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Biogeosciences Discussions

Interactive comment on "The role of endophytic methane oxidizing bacteria in submerged *Sphagnum* in determining methane emissions of Northeastern Siberian tundra" *by* F. J. W. Parmentier et al.

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This content of this response are the same as the previous one, only some formatting errors have been corrected.

Response to Referee 1 (Nathan Basiliko)

General comments: *Parmentier and co authors have (1) measured CH4 ïňĆuxes and environmental variables over 20 days in a ïňĆooded Russian arctic tundra wetland with a focus on comparing CH4 emissions between areas with submerged Sphagnum*

moss present and without Sphagnum moss (the latter having a substantially larger vascular plant cover, but of the same Carex and Eriophorum species as in the Sphagnum plots); (2) incubated Sphagnum plants with apparently non-rate-limiting concentrations of CH4 and O2 and measured CH4 oxidation potentials in vitro and scaled up measurements to the level of in situ net efïňĆux; and (3) performed model sensitivity analysis with a previously described model used by one of the co-authors at this and other sites to help identify if/that presence or absence of Sphagnum mosses plays an important role in CH4 efïňĆux rates observed between these 2 sets of sampling locations. I generally agree with the unsolicited comments from Dr. Knoblauch. I would ïňĄnd it appropriate for the authors to also consider his comments in their revisions (rather than just providing the rebuttal). This is an interesting paper and there is clearly a recent precedent in the literature for conducting this work. I also greatly appreciate that this work was carried out in a representative globally important high latitude site that is logistically challenging to access. Particularly because of the latter point, I ïňĄnd overall merit in the reasonably small scope of ïňĄeld and modelling work that was conducted. I also found the manuscript to be appropriately succinct and generally easy to read. However, even in light of the model sensitivity analysis, I feel that the authors should present their conclusions more cautiously. The study is fundamentally observational and based in only a few measurement locations in one site over a very short period of time. Presence or absence of Sphagnum moss may very well be the key factor driving observed differences, but this, as well as CH4 production, water-table position, and vascular plant cover (that could supply substrate for more rapid methanogenesis as well as represent a potential gas conduit) are not controlled experimentally. I also am also particularly wary of the direct comparison of lab-derived CH4 oxidation potential rates to ïňĄeld-based net CH4 ïňĆux rates.
I provide more speciïňĄc comments below that I hope will prove constructive for the authors as they revise this manuscript. Sincerely, Nate Basiliko (University of Toronto)

We thank the referee for the constructive comments made to this paper. We ac-

knowledge the general observation that our conclusions should be presented more carefully and in the revised manuscript we will do so. The main intention of this article is to show that these endophytic bacteria can be very important in the recycling of methane in submerged areas that are dominated by *Sphagnum*. Unfortunately, by performing in-situ measurements of these emissions in an extremely remote location, we were not able to quantify all necessary parameters as we would have done under laboratory conditions or at a more accessible site. We will rewrite the article to make our conclusions more cautious and better assess the uncertainties involved because of the chosen approach. Here we will use the comments made by the referees and commenter Dr. Knoblauch and discuss the points addressed. The link between the laboratory measurements of methane oxidation and differences observed in the field will be made more cautiously by only providing a calculation that shows that potential rates are in the same order of magnitude as observed in the field, but we will note that these two rates are not comparable with each other due to the vast different differences between laboratory and field conditions.
Furthermore, although the testing of model sensitivity for methane production was part of the original simulation (Van Huissteden et al., 2009), it was not included in the sensitivity analysis in this article due to a communication error. We have now included it, redone the analysis, and we show that although small differences exist in methane production and plant transport between the vegetation types, methane oxidation is still the defining factor that explains the observed differences best.
Furthermore, we have rewritten the introduction, to clarify better clarify the intent of this research. The model description, results and discussion have been altered due to the inclusion of methane production. Also, following the comments made by the referees the discussion and conclusion have been rewritten to a large extent.

P8523 Line 5 (and throughout): How important is this cooperation? Is it a true symbiosis? One might imagine that stable habitat (and perhaps a local O2 supply) for CH4 oxidizing bacteria in these mosses might lead to larger bacterial populations than if living freely in water. However, would moss NPP decline in absence of the *methanotrophs? It is hard to imagine Sphagnum mosses, which often exist above a decomposing organic soil proïňĄle, are typically C-limited and that in absence of CO2 production by bacterial endophytes they could not obtain this amount of CO2 from elsewhere. There is some evidence that elevated CO2 concentrations in peatlands might actually favour vascular plants over Sphagnum mosses (see Fenner et al. Ecosystems 4:635-637)*

The Sphagnum mosses provide a stable habitat and oxygen to the methanotrophs. We incubated water samples as a control for free-living methanotrophs (also described in Kip et al. 2010 and Raghoebarsing et al. 2005). No decrease was observed. Removal of the floating Sphagnum layer from peat cores resulted in a five-fold increase of methane emission (Kip et al. 2010). This all indicates the methanotrophs favor to be in or strongly attached to the mosses instead of free living in the peat water. In this paper, we do not try to establish whether the methanotrophic bacteria that supply CO_2 from CH_4 are truly symbiotic with the plant and we have made it careful to describe the mechanism as a cooperation instead of a true symbiosis, to avoid this discussion. Peat ecosystems are characterized by a slow decay of peat mosses and in general these systems are moderate acidic (pH 4 to 5) and oligotrophic. Although CO2 conditions in pools tend to be higher than in the atmosphere the diffusion of CO2 in water is very slow, which results in carbon limiting conditions (Smolders et al. 2001). *Sphagnum cuspidatum* does not grow at CO2 concentrations below 250 micromol/L and for optimal growth about 850 micromol/L is needed (Paffen and Roelofs, 1991). The anaerobic degradation in the decaying peat results in biogas (ratio carbon dioxide : methane = 1:1) formation. Peat mosses will be able to live without methanotrophic activity but the oxidation of the methane part of the biogas in and on the peat mosses will always lead to increased availability of carbon dioxide for the mosses. Furthermore, Kip et al (Nature, 2010) found in a lab-based experiment through ^{13}C labeling that CO_2 provided by these bacteria provided up to 35 % of the total amount of carbon taken up by varying submerged *Sphagnum* species.

P8523 Line 19: The reference to the response of methanotrophic bacteria to a warmer climate in the last line of the abstract does not really seem to ïňĄt with the scope of the study.

Agreed. In the revised version the abstract has been rewritten and this part has been removed.

P8527 ca. Line 26 (and elsewhere): I don't think that this point can be made conclusively: O2 concentrations were not actually measured, and winds described above in the methods section could create turbulent mixing in this standing water (others, e.g., Strack et al. Global Biogeochemical Cycles GB4003, have reported standing water in a boreal peatland to be a zone of CH4 oxidation). There appears to be a real difference in the water table elevations between these two vegetation communities (perhaps as much as 10cm mean difference) in Figure 3, with a lower (as low as 2-3cm above the surface) and more variable water table in the sampling locations that also had smaller CH4 efïňĆuxes. One might expect water table position to play a role in observed differences in ïňĆux rates. These points are one of the key reasons for my asking the authors to be more cautious in their conclusion about the role of Sphagnum presence or absence as the key driver of differences in observed CH4 efïňĆux.

This is an important point and we have now addressed this in the results and the discussion as described below.

Added to results: In Figure 4, water level, active layer thickness and temperature for the two vegetation classes at each measurement day are shown. From the figure it becomes clear that soil temperature was very similar between the two vegetation types and active layer depth did not differ that much either. However, a significant difference was observed for water level. While water levels were above the surface

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for both vegetation types, the water level in TW4 was somewhat lower from July 23 to July 30. Hereafter, water levels were more similar, although a small difference in water level remained.

Added to the discussion: While this inundation reduced the relative influence of plant transport, it introduces a new issue since water levels were significantly different between vegetation types. Most measurements in July were performed with a higher water table for TW1 than for TW4, as shown in Figure 4. Since this higher water table can lead to lower transport and higher oxidation of methane (?), this could lead to differences in oxidation not related to the methanotrophic bacteria associated with *Sphagnum*. However, this potentially increased oxidation in TW1, the vegetation type without *Sphagnum* and exhibiting the highest emissions, would diminish both methane emissions from TW1 and the difference between the two vegetation types, not increase them. Furthermore, the relative difference between the vegetation types shows no apparent effect of water table. While a large increase in the difference in water level between the vegetation types occurred between July 18 and July 23, the relative difference in emissions did not change. Moreover, the largest relative difference in emissions between the vegetation types was observed for the last 4 measurement days when water levels in both vegetation types were very similar. The observed differences in fluxes are therefore not likely to be due to a difference in surface water level.

P8525 ca. Line 17: What impact could human disturbance associated with collar installation and chamber measurements have made? Were boardwalks present? If not, and perhaps particularly in ïňĆooded conditions, CH4 ebullition could have been triggered by walking (repeatedly) to these measurement areas. Also, do you think that soil compaction occurred during sampling and would this have had any important effects?

Measurements were performed from a boardwalk and great care was taken to avoid soil compaction or other disturbances. Concentration measurements were always checked to see whether they followed a linear pattern, to avoid overestimated fluxes by ebullition. Also, if sites showed a high variation in between days that did not fit in the general pattern of the other plots, the measurement was repeated to make sure that it was not disturbed. We now mention in the text that measurements were performed from a boardwalk.

Page 8529 Line 15 (and elsewhere): "microbiological analysis" is not an appropriate description of what was actually done. Please reword to (some variation of) "CH4 consumption potential measurements".

Agreed. We will change this term into 'incubation study'

Could there have been issues associated with sampling peat in 2008 for these measurements while ïňĆux measurements appear to have been made in 2007? As well as with other concerns I express below, there might be problems with assumptions that the methanotroph community, biomass, and activity rates are stable over time.

The differences observed between the vegetation types (2:1) have been observed in other years as well and we therefore know that this relationship is rather stable. The data of 2007 was used because for that year we had the longest record of methane fluxes measured in these vegetation types. In 2008, this only involved two days (with the same relative differences) which have been omitted to keep the picture simple and straight forward. But we agree that comparing measurements from one year and samples from the other introduces uncertainty. We will address this in the revised version.

Page 8530 Line 11: Were these always linear? (my 'back of the envelope' calculations might be wrong here, but): the in vitro oxidation rates seem quite fast; if only about 50micro mol CH4 was added to each vial and oxidation rates were about 50 micro

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mol/g dry peat/day, I would expect to have seen an exponential decrease in mass of CH4 over the time frame of the incubations. If this was the case, it might be best to calculate linear rates over an earlier portion of the incubation or to ïňĄt a non-linear regression model. Interestingly, the CH4 oxidation potential rates reported are very similar for those that colleagues and I have measured in portions of submerged, but not emergent, Sphagnum majus in an inundated margin of a southern boreal Canadian bog (See Fig 4b in the cited 2004 Wetlands paper).

The rates were indeed very fast. For all rate calculations linear parts of the methane vs time curves were used. Within the range from 50 to 25 micromol of methane in the headspace of the incubation bottles decrease was always linear. Below 25 micromol indeed an exponential decrease was observed.
The two rates presented in Table 2 were determined after the first and second addition of methane and both rates are rather similar for most of the samples.

Page 8530 Lines 12-19 and elsewhere: I really think that it is too far of a stretch to compare CH4 oxidation/consumption potential measurements to ïňĄeld efïňĆux rates in an absolute sense. At best, when performed over short/initial periods of time following sampling, this lab assay can report relative rates of CH4 oxidation given large/nonrate limiting amounts of CH4 and O2. The cited Sundh (1995) paper indicated that this might represent relative viable methanotroph biomass only as I recall (and many other subsequent publications have generally corroborated this). Because "low-afïňĄnity" methanotrophs typically exhibit ïňĄrst-order (substrate-availability controlled-) CH4 oxidation dynamics, the rate observed in the lab where plenty of CH4 and O2 is supplied evenly to mosses can't represent the conditions in the ïňĄeld (that is, the entire mass or volume of the 3-dimensional 0.25m2 "cube" of Sphagnum is not evenly exposed to 10,000ppm CH4 and 200,000ppm O2). Again I strongly feel that these measurements can only be used to illustrate potential relative differences across treatments or environments. This was not done, and the quantitative scaling-up is inappropriate, I am afraid.

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As such, I suspect that the data by themselves are not very useful. Perhaps a comparison could be made to potential rates published using similar techniques in other Sphagnum-dominated wetlands. If not, I suspect that this section should be removed from the paper.

The upscaling provided is mostly meant to show that these rates can be achieved under conditions where ample $CH_4 and O_2$ are available and as such they are not directly comparable to the field. We acknowledge this in the discussion and only use the relative difference measured in the field and not the absolute laboratory rates when we are considering the potential influence of these bacteria. We will however rewrite this part of the paper to show that this is only meant as a back-of-the envelope calculation that indicates that oxidation rates measured in the lab fall within the same order of magnitude, not to compare absolute numbers with each other.

The new text of this paragraph is as follows:

The obtained oxidation rates are determined in mol CH_4 gDW^{-1} day^{-1}, while fluxes in the field are measured in mg CH_4 m^{-2} hr^{-1} and this makes it difficult to compare the two rates. Ideally, the two could be compared by multiplying with the amount of dry weight of *Sphagnum* per m^2. However, oxidation rates from the incubation study were determined under ideal conditions with an ample supply of methane and oxygen, which is unlikely to be the case for field conditions, and concentrations may vary vertically in the field. Nonetheless, by multiplying the incubation rates with the amount of dry weight of Sphagnum per m^2, an indication will be given whether the optimal oxidation rates from the laboratory are in the same order of magnitude as in the field. If this crude, and most likely overestimating, translation of fluxes from the laboratory to the field shows us lower rates than the observed differences, we know that these differences must be due to other factors than oxidation alone. Notably, the reverse does not necessarily hold true but provides a picture of potential oxidation under ideal circumstances.
To apply this crude method, four 0.25 m^2 plots, with the TW4 vegetation type, were selected and all *Sphagnum* was collected. This *Sphagnum* was dried in an oven for

a week at 60C and weighed afterwards. This weight was used to calculate optimal oxidation rates in mg CH_4 m^{-2} hr^{-1}.

And in the discussion: The rates obtained from the incubation study were recalculated to fluxes per m^2 by multiplying the oxidation rates by the amount of dry weight per m^2. Although differences in methane and oxygen concentrations between the lab and the field preclude a direct comparison to differences observed in the field, they do show that there is a very high potential for methane oxidation in submerged *Sphagnum* if viewed under these ideal conditions. The conversion to m^2 gave oxidation rates that were twice as high as in the field and this indicates that the potential for high oxidation in *Sphagnum* is present, although caution has to be expressed to view these numbers in an absolute sense, since they are most likely overestimating field conditions.

P8534 ca. Line 25: Would physically removing Sphagnum be a potential (and easy) means of testing its role more directly/experimentally?

This is a very interesting suggestion and seems like a good starting point for future research. Additionally, removal of vascular plants from the vegetation type without *Sphagnum* may also be considered and compared to the undisturbed vegetation type.

P8535 Lines 1-14: I fully agree that this supports the conclusions of the paper (i.e. that moss cover and not vascular cover in important in observed CH4 emissions), however given the very short duration of the study and that one site with relatively few measurement locations were observed, one must be more cautious in relying on such a coarse relationship (e.g. that % cover would conclusively predict variability in short-term net CH4 emissions across a small spatial scale). Likewise with the model results, they certainly support the conclusions, but are based on a simpliïňĄed/idealized understanding of CH4 dynamics in these systems. Again, I feel that the appropriate way to deal my concerns is to report the conclusions (in the abstract, discussion, and conclusions sec-

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tions) a bit more cautiously.

We concur. The revised conclusions are as follows:

In this study, methane emissions from two inundated vegetation types were compared. Areas dominated by submerged *Sphagnum*, with some sedges, were found to exhibit emissions that were two times lower than inundated vegetation dominated by sedges, but without *Sphagnum*. An incubation study of submerged *Sphagnum* samples showed that very high oxidation rates of methane, even at 4C and on ice, was possible in this vegetation. This suggested that below the water table oxidation in submerged *Sphagnum* is one of the key processes in clarifying the difference between the two studied vegetation types.
To assess the likelihood in which other known parameters such as plant transport and methane production could explain the observed differences, both vegetation types were modeled in detail, together with a sensitivity analysis on the parameters. While this model study showed that methane production and plant transport might be somewhat higher in the vegetation type without *Sphagnum*, possible values largely overlapped and averages were comparable. Furthermore, the model appeared to be much more sensitive to within plant oxidation, which showed average values that were 50% higher in the vegetation type with *Sphagnum*. This reaffirms the importance of the activity of these methanotrophic endophytes in submerged *Sphagnum*.
Since most methane in this tundra type is emitted from the two studied vegetation types, these results are also spatially important. Respective surface cover of the two vegetation types is 7 to 3 for TW1 and TW4 respectively (?) and this means that the vegetation type dominated by submerged *Sphagnum* represents 30% of the methane emitting surface. If we assume a ratio of 2 to 1 in the emissions between the two vegetation types, it can be estimated that oxidation by methanotrophic endophytes plays a large role in 15% of the net methane emission from this tundra site.
We conclude, by combining flux chamber measurements, an incubation study and modeling, that this type of methanotrophic bacteria, that live in a cooperation with

submerged *Sphagnum*, is an important factor in the recycling of methane within this tundra vegetation type. Although other factors such as methane production and plant transport are also important in determining emissions, the activity of these endophytic bacteria adds to a better understanding of the drivers and controls of methane emissions from tundra.

P8535 ca. Line 25 (and elsewhere): The emphasis here seems to be on the potential for vascular plants to allow CH4 to bypass CH4-oxidation, but roots also likely represent a key source of relatively labile C substrate that could increase rates of methanogenesis. I think this could also potentially be important to consider

In the revised model run, we have included methane production which is governed by plant exudates. Also, the model already contained root depth but this parameter was found not be be predictive of the results.

Interactive comment on Biogeosciences Discuss., 7, 8521, 2010.

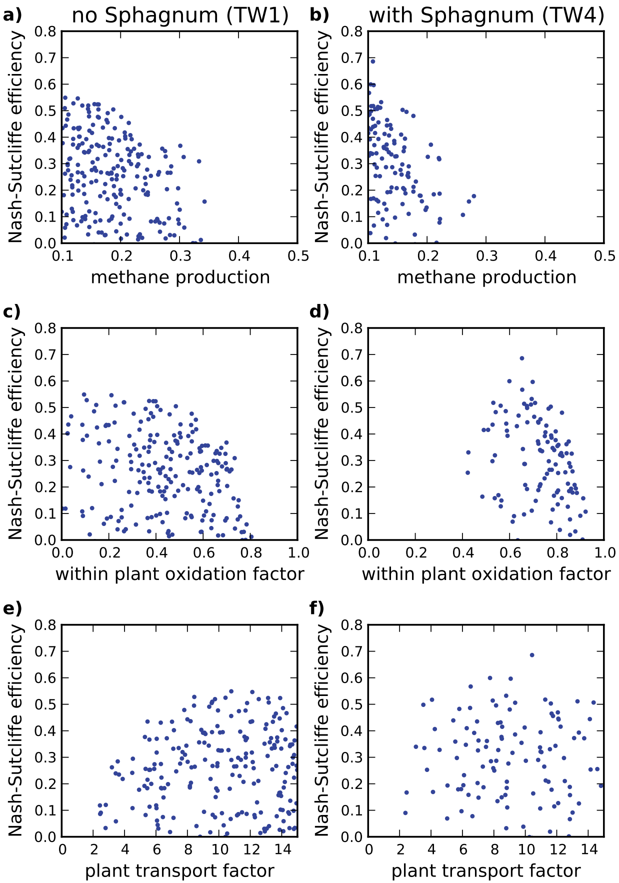

Fig. 1. GLUE analysis of model parameters for both vegetation types, showing Nash-Sutcliffe efficiency.