

## ***Interactive comment on “CO<sub>2</sub> and nutrient-driven changes across multiple levels of organization in *Zostera noltii* ecosystems” by B. Martínez-Crego et al.***

### **Anonymous Referee #1**

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#### GENERAL COMMENTS

The manuscript looks at the interactive effects of multiple stressors (nutrient enrichment and acidification (CO<sub>2</sub> enrichment) on a seagrass ecosystem by investigating responses at both the plant and the associated community level. The manuscript is data rich with measurements taken at many different levels of organisation from plant growth and biochemistry to epiphyte and grazer abundances assemblages and community metabolism through the use of benthic chambers to measure oxygen evolution.

Manipulative studies investigating multiple stressors are extremely valuable because they allow for experimental testing of interactions that are otherwise very difficult to

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predict. One of the restrictions of experimentally testing multiple stressors is the large number of sampling units required to account for multiple levels within treatments with sufficient replication. The current manuscript only looked at 2 levels of nutrient and pH (ambient and enriched) with low levels of replication ( $n = 2$ ). They also looked at these factors in seagrass systems from both low and high nutrient meadows. This design does not allow for determining if responses were linear or tolerance thresholds existed before responses were observed, however it does provide insight into whether additive or synergistic interactions may be at play. Here the authors found that increased CO<sub>2</sub> attenuated the effects of nutrient enrichment. By measuring variables across multiple levels of organisation they were able to determine that adverse responses at the plant level were mediated through the proliferation of epiphytes. Despite the low replication of treatments, the experimental design had the power to detect statistically significant differences for many of the measures.

Overall, studies such as this that study the interactive effects of multiple stressors are valuable additions to the scientific literature.

#### SPECIFIC COMMENTS

The paper is fairly well written although in some areas could be improved and I have highlighted these in my technical corrections below.

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? (Page 5255, Lines 4-9)

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 (Page 5247, Line 22-28).

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## TECHNICAL CORRECTIONS

Page 5243, Line 11: Should read "...assess if they react differently."

Page 5245, Line 28: Should read "...five shoots per pot."

Page 5245, Line 18: It is not clear what three-five leaves means and why it is not standardised to a single number

Page 5245, Line 28: How were epiphytes removed?

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?

Page 5247, Line 19: Greenhouse-Geisser adjustments actually correct the degrees of freedom, not the Significance levels (although significance levels will also change)

Page 5251, Lines 13-14: Should read "...with only purple bacteria appearing in them."

Page 5251, Line 28: Replace "low" with "minor" or "small".

Page 5253, Line 26: Replace "evidenced" with "observed".

Page 5254, Line 6: Remove ", however,".

Page 5255, Line 13: Surely there are more recent papers than (Fenchel 1977) to cite.

Page 5255, Line 19: Replace "stronger" with "greater".

Page 5255, Line 27: Replace "a scarce" with "minimal".

Page 5256, Lines 2-4: The sentence beginning with "To avoid toxicity, . . ." is confusing and the latter part needs revising for clarity.

Page 5256, Line 5: Replace "since" with "as".

Page 5257, Lines 10-11: This belongs in results section.

Page 5257, Line 27: Should read "...meadow supports the hypothesis that

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nutrient. . . . ."

Page 5258, Line 7: The sentence beginning with "Blooms of the, . . ." is confusing and the latter part needs revising for clarity.

Page 5258, Line 19: Should read "...CO<sub>2</sub> or nutrient enrichment. . . . ."

Page 5258, Line 20: Replace "of" with "in".

Page 5258, Line 25: Replace "with no" with "without a".

Page 5262, Line 1: Hughes et al. 2004 – List of minor authors has first names rather than surnames listed (e.g. Susan, L. W. should be Williams, S. L.)

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