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Biogeosciences Discussions

Interactive comment on "Methanotrophic activity and diversity in different Sphagnum magellanicum dominated habitats in the southernmost peat bogs of Patagonia" *by* N. Kip et al.

N. Kip et al.

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Rebuttal: Anonymous Referee #2

General Comment: This is a short but competent study that adds to a growing body of work describing methanotrophs in various soil habitats. Southern hemisphere peatlands are less extensive than those in the northern hemisphere, and therefore until now have received relatively little attention. Kip et al have combined molecular detection techniques with field and laboratory biogeochemical studies to examine methan-

otrophs in a peatland in Patagonia. I think this is a good paper and have only a few minor comments.

Thank you for the compliments.

Specific comments:

- On p.9359 line 20 the authors conclude that ". . .methanotrophic diversity was. . . surprisingly comparable to the methanotrophic diversity in peat mosses from the Northern hemisphere." This is an interesting finding, but I'm not sure why anyone should be surprised. Most microbiologists since Beijerinck have agreed on the ubiquity of bacterial species. According to earlier studies, including Kip et al. (2010), different peat moss species and different peat landforms have little effect on methanotroph community composition. The habitat studied here is geochemically similar to a northern peatland, at least superficially based on temperature and pH. We should therefore expect similar microflorae.

We agree. This conclusion was modified and the word 'surprisingly' was deleted.

- It is interesting that the authors tried to detect verrucomicrobia, since this has not been directly attempted in earlier studies. Given that the known verrucomicrobial methanotrophs are acidophiles, peats might be an appropriate habitat. Unfortunately, because of the limited database for these methanotrophs, there is no way of knowing how effective the designed primers are.

We agree that the Verrucomicrobial database is very limited and this will be emphasized in the manuscript by adding the following sentence to the Discussion section:

"There was no detection of Verrucomicrobial methanotrophs using newly designed primers. This could indicate the absence of these type of methanotrophs or they maintained undetected because of the limited sequence database, which narrows the detection range of the primers."

- The paper combines some flux measurements made in the field with some measure-

ments of potential rates from laboratory incubations. It would therefore be useful if the authors described the rates obtained from lab studies (particularly for methane oxidation) consistently as “potential methane oxidation rates” rather than “methane oxidation rates” or “methane oxidation activity” (e.g. pp. 9366-9367). The authors are obviously aware of the distinction (p. 9367 line 10), but perhaps a casual reader would be helped by more clarity.

To be more clear all the mentioned ‘rates’ will be changed into ‘potential rates’.

-Table 1 and Fig 1. Are the errors presented standard deviations or standard errors? The latter would be better. Please present replication (n) for each data set.

For the potential methane oxidation and production rates standard deviations are given for n=6, which is also indicated in the materials and methods and for the ecological parameters and methane emission data we refer to Fritz et al., 2011.

- p. 9360 line 10 (also Abstract p. 9359 line 2) “Methanotrophs were found to be present on and inside Sphagnum mosses”. This is a misleading statement, since neither of the cited papers clearly demonstrated that methanotrophs were present INSIDE Sphagnum mosses. Kip et al (2010) used washed mosses only in their studies. Raghoebarsing et al (2005) observed bacteria in hollow hyaline cells, however these dead porous cells are not strictly “inside” the plant. Nor has it ever been proven that the bacteria observed by Raghoebarsing et al (2005) are methanotrophs. They are phylogenetically different from any methanotroph species detected in Kip et al (2010) or in the present paper, and do not belong to any recognized methanotrophic cluster (only 93% similar to Methylocella based on 16S rRNA).

Indeed a direct link was not provided yet. However, after intense washing the Sphagnum mosses still show methanotrophic activity. So the methanotrophs have to be somewhere. We suggest to change the sentences to:

“Methanotrophs living inside the dead hyaline cells or on the Sphagnum mosses . . ..”

“Methanotrophs were found to be present inside the dead hyaline cells or on the Sphagnum mosses.”

- p. 9369 line 8 Methylothermus is generally not classified as a type X methanotroph, despite the fact that its pmoA is phylogenetically closest to type X species.

We agree that the sentence is confusing and will change the sentence by removing “Methylothermus” from it.

- p. 9369 line 25 I think the general feeling among scientists who work with methanotrophs is that the available mmoX primer sets are not effective for detecting methanotrophs in situ. For example, we expect all Methylosinus strains and most Methylocystis strains to have mmoX genes as well, so the detection of pmoA genes from these organisms in the peat but the concurrent failure to detect their mmoX genes suggests that pmoA detection systems are more sensitive. Species like Methylocella that have an mmoX but no pmoA may be underappreciated.

We have tested several different mmoX primer sets and did not obtain any PCR product from the DNA isolated from the mosses from these peatlands and a Methylocella palustris species was used as a positive control. See also our answer to the first comment of Referee #1.

- p. 9370 line 22. I do not think the coexistence of potential methanogenesis and potential methanotrophy necessarily indicates that microsites for both organisms are present. This certainly could be true, however it also could be that the methanotrophs are dormant in situ but rapidly activate in the oxidation assays.

There is no way to determine whether methanotrophs are active in situ as we have measured in the oxidation assays. However the mentioned fluctuations of water level are not a matter of hours, but days or weeks and are therefore comparable to the oxidation assays and if they respond in the oxidation assays we assume this can also happen in situ.

- Fig 3. The legend refers to a colour coding bar that indicates signal intensity, however no bar is presented. As the Peat264 probe is highlighted, it should be explained somewhere why this is important and what it detects.

Thank you for pointing out this omission. The colour coding bar was added. The Peat264 probe will be explained in the results section as follows:

"The probe Peat264, targeting a group of uncultivated peat-related alphaproteobacterial methanotrophs also showed a strong hybridization."

Grammatical comments:

p. 9359 line 15-17 Grammatically confused sentence "situated at depths around the water levels"

The word 'situated' will be removed.

p. 9360 line 12 the use of "benthic" here is a little unusual, as it is usually applied to true aquatic systems like lakes and oceans.

'benthic carbon' will be changed to 'carbon of the system'.

p. 9361 line 16 peatlands instead of peat lands

This will be changed as suggested.

p. 9364 line 7 "stands" is an unusual term for a ground-cover plant.

This will be changed to: ". . .. found in samples from patches with living Sphagnum"

p. 9367 line 8 "At even greater depths. . ."

This will be changed as suggested.

p. 9367 line 22 "a threefold increase of methane oxidation potential within . . .

This will be changed as suggested.

p. 9368 line 8 This reads as if northern peatlands are the only ecosystems that have been studied with molecular diversity surveys! Perhaps "Molecular studies of bacterial diversity in peatlands have until now been limited to sites in the Northern Hemisphere"

This sentenced was changed to: "So far bacterial diversity studies in peatlands have been limited to sites in the Northern Hemisphere."

Fig 2. What is the size of the clone library?

The size of the 16S rRNA clone library was 180 clones, this is explained in the Supplementary Material.

Interactive comment on Biogeosciences Discuss., 8, 9357, 2011.