecol. Lett., 13, 1419–1434, 2010.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., and Gattuso, J. P.: Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming, Glob. Change Biol., 19, 1884–1896, 2013.

Kuffner, I. B., Andersson, A. J., Jokiel, P. L., Rodgers, K., and Mackenzie, F. T.: Decreased abundance of crustose coralline algae due to ocean acidification, Nat. Geosci., 1, 114–117, 2008.

Labasque, T., Chaumery, C., Aminot, A., and Kergoat, G.: Spectrophotometric Winkler determination of dissolved oxygen: re-examination of critical factors and reliability, Mar. Chem., 88, 53–60, 2004.

Lambers, H.: Rising CO_2, secondary plant metabolism, plant-herbivore interactions and litter decomposition, Vegetatio/Plant Ecology, 104/105, 263–271, 1993.

Lidbury, I., Johnson, V., Hall-Spencer, J. M., Munn, C. B., and Cunliffe, M.: Community-level response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent ecosystem, Mar. Pollut. Bull., 64, 1063–1066, 2012.

Lindroth, R.: Impacts of elevated atmospheric CO_2 and O_3 on forests: phytochemistry, trophic interactions, and ecosystem dynamics, J. Chem. Ecol., 36, 2–21, 2010.

Liu, J., Weinbauer, M. G., Maier, C., Dai, M., and Gattuso, J. P.: Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning, Aquat. Microb. Ecol., 61, 291–305, 2010.

López, N. I., Duarte, C. M., Vallespinós, F., Romero, J., and Alcoverro, T.: The effect of nutrient additions on bacterial activity in seagrass (*Posidonia oceanica*) sediments, J. Exp. Mar. Biol. Ecol., 224, 155–166, 1998.

Martin, S., Rodolfo-Metalpa, R., Ransome, E., Rowley, S., Buia, M. C., Gattuso, J. P., and Hall-Spencer, J.: Effects of naturally acidified seawater on seagrass calcareous epibionts, Biol. Lett., 4, 689–692, 2008.

Palacios, S. L. and Zimmerman, R. C.: Response of eelgrass *Zostera marina* to CO_2 enrichment: possible impacts of climate change and potential for remediation of coastal habitats, Mar. Ecol.-Prog. Ser., 344, 1–13, 2007.

Peñuelas, J. and Estiarte, M.: Can elevated CO_2 affect secondary metabolism and ecosystem function?, Trends Ecol. Evol., 13, 20–24, 1998.

Peralta, G., Pérez-Lloréns, J. L., Hernández, I., and Vergara, J. J.: Effects of light availability on growth, architecture and nutrient content of the seagrass *Zostera noltii* Hornem, J. Exp. Mar. Biol. Ecol., 269, 9–26, 2002.

Peralta, G., Brun, F. G., Hernández, I., Vergara, J. J., and Pérez-Lloréns, J. L.: Morphometric variations as acclimation mechanisms in *Zostera noltii* beds, Estuar. Coast. Shelf S., 64, 347–356, 2005.

Pidgeon, E.: Carbon sequestration by coastal marine habitats: important missing sinks, in: The Management of Natural Coastal Carbon Sinks, edited by: Laffoley, D. and Grimsditch, G., IUCN, Gland, Switzerland, 47–50, 2009.

Piontek, J., Lunau, M., Händel, N., Borchard, C., Wurst, M., and Engel, A.: Acidification increases microbial polysaccharide degradation in the ocean, Biogeosciences, 7, 1615–1624, doi:10.5194/bg-7-1615-2010, 2010.

Quinn, G. P. and Keough, M. J.: Experimental Design and Data Analysis for Biologists, Cambridge University Press, Cambridge, UK and New York, USA, 2002.

Robinson, E. A., Ryan, G. D., and Newman, J. A.: A meta-analytical review of the effects of elevated CO_2 on plant-arthropod interactions highlights the importance of interacting environmental and biological variables, New Phytol., 194, 321–336, 2012.

Russell, B. D., Thompson, J. I., Falkenberg, L. J., and Connell, S. D.: Synergistic effects of climate change and local stressors: CO_2 and nutrient-driven change in subtidal rocky habitats, Glob. Change Biol., 15, 2153–2162, 2009.

Russell, B. D., Harley, C. D. G., Wernberg, T., Mieszkowska, N., Widdicombe, S., Hall-Spencer, J. M., and Connell, S. D.: Predicting ecosystem shifts requires new approaches that integrate the effects of climate change across entire systems, Biol. Lett., 8, 164–166, 2012.

Russell, B. D., Connell, S. D., Uthicke, S., Muehllehner, N., Fabricius, K. E., and Hall-Spencer, J. M.: Future seagrass beds: can increased productivity lead to increased carbon storage?, Mar. Pollut. Bull., 73, 463–469, 2013.

Santamaría, L., Dias, C., and Hootsmans, M. J. M.: The influence of ammonia on the growth and photosynthesis of *Ruppia drepanensis* Tineo from Doñana National Park (SW Spain), Hydrobiologia, 275–276, 219–231, 1994.

Silyakova, A., Bellerby, R. G. J., Schulz, K. G., Czerny, J., Tanaka, T., Nondal, G., Riebesell, U., Engel, A., De Lange, T., and Ludvig, A.: Pelagic community production and carbon-nutrient stoichiometry under variable ocean acidification in an Arctic fjord, Biogeosciences, 10, 4847–4859, doi:10.5194/bg-10-4847-2013, 2013.

Smith, A. M. and Zeeman, S. C.: Quantification of starch in plant tissues, Nat. Protoc., 1, 1342–1345, 2006.

Spivak, A. C., Canuel, E. A., Duffy, J. E., and Richardson, J. P.: Top-down and bottom-up controls on sediment organic matter composition in an experimental seagrass ecosystem, Limnol. Oceanogr., 52, 2595–2607, 2007.

Stamp, N.: Out of the quagmire of plant defense hypotheses, Q. Rev. Biol., 78, 23–55, 2003.

Stiling, P. and Cornelissen, T.: How does elevated carbon dioxide (CO_2) affect plant-herbivore interactions? A field experiment and meta-analysis of CO_2-mediated changes on plant chemistry and herbivore performance, Glob. Change Biol., 13, 1823–1842, 2007.

van Katwijk, M. M., Vergeer, H. T., Schmitz, H. W., and Roelofs, G. M.: Ammonium toxicity in eelgrass *Zostera marina*, Mar. Ecol.-Prog. Ser., 157, 159–173, 1997.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H., and Wall, D. H.: Ecological linkages between aboveground and belowground biota, Science, 304, 1629–1633, 2004.

Waycott, M., Duarte, C. M., Carruthers, T. J. B., Orth, R. J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Short, F. T., and Williams, S. L.: Accelerating loss of seagrasses across the globe threatens coastal ecosystems, P. Natl. Acad. Sci. USA, 106, 12377–12381, 2009.

Woodward, G., Perkins, D. M., and Brown, L. E.: Climate change and freshwater ecosystems: impacts across multiple levels of organization, Philos. T. R. Soc. B, 365, 2093–2106, 2010.

Zvereva, E. L. and Kozlov, M. V.: Consequences of simultaneous elevation of carbon dioxide and temperature for plant–herbivore interactions: a metaanalysis, Glob. Change Biol., 12, 27–41, 2006.

Table 1. Environmental and *Zostera noltii* community traits that revealed significant differences between the low- and high-nutrient donor meadows during June–August 2011, prior to the start of the experiment, tested using unpaired *t* tests. [a]Sqrt-transformed data to meet normality. [b]Mann–Whitney rank tests were conducted for variables that did not meet normality even after transformation. All measured traits and methods are shown in Appendix A (Supplement).

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

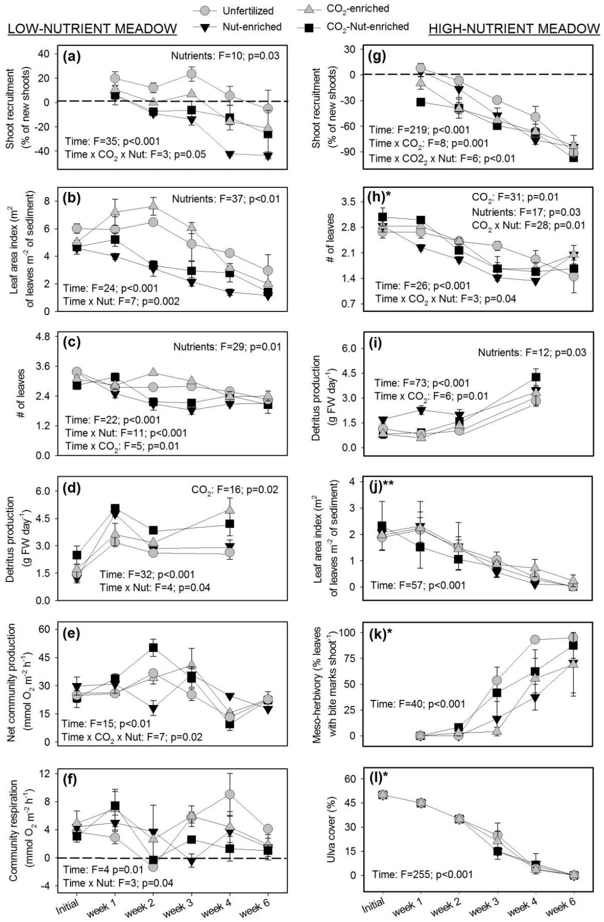

Fig. 1. Caption on next page.

Fig. 1. Effects of CO_2 and nutrient additions on *Zostera noltii* plant-, community-, and ecosystem-level traits from the low-nutrient (left graphs) and high-nutrient meadow (right graphs) through time. Symbols are means (±se, $n = 2$). F statistics and p levels from RM ANOVA tests are shown for among-subject factors (CO_2; Nut: nutrients) and their interaction ($CO_2 \times$ Nut), and for within-subject factor (time) and interactions (time $\times CO_2$, time × Nut, time $\times CO_2 \times$ Nut). Only the significant effects ($p < 0.05$) and useful traits for results interpretation are shown here; the non-significant effects are shown in Appendix C (Supplement). *Variables that did not meet normality after transformation, for which the significance level was more restrictive ($p < 0.03$) to minimize the possibility of Type I error. **Variable sqrt-transformed to meet normality.

BGD
11, 5239–5274, 2014

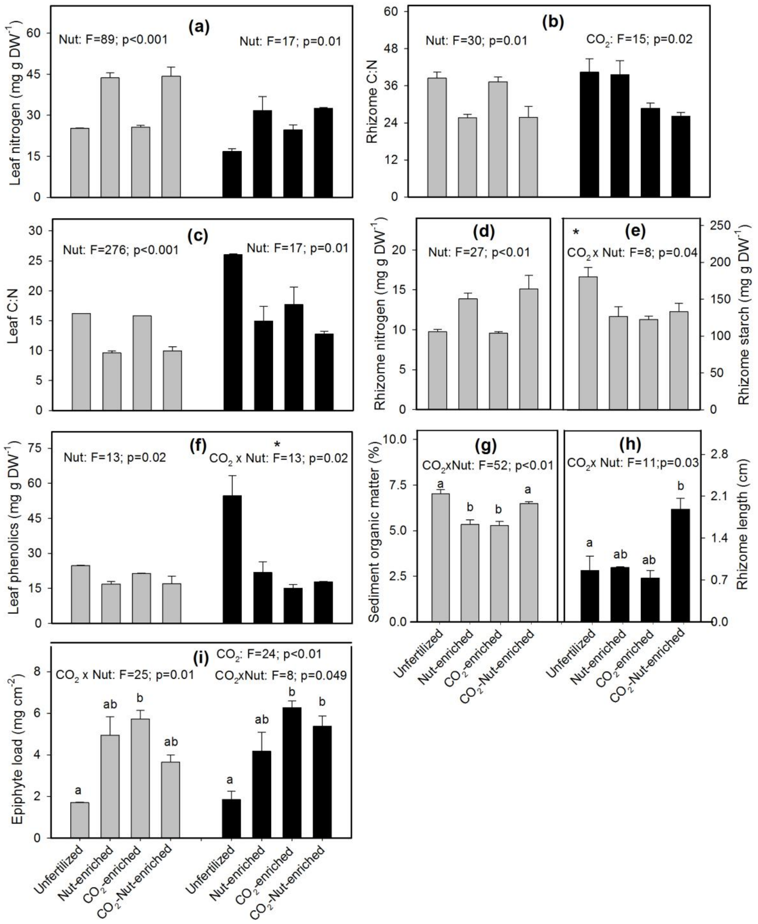

Fig. 2. Caption on next page.

Fig. 2. Effects of CO_2 and nutrient additions on *Zostera noltii* plant-, community-, and ecosystem-level traits measured at the end of the experiment from the low-nutrient (grey bars) and high-nutrient meadow (black bars). Bars are means (±se, $n = 2$). The F statistics and p levels from two-way ANOVA tests are shown for each fixed crossed factor (CO_2; Nut: nutrients) or interaction (CO_2 × Nut). Lowercase letters above bars show significant differences between treatments for significant interactions (t tests, see Methods). Only the significant effects ($p < 0.05$) are shown here; the non-significant effects are shown on Appendix D (Supplement). *Variables showing no significant differences following t tests, for which the significance level was more restrictive ($p < 0.03$) to minimize the possibility of Type I error.

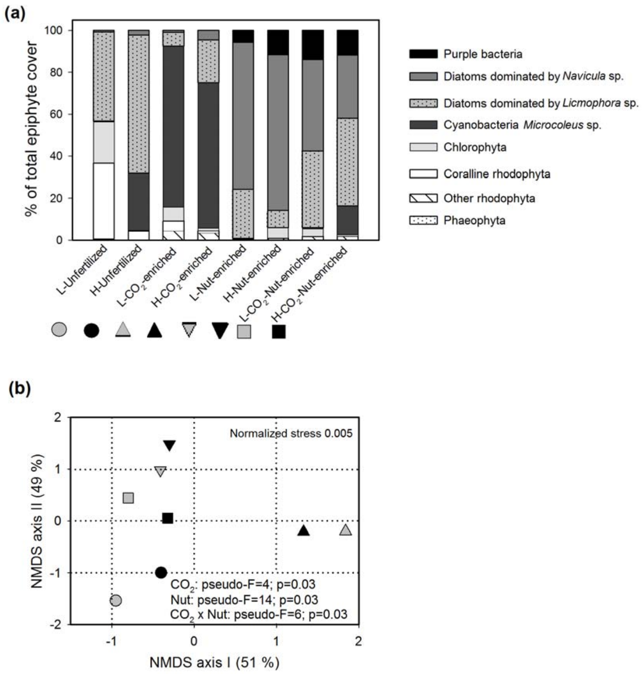

Fig. 3. Responses of *Zostera noltii* epiphyte populations to experimental addition of CO$_2$ and nutrients: **(a)** relative abundance of the main epiphyte taxa of plants exposed to experimental treatments; and **(b)** NMDS ordination of experimental treatments (see symbol legend below treatment names in Fig. 3a) based on leaf epiphytes of plants from the low-nutrient (L; grey symbols) and high-nutrient meadow (H; black symbols). Pseudo-F statistics and p-levels from two-way PERMANOVA test are shown in the NMDS diagram for each fixed crossed factor (CO$_2$; Nut: nutrients) and their interaction (CO$_2$ × Nut).

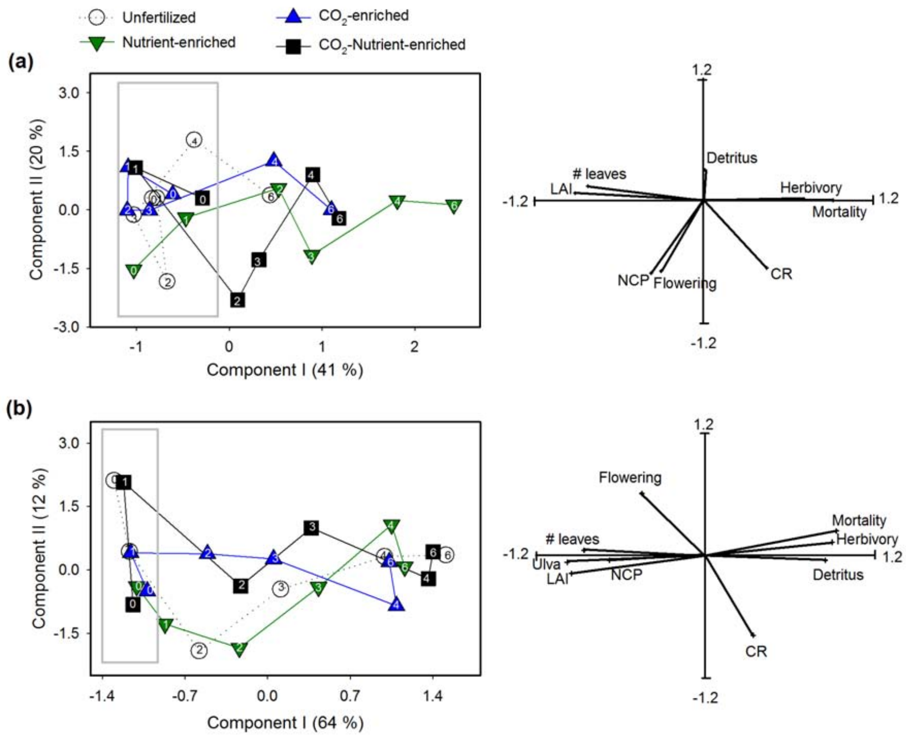

Fig. 4. Principal components analysis of *Zostera noltii* plant-, community-, and ecosystem- level responses to treatments through time: **(a)** low-nutrient and **(b)** high-nutrient meadow. Numbers inside the symbols indicate sampling weeks from 0 to 6. The initial variability (week 0) along component I is incorporated within a grey rectangle to highlight the range of initial natural variability. Variable loadings on the two principal components are depicted in right graphs. LAI refers to leaf area index, NCP to net community production and CR to community respiration.

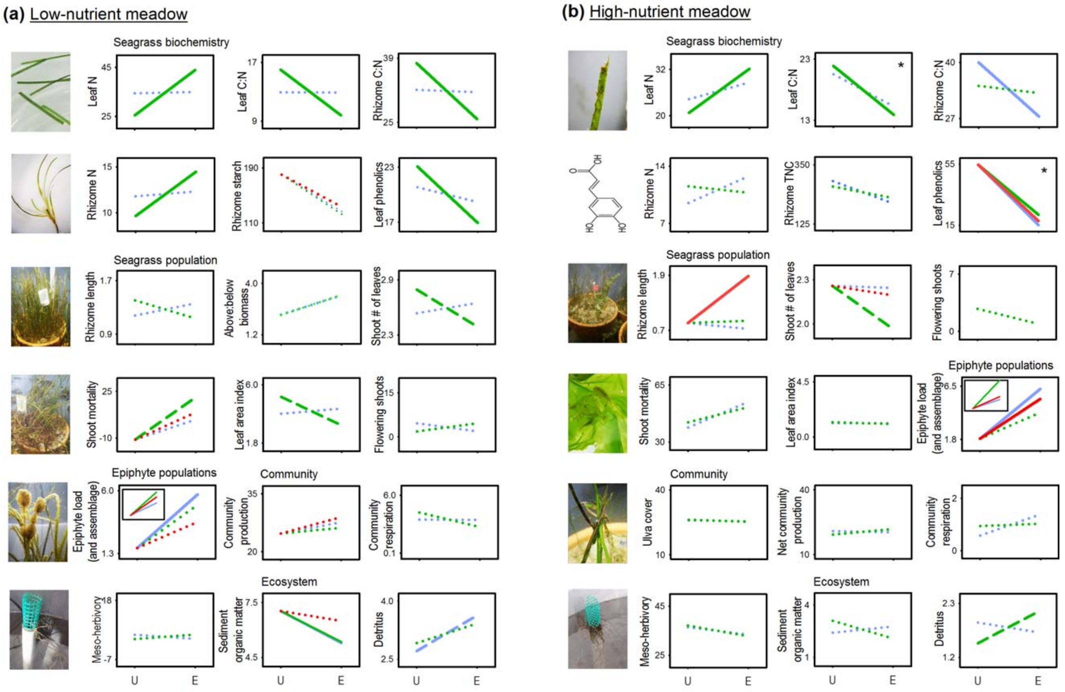

Fig. 5. Caption on next page.

Fig. 5. Summary of the effects of CO_2 (blue line) and nutrient (green line) additions, and when significant of their interaction (red line), on *Zostera noltii* plant-, community-, and ecosystem-level traits of low-nutrient **(a)** and high-nutrient **(b)** meadows. Solid lines indicate significant effects on variables measured at the end of the experiment and dashed lines on variables measured through time. Dotted lines indicate no significant effects. Letters below the *x*-axis denote unfertilized (U) and enriched (E) treatment, which is the pooled mean response to the respective enrichment over the other when there was no significant interaction (no red line). For shoot mortality and net community production (NCP) in the low-nutrient meadow a significant Time × CO_2 × Nutrients interaction was detected and values were represented as for CO_2 × Nutrients significant interactions. *Apparent effect due to a drastic increase in unfertilized plants in relation to the initial field conditions (see results).