

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

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Received and published: 20 September 2018

### GENERAL COMMENTS

The ms by Garcias-Bonet et al. addresses a relevant novel scientific question, which is the response of N<sub>2</sub> fixation in vegetated marine sediments to warming. It is also valuable that the paper includes the sediments of, not only the well-studied seagrass species *P. oceanica*, but also the less studied seagrass species *C. nodosa* and the green macroalgae *C. prolifera*. The scientific question fits well in the scope of BG since N<sub>2</sub> fixation is a relevant metabolic process in marine sediments and, in the particular case of the Mediterranean sea, it supports the primary production of seagrass *P. oceanica*.

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The conclusions of the ms are based on N<sub>2</sub> fixation rates measured in triplicate sediment samples taken from one single location in the Mediterranean Sea and exposed to five meaningful experimental temperatures, from 25 to 33°C. The authors reach two clear and relevant conclusions: first, N<sub>2</sub> fixation is higher in the sediment colonised with marine macrophytes than unvegetated sediments, and second, warming up to 31°C is expected to increase the N<sub>2</sub> fixation rates in the sediment of marine macrophytes, but above 33°C the rate will decrease. The methods and experimental design are sound, but authors should encompass the limitations of the study when interpreting the results: first, rates were measured in sediments collected in just one site and, second, the study does not account for synergic or antagonistic effects with other environmental drivers.

The overall presentation of the ms is clearly structured and ideas and paragraphs are presented in a logical way. Language is fluent and precise, with only some suggested corrections to improve readability and clarity (see technical comments). In general, methods are clearly outlined and described, but I have a relevant comment regarding the inclusion or not of the vegetation (above- and/or below-ground) during the cores extraction and later on in the sediment incubations (see specific comments). This should be clearly stated throughout the ms because in the introduction they explain that endophytic nitrogen-fixing bacteria have been detected in association to *P. oceanica* roots and leaves, so it is not clear if the N<sub>2</sub> rates measured are solely due to the sediment behind or close to the marine macrophytes or if they are also due to the bacteria found on the surface of the macrophytes. I have other specific questions listed below.

## SPECIFIC COMMENTS

Introduction.

Authors should reinforce the background on the effects of warming on *C. prolifera* and *C. nodosa*, since most of the information given in the present version is focus on *P.*

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oceanica. Also, they should explain their hypothesis behind the comparison of the sediments of the three marine macrophyte types (L83-86).

#### Materials and Methods.

Regarding the sediment samples: a) how many cores were taken in the field for each vegetation type and in which part of the patch (edge or centre)?; b) did the sediment cores included the above- and/or below-ground vegetation or not?; c) Why were the top 10-cm selected for each core (rhizosphere depth varies between the three species)?

The text explaining how the experimental temperatures were selected (L124-133) is confusing, in particular when comparing how values are given in figure 1 (“average summer median”, L331), table 1 (many statistical descriptors) and the text (“average summer mean”, L126). Also, the 29°C and 31°C treatments were selected as the current summer mean SST (26.54 +/- 0.17°C) plus the projected mean SST increase (2.8 +/- 1.1 °C) (L127-130). How did you yield the 29 and 31°C? My best guess is based on the errors reported, but this should be confirmed and explained:  $26.54 + 0.17 + 2.8 + 1.1 = 30.61^{\circ}\text{C}$  and:  $26.54 - 0.17 + 2.8 - 1.1 = 29.34^{\circ}\text{C}$ .

Authors explained that negative controls were run (L160-163), so they should report somewhere the results of the controls and if they were used to correct the rates calculated in the sample incubations.

Was the Arrhenius function fitted with a linear regression? (L179-180).

#### Results.

Results are presented by 3 groups of temperatures (e.g. Fig. 2), although in L176 authors explain that statistical differences were tested by temperature treatment (5 levels). Please, check that figures, statistical tests and text should report results in the same way.

In figure 3, authors pooled the 3 types of vegetated sediment to see how the fixation rate varies with temperature. However, the rates, when expressed in a dw basis as it is

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in Fig. 3, differed statistically among the 3 types (L200-202). I think it would be more appropriate to assess this relationship using fixation rates by unit of area, because no differences were found among vegetated sediment types (L202-203).

Discussion.

Authors should include in the discussion section the limitations of doing estimations based on results from just one location. Also, there could be many other environmental factors acting in synergy or antagonistically with temperature and affecting N<sub>2</sub> fixation rates in the sediments.

Authors should include in the conclusion paragraph (L308-312) that there is a reversal in the thermal response of N<sub>2</sub> fixation rate in vegetated sediments after 31 °C, and state briefly that the conclusions have limitations.

TECHNICAL COMMENTS.

L27. Should be “on N<sub>2</sub> fixation rates in the sediment of three key marine macrophytes”.

L28-31. Authors should include the experimental temperature values in or at least the tested range (25-33°C).

L35. Write “showed” for consistency with the tense used before.

L39. Explain the meaning of the lower values of Q<sub>10</sub> and activation energy in the vegetated sediments (e.g. parameters of temperature dependence of N<sub>2</sub> fixation was...). Otherwise remove that sentence.

L79. Add space after semi-colon in the citation.

L88. Add “vegetated” before “ecosystems to warming” to be specific.

L91-92. Without reading the M&M section, it is surprising to find two temperature values for a summer mean. This needs clarification (e.g. by saying “summer mean range” or similar).

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L100. Add “macrophytes” before “community” to be specific.

L117. Say which years are “the last years”.

L147. Remove “The incubation lasted 24h”, it is already said in L145.

L140-147. Text explaining how the temperature treatments were attained in the lab would fit better at the end of the first paragraph of this subsection (L133), since it seems logical to present the temperature methods after the explanation of the temperatures chosen, and then follow up with the detailed explanation of the acetylene reduction assay.

L168-170. It is written that rates “were standardised to surface area integrated over 10 cm sediment depth”, but they later show the results in terms of mol N gDW<sup>-1</sup> h<sup>-1</sup>. Include the standardisation in terms of dw in these lines as well.

L182. The Q10 parameter is not properly explained and I think that authors should show the formula for the calculation (it may be not be a familiar concept for the BG readers).

L193. Should be “table 3”.

L195-205. For which temperature level are the values given in this paragraph?

L197-200. Give also the values of fixation rate for *P. oceanica*, as it is given for the other two types of vegetated sediments.

L204. Statistical information given for the correlation of OM and N<sub>2</sub> rates is incomplete. Authors should give the Pearson’s coefficient, df, and p.

L209. Refer to Fig. 2 at the end of the sentence.

L235-236. Check the text “... supported 3 to 4-fold higher H<sub>2</sub> fixation rates”, because in L196 is written “3-fold” and in L208 “twice”.

L264. Delete the extra parenthesis.

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L266. Delete the extra parenthesis.

L376. “Sediment” is repeated twice in the sentence, remove one of them.

L377. Add “Mallorca” after Pollença Bay, for consistency with the other legends.

L378. Write the sample size of the OM and DBD values.

Table 1. I suggest to remove the lines of years 2014 and 2015 in the table and explain in the legend that data are not available for those years. Then remove the \* footnote as well.

Table 2. Write the sample size for the temperature measurements.

Figure 2. Explain what the box plots show (box, whiskers, “+” symbol, in-box line, dots), since it may vary among statistical softwares. Explain what asterisks on top of the box plots mean.

Figure 4. Explain what solid lines mean. Add units in the x-axis.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2018-373>, 2018.

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