

## ***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.***

**Anonymous Referee #2**

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

Specific comments: -On p 9359 line 20 the authors conclude that “. . .methanotrophic  
C4316

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.

-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.

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

-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.

-p 9360 line 10 (also Abstract 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. Raghoebarasing 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).

-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.

-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.

-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.

-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.

Grammatical comments: p 9359 line 15-17 Grammatically confused sentence “situated at depths around the water levels” 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. 9361 line 16 peatlands instead of peat lands 9364 line 7 “stands” is an unusual term for a ground-cover plant. 9367 line 8 “At even greater depths...” 9367 line 22 “a threefold increase of methane oxidation potential within ...” 9368 line 8 This reads as if northern

C4318

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

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