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Biogeosciences Discussions

Interactive comment on "Anaerobic oxidation of methane in grassland soils used for cattle husbandry" *by* A. Bannert et al.

Anonymous Referee #2

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General comments

The paper "Anaerobic oxidation of methane in grassland soils used for cattle husbandry" reports interesting laboratory data showing methane oxidation in an agricultural soil under anaerobic conditions. Moreover, by using 13CH4 the authors are able to trace the carbon into PLFA and make some interpretation on methanotrophic sources. In concert with geochemical data and calculations, it appears plausible that methane oxidation was nitrate-dependent, which – to the best of my knowledge – is the first good evidence for the occurrence of this process in soils. Studying methanotrophic processes, in general, is a hot topic and suitable for Biogeosciences. The paper is generally well written, includes interesting data and – except for some points mentioned below – conclusions are supported by the data. Before publication in Biogeosciences,

however, I have one main and few specific points, which should be addressed in the revised version (minor revision).

My main concern relates to the chemotaxonomic interpretation of PLFA, particularly of 16:1ω7. 16:1ω7 (syn. 16:1Δ9) is in fact a ubiquitous FA in microbial systems and can not serve as biomarker strictu sensu. Anyway, despite the fact that some of the conclusions made by Raghoebarsing et al. (2006) were wrong (meaning the co-existence between archaea and bacteria), the result, which they gained from their 13CH4 labelling experiment, can be used also for the interpretation of the data presented here. Although 16:1ω7 is very common, it was also observed as most enriched FA after 13CH4 addition by Raghoebarsing et al (similarities hold also for n14:0, 18:1ω7 etc.). This can be used as a support for nitrate/nitrite based AOM and the very weak line of evidence favoring growth of aerobic MO should be omitted (as other marker PLFA of type I and type II methanotrophs – e.g. 16:1ω8 and 18:1ω8 - are obviously lacking). The following paragraphs should be modified according to my concern.

4921, line 14 ff.: to draw a relationship between aerobic and anaerobic methanotrophs from the 13C-incorporation into the unspecific 16:1ω7 is not correct. An uptake into an unspecific PLFA, which, however, in concert with the decrease in nitrate and the publication of Raghoebarsing et al. 2006 can be read as indication for the anaerobic oxidation of methane by relatives of candidatus Methylomirabilis oxyfera.

Page 4932, line 15 ff. This paragraph must be rewritten according to the general remark mentioned above. As described, particularly the 16:1ω7 is ubiquitous and has, strictly spoken, no biomarker value. I would here rather use the absence of significant uptakes into 16:1ω8 or 18:1ω8 (e.g. Bowman et al., 1991) as indication that common aerobic methanotrophs of the type I and II cluster are unlikely to explain the observed uptake pattern. In the end the data of Raghoebarsing et al. 2006 can be carefully used that they are mostly in line with the high uptakes observed here.

4934, line 4 ff: this paragraph should be rewritten according to the comment above.

Table 2: I know that comparable lists are commonly used in respective publications, but think they are not helpful and often, here as well, biased. There are indeed several PLFA with biomarker value, but others lack a biosignature function. For instance, the list for 16:1ω7 sources includes aerobes, anaerobes, type I methanotrophs, Methylococcus (which is in fact a type I methanotroph), etc. This is not helpful information and the authors should seriously take into account omitting this biased selection and the Table at all.

My second point is more a comment, perhaps to be considered in future experiments. Denitrification rates were obviously similar independent of the addition of methane. In fact, this does not exclude that nitrate-based AOM was occurring, but it calls for a careful interpretation in terms of other explanations and/or modifications of the experimental protocol. One option would be to also analyse nitrate (and nitrite) concentrations obtained during the experiments. The other would be to integrate genetic work or stable isotope probing using RNA-SIP to find other indications for nitrate-dependent AOM in soils.

Specific comments

To complete the still short list of publications dealing with the anaerobic oxidation of methane, the authors should also add Rasigraf et al. (2012) and include the main result in the introduction.

A recent and non-cited publication deals with changes in microbial compositions as response of cattle husbandry (Elhottova et al. 2012; Applied Soil Ecology). Are these data related to the study here? If yes, it should be discussed in the light of the findings of the current study.

One recommendation for future experiments is to also calculate uptake rates (as concentrations were also analysed). If just taking $\Delta\delta$13C-values, changes in the microbial community composition resulting in changing PLFA abundances are not sufficiently expressed. In other words, a compound with low concentration, where the source or-

ganism is triggered by the substrate addition will show a high change in δ13C-values, while real uptakes can be in fact low. On the other hand, a compound with high abundances and low turnover time, will show only slight changes in δ13C although 'real' uptakes can be in fact high (expressed as compound specific e.g. (13)C uptake in ng g-1 soil (dw)).

4922, line 5: change "methanogenic" to "methanotrophic"

4926, line 23: I tried to understand the definition for "unsaponifiable non-ester linked fatty acids", but it remained unclear to me. Is it a plasmalogen or a free fatty acid? Please clarify if possible

4927, line 4 ff: Please clarify. Double bond positions can not be identified by EI mass spectra (if DMDS- or other derivatives were not used; see Buser et al. 1983). Did the authors perform co-elution experiments using commercially available standards (e.g. Supelco FAME 37, or BAME)?

4930, line 11ff. How can methanogenesis and methanotrophy be separated? With the data shown, it allows to characterise methanotrophy in the soil.

Line 4931, line 2: The detection limit for sulphate is different here to that given in the result section. Please correct where necessary.

4932, line 13: The authors should remain at discussing PLFA, which also demonstrated 13C-enrichments and not only increases (the latter is not easy to be explained if only methane addition is the difference between both approaches). This relates for instance to the 22 and 24 FAs, which were not enriched (Fig. 4) and there is therefore no need to discuss them. Even if the source was autotrophic and use the CO2 they should be enriched through cross-feeding (from 13C-enriched CO2 from methanotrophy). Can heterogeneities really excluded (also to explain the concentration increase in 18:1ω9)? Is biomass increase in that amount feasible (considering the often low doubling times of anaerob methanotrophs; e.g. for NC10 Ettwig et al. 2010 described it with 1-2 weeks)?

4932, line 16-18: What is the difference between "type I and II methanotrophs“ and "methanotrophic bacteria“? Both terms are used as synonyms with different specificity!? Anyway, the authors should modify this paragraph according to my comment above.

4932, line 23: I can’t find information about the length of hydroxy-FA in archaea (in Gattinger et al., 2002) and considering the lack of 13C-uptake the statement should be deleted here. To test for archaeal growth the authors should consider changing their protocol, which also allows for analysing GC-amenable archaeal lipids (e.g. archaeol).

4933, paragraph starting at line 19 better should be omitted, as this is very speculative. Or the authors should add a reference demonstrating metabolism without substrate uptake (or that of the resulting 13C-enriched CO2).

4934, line 1 and 3: exchange “nutrient” with “carbon”

Figure 4: Just a comment. The high $\Delta\delta$13C-values for NEL-ubr16:0 are (perhaps) an example for a situation I have described above. Concentrations appear to be low (Fig. 3) and thus a push in the growth of an originally minor abundant source organism would have shifted the δ13C considerably, although the uptake in 13C-carbon ng g-1 soil (dw) could have been low!?

Figure 3: If possible, data points should be slightly shifted from the fatty acid names (they appear to overlap). Some of the names look also odd.

Figure 4: Please avoid showing two decimal places for data (one is sufficient), which were obtained with an accuracy not higher than 0.2 (which is the case for δ13C analyses).

References Bowman, J. P., Skerratt, J. H., Nichols, P. D., and Sly, L. I., 1991. Phospholipid fatty acid and lipopolysaccharide fatty acid signature lipids in methane-utilizing bacteria. FEMS Microbiol. Ecol. 85, 15-22.

Buser, H. R., Arn, H., Guerin, P., and Rauscher, S., 1983. Determination of double

bond position in mono-unsaturated acetates by mass spectrometry of dimethyl disulfides adducts. Anal. Chem. 55, 818-822.

Rasigraf, O., Vogt, C., Richnow, H.-H., Jetten, M. S. M., and Ettwig, K. F., 2012. Carbon and hydrogen isotope fractionation during nitrite-dependent anaerobic methane oxidation by Methylomirabilis oxyfera. Geochimica et Cosmochimica Acta 89, 256-264.

Interactive comment on Biogeosciences Discuss., 9, 4919, 2012.