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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.

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We would like to thank the Referee for his/her valuable and critical comments. We considered all points mentioned by him/her as outlined below and hope that we addressed them adequately.

General comments:

"The influence of microtopography on GHG production and emission has been well investigated by numerous studies. It is well known that both GHG production and the dominance of acetoclastic methanogenesis would decrease with peat depth. The authors need to place this study in proper context and point out the novel findings in light of the previous work done."

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-Referee is right when saying the influence of microtopography on GHG production and emission has been well investigated by numerous studies (even for the same peatland). However, the hypothesis on the decrease of the contribution of acetoclastic methanogenesis with peat depth has never been tested under laboratory conditions for the studied peatland (Salmisuo). A conclusion about increased contribution of hydrogenotrophic (CO2-reduction) pathway of methanogenesis with depth was done mostly based on in situ δ13C-CH4 measurements (e.g. Dorodnikov et al., 2013). Moreover, contrasting results were reported on the distribution of methanogenic microbial communities with the peat depth at the same experimental site (Galand et al., 2002). Thus, the main microbial groups present in the upper layer (study defined 10-40 cm) were most related to the hydrogen utilizing methanogens – Methanomicrobiales (i.e hydrogenotrophic pathway), whereas with depth (up to studied 100 cm), the dominant groups were most related to Methanosarcinale, which can perform both methanogenesis pathways. Therefore, the stated hypothesis on change of methanogenesis type with peat depth aimed to answer two questions (i) are the field and laboratory studies yield similar conclusions, (2) do the molecular analyses on microbial community structure (Galand et al., 2002) or pure cultures (BES study of Whiticar et al., 1986) fully represent the microbial ecology? Furthermore, as we already explained to the Referee #1, our study included the whole peat profile down to 200 cm depth (e.g. Saarnio et al. 1997 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 slowly to detect them by now. Other reasonings can be found in the response to the comments of Referee #1 (see page C3).

“The methodology used in this study appears to have a number of flaws, which would adversely affect the reliability of data collected and validity of the findings generated. For example, the authors suggested that oxygen might be present in the glass jars after N2 flushing, leading to suppression of CH4 production in the controls. “

-We understand the concern of the Referee mentioned the shortcomings of our study in the text (lines 153-154; 204-209; 304-306). However, the possible presence of oxygen was not related to the N2 flushing (as stated by the Referee), rather than to the N2 bubbling of the milli-Q water which was added to the controls similarly as in BES treatment, where it served as solvent for BES (lines 195-196). We apologize for the misunderstanding and will rephrase the sentence for more clarification (see *1). Although, the addition of milli-Q water to the controls was accompanied by a decrease in CH4 production, the decrease was not as substantial as in the BES treatments (Fig 2.). Furthermore, when calculating the inhibition effect of BES, the possible effect of the milli-Q water on the decrease of CH4 production was subtracted from the overall inhibition effect (lines 167-170; Fig.3).

*1 This was probably due to trace amounts of dissolved oxygen left after the N2 – bubbling of the milli-Q water which was carried out prior to the addition.

“The soil was not flooded in the anaerobic incubation, and the addition of small volume of BES to soil samples without mixing raise concern about the degree of completion of the intended inhibition.”

-In the experiment, we used field moisture content of peats which comprised 88 to 96 % from the total weight. Therefore, there was enough water in samples through which BES as a solution was diffused. The most important, the solution was dropped evenly over the surface of microcosms, so the achieved effect demonstrated the approach was effective enough. The final estimated BES effect up to 68% as compared to the initial level (according to calculations at lines 167-170; Fig. 3) confirmed the efficiency of the method of inhibitor amendment.

“Low CH4 concentrations also led to a number of samples being failed to be analyzed for 13C with IRMS, resulting in a limited sample size.”

-It is true that the CH4 concentration was too low for the 13C analysis in some samples. However, the pattern of more depleted CH4 in 13C with depth was clearly visible and

statistically significant (Fig. 4b).

“The manuscript also suffers from a lack of some essential information on site conditions and research methodology for readers to interpret the results. Some methods were not applied consistently (e.g. sampling period), and more detailed explanations/ justifications are required. “

-We are thankful for the Referee′s comment and we will add more information about the site conditions to the text (water table position in relation to hummocks and hollows) (*see 2). Regarding the inconsistency of sampling, this was related to technical issues and analytical routine of the laboratory. However, we believe it would not affect the final conclusions derived from the study. Anyway, we mention this shortcoming in the text (lines 124-125).

*2 The surface of the sampling sites was subdivided into three main microforms in accordance with the topography, water table level and vegetation communities: 1) elevated dry hummocks with an average water table between 15 to 20 cm below the peat surface and Eriophorum vaginatum, Pinus sylvestris, Andromeda polifolia, Sphagnum fuscum as dominat plant species , 2) intermediate lawns with an average water table from 5 to 15 cm below the peat surface and Eriophorum vaginatum, Sphagnum balticum, Sphagnum papillosum as dominant plant species 3) depressed wet hollows with an average water table between 0 and 5 cm above the peat surface and dominant plant species Scheuchzeria palustris, Sphagnum balticum (Becker et al., 2008), whereby the two contrasting microform types – hummocks and hollows – were tested in this study.

“Overall, the discussion of this manuscript is not strong, and is over-speculative without strong support of ancillary data. Given this research site has been quite well studied with regards to GHG dynamics, relevant literature and/or data should be included for a more elaborate interpretation of the results.”

-We followed all recommendations of both Referees to improve the discussion of our

manuscript. Along with studies mentioned (e.g. Becker et al., 2008; Dorodnikov et al., 2013; Saarnio et al., 1997) we will incorporated the study of Galand et al. (2002) on the distribution of methanogenic microbial communities with depth on the same research site.

Specific comments:

“Title – Only CH4 formation is mentioned, but CO2 production actually constitutes a considerable portion of the manuscript. The title should be revised to better indicate the actual contents covered in the manuscript.”

-According to recommendation, we will revise the title of our manuscript to better indicate the actual contents: “Microtopography and depth matters for CO2 and CH4 formation in a peat soil: a combined inhibitor and 13C study”

“L27-28 – Please specify the difference in % contribution.”

-Unfortunately, we could not provide such a quantitative measure, as the contribution of either of pathways was assessed based on δ13C signature of CO2 and CH4 in hummocks and hollows. Therefore, we may only refer to relative difference in isotope signatures between microforms. If necessary we could add the following statement: “averaging across depths, δ13C-CH4 was ca. 40% more depleted, whereas δ13C-CO2 was ca. 15% more enriched in hollows as compared to hummocks”.

“L39 – “release” and “fluxes” are redundant. Use either “potential of releasing large CO2 and CH4: : :” or “potential of large CO2 and CH4 fluxes: : :”.”

-Corrected: “. . .but also revealed their potential of releasing large amounts of CO2 and methane (CH4) to the atmosphere...”

“L40 – Add “CO2 and CH4” between “both” and “are”.”

-Will be corrected.

“L47-55 – Include a more detailed review of previous findings on the effects of microto-

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pography on peatland GHG dynamics here."

-We appreciate the suggestion from the Referee and we will extend the text passage at line 54 about the effects of microtopography on peatland GHG dynamics (see *3).

*3 Most studies so far focused on aboveground GHG flux measurements to the atmosphere as related to the types of microforms (Bubier et al., 1993; Waddington and Roulet, 1996; Saarnio et al., 1997; Dalva et al., 2001; Forbrich et al., 2010; Aleina et al., 2016). Studies which used closed chamber methods reported CH4 fluxes to decrease in the order hollows>lawns>hummocks and explained the higher fluxes of hollows either by higher CH4 production rates in hollows or higher CH4 oxidation rates in hummocks (Bubier et al., 1993; Waddington and Roulet, 1996; Becker et al., 2008; Forbrich et al., 2010; Dorodnikov et al., 2013). Contrary to the CH4 fluxes, CO2 respiration rates were reported to decrease in the order hummocks>lawns>hollows (Dalva et al., 2001; Becker et al., 2008).

"L48 – Do you mean "depressed hollows"?"

-Yes, we will correct the phrase accordingly.

"L60 – Give the full term of SOM before using abbreviation."

-The full term will be added to the text.

"L67 – A bit confusing to write "metals (Fe)" as Fe is just one of the many metals that exist in nature."

-The sentence will be rephrased as following: "...metals, e.g. iron (Fe)..."

"L93-94 – Replace "more wet hollows" with "wetter hollows". The deeper peat layers in both hummocks and hollows were inundated – how would that affect your hypothesis?"

-The sentence will be rephrased as suggested from the Referee. Regarding the inundated deep layers below hummocks and hollows, we expected to observe difference in a methanogenesis pathway as compared to shallower peat layers (see the hypothesis

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3), rather than total GHG production between microforms.

“L97-98 – This hypothesis needs to be revised such that it could be tested scientifically – specify which pathway would be dominant in hummocks and hollows. The authors hypothesize here that the difference will be caused by substrate quality, yet the quality of peat in the two sites has not been adequately characterized in this study.”

-The hypothesis will be rephrased as following: ”Due to higher availability of fresh plant-derived deposits in the upper vs. deeper peat layers, the contribution of acetoclastic pathway to the total methanogenesis should decrease with depth below both microform types; between the microforms, more intensive rhizodeposition in hollows should promote contribution of acetoclastic pathway, as compared to hummocks, at least in the topsoil”. Additional information will be added to line 54 of the introduction to support the hypothesis (see *4).

*4 Thus, based on radioactive labeling (14C-CO2), it was found that Scheuchzeria-dominated hollows had higher rates of transported CH4 from the peat column to the atmosphere than Eriophorum-dominated hummocks due to a higher contribution of recent plant photosynthates to methanogenesis (Dorodnikov et al., 2011).

“L106-108 – Given that the site has three main microforms, why the authors have decided to include only two microforms in this study but not all three for more comprehensive investigation? In another paper by the same group of researchers, three microforms were included in the analysis (Lozanovska et al., 2016). Moreover, some important site information (e.g. average water table, dominant plant community, etc.) associated with the two microforms should also be added for proper interpretation of results.”

-We included only two microforms due to limited time and resources during the experiment (especially due to the time consuming flux measurements). Thus, we have chosen the two most contrasting microform types – hollows and hummocks. Information about the water table level and plant communities will be added to the text (see

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*2)

“L111 – Any reasons for choosing these 5 depths for investigation? Given that the water table at hollow is rather shallow, the upper peat profile should be studied in greater resolution as this is the zone where the hydrologic regime is most distinctively different between the two microforms?”

-The Referee is completely right; the greater resolution of sampling at the upper peat profile would provide more comprehensive evidence on processes of interest. However, by the time of sampling we aimed to cover the full peat profile and were restricted by time and resources. Five depths were chosen as a compromise and we conducted sampling in a similar way for all microforms. But, sure, future studies should consider the uppermost dynamical peat layer of microforms in more detail.

“L112 – The meaning of “middle 10 cm section” is not clear. For example, for the depth of 15 cm, do the authors sample from the layer of 10-20 cm to represent this specific depth?”

-Yes, exactly, to represent 15 cm depth level, 10-20 cm peat layer was extracted. We will incorporate this information to the text

“L123-128 – Why was gas production measured by sampling over a relatively short period of _2 hours for 8 times throughout the whole study period? What was the rationale of determining production rate over such a short period, compared to determining an overall production rate over the whole study period by sampling gas periodically? Also, why were sampling frequency and duration differ between hummocks and hollows? How many jars exactly have been treated with BES, and those without as controls? Reasons should be given to support the chosen methodology.”

-We decided to measure gas production potential in our microcosms according to a standard flux measurement procedure: (1) flushing of the headspace to remove the accumulated gas from the previous measurement (then there is an establishing gradient

of concentrations, which allows proper diffusion of gas from soil into the headspace); (2) sampling of headspace gas over time to detect an increase in concentration (two hours were empirically determined as sufficient time to achieve sound CH4 increase, whereas for CO2 it could be shorter, e.g. 30 min; however, we kept uniform timing to omit undesired oxygen contamination through too often sampling); (3) calculation of gas flux as the slope of concentration increase over time. As proposed by the Referee, continuous incubation will inevitably bring to the saturation of the headspace with gas thereby changing the concertation gradients and hence adequate flux rates. Regarding the duration of measurements for hollows and hummocks, please, refer to our response to general comments above. We treated three replicates with BES and two as controls. Before addition of BES, all our microcosms were controls, where we measured initial CH4 production potentials (lines 118-125). We have randomly chosen BES treatments and the rest was continuously measured as controls.

"L130-132 – What exactly was meant by "comparatively effective"? Give more concrete figures. Why the BES concentration was chosen to be 1 mM? A higher concentration might ensure a more complete inhibition and thus be more conservative. Was there any disadvantage of using a higher concentration? Only 2 ml of BES was added to 15 g of soils – was this small volume enough to ensure saturation and hence complete inhibition? There was no shaking or other measures to ensure a good mix of soil and inhibitor neither."

-Prior to the main incubation experiment we tested several concentrations of BES (1, 10 and 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 (see supplementary). Thus, the lowest BES concentration was chosen in the main experiment because we aimed to only block the acetoclastic pathway of methanogenesis which was proposed to be achieved with the lowest tested concentration of 1 mM (after Zinder et al., 1984). About the effectiveness of BES amendments, please see our response above in the General comments section.

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"L134-135 – No difference in CH4 production initially after BES amendment – does that mean no inhibitory effect? Why measurements only began 9 days after BES addition? Not sure about the linkage here."

-Measurement of gas fluxes after BES addition was made on the same day for hollows. This sampling revealed no change in gas fluxes as compared to the flux before BES addition. Due to the fact that we did not have slurry and we did not shake our microcosms, longer time was necessary for BES to distribute in the soil and start to function. Therefore, for hummocks, we conducted the 1st sampling after BES addition later as compared to hollows.

"L141-142 – What was the dilution rate? What exactly were the "suitable concentrations" chosen? Why was the number of measurements different between microforms? Further justification is needed."

-For each 13C-CO2 measurement the dilution rate of the gas samples and pure N2 was 1:60, respectively. With "suitable concentrations" we meant concentrations which were between the recommended minimum and maximum threshold for the CRDS Picarro (380 - 2000 ppm). We will add this information to the text. The different number of measurements between microforms, we can explain by technical issues and analytical routine of the laboratory. However, as already stated above, we are convinced it would not affect the final conclusions derived from the study.

"L155 – The ratio is weight to volume?"

-The ratio was volume to volume, i.e. 15 g fresh peat was ca. 15-20 ml volume depending on a depth horizon (less decomposed material was larger by volume), so 30-40 ml of DI-H2O was used for extraction.

"L155-163 – Why did the authors only measure dissolved N but not other chemical species? Why did the authors use H2O for extraction, but not the common reagent KCl used in soil analysis? How was total dissolved N be determined? By acid digestion?"

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-The amount of extracts was enough for dissolved N analyses, while larger volumes were necessary for analyses of other chemical species. We used DI-H2O as an extractant as the KCl would result in very high yield of DOC from organic material which could interfere the analysis of dissolved N species with our analytical equipment. Continuous-Flow-Analysis with multichannel peristaltic pumps (Cenco Instrumenten, Mij. N.V.Breda, Netherlands) is a photometer which measures dissolved Nt, NO3- and NH4+ simultaneously based on color-developing chemical reactions with sulfanilamide and N-(1-Naphthyl) ethylenediamine dihydrochloride (Nt and NO3-) as well as with Na-Salicylate and Na-nitroprusside dehydrate (NH4+).

“L169 – What was this “weighting”, and how was it exactly done? Please give more information.”

-The weighting was done separately for each microform type (hollows, hummocks) by dividing the mean CH4 production rate of each depth (e.g. 15 cm) with the sum of the mean CH4 production rates of all depths (15 cm + 50 cm + 100 cm + 150 cm + 200 cm). The result is the contribution of CH4 production of each depth to the total CH4 production within each microform type. Next, the result was multiplied with the difference of the mean CH4 production rate before and after adding BES. The weighting was done to allow comparison of the dimension of inhibition between the different depth layers (without the weighting it could have been misleading to show a BES effect of e.g. 2000% in hollows 200 cm depth with production rates around “0” and a BES effect in hollows 15 cm depth, where most of the CH4 is produced, of e.g. 200%).

“L179 – Give the mean and p values.”

-The mean higher CH4 production rate of hollows as compared to hummocks was ca. 22.4 ng g soil-1 hour-1 (p-value 0.0036).

“L191-192 – Why would CH4 production increase over time? This was not addressed in the discussion. One would expect CH4 production to decline with time as substrates become increasingly depleted.”

-It is true that over time the substrates available for methanogenesis decrease (at least products of fermentation, i.e. acetates, whereas CO2 reduction will depend on availability of H+) which would lead to a decreased CH4 production over long-term time span. However, within the relatively short-term period of our experiment the competition of methanogens with other microbial groups (e.g. denitifiers, Fe, S- reducers) for energetically favorable alternate electron acceptors (see lines 273-277) in the incubation jars, seemed to be of higher importance than the depletion of substrates. Thus, the production of CH4 should increase as soon as the electron acceptors are exhausted and methanogenesis becomes energetically efficient.

“L194-196 – If there was indeed oxygen left in the jar after N2 bubbling, then perhaps the whole experimental setup was flawed? If this is true, then I would expect CH4 production to be underestimated in the BES treatment as well?”

-We assumed that the decrease of CH4 production after 1-time water addition (control) and BES solution (BES treatment) could be partly related to tracers of oxygen left after N2-bubbling of DI-H2O. However, we are stressing that the headspace was periodically flushed with N2, thus if not consumed in numerous metabolic reactions, the O2 tracers would anyway be removed during later flushing procedures. Therefore, we are convinced such an occasional effect did not flaw the whole experiment. Moreover, the anaerobic indicator stripes (see line 116) showed no evidence of O2 present in the jars throughout the whole incubation period.

“L216 – I do not think it is appropriate to put “0” for concentrations under the detection limit – should use “trace” to represent instead. Was there significant difference in N concentration among depths or microforms?”

-“0” will be replaced with “trace”. No significant difference was observed between microforms at each single depth layer. In microform type hollows there was significant increase of extractable nitrogen and ammonium with depth. In hummocks the ammonium concentrations were significantly different between the 50-100 and 150-200 cm

depth layers. For the extractable nitrogen concentrations in hummocks, the depths of 50, 150 and 200 cm were significantly different (Fig. 5).

“L230 – Did the Becker et al study measure in situ CO2 emission from soil surface rather than CO2 production? If so, then the reported values would not be comparable with those of production determined in this study.”

-We thank the Referee for the notice. Yes, Becker et al. (2008) reported emission rates and not the CO2 production. We will improve it in the text. To omit misunderstanding, we referred to the field study to highlight the observed differences in CO2 fluxes between microforms in situ, which were different from the patterns measured in the lab studies. We did not aim to directly compare values of two kinds of studies.

“L234 – What were the “differences in SOM properties”? If hollows had more labile C (not sure if this was the case as no details were provided), one would expect CH4 production should be higher there?”

-According to data available, hummocks and hollows did not differ between each other in organic C content at any depth, whereas peat N (all depths) and P (50, 200 cm) were significantly higher in hollows as compared to hummocks, but S content was significantly higher in hummocks at 15 and 50 cm as compared to respective depths of hollows (this information will be incorporated as a Table). We assumed these factors could have effect on CH4 production potential (Fig. 6) but we did not fully expect them to affect CO2 production (as related to the text mentioned by Referee here). To omit misunderstanding we will delete the controversial expression.

“L237-240 – Please further explain this sentence. How would the presence of aerobe and facultative anaerobe in hollows lead to the same CO2 production rate between the two microforms? I could not see a clear linkage here.”

-Non-significant differences in CO2 production between hummocks and hollows did not support our hypothesized pattern, due to, indeed, expected differences in micro-

bial communities between microforms (e.g. Galand et al., 2002; Kotiaho et al., 2013; Deng et al., 2014). Interestingly, incubation under aerobic conditions revealed similar (no difference between microforms) CO2 production results (Lozanovska et al. 2016). Both findings contradict to in situ CO2 emissions 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 in nature) 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).

"L276-280 – There was no mentioning of measuring these elements/compounds in the methodology section – please add these missing information. Why did the authors analyze total Fe, S and NH4 contents, but not the alternative electron acceptors (e.g. NO3, SO42-) that have been widely known to play a role in governing CH4 dynamics? That would give more meaningful analysis, and the Lozanovska et al (2016) paper published by their group should be relevant here. Figure 6 should first be presented in the results section."

-We add the missing information to the methodology section (*3). The nitrate (NO3-) concentration was analyzed but appeared below the detection limit in all microforms and depths (see lines 217-218). Unfortunately, it was not methodologically possible for us to measure SO42- in water extracts. Instead, we have information on the total S content in peat material. The article of Lozanovska et al. (2016) investigated the effect of nitrate and sulfate addition on CO2, N2O production and CH4 oxidation under aerobic condition. In the context of the current study (anaerobic conditions), we do not think it is appropriate to directly compare it with Lozanovska et al. (2016). Regarding

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the Fig. 6, we prefer to keep it for the discussion as it directly relates to the text there. However, we will extend the results section and add information on other chemical properties measured (S, Fe) as a table.

*3 For the total sulfur (S) and iron (Fe) measurement, peat samples were dried (60âŰęC, 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).

“L288-289 – Not sure if other studies involving delta 13C-CH4 have the same issue with the low concentration of CH4 for analysis?”

-Measurement of δ13C in low CH4 concentrations is possible with the utilization of Precon unit connected to an IRMS (it applies a liquid N trap, which repeatedly concentrates CH4 sample via freezing). Unfortunately, we did not have such equipment at our disposal and could analyze samples only with CH4 concentrations above 800-1000 ppm.

“L292-293 – Again cannot see the linkage – why inhibition by BES was not selective when the hydrogenotrophic pathway was dominant before BES addition?”

-The addition of BES resulted in a substantial decrease of CH4 production in all peat samples (in all depths of hummocks and hollows with a measurable CH4 production). This included the samples with a dominating acetoclastic and also hydrogenothrophic pathway of methanogenesis. Thus, BES was not only blocking the acetoclastic- but also the hydrogenothrophic methanogenesis. This means that BES was not selective (only blocking the acetoclastic methanogenesis).

“L295 – Replace typo “culturs” with “cultures” “

-Will be corrected.

“L314-316 – Why would the hydrogenotrophic pathway be more dominant in hollows? Were there any possible causes to account for this biogeochemical difference? Would there be differences in the amount and quality of root exudates from peatland vegetation, for example?”

-We are very grateful to the Referee for such an important question. First of all, according to our earlier studies, indeed, plant communities dominating on selected microforms affect methanogenesis differently. Thus, Scheuchzeria palustris dominated on hollows showed 4 times higher contribution of recent photosynthates to methanogenesis (estimated based on 14C labeling) as compared to Eriophorum vaginatum which dominated on hummocks and lawns (Dorodnikov et al., 2011). Based on this, we may expect higher methanogenesis in hollows as compared to hummocks under equivalent temperature and aeration regimes. In this case, CH4 should predominately be produced via acetoclastic pathway as the rhizodeposits would be quickly converted to acetic acid (acetates) due to fermentation under anaerobic conditions. In the current study, we can relate CH4 at 15 cm depth of hollows to be produced via acetoclastic pathway (Fig. 7) what is in agreement with the expected pattern. However, we couldn’t compare it with hummocks, as there was not enough CH4 for an isotope analysis. Interestingly, in deeper layers (50, 100 cm) the pattern changed: hydrogenotrophic pathway contributed more to the total methanogenesis in hollows vs. hummocks (Fig. 7). We can explain this by the lacking regulatory effect of living vegetation which roots naturally grow deeper as 50 cm and decreased availability of fresh plant-derived debris.

“Figure 2 – Should show the data points and error bars for the control also. Y-axis should be “CH4 production rate” rather than “CH4 rate”. Would CH4 production in the control for hummock at 50 cm depth on day 63 be an artefact? If this data point is removed, CH4 rate actually had little change over the whole incubation period.”

-We did not show the error bars for control treatment as they were too small from a du-

plicate measurement. Nevertheless, the overall control was statistically different from the BES treatment since before addition of BES, all our microcosms were controls, where we measured initial CH4 production potentials (lines 118-125). We have randomly chosen BES treatments and the rest was continuously measured as controls. This made us possible to compare all treatments thereafter. The title of the Y-axis will be changed to "CH4 production rate". Even though the CH4 production in the control for hummock at 50 cm depth on day 63 might be an artefact, the CH4 production would be still more than 2 folds higher than in the BES treatment of the same depth. This illustrates the effective suppression of CH4 production by BES.

"Figure 5 – Soil N should be expressed in per unit mass of soil?"

-Since we measured water-extractable (dissolved) forms of N we decided to present it as per L of solute. Changing values as per gram of soil will not affect the pattern of concentrations distribution, i.e. the observed differences remain unchanged. If the Referee would insist we can recalculate values on a soil dry weight basis.

"Figure 6 – Why only three depths were chosen for presentation here? The N size seems quite small for establishing relationships between the two variables. Was the fitted line statistically significant?"

-The data of S and Fe were adopted from another study with the same soil samples but from three depths only (15, 50, 200 cm). We used the available information to see a relationship between CH4 production potential and the chemical composition of peat extracts (N) and solids (Fe, S). Though just three depths were measured, each was done in triplicate. Still, the correlation of CH4 production potential was significant for NH4+ and S data of hollows, whereas correlation between CH4 production and Fe in hollows as well as all correlations in hummocks were not significant.

Literature:

Deng, Y., Cui, X., Hernandez, M., Dumont, M. G.: Microbial Diversity in Hummock and

Hollow Soils of Three Wetlands on the Qinghai-Tibetan Plateau Revealed by 16S rRNA Pyrosequencing, Plos One, 9 e103115, 2014.

Galand, P. E., Saarnio, S., Fritze, H., Yrjälä, K.: Depth related diversity of methanogen Archaea in Finnish oligotrophic fen, Microbial Ecology, 42, 441-449, 2002.

Kotiaho, M., Fritze, H., Merilä, P., et al.: Actinobacteria community structure in the peat profile of boreal bogs follows a variation in the microtopographical gradient similar to vegetation, Plant and Soil, 369, 103-114, 2013.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2016-162/bg-2016-162-AC2-supplement.pdf

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

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