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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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General comments: *1) In their manuscript, Parmentier and co-authors address the capacity of methanotrophic endophytes of submerged Sphagnum species to reduce methane emissions from a Northeastern Siberian tundra environment. The authors measured methane ïňĆuxes from mid of July to beginning of August, 2007, from two similar sites (TW1 and TW4) one vegetated with Sphagnum and vascular plants and one site without Sphagnum. Also, potential methane oxidation rates were determined at different temperatures in samples from two additional sites also covered with Sphag-*

num. The obtained potential methane oxidation rates were up-scaled and compared with ïňĄeld methane emissions. In addition, process based modelling was carried out aiming to understand if the observed differences in methane ïňĆuxes from the two sites could be explained by methane oxidation through moss associated bacteria. The manuscript is well written, clear and not overlong. A couple of very interesting papers on the particular interaction between methanotrophs and Sphagnum have recently been published either dealing with factors possibly inïňĆuencing methane oxidation in Sphagnum (such as water level and moss species) or dealing with the community structure of those endophytic methanotrophs. A quantiïňĄcation of the methane sink provided through methanotrophs in Sphagnum, however, has not yet been approached so that our knowledge on the importance of this process for the greenhouse gas budget of tundra landscapes is little. Thus, the objective addressed by Parmentier et al. is, in my opinion, highly valuable, the more since the authors included methane ïňĆux modeling. I generally agree with the comments by Mr. Knoblauch and Mr. Basiliko and consider it unfortunate that the major outcome of this paper (that methane oxidation in Sphagnum is mainly responsible for the 50% lower methane ïňĆux from a Sphagnum/sedge site compared to a sedge site without Sphagnum) is not conclusively made. It is well known that typical vascular wetland plants such as Carex and Eriophorum enhance methane emissions. This effect is not only a result of the transport of methane through the aerenchyma but also due to root exudation delivering, directly and indirectly, substrates for methanogenesis. In the manuscript (page 8525, line 1-5), the authors state that methane production is higher in the presence of sedges such as Eriophorum (which, by the way, is absent in the Sphagnum site TW4 according to Tab. 1). So I wonder why it should be surprising that less methane is emitted from a site with a clearly lower vascular plant cover (20-30% compared to 40-90% as shown in Tab.1). Neither data are provided on the below-ground methane stock nor on the methane production rates of both sites, so the differences in methane emissions could as well be a result of higher methane production rates in site TW1. Here I fully agree with the comments by Mr. Knoblauch. It appears to me that the process of methane production

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is also not included in the model applied and can thus not be excluded as a possible explanation for the different ïňĆuxes. How would the model perform, if differences in methane production were considered? In my opinion, the authors should reconsider the interpretation of their data and draw their conclusions with more caution.

We thank the referee for these constructive comments and we acknowledge the main points addressed. Since the initial submission however, we too found that methane production was missing from the GLUE analysis due to a communication error and we regret this unfortunate situation. However, although it is missing from the GLUE analysis, the process was included in the model. To rectify this mistake, we include in this reply a graph that shows the results of the GLUE analysis that includes methane production. Because the value for methane production now varies within in the GLUE analysis, results for the other parameters also change somewhat and therefore the graph is different from the initial submission. The main conclusion however, remains the unchanged.
This new analysis shows that the ranges of methane production for both vegetation types is comparable (0.1-0.3 for TW1 and 0.1-0.35 for TW4), as well as for plant transport (2-15 for both vegetation types), while oxidation shows clearly different ranges (0-0.8 and 0.4-0.9 for TW1 and TW4 respectively). It must be acknowledged that in this new analysis significant differences exists for the averages of the parameters. Average methane production is 0.19 and 0.15 M hr^{-1} (p0.001) and average plant transport is 10.1 and 9.0 for TW1 and TW4 respectively (p 0.1). However, average oxidation shows much larger differences (0.44 and 0.73 for TW1 and TW4, p1000). Thus, although small differences in methane production and plant transport are likely to occur, when methane production is included in the GLUE analysis, oxidation remains the most identifiable parameter to explain the observed differences.
We will include this new information in the manuscript and we regret that this was omitted in our initial submission. Also, Table 1 shows that TW4 has no *Eriophorum* present. In practice some *Eriophorum* did occur in the *Sphagnum* dominated vegetation type (although less than *Carex*) and this is described in the text in section 2.2.

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However, the description in Table 1 was kept the same as in the original article of van Huissteden et al (2005), where *Eriophorum* is not mentioned. We acknowledge that this might lead to some confusion and will add *Eriophorum* to the table.
Apart from the changes mentioned above, the introduction has been rewritten, to better clarify the intent of this research. Also, the model description, results and discussion have been altered due to the inclusion of methane production and the discussion and conclusion have been rewritten according to comments given by the referees.

SpeciïňĄc comments: *2) The potential methane oxidation rates presented by Parmentier et al. validate that methanotrophs associated to submerged Sphagnum species are actively oxidizing methane. Given the very remote character, the-in terms of greenhouse gas ïňĆuxes importance of Siberian tundra, and the widespread distribution of Sphagnum in Arctic wetlands, this result is certainly interesting. However, in order to quantify by how much methane emissions are reduced through methanotrophs in Sphagnum (which in my opinion is the interesting and new part), a different and more experimentally de- ïňĄned approach less afïňĆicted with spatial heterogeneities should have been explored. The process of methane oxidation, for example, could be inhibited in the ïňĄeld or in mesocosms using CH2F2. The difference in methane ïňĆuxes with and without inhibition would give a more realistic picture on the actual methane oxidation capacity of the bacteria associated to Sphagnum. Alternatively, Sphagnum could be removed from the ïňĄeld site as already suggested by Mr. Basiliko.*

We acknowledge these suggestions and in future research this should definitely be considered. Unfortunately it is very difficult to transport CH2F2 to the site. In the past, Russian customs have made it near to impossible to import even non-flammable gases such as CO2 or compressed air since all transport has to go through the air. Because of this difficulty we have not applied this method up to this date. Transporting a monolith back to the Netherlands for analysis is also impossible because of legislative restric-

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tions. Also, *Sphagnum* removal, as suggested by Mr Basiliko, or removal of vascular plants from TW1, are both options that should be considered in future research.

3) I agree with Mr. Knoblauch that potentially high methane oxidation rates of TW1, the site without Sphagnum, cannot be excluded since this was not measured.

See point nr. 1. High oxidation rates in TW1 are possible according to our GLUE analysis but this same analysis makes it very likely that these rates are much higher in TW4.

4) I do not consider it appropriate to compare potential methane oxidation rates based on artiïňĄcial lab conditions with ïňĆuxes measured in the ïňĄeld (page 8530, line 12-19). Thus, I do not think the up-scaling of methane oxidation rates to ïňĄeld methane ïňĆuxes makes very much sense. In addition to the points already made by Mr. Basiliko in this respect, in-situ methanotrophic activity in the moss is likely substrate limited due to the very slow diffusion of methane through water. Methane concentration proïňĄles could have shed light on the actual ïňĆux of methane into the moss layer.

We agree and following the comments of both Mr. Basiliko and this referee, we have changed the text accordingly. The point of this paragraph is to show how ideal oxidation rates would be expressed in the same units as in the field, not to use these absolute numbers for upscaling. We hope this text includes all of the concerns of the referees. It now reads:

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

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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.

5) Page 8524, line 14-19: The authors state that methane emissions are sensitive to the position of the water table. Though in their study both sites are inundated, the water table still differs by up to 10 cm (Fig.3 e.g. on 23rd, 25th, 26th of July). This certainly has an effect on methane emissions (e.g. depth of the water column determines how far the gas needs to diffuse; refer, for example, to the paper by Sachs et al., 2010, Global Change Biology and the papers mentioned within). Potential effects of the different water table positions of TW1 and TW4 need to be discussed

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throughout the manuscript.

In the revised manuscript, we have added the following paragraph to the results and the discussion to address this issue.

Added to results: In Figure 5, 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 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 5. 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

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therefore not likely to be due to a difference in surface water level.

6) Page 8528, line 17-18 and page 8529, line 16: What is the reason for the different years of chamber measurements (2007) and the determination of potential oxidation rates (samples from 2008)? Even more importantly: why were the samples for the determination of potential methane oxidation rates not obtained from site TW4 where the ïňĆux measurements were conducted but from sites NS1 and NS2? Is there a particular reason for this approach? Also, what means 'similar vegetation distribution' here (same page line 17)? Considering the different years and sites for ïňĆux measurements and the determination of potential methane oxidation rates, the up-scaling approach appears to be even less reasonable (see comment 4).

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 have addressed this in the method section of the revised version.
Also, we sampled Sphagnum from the two additional sites, NS1 and NS2, to avoid disturbance of the methane plots and allow future measurements in the same location. We concur that this is not clear in the text right now and we have changed this paragraph into the following:

In July 2008, two additional sites, NS1 and NS2, with a similar vegetation distribution as the TW4 flux sites, were selected for sampling *Sphagnum*. This was done to avoid disturbance of the methane measurement plots, allowing for future measurements in the same locations. The samples were brought back to the Netherlands in

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closed plastic bags and kept cool as much as possible. During transits by airplane they were no longer than 20 hours without active cooling. Incubation experiments were performed in September 2008. While flux measurements were performed in the previous year, ? showed that the difference between the studied vegetation types is quite similar inter-annually, by using a roving method throughout the studied area in the years preceding this study.

7) Page 8535, line 15-17: Again, what about methane production? Was this considered, too?
See our comments at point nr. 1

8) Page 8538, line 4-6: Here I am lost. What is the study site the authors are talking about? On page 8527, line 21-24 (method of methane ïňĆux measurements) it is referred to Huissteden et al., 2005. There, 12 different landscape classes were identiïňĄed with ïňĆoodplains emitting most of the methane. Why are now only tundra sites (TW1 and TW4) mentioned to emit methane? Even excluding the ïňĆoodplains, also TW2 and TW3 were previously reported to emit methane?
The studies site is the same site, we will clarify this in the text. The rationale for only comparing vegetation types TW1 and TW4 is discussed in section 2.2. We do not consider TW2 and TW3, since these type of sites are located in the floodplain, not the tundra terrace. From the vegetation types in the tundra terrace, TW1 and TW4 are responsible for almost all emissions. We have now added to the method section that the floodplain is a different area that is not considered in this study.

9) Page 8524, line 2: Please deïňĄne 'pressure'. What 'pressure' for tundra ecosystem has already occurred due to rising temperatures? Give references here.
In the revised article the introduction has been rewritten, and this sentence has been removed.

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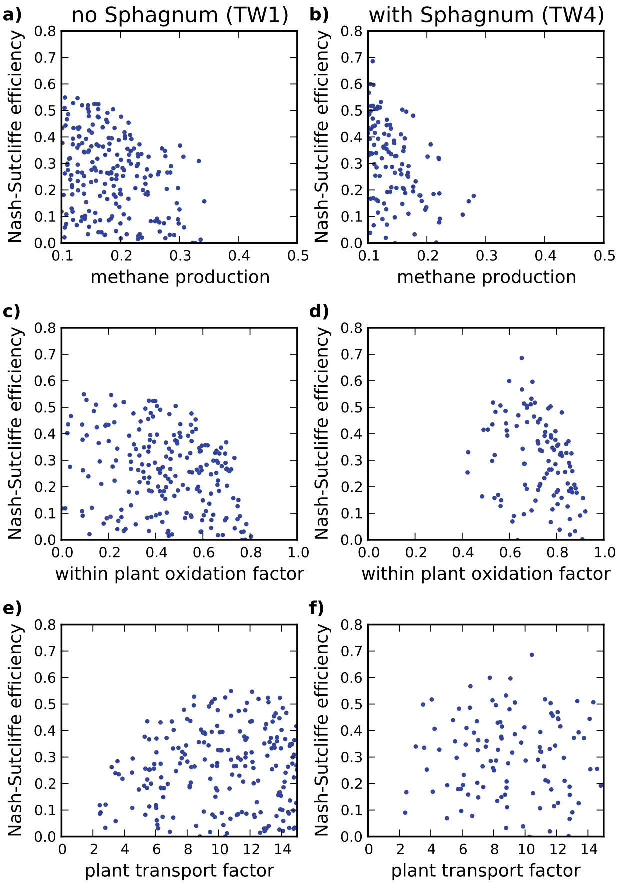

Fig. 1. GLUE analysis of model parameters for both vegetation types, showing Nash-Sutcliffe efficiency.

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