Biogeosciences Discuss.,
doi:10.5194/bg-2016-297-AC1, 2016

Interactive comment on "Rooting and plant density strongly determine greenhouse gas budget of water hyacinth (*Eichhornia crassipes*) mats" *by* Ernandes Sobreira Oliveira Junior et al.

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Received and published: 18 November 2016

We would like to thank Referee 1 for her/his comments and suggestions. Please find our itemized list of responses below, as well as our revised manuscript (with tracked changes). Our responses are structured as follows: (1) comments from referees/public, (2) authors' response, and (3) authors' changes in manuscript indicating the page and line of the changes when applicable.

1) The link between trace gas emissions and biological invasions is still poorly explored and deserves attention from the scientific community. In this paper, the authors try to elucidate the effect of water hyacinth density on net GHG emissions, by using a mesocosms approach and by measuring net CO2 and CH4 diffusive and ebullitive

fluxes under controlled conditions. My general opinion is that, even if the topic could be attractive to the readers, the experimental design, the initial hypotheses and final results here presented are not enough substantial to be published in Biogeosciences. My decision is therefore to reject, and I encourage the authors to resubmit in a minor journal. Please find some comments that I hope will help in revising the manuscript.

2) Thank you for agreeing that the link between biota and GHG emissions is a topic that deserves attention and further study. We would like to stress that before our manuscript was placed online as a discussion paper, the associated editor already judged that the manuscript was within the scope of the journal. We agree with him that our study matches the scope of Biogeosciences as it encompasses "all aspects of the interactions between the biological, chemical, and physical processes in terrestrial (...) with the geosphere, hydrosphere, and atmosphere". Our study also fits perfectly into two core fields identified by the Biogeosciences editorial board (biogeochemistry and gas exchange; and plant–soil interactions). We also would like to point out that experimental approaches are explicitly welcomed by the journal.

1) TITLE The term 'mats' is not appropriate, since you are working in small mesocosms. I would not use the word "strongly", as only a part of your results is statistically significant. All along the paper, I'd rather talk about biomass and not density, as density takes into account the weight of a single plant, and this can be very variable.

2) We removed "Strongly" and "mats" from the title. It now reads "Rooting and plant coverage determine greenhouse gas budget of water hyacinth (Eichhornia crassipes)". Throughout the manuscript we changed the word "density" to "coverage".

3) Page 1 lines 1-2.

1) INTRODUCTION In general, the introduction is excessively focused on the water hyacinth; you could improve the text by citing other studies carried out on other weaklyanchored hydrophytes, such as Trapa natans, or other floating-leaved rhizophytes (Nuphar spp.). Your results from not-rooted mesocosms could even be com-

pared to those found within free-floating plants (Lemna spp. mats, or Azolla spp.). The following studies could give the reader some insights into the topic: - Bolpagni, R., Pierobon, E., Longhi, D., Nizzoli, D., Bartoli, M., Tomaselli, M., Viaroli, P. (2007). Diurnal exchanges of CO2 and CH4 across the water–atmosphere interface in a water chestnut meadow (Trapa natans L.). Aquatic botany, 87(1), 43-48 - Grasset, C., Abril, G., Guillard, L., Delolme, C., Bornette, G. (2016). Carbon emission along a eutrophication gradient in temperate riverine wetlands: effect of primary productivity and plant community composition. Freshwater Biology - Caraco, N. F., Cole, J. J. (2002). Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river. Ecological Applications, 12(5), 1496-1509 - Ribaudo, C., Bartoli, M., Longhi, D., Castaldi, S., Neubauer, S. C., Viaroli, P. (2012). CO2 and CH4 fluxes across a Nuphar lutea (L.) Sm. stand. Journal of Limnology, 71(1), 21.

2) Some members of our team have recently published a paper focussing on the fate of methane in floating plant dominated systems (Kosten et al. 2016). In that paper, various floating plants were compared (and the Ribaudo et al. paper was, for instance, included in that study). We therefore chose to focus on water hyacinth only in this paper. The fact that water hyacinth is a widespread, invasive, highly problematic but also often used species, merits, to our opinion, a paper focussing on this single species. We aimed to elucidate this in the introduction. We do agree, however, that it may indeed be informative for the reader to include references to other floating plant species. We therefore now explicitly refer to the overview in Kosten et al (Page 5 lines 11-14) in the introduction, and added the suggested references to provide a broader context (Page 4 lines 12-15).

Bolpagni, R., Pierobon, E., Longhi, D., Nizzoli, D., Bartoli, M., Tomaselli, M., Viaroli, P. (2007). Diurnal exchanges of CO2 and CH4 across the water–atmosphere interface in a water chestnut meadow (Trapa natans L.). Aquatic botany, 87(1), 43-48

Caraco, N. F., and J. J. Cole. 2002. Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river. Ecol. Appl 12: 1496–1509 Grasset, C., Abril, G., Guillard, L., Delolme, C., Bornette, G. (2016). Carbon emission along a eutrophication gradient in temperate riverine wetlands: effect of primary productivity and plant community composition. Freshwater Biology. 61 (9), 1405–1420.

Kosten, S., M. Piñeiro, E. de Goede, J. de Klein, L. P. M. Lamers, and K. Ettwig. 2016. Fate of methane in aquatic systems dominated by free-floating plants. Water Research 104: 200-207.

Ribaudo, C., Bartoli, M., Longhi, D., Castaldi, S., Neubauer, S. C., Viaroli, P. (2012). CO2 and CH4 fluxes across a Nuphar lutea (L.) Sm. stand. Journal of Limnology, 71(1), 21.

3) Page 5 line 11-14; page 4 lines 12-15.

1) page 4 line 17: I believe that the expected effect of rooting on GHG emission should be better explained and justified. I do not think that 'chimney effect' is the right term for the ecophysiological mechanism you are referring to. Indeed, the mechanism of gas transport through the macrophytes aerenchyma is widely defined as 'convective flow' or 'pressurized flow' and implies complex interactions between aerenchyma structure, internal pressure and gas concentration. I do not think that Bastviken (2009) is the right reference, try instead with: -Grosse, W., Armstrong, J., Armstrong, W. (1996). A history of pressurised gas-flow studies in plants. Aquatic Botany, 54(2), 87-100 -Konnerup, D., Sorrell, B. K., Brix, H. (2011). Do tropical wetland plants possess convective gas flow mechanisms?. New Phytologist, 190(2), 379-386 *page 5 line 6:

2) Although in literature plant-mediated transport of methane is sometimes referred to as the chimney effect (eg. (Bhullar et al. 2013; Journal of Plant Ecology 6: 298-304)) we fully agree to change it to a more self-explanatory and explicit term. We therefore now use 'plant-mediated transport' which may include different types of gas transport including pressurized flow (convective flow), and passive molecular diffusion (Grosse et al. 1996; Cronk and Fennessy 2016; Konnerup et al. 2011). This information was added in the text (Page 4 lines 19-22 and page 5 lines 1-2).

We added the folowing references:

Cronk, J. K., and M. S. Fennessy. 2016. Wetland plants: biology and ecology. CRC press.

Dacey, J., and M. Klug. 1979. Methane efflux from lake sediments through water lilies. Science 203: 1253-1255.

Grosse, W., Armstrong, J., Armstrong, W. (1996). A history of pressurised gas-flow studies in plants. Aquatic Botany, 54(2), 87-100.

Konnerup, D., Sorrell, B. K., Brix, H. (2011). Do tropical wetland plants possess convective gas flow mechanisms?. New Phytologist, 190(2), 379-386

3) Page 5 line 2-6.

1) The hypotheses of the study are not clearly stated; what are you expecting as a result of the experiment?

2) By describing all possible (including contrasting) effects of density and rooting on CO_2 and CH_4 fluxes we aimed to explain that the outcome of the experiment is not straightforward a priory especially regarding CH_4. To highlight this, we now added a phrase explicitly stating this (Page 5 lines 18-22). We also added a hypothesis regarding CO_2 (Page 6 lines 12-13).

3) Page 5 lines 18-22; page 6 line 6-7.

1) METHODS I think the main weakness of the experimental design is the lack of replicates. Each treatment is tested only on n=4, which is not adequate in order to obtain a robust result.

2) We of course agree a higher number of replicates would make the outcome more robust. The number of replicates was – as it is always - a trade-off between logistic-feasibility and statistics and n=4 (with a total of 24 aquaria) is often used in biological mesocosms experiments. The fact that we still found statistical differences supports

our conclusions.

1) From the description, I understand that you measured fluxes on 3 different dates and then pooled the results together and expressed as mean ± error. Well, this makes 12 pseudo-replicates, not factual replicates.

2) We indeed used 4 replicates in three different times, and fully agree with the referee. This pseudo-replication issue has now been corrected by including time as an independent variable in our mixed effect model. We changed our model from model.target<-lme(CH4 plant*mesh,data=GWP, random= 1|code,na.action = na.omit) to model.target<-lme(CH4 time*plant*mesh,data=GWP,random= 1|code,na.action = na.omit) We incorporated the new statistical outcomes throughout the entire manuscript. We found minor changes in the X^2 value and in the Tukey test results, and the conclusions therefore remain the same.

3) E.g. page 11 line 11.

1) page 6 line 3: ": : :phosphorous propagules." I think the verb is missing in the sentence.

2) Indeed the verb was missing. Changed into 'phosphorus granules were added.'

3) Page 7 line 5.

1) page 3 line 9: If 100

2) We think here you refer to page 6 line 9 (original manuscript). We totally agree, and changed the 'density' into 'coverage', which relates to biomass and density.

3) Changes were made throughout the manuscript, e.g. page 6 line 2.

1) Paragraph 2.3. This paragraph needs to be more explicit and detailed. Maybe I'm missing some important information, but I do not see how you can be sure that no CH4 bubbles were emitted while you measured "diffusive" fluxes. Also, how long the incubations lasted, during the day and during the night? It would be interesting for the

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Discussion paper

reader to see the possible difference between day and night results, especially for CO2 fluxes. In general, it would be interesting for the reader to see the values corresponding to each date of measurement, for both diffusive and ebullitive fluxes.

2) The occurrence of bubbles can be observed almost real-time in the output (screen and file) of the NIRS-CRD gas analyser as sudden increases (spikes) in CH4 concentrations. When bubbles occurred, the aquaria lid was removed and flushed to restore atmospheric levels after which the diffusive flux measurement was started again. This method determining diffusive fluxes excluding bubbles is described in Bastviken et al (2004). We now included this additional information in the method section to prevent confusion (page 8 line 21 and page 9 line 1). The magnitude of the diffusive flux calculated is based on the linear increase in CH4 concentration in time for a time span without CH4 spikes. The exact calculation is described in detail in Almeida et al (2016) to which we refer now (page 9 lines 1-4). We left the aquaria closed for 5 minutes during the day and also during the night. Thank you for suggesting to include the graphs of the day and night fluxes, which is indeed informative. We now included these in the supplementary material (Fig. S3) and in the main text (page 13 line 1). We chose not to present an emission versus time graph as there was no significant relationship between gas emissions and time (X^2=0.87; P>0.05 for CH4; and X^2=4.99; P>0.05 for GWP), except for CO2 (X^2=30.80; P>0.05 for CO2) (Page 13 line 2-3).

Additional references:

Almeida, R. et al. 2016. High Primary Production Contrasts with Intense Carbon Emission in a Eutrophic Tropical Reservoir. Frontiers in Microbiology. 7, (2016), 717.

Bastviken, D., Cole, J., Pace, M. and Tranvik, L. 2004. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. Global Biogeochemical Cycles. 18, 4 (2004)

3) Page 9 lines 1-4; page 13 line1; page 13 line 2-3.

1) I have a serious concern about the length of your incubation for measuring ebullitive fluxes. In literature, you can find many papers demonstrating that small volumes of air in the headspace (around 6 liters above the water, in your case) can be quickly saturated in CH4. Thus, if you measured only T0 and Tf after 24h, you most probably underestimated your flux, because the slope of your regression was affected by saturation in the headspace. Of course, the degree of saturation in the headspace would depend on the CH4 concentration in the water; I think that it will help to show the dynamic of CH4 in the water throughout the duration of the experiment.

2) We agree that diffusion back into the water may lead to an underestimation of the ebullitive flux when the concentration in the headspace becomes higher than the concentration in the water. We calculated the methane concentration in the water based on the diffusive flux and a gas transfer velocity of 0.05 m/d (Typically the gas transfer velocity in standing waters in aquaria is around 0.05m/d - 0.1 m/d and the addition of plants further decreases this (Kosten et al (2016)). We found that fluxes back into the water only occurred in the controls and in some aquaria with 50

3) Page 12 line 16-19.

1) *page 7 line 21: how did you sample the headspace? Were the samples transferred to vials or directly injected in the GC? Which volume of injection? Please be more precise.

2) Samples from the headspace were taken in duplicate using a 1ml plastic syringe. 0.5 ml Samples were directly injected in the Gas Chromatograph. We now added this information to the method section.

3) Page 9 lines 7-11.

1) *page 8 line 12: Is it a total biomass (leaves+petioles+roots)? How did you measure the fresh weight? Is it a standard error or deviation? As I can understand, this is the weight of a single plant. But in Methods section you indicated a plant weight was 160

g (as FW or DW? Error?). Did the weight of a single plant changed throughout the 50 days experiment?

2) We agree that this may be confusing, and now explicitly mention the way that the plant was measured. The total biomass was measured using the fresh weight using a paper towel to carefully blot the plants and dry the excess of water attached and immediately measured on the digital scale (page 7 line 13-14). The numbers are standard deviation. Four extra plants were taken for the initial chemical analysis and they are represented by FW and SD (page 10 lines 6-7). We did not show data for the weight of a single plant in the manuscript at the end of the experiment.

3) Page 7 lines 13-14; page 10 lines 6-7.

1) *page 8 line 21: replace by “Statistical analyses”

2) We replaced for the right term.

3) Page 10 line 15.

1) *page 9 line 13: To which water sample does this sentence refer to?

2) We are not sure if we understood this question (as no samples are mentioned in this line). Concerning the oxygen measurement: we did not sample water for oxygen concentration, instead we used a Hach HQd field probe to directly measure the oxygen concentration 20 cm below the surface (as explained in the Methods section).

1) *page 10 line 5: there should be a mistake relating the name given to the figures (S1A, S2: : :).

2) We refer to the supplementary figures here (they are correctly mentioned).

1) *page 10 line 18: If some results are not significant, then do not give importance to them. Statistical analyses did say they are not significant.

2) We agree and have removed this sentence.

1) DISCUSSION This section is confusing and should be reorganized. Before doing that, ayou should clearly state your hypotheses in the Introduction section as simple questions which can be answered by yes/no or true/false. Then reorganize your discussion answering those questions. At present, the discussion contains many elements which are not pertinent. Many references are not appropriate. I also think that you should spend some considerations on the impact that high productive plants have on sedimentary processes in correspondence of periods of senescence of plants. That is, in late summer and fall, the sedimentary oxygen demand could increase and enhance CH_4 and CO_2 benthic fluxes. What is happening when the plants are not fixing as much carbon as in summer? What about the build-up of dead biomass on the bottom? Which could be the real C budget on a complete year?) Please do not put again the reference to the figures in Discussion section.

2) We rephrased the first paragraph of the discussion referring to our (newly included) hypotheses (page 13 lines 6-11). The subsequent paragraphs refer to the different processes involved in the GHG budget and as such form part of the discussion of the research question. Now that we explicitly mentioned the hypotheses, we trust this is clear. We carefully re-checked all references and replaced them when pertinent (e.g. page 13 line 20). We indeed agree that senescence and subsequent decomposition will affect the overall GHG budget. The magnitude of the impact decaying plant material will have on the total GHG budget will depend on the relative importance of this organic matter source to the sediment (with respect to other OM sources). The sediment we used in our experiment already has a relatively high CO_2 production rate (as compared to sediments presented in for instance (Cardoso et al. 2014). As the impact of decaying plant material on the overall GHG budget is important (and complex as the species generally grow in tropical regions where year round high production rates can be achieved but hydrological regimes may cause that decay takes place at different locations than production), we now included this information in the discussion (page 16 lines 5-7). We personally prefer to include references to figures in the discussion section to guide readers that focus on the discussion alone, but if the Editor prefers to

remove them, we are of course willing to remove these references.

Cardoso, S. J., A. Enrich-Prast, M. L. Pace, and F. Roland. 2014. Do models of organic carbon mineralization extrapolate to warmer tropical sediments? Limnol. Oceanogr. 59: 48-54.

3) Page 13 lines 6-11; page 13 line 20; page 16 lines 5-7

1) *page 12 line 5: the reference to a submitted article is not suitable.

2) The paper has now been published and the full reference has been added.

3) Page 20 line 9-10.

1) *page 12 line 12-15: those sentences are not supported by results, please remove them. If you really expect what you wrote, please support those sentences with references and put them in the Introduction section as a part of your initial hypotheses.

2) We now included the phrase in the introduction.

3) Page 6 lines 1-9.

1) *page 13 line 15: Typha domingensis is a helophyte. You'd rather talk about weaklyanchored or free-floating hydrophytes in your discussion.

2) We changed the phrase including data from water hyacinth.

3) Page 16 lines 1-2.

1) *page 13 line 21: Based on your results, obtained from small mesocosms, you cannot extrapolate to "open waters".

2) The sentence was rephrased to avoid confusion.

3) Page 16 lines 4-5.

1) Tables: Authors must use standard deviation and not standard error of mean, the former referring to data variability around mean of a sample of population (this is your

case), the latter referring to precision for an estimated population mean (this is not your case).

2) We chose SE based on visual considerations. However, we agree on the suggestion and changed the SE for Standard deviation (SD) throughout the manuscript.

3) E.g. Page 22 line 2-5.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2016-297/bg-2016-297-AC1-supplement.pdf

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Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-297, 2016.