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Interactive comment on "CO_2 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 1 COMMENTS

We greatly appreciate the comments and detailed suggestions by the anonymous referee. The referee provided two major comments, two specific comments, and several technical corrections for manuscript improvement or for detail clarifications.

Regarding the general comments, as the referee correctly points out, our experimental design allows for determining the nature of the interaction between stressors (synergistic or antagonistic) but not the existence of thresholds or linear responses. This latter point was out of the scope of our study. The referee acknowledges that, in spite of

the low replication of treatments, significant differences were detected that provided a general insight on the seagrass ecosystem responses at several levels of the biological organization. This general view is the major aim of the present work and it suggests ways forward for future research.

Referee's specific comment – "The authors attribute the lack of grazer control of epiphyte growth to either an imbalance between epiphyte growth and grazing activity or a non-specialist grazer being the most abundant. Is it possible that the shift in epiphyte assemblages towards cyanobacteria which were less palatable for the grazers present?"

This would be a particular case in which the most abundant mesograzer in our experiment, Cymadusa filosa, was not targeting the epiphytes that were mostly composed by cyanobacteria in the CO2-enriched treatment. However, cyanobacteria are palatable and structurally accessible food for mesograzers (e.g. Vargas et al. 1998- J. Phycol. 34, 812–817; Nagarkar et al. 2004- Hydrobiologia 512: 89–95). At the same time, as stated in this paragraph, small invertebrate grazers (amphipods, isopods, gastropods) have been widely recognized as controllers of epiphyte overgrowth under nutrient enrichments (Hughes et al., 2004-Mar Ecol Prog Ser 282, 87-99), including cyanobacteria blooms (Neckles et al. 1993-Oecologia, 93, 285-295). We have revised the paragraph according the referee's suggestion, considering the particular control of the cyanobacteria overgrowth by the amphipod Cymadusa filosa rather than a general epiphyte control by grazers.

Referee's specific comment – "Overall, the statistical analysis is sound, however I would question why t-tests were used instead of post-hoc analysis (e.g. SNK, Tukeys or an appropriate test if variances were heterogeneous)? The ANOVA is the appropriate test to use and does not need to be confirmed by t tests. Nor do t tests tell you anything about interactions."

We agree with the referee that the ANOVA is the appropriate test and does not need

to be confirmed by t tests. We have revised this paragraph to incorporate the remarks made by the referee. We have clarified that within the design of the two-way ANOVA (two fixed crossed factors), a normal distribution with unequal variances was found for all variables, which is usual when the sample size is small. Following recommendation by Quinn and Keough (2002), we proceeded with the analyses but making significance level more restrictive to minimize the possibility of Type I error (mistakenly detection of differences). Fig. 2 and 5 (and their legends), Appendix D, and text in the manuscript have been modified accordantly. Regarding the first part of this remark, post hoc comparisons were not possible in the factorial design because we only have two levels (unfertilized and enriched) within each factor (CO2, Nutrients). When significant interactions were detected, we needed another test to detect significant differences between the 4 treatments separately considered (Unfertilized, CO2-enrichmed, Nut-enriched, and CO2-Nut-enriched). Given the unequal variances found for all variables under this design (1 factor: treatment, with 4 levels) and that the homogeneity of variances is an important assumption in the ANOVA (see Quinn and Keough 2002), we chose to use Welch's t test (robust against unequal variances) instead SNK or Tukey's comparisons after one-way ANOVA.

Regarding the technical corrections suggested by the referee, we have either accepted all edits and minor corrections or have added most suggested clarifications. Clarifications are addressed in detail as follows:

"Page 5245, Line 18: It is not clear what three-five leaves means and why it is not standardised to a single number" - Five shoots were used to quantify the number of leaves and the LAI at the beginning and at the end of the experiment, while three shoots were used in between sampling events. We have revised this paragraph to clarify this referee's remark.

"Page 5245, Line 28: How were epiphytes removed?" - Epiphytes were removed with a glass slide to minimize damage on leaf surface. This has been clarified in the revised version.

"Page 5247, Line 8: I don't think export is the correct term. How do you know this carbon would not have settled back to the sediment within the bed?" – The referee is right. In seagrass meadows, detritus production can be exported by currents and waves to neighboring systems (e.g. deep systems or beaches), enter in the trophic web through decomposers or detritivores, or be buried under the meadow becoming part of the carbon sink (Pergent et al. 1994-Mar Ecol Prog Ser 106, 139-146; Cebrián and Duarte 2001-Aquatic Botany, 70, 295-309; see also references already cited in the manuscript: Pidgeon 2009, Fourqurean et al. 2012). To avoid any confusion, we have removed the allusion of detritus production as indicator of carbon export.

"Page 5247, Line 19: Greenhouse-Geisser adjustments actually correct the degrees of freedom, not the Significance levels (although significance levels will also change)" - The referee is right. We have corrected this sentence.

"Page 5251, Lines 13-14: Should read "...with only purple bacteria appearing in them." - This change was not accepted because it would alter the meaning of the sentence. Purple bacteria only appeared in the nutrient- and CO2-and-nutrient- treatments, but they were not the only group appearing in these treatments.

"Page 5255, Line 13: Surely there are more recent papers than (Fenchel 1977) to cite." - Fenchel 1977 has been replaced by more recent references that highlight the relevance of detritus-based food webs in seagrass meadows (i.e. Pergent et al. 1994-Mar Ecol Prog Ser 106, 139-146; Moore and Fairweather 2006-Oikos 114, 329-337).

"Page 5256, Lines 2-4: The sentence beginning with "To avoid toxicity, ..." is confusing and the latter part needs revising for clarity." - The paragraph beginning with this sentence has been rewritten and made clear in the revised version as follows: 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), although it can be avoided by a rapid assimilation of the excess ammonium into amino acids causing a reduction of carbohydrate reserves (Brun et al., 2002; Invers et al., 2004). However, we did not

find any indications of the occurrence of this toxicity, as no significant plant mortality or carbohydrate decrease were detected.

“Page 5257, Lines 10-11: “The effect of nutrient enrichment was higher in the low-nutrient than in the high-nutrient meadow (Fig. 5a vs. b).” This belongs in results section.” - The referee seems confused because we cited here the Figure 5. This figure provides a summary of all previous results already described in the Results section (i.e. all CO2 and nutrient effects across the studied organization levels) and it helps to follow the comparison of the low-nutrient versus the high-nutrient meadow as well as the entire discussion. For this reason this figure is deliberately cited in the Discussion rather than in the Results section.

“Page 5258, Line 7: The sentence beginning with “Blooms of the, ...” is confusing and the latter part needs revising for clarity.” - The sentence has been rewritten and the confusing part has been removed.

Interactive comment on Biogeosciences Discuss., 11, 5239, 2014.