Biogeosciences Discuss.,
doi:10.5194/bg-2016-162-AC1, 2016

Biogeosciences Discussions
Open Access
EGU

Interactive comment on "Microtopography matters for CH_4 formation in a peat soil: a combined inhibitor and ^{13}C study" *by* Johannes Krohn et al.

Johannes Krohn et al.

Johannes.Krohn@gmx.de

Received and published: 14 July 2016

We acknowledge the valuable comments of the Referee #1. We considered all points mentioned by him/her as outlined below and hope that we addressed them adequately.

General comments:

"Unfortunately, mostly due to the selected inhibition method and other shortcomings, the data presented here do not allow any conclusions on this question. Some of the isotope data point to a difference in pathways between hollows and hummocks (Fig.4), but this difference could actually represent the depth variation of the pathways. No information is given on water table position in the hummocks, but because hummocks are elevated, the sampled depths in hummocks will be higher in respect to the water level than in hollows. Therefore it is problematic to compare the depth of 15 cm in

Printer-friendly version

Discussion paper

hollows (water-logged, optimal depth for CH4 production) to depth 15 cm in hummocks (most likely above water level, aerobic and not the optimal depth for CH4 production). It seems in the hummocks the depth of 50 cm is closer to the depth of highest CH4 production (Fig. 1a) and thus more appropriate for comparison with 15 cm of hollows."

- We understand the concern of the Referee and see the shortcomings of our study which were considered and acknowledged in the text (lines 146-147; 194-197; 288-290). However, the idea of using the BES at a certain concentrations to partition methanogenesis pathways was addressed to the fact that studies on similar topic have been done only with pure microbial cultures. As a result, we demonstrated that, unfortunately, it is very difficult (if at all possible) to achieve a comparable result on complex natural objects, namely peat soil in our case. Thus, the mentioned "lack of any conclusion" is additional evidence on the existing differences between "artificial" (microbial cultures) and "natural" objects which have to be considered in other studies. Regarding the depth positions of peatland microforms, we agree with the Referee that in situ environmental conditions are strongly variable in both microform types, at least in the topsoil (15 to 50 cm in the studied peatland). Indeed, water table position in hummocks was on average 15 to 20 cm below the surface as compared to hollows (this information will be added to the text, see *1). Actually, these features of microforms were key points while proposing hypotheses (lines 93-98) and we planned to conduct relative differences between microforms with depth. Secondly, we kept uniform conditions during the incubation experiments for all peat samples, thus we revealed the in situ inherited properties under laboratory conditions as well.

*1 The surface of the sampling sites was subdivided into three main microforms according to the topography and water table level: 1) elevated dry hummocks with an average water table between 15 to 20 cm below the peat surface , 2) intermediate lawns with an average water table from 5 to 15 cm below the peat surface and 3) depressed wet hollows with an average water table between 0 and 5 cm above the peat surface (Becker et al., 2008), whereby the two contrasting microform types – hummocks and hollows –

Printer-friendly version

Discussion paper

were tested in this study.

“The conclusions that are well-supported by the data concern differences in CH4 and CO2 production and pathway of methanogenesis with depth in the peat profile. However, decrease of production and increased contribution of hydrogenotrophic methanogenesis with peat depth are well-described already by several studies. Methane production with peat depth and between microforms has even been reported in the same peatland (Saarnio et al. 1997 cited in the manuscript).”

- Referee is fully correct when saying the pattern of methanogenesis type with peat depth was previously reported even for the same peatland. However, we have to stress that our study included the whole peat profile down to the mineral soil horizon in ca. 200 cm depth, whereas the study of Saarnio et al. 1997 just considered a peat profile of the top 100 cm. Moreover, although we did not aim initially to test effect of time on our results on methanogenic pathways for the studied site, it appeared the main patterns of methane production discovered ca. 20 years ago (e.g. Saarnio et al., 1997) are still actual. This stresses the conservative conditions on site or the changes occur too slow to detect them by now.

“There are several concerns about the inhibition method used. It is difficult to consider the addition of 1 mM BES as a justified approach to study the contribution of methanogenetic pathways. The use of this low concentration to inhibit acetoclastic methanogenesis is based on a single study on digestor sludge (Zinder et al. 1984), where the authors warn that the results should not be generalized to other systems or to longer incubations times (as in this study). Very variable and contradicting BES concentrations have been shown to inhibit all methanogenesis. The authors conclude that the method was not specific for acetoclastic methanogenesis, but this could already have been anticipated based on literature, and it could have been obvious from the preliminary tests with different concentrations (l. 128-129).”

- We completely agree with the Referee that the effective concentration of BES cannot

be based solely on one reported concentration of a study (e.g., Zinder et al. 1984). It has rather to be tested for every incubation experiment. Therefore, we tested several concentrations of BES (1 mM, 10 mM, 100 mM) on samples of the same soil. The suppression of CH4 formation with 1 mM of BES was comparatively effective as by 10 and 100 mM (Fig. 1). Thus, the lowest BES concentration was chosen in the main experiment. We regret that the overall suppressed CH4 production was too high to quantitatively measure δ13C with our set-up to support or reject proposed hypothesis, but it does not ultimately mean the partitioning was false. We believe the results could help other researchers to design their experiments.

“BES was also added in a small volume (2 ml) to solid peat, not a slurry - did it spread throughout the peat? Considering this issue, the level of inhibition is actually very surprising.”

- The moisture content of the peat samples ranged from 88 to 96 % of the total peat weight (close to saturation) and therefore, we did not want to oversaturate the samples. With this high moisture content we assumed the added 2 ml solution spread through the whole sample. The most important, the solution was dropped evenly over the surface of microcosms, so the achieved effect demonstrated the approach was effective enough.

“An additional problem is that the controls appear to have not been done in triplicate like the treatment (l. 132-33, no error bars in Fig. 2). “

- We thank the Referee for the critical comment, and can explain the lack triplicate in controls with our experimental set-up. Before addition of BES, all our microcosms were controls, where we measured initial CH4 production potentials (lines 118-125). We randomly have chosen BES treatments and the rest was continuously measured as controls. This made us possible to compare all treatments thereafter.

“There is also a more commonly used inhibitor for acetoclastic methanogenesis (methyl fluoride) available.”

Printer-friendly version

Discussion paper

- Indeed, CH3F is an effective inhibitor of methanogenesis, but we would like to mention, that the compound is a gas. Thus, we were not sure that with such a high moisture content of the peat soils, the gases diffusion would be good enough. Furthermore, the admixture of CH3F would affect δ13C-CH4 analyses and should be used in accordance with the set-up of an experiment. Anyhow, we are thankful for the information and will consider it in the future experiments (without C isotope applications).

"It is difficult to see how the data on N, S, and Fe (Fig. 5, Fig. 6) contribute to the main hypotheses. Were the correlations with CH4 production expected to vary between hummock and hollow? Moreover, the data on Fe and S Fig. 6 are not mentioned at all in the methods or the results."

- It is widely reported that denitrification (Schlesinger and Bernhart, 2013; Rubol et al., 2012; and references therein), sulfate reduction (Lovley and Klug, 1983; Pester et al., 2012) and iron reduction (Lovley et al., 1996; Cervantes et al., 2002) are energetically more efficient processes which outcompete methanogenesis. Unfortunately, it was not possible to measure the mentioned processes concurrently during the experiment, but we attempted to link CH4 data with the chemical composition of peat extracts (N, NH4+) and solids (S, Fe) to explain the observed differences between microforms and depths. We did not expect initially any substantial differences in chemical composition of peat from hummocks and lawns. Information about the data on Fe and S (Fig. 6) will be added to the Materials and Methods (see *2) and Results (see *3) sections.

*2 For the total sulfur (S) and iron (Fe) measurement, peat samples were dried (60 degree Celsius, 2-3 days) and grinded to fine powder by a Fritsch Pulverisette (type 00.502, Oberstein, Germany) equipped with an agate pocket and ball mill. Total S content was then determined with an ICP spectrophotometer (iCAP 6000 series, ASX-520 AutoSampler, Thermo Scientific,USA) after digestion of the samples in a mixture of nitric and hydrochloric acid (2:1 v:v) by a Digestore Milestone MLS 1200 (Microwave Laboratory System, Sorisole BG, Italy).

Printer-friendly version

Discussion paper

*3 S concentration for hollows were 0.41 ± 0.02 (mean ± SE) mg g d.w.-1 in the top and 1.57 ± 0.01 mg g d.w.-1 in the deepest soil layer, whereas for hummock it was 0.45 ± 0.04 and 1.43 ± 0.005 mg g d.w.-1, respectively. Fe concentration ranged from 0.31 ± 0.05 to 4.16 ± 0.12 mg g d.w.-1 in hollows and 0.84 ± 0.12 to 3.16 ± 0.05 mg g d.w.-1 in hummocks from the top to the deepest soil layer, respectively.

"It is true that there is quite little information on microform effects on CH4 and CO2 production (lines 55-56), but the studies that have already addressed this question should be mentioned here or in the discussion (for example Bubier et al. 1993, Saarnio et al. 1997 cited in the manuscript)."

- We are very thankful to the Referee and will include the studies of Forbrich et al., 2010 and Aleina et al. 2016 to the text (see *4).

*4 Forbrich, I., Kutzbach, L., Hormann, A., Wilmking, M.: A comparison of linear and exponential regression for estimating diffusive CH4 fluxes by closed-chambers in peatlands, Soil Biology & Biochemistry, 42, 507-515, 2010.

Aleina, F., C., Runkle, B., R., K., Brücher, T., Kleinen, T., Brovkin, V.: Upscaling methane emission hotspots in boreal peatlands, Geoscientific Model Development, 9, 915-926, 2016.

Specific comments:

"1. lines 71-72 What do you mean with 'competitive effects between methanotrophs and methanogens for electron donors'? Aerobic methanotrophs and anaerobic methanogens do not compete for the same electron donors."

- We fully agree and apologize for inconvenience, we meant "methanogens" and "other microorganisms" (responsible for denitrification, sulfate and iron reduction/oxidation, etc.).

"2. l. 75 Methyl-coenzyme M is required for methanogenesis, not responsible for methanogenesis."

- We agree and correct it.

“3. l. 230 Do you mean CO2 emission rather than production?”

- We would like to differentiate both terms: “emission” in our understanding is mainly the flux of CO2 measured in the field, whereas CO2 evolved in microcosms during incubation under controlled and predominately favorable conditions (at least in terms of temperature and moisture parameters) is the “production potential”. We will change this definition as suggested from the Referee to CO2 emission.

“4. l. 236-240 This reasoning is difficult to follow. How does possible higher occurrence of aerobic and facultative microbes in hummocks than in hollows lead to similar CO2 production rates under anoxic conditions?”

- Non-significant differences in CO2 production between hummocks and hollows did not support our hypothesized pattern, due to, indeed, expected differences in microbial communities between microforms. Interestingly, incubation under aerobic conditions revealed similar (no difference between microforms) CO2 production results. Both findings contradict to in situ CO2 fluxes with higher rates from hummocks as compared to hollows (e.g. Becker et al., 2009). Since we did not measure microbial community structure in this experiment, we can’t conclude about dominance of any aerobic/anaerobic/facultative groups of microorganisms in studied microforms. Probably, even in case of distinct community structure, its adaptation mechanisms to contrasting conditions (occurring under natural conditions) did not allow to distinguish between CO2 production from hollows and hummocks neither with nor without oxygen availability. Pronounced differences observed under field conditions may reflect contribution of root respiration or any other effect of vegetation. This possible explanation was mentioned in the text (lines 230-232).

“5. l. 256-258 What support is there for the statement that single plant species directly contribute to methane production? Please rephrase and also consider that in addition to the plant community, peat quality can differ between hollows and hummocks due to

different exposure of organic matter to aerobic decomposition."

- The study of Dorodnikov et al. (2011) reported higher contribution of recent plant photosynthates (based on 14C incorporation) to methanogenesis from hollows-dominating Scheuchzeria palustris as compared with Eriophorum vaginatum typical for hummocks and lawns (lines 256-258). We agree that the peat quality can differ between hollows and hummocks among other factors also due to different exposure of organic matter to aerobic decomposition. This information will be added to the text (see *5).

*5 The overall higher CH4 production from hollows vs. hummocks (Fig. 1a) depends on SOM quality, which in turn is affected by aboveground plant communities and by the period of aeration controlling organic matter decomposition.

"6. l. 271 Please rephrase - there is no data on higher active biomass of methanogens in hollows than in hummocks."

- The sentence will be rephrased (see *6).

*6 Moreover, Yavitt and Seidman-Zager (2006) suggested a greater frequency and duration of anaerobic conditions to be responsible for a larger active biomass of methanogens in hollows than in hummocks. Future analyses of the microbial community structure of hollows and hummocks would therefore be useful to understand the CH4 production patterns.

"7. l. 320 The low CH4 production may simply indicate very low numbers of methanogens and low substrate availability in the highly decomposed deep peat layers"

- Yes, we completely agree with the Referee, that the lowest CH4 production potential in the deepest peat horizons was most probably related to the quality of OM in the highly decomposed peat soil. However, we could not conclude about the abundance (numbers) of methanogens as we did not measure this parameter.

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016.

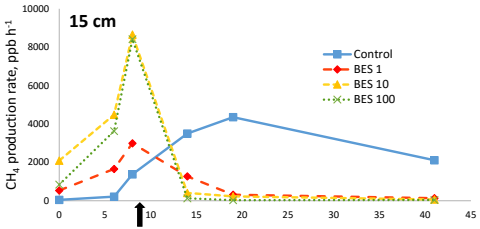

Fig. 1. CH_4 production rates (ppb $hour^{-1}$) at 15 cm depth of the microform type hollow before and after the addition of specific inhibitor BES at increasing concentrations (1, 10, 100 mM). Black arrow: date of BES addition. The same BES concentrations were tested for the soil from 100 and 200 cm depths but the CH_4 production was too low to be measured accurately.

Fig. 1.

Printer-friendly version

Discussion paper