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Biogeosciences Discussions

Interactive comment on "Wetland restoration and methanogenesis: the activity of microbial populations and competition for substrates at different temperatures" *by* V. Jerman et al.

Anonymous Referee #2

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This study investigated the potential physiological capabilities and limitations of methanogenic and Fe(III) reducing activities in a drained peatland intended to be re-flooded. Methane formation in anoxic incubations started after a prolonged lag phase and emission of methane, due to methanotrophic activity, is expected to be negligible. The authors suggests that methanogenesis was limited by competition with microbial Fe(III) reduction for substrates like acetate or H2.

The manuscript could be strengthened if clarification of material and methods, and discussion of the broader implications of the results is provided.

I have the following general concerns that should be addressed before this paper would

fit well into Biogeosciences:

(1) The paper is in part a bit sloppy, i.e., missing parenthesis at citations in the text, inconsistent figure layout concerning the order of accession numbers and species name (Fig. 7 vs. Fig. 6 and 8), the obtained accession numbers should be included in the trees, although they are given in the material and methods section, the figure order has to be changed (Fig. 5 is mentioned after Fig. 6).

(2) The length, particularly of the site description and site history in the introduction and methods section, needs to be shortened and combined. As your samples were obtained at just one sampling site and your investigations focused on process studies, a detailed explanation of the Ljubljana marsh area is redundant.

(3) As the main focus in the introduction section is on methanogenesis, the competition with Fe(III) reducers for substrates has to be introduced in more detail and supporting literature would be helpful. In addition, there are suggestions that methanogens may transfer electrons to Fe(III).

(4) A brief introduction of Fe(III) reducing processes in hydrological unstable environments would support your story. It is not clearly stated in the introduction why Geobacteraceae were investigated as a representative group of Fe(III) reducing microorganisms.

(5) Please provide more information about GC and HPLC analysis (column material; especially for measurement of organic acids and alcohols).

(6) I have concerns with your general conclusion that the formation of CH4 was limited by competition for substrates, mainly for acetate (2372:20-21). From your accumulation data obtained 115 days after incubation (Fig. 2) it can not be stated that Fe(III) reduction and methanogenesis are competing processes. I agree that the correlation for acetate and CH4 is quite as good. However, concomitant accumulation of acetate and Fe(II) at around 15°C suggesting a) no potential substrate limitation for methano-

genes and b) low acetate consumption of Fe(III) reducers. In addition, the pool of microbially available Fe(III) can be reduced within 70 days at 25°C (Fig. 4). Thus, accumulation data of redox sensitive processes and metabolic intermediates obtained after an incubation period of 115 days could be misinterpreted. Data for acetate during your incubations presented in parts in Fig. 4 could be strengthening the suggestion for competing processes. In addition, incubation experiments with additional acetate or H2/CO2 could also support this conclusion. Right now, I would suggest weakening your statement or add supportive data.

(7) The authors suggest that a fluctuating water table would enhance the redox cycling of iron for this peatland and decrease potential emission of CH4 (2372:20-2373:2). I agree with this statement, but I think it would be worthwhile to consider the implications of the findings beyond this study site. Would these results apply to all peatlands of the same type that are intended to be reflooded? As methanogenesis was restricted to temperatures above 15°C do you expect any methanogenic activity in-situ (or maybe just in the summer season)? Do you think that changing redox conditions would enhance the mineralization and emission of carbon for this peatland?

Some specific suggestions for improvement are given below (usually by page: line):

2358:11 How long was the lag period? In general, values or ranges for lag periods should be mentioned in the manuscript.

2358:18 I like that the abstract is succinct but a link from methanogens to Geobacteraceae as a representative group of Fe(III) reducers would be helpful.

2359:1-2 Since you focused on a peatland and the potential release of methane you should mention the important function of peatlands to store carbon than the ability to store and clean water.

2359:27 Add the temperature optimum for mineralization.

2361:22 Add the range of these fluctuations.

2361:24 How were the cores pooled? Were all of the ten cores pooled together or just the individual cores? Be more specific.

2362:5 Did you measure the moisture content of your soil incubations during the first incubation period of 65 days? Was it still at 90% water holding capacity although you used open microcosms? Add a comment.

2362:9-12 When did you add the difluormethane? Did you add this inhibitor to all incubations at every sampling or just at the end of incubation? Add this information to your material and methods section.

2362:13 What anaerobic processes are you referring to? Be more specific.

2362:13-15 The writing needs to be improved! Separate both experiments to be more specific. Right now, it is difficult to follow your instructions.

2362:21 Add temperature steps (2.5°C?).

2362:22-24 Replace “after the tubes were opened” with “at the end of the experiment”.

2362:25 Add “potential” methanogenic activity/pathways, because incubations temperatures were not representative for field conditions.

2363:3 Replace the first sentence.

2363:3-9 Add more details, i.e. detection limit for gases (concerning 2362:7), column material. Add a definition for “low” concentrations?

2363:12 Again, add more details (HPLC, GC).

2364:8 Add a definition of labile and recalcitrant organic fractions in this case (extractable, thermal, microbial degradable)?

2365:3-5 Add primer names.

2365:26 Is it important to give the number (two) of the DGGE gels? It’s confusing because you never mentioned different DGGE gels. Which bands from which incubation

temperature did you excise? Be more specific.

2366:18 Always add ranges or values for the lag phases.

2366:27 – 2367:8 Did you consider that at higher temperatures (up to 80°C) volatile carbon compounds, i.e. alcohols and fatty acids, as a part of the labile fraction may disappear abiotically from the soil?

2367:3 There are no half-life data calculated from methanogenesis in Fig. 3a!

2367:9 Are the data for H2, alcohols, fatty acids, ammonia and Fe(II) also caused by non-linear increase and therefore given as accumulation during the experiment? For easier comparison, change units for acetate and H2 to μmol per g dry wt.

2367:10 There is just one visible temperature optimum at 65°C.

2367:15 Add the temperature optimum.

2367:16 In Figure 2d the rate of potential Fe(II) accumulation is not decreasing at 15°C. "No further increase" would be more precise. Furthermore, total content of iron data would be helpful for comparison of Fe(II) accumulation.

2368:2-3 Add real data for "high levels" of H2, alcohols and fatty acids. However, H2 and acetate had their highest concentrations at 70°C (Fig. 2)!

2368:6 As a real plateau phase for Fe(II) concentrations is not shown in Figure 4, amount of data points are not sufficient enough to compare "maximum" Fe(II) concentrations with total iron content. Add concentrations for total iron.

2368:7 The method for the determination of chemical reducible iron or total iron is missing in the M&M section.

2368:10-11 Add results for methanogenesis at 47 and 60°C even there was also no activity.

2368:22 Unit of T-RFs is missing.

2368:23-24 Be consistent (see value for PCR products at 2368 15-16).

2368:27-2369:2 The interpretation for the T-RF 185bp fragment should be combined in one sentence.

2369:3 What is the optimal temperature you are referring to? Did you mean the optimum temperature for acetoclastical methanogenesis or methanogenesis in general? Which methanogenic slurry did you use for this analysis (incubation temperature and time)?

2369:6 Since not all PCR products belonging to Geobacteraceae (in comparison with 2369:13-15), be careful using "Geobacteraceae-specific PCR".

2370:13 As far as I know, some Archaea are also able to reduce Fe(III). Use a more general term instead "bacteria".

2371:6 Change "onset of iron reduction and methanogenesis" to "ongoing iron reduction and onset of methanogenesis".

2371:9 Which sequences are you referring to? Be more precise!

Table 1 For better comparison, add corresponding sequence similarities and names of clones, representative for the groups or bands at the specific incubation temperature.

Phylogenetic trees: Add bootstrap values for the trees and mention the number of replicates for the phylogentic analyses.

Interactive comment on Biogeosciences Discuss., 6, 2357, 2009.