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Biogeosciences Discussions

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

Anonymous Referee #1

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The paper is a continuation of the work by Hurkuck et al. (Chemosphere 86, 2012, 684), which was recently published by the Keppler-group. Now, they incubated soil and peat samples under oxic conditions at different temperatures, after drying-wetting cycles, in the presence of different concentrations of hydrogen peroxide, and under irradiation with UV light. Under all these conditions, CH4 production was observed at low rates. Methane production was also observed in peat after Gamma-irradiation, which presumably sterilized the material. The authors conclude that this is evidence for non-microbial methane formation, which has so far not been shown for soil samples. In the Introduction, they present a comprehensive overview of similar CH4 production from plant material.

The experiments are well done and the data are, as I believe, robust. Personally, I got quite convinced that there is indeed a potential for abiotic CH4 production in soil and peat. However, much of the conclusion is based on comparison with CH4

production from plant material or from compounds such as lignin or pectin. Such CH4 production can of course only be due to chemical reactions. However, the analogy is not necessarily a proof for the same reactions occurring in soil, which is much more complex than plant material or chemical compounds. Therefore, the issue is not yet unambiguously proven. Nevertheless, I think that the research of the present paper is an important step forward, in particular if the Discussion is addressing the concerns mentioned below.

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) were not tested.

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.

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., 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.

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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.pone. 0020453, 2011).

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 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.

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.

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