Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2018-373-RC2, 2018

Biogeosciences Discussions
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EGU

Interactive comment on "Warming effect on nitrogen fixation in Mediterranean macrophyte sediments" *by* Neus Garcias-Bonet et al.

Anonymous Referee #2

Received and published: 25 September 2018

General comments: The MS addresses changes in nitrogen fixation rates by diazotrophic bacteria in vegetated and unvegetated sediments in the Mediterranean Sea in face of the global warming. The topic is relevant considering the actual scenario of climate changes. The experimental methods are appropriate and the results are quite interesting, making a significant contribution to the study of biogeochemical cycles in seagrasses and macroalgae habitats. However, I think the MS would benefit from adding more information in the Introduction and Discussion. Some methods also need clarification. Finally, I have some doubts/suggestions regarding the statistical analyses and results presentation. Please find my comments and corrections below.

Specific comments:

Lines 60-63 and 83-85: please add more information about the ecological role of C.

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nodosa, C. prolifera and P. oceanica as “key macrophytes” in the study area;

Line 100: Plant communities? Benthic communities?

Lines 107-108: I wonder if benthic macrofauna was also sampled, which could influence microbial community (?). Please also provide more information whether samples were obtained from homogeneous patches or some kind of abundance quantification;

Lines 111-119: I feel that these temperature results are just background information for your experimental set up. Although I recognize their importance, I think they (both Figure 1 and Tables 1 and 2) should go as supplementary material;

Lines 122-124: so you used a full factorial experimental design testing the factors “sediment type” and “temperature” and their interactive effects?

Lines 134: were seagrass plants “transplanted” along with sediments to the laboratory? As you are talking about endophytic bacteria, I believe at least the belowground biomass was present. . .

Lines 122-159: I would recommend first describing sediment sampling/preparation, then the experimental treatments followed by the description of N2 determination procedures;

Line 147: as the incubations lasted only 24 h, I think you are investigating the impacts of heatwaves (short-term events) rather than warming per se (long-term warming) as your MS tittle suggests;

Lines 166-170: please provide more information on why measuring the sedimentary OM (a proxy for belowground biomass?). Also clarify the difference between using sediment dry weight and standardized fixation rates in your results;

Lines 168-170: Speaking somewhat naïvely here but it seems to me that, if you have nutrient stocks in mind, you should focus on results standardized by sediment bulk density. If dry bulk density is the best choice here, results shouldn’t be expressed in

mg N m-3?

Lines 173-178: it appears to me that a factorial two-way ANOVA for all variables measured is the most informative analysis in your case. Has the data gone through some transformation? Or have you considered using a generalized linear model model with gamma or lognormal data distributions? I also could not understand why using a Friedman test (a non-parametric alternative for repeated-measures ANOVA) followed by a Mann-Whitney U test (non-parametric comparison between two populations). I think maybe you have an unbalanced design in the latter case (N = 9 for vegetated and N = 3 for bare sediment). If possible, try a one-way ANOVA partitioning the sum of squares into an a priori contrast between the unvegetated sediments and all pooled vegetated sediments (see more details in Bruno et al., 2005 for instance). My main point here is: whenever possible, use a more robust and informative single analysis where you can determine the interactive effects sediment types X temperature;

Lines 181: please provide more information on Q10;

Line 192: Table 3 instead of 2;

Lines 195-202: could you please provide a figure?

Lines 195-217: Maybe I missed it, but you performed an experiment under five different temperatures, determining N2 fixation rates at 12,17, 20 and 24 h. I couldn′t find all these results in Figure 2.

Lines 218-221: I don′t think a linear regression is the best choice here, as highlighted by your low R2 value. Have you tried to fit your data to distinct models (e.g., a polynomial one)?

Lines 209-215: this sentence is too long;

Lines 227-233: please revise this sentence;

Lines 244-248: Do you mean variability in seagrasses habitats among sites?

Lines 248-259: seagrasses and macroalgae have distinct C/N ratios and, consequently, different biomass turnover rates and consumption susceptibility. Such differences determine their role as carbon (e.g., Krause-Jensen et al. 2018) and nutrient stocks (e.g., Lanari et al. 2018). Increasing temperatures may also enhance organic matter remineralization, which may counteract increasing N fixation rates in an ecosystem functioning perspective. These topics could be further explored here;

Lines 274-278: please clarify this sentence;

Lines 274-305: considering your MS tittle, I think the discussion on warming effects on N2 fixation can be enriched. For instance, effects of warming are also reported for other biogeochemical cycles, such as carbon stocks (e.g., Arias-Ortiz et al. 2018);

Lines 302-305: this argument was also presented in the Introduction. If P. oceanica is predicted to be extinct in 2049, why test it under scenarios expected by the end of the century?

Lines 307-308: I think here (and in your Discussion) you could explore more the significance of your results on local biogeochemical cycles. Moreover, in the lines 311-312, it was found that N fixation rates in vegetated sediments decreased at 33°C.

Technical corrections:

Although I am not a native English speaker, I think the MS would benefit from a further English revision (e.g. Lines 124-125: please be consistent with the use of the past tense in the methods description);

Lines 238-239: correct the citation Agawin et al.

Figure 2: in my opinion, such results should be presented as in Figure 3 (i.e., temperature values in x-axis and distinct lines representing different sediment types);

Figure 3: also present the results from distinct vegetation types (maybe panel A and B in the figure?)

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Figures 2 and 3: please insert in the legend whether results refer to dry weight or bulk density. Insert N values. Please highlight the meaning of the asterisks. Some redundant information (e.g., ". . .under RCP6.0 scenario") can be removed. Explain the boxplots (mean, quartiles, etc).

Suggested references:

Arias-Ortiz et al. 2018. A marine heatwave drives massive losses from the world′s largest seagrass carbon stocks. Nature Climate Change 8:338-344.

Bruno, J.F., Boyer, K.E., Duffy, J.E., Lee, S.C., Kertesz, J.S., 2005. Effects of macroalgal species identity and richness on primary production in benthic marine communities. Ecol. Lett. 8, 1165–1174.

Krause-jensen, D., Lavery, P., Serrano, O., Masque, P., Duarte, C.M., 2018. Sequestration of macroalgal carbonâĂŕ: the elephant in the Blue Carbon room. Biol. Lett. 14, 20180236.

Lanari, M., Coelho Claudino, M., Miranda Garcia, A., da Silva Copertino, M., 2018. Changes in the elemental (C, N) and isotopic (δ13C, δ15N) composition of estuarine plants during diagenesis and implications for ecological studies. J. Exp. Mar. Bio. Ecol. 500, 46–54.

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