Response to Short Comment of P. Bodelier, 20. 02. 2012

Many thanks to the reviewer for the helpful suggestions. Please find our answers to the comments below.

1: It is a pity that the authors did not assess methanogens in parallel, for e.g. by looking at the mcrA gene. Maybe this was done? If yes, I would suggest adding this information to this paper.

The additional assessment of methanogens would certainly have been a valuable addition to this paper. However, for the design of the study we initially hypothesized to primarily encounter oxidation of atmospheric methane, with rare occurrence of microsite methanogenesis (p.1263, ln.22-23). The deep-soil methane source was discovered during the latter phase of the sampling period, when an adaptation of the sampling scheme could not be considered anymore. Nevertheless we will assess methanogenic diversity in a follow-up study.

2: I think the authors should give a more up to date and comprehensive overview of methanotrophs in the introduction. Mention anaerobic methane oxidation as well, as also Verrucomicrobial MOB, NC10 phylum and the filamentous MOB (Crenothrix). Also in the introduction a statement is made which I think is neither completely correct nor relevant (line 20/21). Aerobic MOB have also been isolated from low methane habitats after enrichment using high methane concentrations. The classical low affinity MOB are also present in low methane habitats.

We will consider these points in the revised manuscript.

3: Why did the authors not assess sMMO in these samples? Do the authors expect that sMMO or sMMO containing MOB will not play a major role in these habitats?

In general, we cannot fully exclude the possibility of sMMO playing a role in the environment investigated. However, considering i) the prevalence of the pMMO in almost all known MOB (reviewed in McDonald et al., Appl. Environ. Microb. 74, 1305–1315, 2008), ii) the indications that cells expressing pMMO might have a competitive advantage at lower methane concentrations (Lontoh and Semrau, Appl. Environ. Microb. 64, 1106–1114, 1998), and iii) that pMMO variants might even be responsible for atmospheric methane consumption (Baani and Liesack, P. Natl. Acad. Sci. USA, 105, 10203–10208, 2008.), we believe that the focus on *pmoA*-based diversity analysis did not significantly limit the validity of our results.

4: I think the authors should give an explanation why the amplification with the primers designed for the pmoA2 was so successful. As far as I know there are no MOB who contain the pmoA2 exclusively. Hence, the MOB amplified know should also contain the pmoA1. Hence, I am puzzled by the results obtained. I think this needs some more in depth explanation.

Apparently, the reviewer misunderstand our results description, which may be due to the fact that we have not made sufficiently clear that our information given on p. 1274 (ln. 5-13) relates to *pmoA1* (although this can be deduced from the *pmoA* tree to which we refer

[Figure 3]). The dominant *Methylocystis*-related 243-bp T-RF represents *pmoA1*, while the 350-bp T-RF is indicative of *pmoA2* (Figure 4). This is clearly stated on p. 1280, ln. 26-29. The assignment of the 243-bp T-RF to *pmoA1* of *Methylocystis* was achieved experimentally, while the 350-bp T-RF was assigned to *pmoA2* (*Methylocystis*) by computational analysis. We observed a strict co-occurrence between the dominant 243-bp T-RF (*pmoA1*) and the 350-bp T-RF (*pmoA2*) in the T-RFLP patterns, providing strong support for the computational assignment of the 350-bp T-RF to *pmoA2*. In the revised version, we will make a clear distinction between *pmoA1* and *pmoA2* of *Methylocystis* throughout the text.

We also would like to point out that we tested various *pmoA* PCR primer combinations in first- and second-round PCR. None of the primer combinations consistently produced *pmoA* amplicons from all 27 samples in first-round PCR, but the use of the primer set A189f-A682r resulted in detectable amplicons from 17 DNA extracts. In second-round PCR, pmoA206f-mb661 was the only primer combination that consistently produced *pmoA* amplicons for all 27 samples. Thus, our decision to use the primer set pmoA206f-mb661 in second-round PCR was based on our experimental survey of different primer combinations. The survey is described in detail on pages 1267 (ln. 19-27) and 1268 (ln. 1-11); 1273 (ln. 15-27) and 1274 (ln. 1-4); and 1279 (ln. 15-25).

Apparently, the primer combination pmoA206f-mb661 was most suitable to detect USCγ *pmoA* sequence types in second-round PCR. Here, we refer to our discussion on p. 1279, ln. 15-25. It should be noted that USCγ *pmoA* was also detected as the dominant T-RF in most of the T-RFLP fingerprint patterns generated from first-round PCR amplicons. Primer pmoA206f is an extended version of A189f, originally designed to favor PCR amplification of *pmoA2* (Tchawa Yimga et al., AEM 2003). This explains why we detected *pmoA2* in second-round PCR, but not in first-round PCR. The first-round primer set A189f-A682r favors detection of *pmoA1* from *Methylocystis*, but not the detection of *pmoA2* (GRF1, GRF2, and WIL1 in Fig. S1). Under the PCR conditions used in this study, pmoA206f-mb661, however, supports the detection of both *pmoA1* and *pmoA2* of *Methylocystis* (GRF1, GRF2, KLG 4, WIL 1, and WIL 5; Fig. 4).

5: in the methods section the authors do not say anything about the method to assess quantity and quality of the DNA extracted. Also no information is given on amount of DNA put into the PCR as possible test for PCR inhibition. Please, provide these data.

DNA quantity and quality was checked using a *Nanodrop*® ND-1000 *Spectrophotometer* (NanoDrop Technologies, USA). Using the method described on p. 1267 (ln. 8-18) for DNA extraction, the 260/280 ratio was for all DNA extracts between 1.6 and 1.8. Thus, the environmental DNA was of sufficient purity for further applications, as confirmed by the fact that PCR of bacterial 16S rRNA genes was successful for all the DNA extracts. PCR of bacterial 16S rRNA genes had been included in all PCR experiments as a positive control and generally resulted in strong signals. Information that we used (i) NanoDrop to determine DNA quantity and quality and (ii) positive controls will be added to the revised version. A need for more detailed assessment of DNA quality and possible inhibitory effects may have been required if we would have applied quantitative PCR. However, we did not use qPCR methods in this study.

6: Reviewing this paper I would suggest a different title. Considering the fact that the obtained soil methane profiles can not be linked directly to the MOB observed in combination with the fact that DNA based analyses has been used, I would refrain in this case from using activity in the title: Alternative: Soil methane cycling and microbes involved in glacier forefields on siliceous and calcareous bedrock.

We acknowledge the reviewer's reservation in using "activity" in the title. Indeed we did not provide a direct molecular link between methane oxidation activity and investigated MOB. To some readers it might also implicate that analyses of transcripts were performed. However, we think that a soil methane sink, as quantified in our paper, is an unmistakable sign of the activity of MOB. Also, the soil samples for molecular analyses were taken in close vicinity (max. 10-20 cm) of the soil-gas extraction rod, and the zone of soil and soil-gas sampling has reasonable overlap. Hence, we think that a strong indirect link is given and the term "activity" in the title is justified. Moreover, the reviewer's suggested alternative implies that methanogens were investigated, which was not the case.