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Biogeosciences Discussions

Interactive comment on "Non-microbial methane formation in oxic soils" *by* A. Jugold et al.

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The authors wish to thank referee 1 for his/her efforts in reviewing our manuscript and for the helpful and constructive comments provided. Below are our point by point responses to all issues raised by the referee. The manuscript has been revised accordingly.

Referee: comment 1. It is notoriously difficult to prepare sterile soil samples (see: Brock, T.D., The poisoned control in biogeochemical investigations. In: Environmental Biogeochemistry and Geomicrobiology. Volume 3: Methods, Metals and Assessment, edited by W. E. Krumbein, Ann Arbor, MI, 1978, p. 717). The process of sterilization and its efficiency is not described in the present paper, only Gamma irradiation (p.11969, L.23) is mentioned. Most of the experiments were anyway done with non-sterile samples. Inhibitors of methanogenic microorganisms (e.g., BES, chloroform)

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were not tested.

Authors: The reviewer is correct that we cannot fully rule out the possibility of some methanogenic microorganisms, although under the experimental conditions employed in this study suggest that it would be extremely low. However, encouraged by this comment, we performed an additional set of experiments to investigate the effect of two inhibitors, BES and chloromethane, of methanogenic microorganisms. We tested two samples (lignin and peat) at a temperature of 50°C. No statistically significant differences were found between the treated (with inhibitors) and untreated samples (controls) indicating that methanogens did not contribute to the observed methane formation. These results have been included and discussed in the revised manuscript (new inserted paragraph 3.3 “Influence of methanotrophic and methanogenic microorganisms on CH4 formation”). Details of the soil treatment with gamma radiation have been added to the Materials and Methods section.

Referee: comment 2. The relatively large carbon isotope fractionation (difference in δ13C of organic carbon and CH4) would be consistent with CH4 formation by methanogenic microorganisms, which exhibit fractionation in this range. Of course it is no prove for methanogenesis, but it also does not disprove it.

Authors: The stable carbon isotopes signatures of methane released from aerobic soils in our study cannot be used to distinguish between microbial and non-microbial sources. However, we think it is most important to present these data because they suggest that non-microbial methane sources can show carbon isotope fractionations that are in the same range as those found for microbial sources. Interestingly this has already also been shown for plants and also for extraterrestrial matter (photocatalytical methane formation from meteorites).

Referee: comment 3. The exponential increase with temperature might be an unambiguous indication for a chemical process, since biological reactions generally exhibit a temperature optimum. Unfortunately, however, methanogenic microorganisms (e.g.,

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Methanopyrus) do exist that have a temperature optimum above 90°C, so that a temperature range up to 90°C is not sufficient to prove the absence of activity of such hyperthermophilic methanogenic microbes. I personally think that it is quite unlikely that such hyperthermophilic methanogens were present in the soil and peat samples (so far they have never been demonstrated in such samples), but we should be aware that more than 99% of the microorganisms in the environment still await discovery.

Authors: We agree with the referee.

Referee: comment 4. There is recent literature demonstrating the presence of methanogenic microorganisms in oxic soils, even in desert soils (Angel et al., ISME J. 6, 2012, 847). Therefore, drying-wetting cycles are also not a strict prove for the absence of microbial activity. In fact, some of the methanogenic microorganisms have been recognized as being amazingly recalcitrant against desiccation and aeration stress, and even express hydrogen peroxide-destroying enzymes (Angel et al., PloS ONE 6, e20453, doi:10.1371/journal.pone0020453, 2011).

Authors: In our original manuscript we did not intend to use the results of the drying-wetting cycles to prove the absence of microbial activity. The drying-wetting cycles show large differences of methane emissions between dry and wet soils. Furthermore, no decline in methane release was observed when samples were dried and rewetted. This is certainly no proof for the absence of microbial activity. However, in the light of the recently conducted inhibition experiments (please refer to response to comment 1 above) and the enrichment culture experiment (please refer to comment below, answer to comment 5) we can exclude methanogens as the source for the observed methane emissions.

Referee: comment 5. The paper lacks any microbiological approach. The efficiency of Gamma irradiation was not tested (perhaps it was, but not mentioned). Demonstration of the absence of microbial methanogenic activity or absence of appropriate genetic material was not attempted, although this would have been relatively easy. One could

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test for the absence or presence of genes encoding methyl coenzyme M reductase (mcrA), an enzyme specific for methanogenic microorganisms. It would even be possible to test for expression of such genes. Demonstration of absence of mcrA would render more credibility to the experiments on effects of drying, UV, temperature. The likelihood is large that mcrA genes were indeed absent, but this concern should at least be discussed on the basis of literature data.

Authors: We conducted a set of new experiments using inhibitors of methanogenesis (please see response to comment 1 above). These results have been included and discussed in the revised manuscript. To further prove the absence of microbial methanogenic activity we performed another experiment. Peat or lignin was added to an enrichment culture known to enhance the growth of methanogenic archaea. When samples with or without enrichment culture were compared no difference in CH4 formation was measured after an incubation period of 4 days at a temperature of 25°C. No further increase in CH4 formation was measured when samples were incubated for a longer time period. Both experiments (inhibitors and enrichment culture) strongly support the absence of microbial methanogenic activity in the investigated soils and that in our experiments methane formation was solely driven by a chemical process. Thus, we have not conducted further experiments to test for the absence of appropriate genetic material.

Referee: comment 6. Nature Communications (3:1046, doi:10.1038/ncomms2049, 2012) just published another paper from the Keppler-group in which they show that saprophytic fungi can produce small amounts of CH4 from methionine as precursor. Since this paper is now published, it should also be discussed in the present paper. Important is the context of which processes are eventually more important for CH4 production in aerated soils, the presumable abiotic reactions, the saprophytic fungi, or anoxic micropockets with canonical methanogens such as in biological soil crusts.

Authors: According to the referee's suggestions a new paragraph has been inserted in the outlook section of the revised manuscript. It reads as follows: " The chemical CH4

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formation from organic soil components observed in this study might be only one of several CH4 formation pathways that occur in aerated soils. Further sources involve the degradation of organic matter by saprophytic fungi (Lenhart et al. 2012), methanogenic archaea in anoxic microsites (Kammann et al. 2009), and biological soil crusts (Angel et al. 2011). However, presently our knowledge on the (bio)chemical CH4 formation processes behind all identified sources are limited, therefore it is much too early to speculate about the contribution of the various sources to the release of CH4 to the atmosphere. The amount emitted by various sources released to the atmosphere will be affected to a different extent by chemical, physical and biochemical environmental factors like UV radiation, temperature and moisture. For example, soil moisture will not only affect the CH4 release from chemical degradation of organic soil compounds and from fungi but will also affect oxygen concentration and therefore anoxic microsites where methanogenesis takes place. Thus, it will be a challenge to differentiate between the microbial and non-microbial sources of oxic soils in the field."

Interactive comment on Biogeosciences Discuss., 9, 11961, 2012.