

Interactive comment on “CO₂ and nutrient-driven changes across multiple levels of organization in *Zostera noltii* ecosystems” by B. Martínez-Crego et al.

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REPLY TO REVIEWER#2 COMMENTS

We thank the reviewer for the useful comments, which helped to improve the manuscript by flushing out our oversights and clarifying key aspects. The comments are presented below, followed by our responses.

Referee's specific comment #1 - Lines 8-10, p5241: Please provide citations to support these statements.

The reviewer refers to the sentence “This body of research has revealed that ocean

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acidification can be detrimental to most marine calcifying organisms, while increasing carbon dioxide (CO₂) concentration can benefit primary productivity of phytoplankton, cyanobacteria, fleshy algae, and seagrasses”. This sentence is connected to the previous one, in which the reviews by Doney et al. (2009) and Kroeker et al. (2010) are cited for supporting the statements. No change done.

Referee's specific comment #2- Lines 27-28, p5241: Please provide citations to support these statements.

A reference has been added (Kroeker et al. 2013b-Nature Clim. Change 3, 156-159).

Referee's specific comment #3 - Line 1, p5254: Any reason why all effects on epiphytes and sediments were attenuated under both CO₂ and nutrient enrichment.

Regarding changes in the epiphyte community, we found that the interactive effect resulted in the attenuation of both, the nutrient-induced changes in the epiphyte assemblages and the CO₂-induced bloom of the cyanobacterium *Microcoleus* spp. The most likely explanation is that the interspecific competition between the species that dominated the epiphyte community under high CO₂ (the cyanobacterium *Microcoleus* spp.) and under high nutrient levels (diatoms of the genus *Navicula*) resulted in the observed attenuation under simultaneous addition of CO₂ and nutrients of the direct effects of individual stressors on certain taxa. Negative effects of interspecific competition on the involved species (i.e. symmetrical competition) have long been reported by ecologists (Connel, 1983) and support this explanation.

Regarding changes in the sediments of the low-nutrient meadow, we observed similar organic matter content in the sediment under simultaneous CO₂ and nutrient addition and control conditions, which were lower than under individual enrichments. The simultaneous addition of CO₂ and nutrients might keep microbial decomposition rates in the sediment at control levels, as opposed to the probable acceleration observed under independent CO₂ or nutrient enrichment. Our results under individual enrichments contrast with previous studies conducted in situ with different seagrass species. An-

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tón et al. (2011) found that meadow carbon sequestration was unaffected by nutrient addition, whereas Russell et al. (2013) observed increased carbon sequestration in response to CO₂ enrichment. These authors, however, did not assess the interactive effect of nutrients and CO₂ on carbon sequestration. Moreover, they used whole-community metabolism instead organic matter in the sediment as indicator of carbon sequestration.

We have better explained the responses to the simultaneous CO₂ and nutrient additions in the Discussion, highlighting the key role of species interactions in the attenuation of direct effects of individual stressors. The first paragraph of the discussion, to which the reviewer refers here, has been removed from this position, incorporated into the Abstract and discussed in each appropriate sub-section of the Discussion (see comments below). In addition, we have highlighted the role of species interactions in mediating the individual and interactive effects of eutrophication and acidification in ecosystem functioning through the manuscript (Discussion, Results, and Abstract).

Referee's specific comments #4 and #8- Line 11, p5254: However, this finding is in contradiction to much empirical research for seagrasses. Please address. (Jiang et al. 2010, Campbell & Fourqurean 2013). - Line 23, p.5254: Yet note that in a subsequent study Campbell 2013 Mar Biol document increases in carbohydrate content with elevated CO₂, along with Jiang et al 2010, Palacios 2007, and Zimmerman et al 1997. Clearly both nutrient regime and CO₂ levels can have an effect on carbohydrate levels and should be acknowledged. (Zimmerman et al. 1997, Jiang et al. 2010, Campbell & Fourqurean 2013)

These two specific comments refer to the same paragraph and are interlinked, so we answered and addressed them together. We found that CO₂ enrichment had no direct effects on *Z. noltii* biochemistry, with no significant changes on the total carbohydrate reserves. This finding contrasts with observations in the seagrass *Thalassia hemprichii* (Jiang et al., 2010) and *T. testudinum* (Campbell and Fourqurean, 2013).

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We have rewritten this entire paragraph to acknowledge that our results are different from others and to reinforce the idea that the plant responses to elevated CO₂ levels are highly context- and species-specific, and are not as readily consistent and predictable as resource availability hypotheses would suggest. References have been modified accordingly. The effects of nutrient regime on the carbohydrate accumulation have been addressed in the sub-section 'High- vs. low- nutrient meadows', where we discuss that the accretion of carbon based-compounds in *Z. noltii* might be driven by nutrient deficiency, thus, reinforcing the idea of the context-dependence of seagrass response to high CO₂.

Referee's specific comments #5 to #7 - Line 12, p.5254: So the seagrasses from the low-nutrient meadows were not nutrient limited? What evidence do you have to suggest a trade-off between phenolic production and growth? I currently don't see data to support this conclusion. - Line 16, p.5254: Did CO₂ enrichment actually enhance LAI? Is this supported by your statistical analyses. - Line 20, p.5254: Were your plants really not under any degree of light limitation? Didn't the excessive epiphyte loading reduce light levels?

Again, these three specific comments refer to the same paragraph than the previous remarks and are interlinked. We agree with the referee. As stated in the Results section, Figure 1b suggests that the *Z. noltii* leaf area index (LAI) tended to increase with CO₂ enrichment until the third week of the experiment. However, this trend was not statistically significant (no significant CO₂ effect or CO₂ x Time interaction in the RM ANOVA). Consequently, our results did not provide significant arguments to support a trade-off between phenolic production and growth. As the referee suggests, the lack of significant CO₂-induced changes in *Z. noltii* productivity can be explained by nutrient or light limitation to seagrass growth. Given the huge epiphyte overgrowth induced by CO₂ enrichment, we pointed out to light limitation mediated by epiphyte shading as the most likely reason.

We have revised and rewritten this entire paragraph to accommodate these and previ-

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ous remarks. We highlight that we found no significant increase of seagrass productivity under CO₂ enrichment to support the trade-off between phenolic accumulation and plant growth.

Referee's specific comment #9 - Line 2, p.5255: Cite (Campbell & Fourqurean 2014)

The reference has been added.

Referee's specific comment #10 - Line 5, p. 5256: But don't you document declines in shoot recruitment and LAI?

The referee is right. The paragraph has been modified and combined with the next paragraph to explain that the increased shoot mortality under nutrient enrichment can be linked to ammonium toxicity and/or to a reduction in light availability caused by the dense epiphytic layer of pennate diatoms and purple bacteria. References have been modified accordingly. This change also addresses Reviewer#1 remark about this paragraph.

Referee's specific comment #11 - Line 17 p. 5256: Any chance that this excess organic matter was simply exported out of the mesocosms due to the experimental set up?

No chance. Any export of organic matter should equally affect all treatments, but it was not apparent in the control conditions as reflected by the relatively high organic matter content in the sediment of the unfertilized mesocosms.

Referee's specific comment #12 - Line 22 p.5256: Any explanation for this statement? The first sentence of this paragraph needs clarification / explanation.

This paragraph has been modified also in reply to the referee's comment #3. As explained above, the simultaneous addition of CO₂ and nutrients attenuated the nutrient-induced changes in the epiphyte assemblages and the CO₂-induced increase in the total epiphyte load through the alteration of competitive dynamics and species interactions within the epiphyte community.

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Referee's specific comment #13 - Line 4- 17 p.5257: This paragraph reads more like the results section. Please revise.

Reviewer#1 also made a similar comment for lines 10-11 in p5257. We have revised the entire paragraph to incorporate a proper discussion and contextualization of the results as Reviewer#1 and #2 requested.

Referee's specific comment #14 - Line 19 p.5258: But if I understand correctly, combined CO₂ and nutrients had no effect on carbon sink capacity. Why might this be the case?

See above answer to comment #3.

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