Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

Biogeosciences Discussions Open Access EGU

Microtopography matters for CH_4 formation in a peat soil: a combined inhibitor and ^{13}C study

Johannes Krohn[1], Ivana Lozanovska[1,3], Yakov Kuzyakov[1,2] and Maxim Dorodnikov[1]

[1]Department of Soil Science of Temperate Ecosystems, Faculty of Forest Sciences and Forest Ecology, Georg-August University Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
[2]Department of Agricultural Soil Science, Georg-August University Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
[3]Present adress: Macedonian Ecological Society, Arhimedova 5, 1000 Skopje, Macedonia

Correspondence to: Dr. Maxim Dorodnikov (mdorodn@uni-goettingen.de)

Abstract. Peatlands' microtopography units – hummocks and hollows – are mainly differing by hydrological characteristics (water table level, *i.e.* oxic-anoxic conditions) and vegetation communities. These factors affect the fluxes of key greenhouse gases (GHG) - methane (CH_4) and carbon dioxide (CO_2). However, the effects of microrelief forms on belowground CO_2 and CH_4 production and pathways of methanogenesis need deeper understanding. We hypothesized increasing CH_4 and CO_2 production potentials from naturally drier hummocks to more wet hollows during anaerobic incubation. GHG production in peat was expected to decrease with depth (decreasing inputs of recent plant-derived deposits) but the contribution of hydrogenotrophic vs. acetoclastic pathway to the total methanogenesis should be higher in deeper peat layers as compared to upper layers. To test the hypotheses, we measured CH_4 and CO_2 productions together with the respective $\delta^{13}C$ values under controlled anaerobic conditions with- and without addition of specific inhibitor of methanogenesis (2-bromo-ethane sulfonate, BES) in a peat soil of hummocks and hollows of five depths (15, 50, 100, 150 and 200 cm). The concentration of BES (1 mM) aimed to block acetoclastic but not the hydrogenotrophic pathway of methanogenesis.

As expected, CH_4 production was ca. 2 times higher in hollows than in hummocks, though no differences in CO_2 were measured between the microforms. With depth, CO_2 production rates decreased by 77% (15 cm vs. 200 cm) in both microforms, whereby the highest CH_4 production was measured at 15 cm in hollows (91% of total produced CH_4) and at 50 cm in hummocks (82%). Noteworthy, at 15 cm of hummocks less than 1% of total CH_4 production was observed. Decreasing GHG production rates with depth positively correlated to an increase in the extractable total N and NH_4^+ concentrations. The hydrogenotrophic pathway of methanogenesis in deep vs. surface layers was depicted by lower (more negative) $\delta^{13}C$-CH_4 and higher $\delta^{13}C$-CO_2 values, respectively. Between the microforms, overall higher contribution of hydrogenotrophic vs.

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

acetoclastic methanogenesis corresponded to hollows as compared to hummocks. Contrary to the expectation, the addition of 1mM BES was not selective and inhibited both pathways of methanogenesis. Concluding, peatlands' microrelief is an important factor regulating the GHG fluxes. However, the effects of microforms on the production of CH_4 and pathways of methanogenesis were pronounced for the upper 50 cm layer. Finally, inhibition with BES appeared to be less effective tool for the partitioning between pathways of methanogenesis as compared with the isotope method.

Key words: Greenhouse gases, boreal peatland, microtopography, stable carbon isotope method, methanogenesis inhibitor, 2-bromo-ethane sulfonate

1 Introduction

Northern peatlands historically have been a sink of atmospheric carbon dioxide (CO_2) but also revealed their potential of releasing large CO_2 and methane (CH_4) fluxes to the atmosphere as a result of environmental or anthropogenic forcing (Limpens et al., 2008). Both are important greenhouse gases (GHG, IPCC, 2014) which balance in peatland ecosystems is regulated by multiple environmental factors. Among them the water table level, which controls the aeration status of the peat (Moore and Knowles, 1989; Moore and Roulet, 1993; Kettunen 2003), the peat quality, which reflects the decomposability of constituent substances (Svensson and Sundh 1992; Yavitt et al., 2000), the vegetation, which regulates the peat quality, transfer of gases belowground and to the atmosphere (Whiting and Chanton, 1993; Bubier et al., 1995) and the temperature, which controls the metabolic rate of microorganisms (Crill et al., 1993; Granberg et al., 2001). Another important factor is the peatlands' microtopography, which highlights the role of location with specific physical and biochemical conditions, and stipulates the interaction between the atmosphere, vegetation and the subjacent peat (Dorodnikov et al., 2011). Thus, depending on a surface elevation three microrelief forms (microforms) are distinguished: elevated hummocks, depressed lawns and intermediate lawns (Bubier et al., 1993). Two contrasting microforms – hummocks and hollows – distinctly differ by the water table level, *i.e.* subsurface of water logged hollows is typically anaerobic as compared to drier hummocks thereby stressing the difference in redox processes between two microforms (Kettunen, 2003). Furthermore, the plant species composition is closely connected with the water table and moisture conditions (Waddington and Roulet, 1997). This controls the input of plant-

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

derived deposits in the microforms, hence affects the carbon turnover and the formation and emission of GHG (Ström et al., 2005). Most studies so far focused on aboveground GHG flux measurements to the atmosphere as related to the types of microforms (Bubier et al., 1993; Dalva and Moore, 2001; Baird et al., 2009). But there is still not enough understanding of the mechanisms controlling belowground CH_4 and CO_2 dynamics in profile layers below subsurface of microforms.

Generally, the CH_4 cycling in peatlands consists of CH_4 production (methanogenesis) in the anoxic parts of the soil by microorganisms of the archaea type (methanogens) and CH_4 oxidation (methanotrophy) in presumably oxic layers (Lai, 2009). The process of methanogenesis involves two main pathways: (1) acetate cleavage (acetoclastic pathway), which mostly occurs in the presence of fresh SOM and (2) CO_2 reduction with hydrogen (H_2) (hydrogenotrophic pathway) when other substrates for methanogenesis are scarce (Hornibrook et al., 1997; Popp et al., 1999). CO_2 production occurs during both anaerobic SOM fermentation and methanogenesis, as well as in the oxic part of the soil by plant- and microbial respiration, together with methanotrophy. As described above, peatland's microforms are distinct by the thickness of aeration zone of peat and plant communities which supply microorganisms with organic substrates. This in turn may affect the proportion of two methanogenesis types between, e.g. hummocks and hollows and especially with depth (Dorodnikov et al., 2013).

Among other factors controlling CO_2 and CH_4 production in peatlands, deposition of some anions, such as ammonium (NH_4^+), nitrate (NO_3^-), sulfate (SO_4^{2-}), metals (Fe) could alter GHG fluxes (Eriksson et al., 2010; Sutton-Grier et. al., 2011). Supply of peatlands with N and S compounds occurs mainly through the anthropogenic eutrophication of inland waters and/or acidic deposition from the atmosphere (Sutton-Grier et. al., 2011). Along with the nutrition effect of N, S, Fe compounds for the plant- and microbial communities, they participate in redox reactions as alternative electron acceptors (AEAs) when oxygen availability is low. The presence of AEAs can reduce CH_4 production due to a combination of inhibition and competitive effects between methanotrophs and methanogens for electron donors (Bodegom and Stams, 1999; Eriksson et al., 2010).

Under laboratory conditions, the mechanisms involved in CH_4 and related CO_2 dynamics can be studied using an approach involving a specific inhibitor of methanogenesis, 2-bromo-ethane sulfonate (BES). BES is known to inhibit the reductive demethylation of methyl-Coenzyme M (Müller et al., 1993), a coenzyme responsible for methanogenesis. BES added at a certain concentration reportedly inhibits the acetoclastic – but not the hydrogenotrophic pathway – of CH_4 production (Zinder et al., 1984). Therefore, amendment of peat soil with BES may help to reveal the distribution of methanogenesis pathways

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

between microforms and with the depth. Another method is based on stable C isotope signatures (represented as $\delta^{13}C$ values) of CH_4 and CO_2 which reflect the CH_4 pathway formation (Whiticar, 1999; Conrad, 2005). Thus, CH_4 produced by the acetoclactic pathway is less ^{13}C depleted (e.g. shows higher $\delta^{13}C$ values) than CH_4 produced by the hydrogenotrophic pathway (lower $\delta^{13}C$ values) because of stronger discrimination against heavier ^{13}C during the latter process (Whiticar et al., 1986; Avery et al., 1999). The combination of both methods is assumed to provide strong evidence for the respective methanogenic pathway. If the inhibitor BES is blocking CH_4 production by the acetoclastic pathway, then the respective $\delta^{13}C$-CH_4 signature should decrease due to a higher contribution of ^{13}C-depleted CH_4 produced by the hydrogenotrophic pathway as compared to the control (without inhibitor). Nonetheless, other important factors influencing $\delta^{13}C$ in CO_2 and CH_4 (e.g. the $\delta^{13}C$ value of the organic substrate) have to be considered. Avery et al. (1999) and Steinmann et al. (2008) gained valuable information about vertical and seasonal changes in isotopic composition of CH_4 in peat profiles. We still, however, have very little information about the effect of peatland microtopography on the patterns of CH_4 and CO_2 isotopic signatures (Dorodnikov et al., 2013).

This study was designed to cover two aspects. Firstly, to estimate the production potential of CH_4 and CO_2 in depth profiles down to 200 cm below two contrasting microforms – wet hollows and dry hummocks. Secondly, to identify the contribution of the two pathways of methanogenesis in peat depth layers below both microforms by adding BES and measuring $\delta^{13}C$ in CH_4, CO_2 and peat soil. The following hypotheses were tested:

I. Naturally more wet hollows will show a higher CH_4 and CO_2 production potential (microbial communities will be better adapted to *in vitro* anaerobic conditions) as compared with drier hummocks.

II. Upper peat layers, which contain less decomposed organic matter, will show higher CH_4 and CO_2 production potentials in contrast to deeper, more decomposed layers.

III. Peat layers of hollows and hummocks will be dominated by different pathways of methanogenesis due to differences in substrate quality.

Finally, our research question was, whether the CO_2 and CH_4 production potentials of the tested peat soils could be linked to their intrinsic chemical composition.

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

2 Materials and Methods

2.1 Experimental site and peat soil collection

The experimental site is a central part of a natural minerogenic, oligotrophic low-sedge pine fen Salmisuo, located in the North Karelian Biosphere Reserve (62°47´N, 30°56`E) in eastern Finland. A detailed description of the site is provided by several authors (Saarnio et al., 1997; Alm et al., 1999; Becker et al., 2008; Jager et al., 2009). The surface of the sampling sites was subdivided into three main microforms: 1) elevated dry hummocks, 2) depressed wet hollows and 3) intermediate lawns (Becker et al., 2008), whereby the two contrasting microform types – hummocks and hollows – were tested in this study. Peat samples were taken with a peat auger (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) – a stainless steel half-cylindrical sampler (50 cm long, 6 cm wide) with a massive cone and a cutting edge, sealed off by a hooked blade. Soil was sampled from both microform types and five depths: 15, 50, 100, 150 and 200 cm. Each true replicate consisted of a minimum of three randomly picked cores, of which a middle 10 cm section was collected and aggregated. Each microform type and depth horizon was sampled in triplicate.

2.2 CH_4, CO_2 production measurements and inhibition of methanogenesis

Aggregated peat soil samples from each depth and microform type (true replicates, n=3) were split for 5-6 pseudo replicates of 15 g fresh weight and placed together with anaerobic indicator stripes (Microbiology Anaerotest, Merck, Darmstadt, Germany) in 150 ml Mason jars, which were closed by butyl rubber septa and screw caps.

To create anaerobic conditions, the jars were connected to an evacuation line via needles with 3-way-stopcocks and flushed with pure N_2 for 20-30 min. After flushing, the jars were equilibrated to atmospheric pressure through a water lock and immediately filled with 15 ml pure N_2 to prevent air diffusion into the headspace and to enable subsequent sampling. The same procedure was repeated every time before gas production measurements.

To collect gas samples, a 60-ml gas-tight syringe was used to sample ca. 20 ml headspace gas, which was immediately transferred to a 12-ml pre-evacuated glass vial with overpressure. For a gas production measurement, four gas samples were

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

taken with time intervals of 30-60 min after "zeroing" (headspace flush with N_2). The overall sampling period lasted 49 days for hollow and 79 days for hummock, with eight measurements for each microform type.

After the first four gas production measurements (at day 37 for microform type hollow and at day 54 for hummock), 2 ml of the inhibitor BES (predissolved in O_2-free milli-Q water) were added to respective BES-treated jars, whereas the rest of jars served as controls. The effective BES concentration was determined prior in a testing experiment of CH_4 production from the same soil amended with 1, 10 and 100, mM of BES after Zinder *et al*. (1984) and Smemo and Yavitt (2007). The suppression of CH_4 formation with 1 mM concentration of BES was comparatively effective as by 10 and 100 mM (data not shown). Thus, the lowest BES concentration was chosen in the main experiment. 1 mM BES was added through the 3-way-stopcocks (without opening the jars) to three replicates of each depth. The same volume (2 ml) of O_2-free milli-Q water was added to the remaining control. The subsequent gas production measurements were performed in the same manner as before addition of inhibitor and milli-Q water. Within the first days after BES amendment of hollows, no detectable difference in CH_4 production was observed; therefore, for hummocks, the GHG measurements started 9 days after adding BES.

During the experiment, all jars were stored at room temperature (about 22°C) in the dark to avoid any possible production of oxygen by algae. CH_4 and CO_2 concentrations were measured on a gas chromatograph GC 6000 VEGASERIES 2 (Carlo Erba Instruments) equipped with a flame ionization detector, an electron capture detector and a pressure-controlled autosampler for 64 samples. Detailed information on the equipment can be found in Loftfield et al. (1997).

2.3 $\delta^{13}C$ analyses

To measure the stable C isotope composition in CO_2 (shown as $\delta^{13}C\text{-}CO_2$), 1 ml headspace gas sample was taken as described above and diluted with pure N_2 to obtain suitable concentrations for the analysis. The number of measurements was three for hollow (all after addition of BES) and four for hummock (two before adding and two after adding BES). The diluted gas samples were measured for $\delta^{13}C\text{-}CO_2$ with a Cavity ring-down spectroscope (CRDS), Picarro G2131-i (Picarro, Inc., Santa Clara, CA, USA).

Due to the requirements of the Isotope Ratio Mass Spectrometer (IRMS) for certain minimal CH_4 concentrations, $\delta^{13}C\text{-}CH_4$ could be measured only in three soil layers (15, 50 and 100 cm) of both microform types. A headspace gas sample of 15 ml

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

was taken as described above and transferred to a 12-ml pre-evacuated glass vial for $\delta^{13}C$-CH_4 measurement on a IRMS Delta C with a Conflo III interface (both from Thermo Fischer Scientific, Bremen, Germany) at the Centre for Stable Isotope Research and Analysis (KOSI), Büsgen-Institute, Georg August University Göttingen, Germany.

To measure $\delta^{13}C$ in solid samples, the peat soil was dried at 40°C during several days, ball milled and weighed in tin caps. Samples were combusted in a Flash 2000 elemental analyzer (Thermo Fisher Scientific, Cambridge, UK) and the $^{13}C/^{12}C$ ratio was measured on a Delta V Advantage IRMS with the Conflo III interface (Thermo Electron, Bremen, Germany) at KOSI.

2.4 Measurement of dissolved total N, NO_3^- and NH_4^+

Peat samples from both microforms and all depths were amended with DI-H_2O in a proportion of 2:1 and shaken for 1.5 h. The obtained peat extracts were thoroughly filtrated several times: first, through a coarse paper filter (595 ½, Whatman) into 50 ml centrifuge tubings. Then, to increase the output of solution from solid remnants, the latter were centrifuged at 2000 rpm for 5 min in containers with porous bottom and glass fiber filters. The extra solution was filtrated again through a paper filter and mixed with previously filtrated solution. The second filtration was done through fine syringe filters (Sartorius 0.20 µm pore size with Luer lock, Göttingen, Germany) into 15-ml plastic centrifuge tubings. All filtrates were kept in a cold storage room at a temperature of 4-6 °C prior to analysis. The concentrations of extractable N, NO_3^- and NH_4^+ were measured photometrically via Continuous-Flow-Analysis using multichannel peristaltic pumps (Cenco Instrumenten, Mij. N.V.Breda, Netherlands).

2.5 Calculation of gas production, effects of BES and statistical analysis

To calculate a gas production rate, four CH_4 and CO_2 concentrations (as ppm and ppb values) measured in each soil sample within 240-250 min were linearly approximated and the Ideal Gas Law was used to convert the concentration from ppm/ppb to mass units per gram soil on a dry weight basis (ng g $soil^{-1}$). The BES effect was determined for each microform and depth by calculating the difference (in %) of the mean CH_4 production rate before and after adding BES. The difference was then corrected with respective control treatments and "weighted" against each other according to their contribution to the overall CH_4 production.

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

The differences in CH_4 productions between microforms and depths, as well as before and after adding BES, were evaluated with two-way ANOVA and Fischer´s LSD test using STATISTICA 10.0 (StatSoft, USA). The required normality and homogeneity of the data were checked with the Kolmogorov-Smirnov and the Levene´s test, respectively. The variables were treated as independent for all depths below a microform type and for a certain depth between microforms. The significance of differences was determined at $P<0.05$ level.

3 Results

3.1 CH_4 and CO_2 production depending on microforms and depth

Based on the sum of CH_4 production from all depths, hollows showed a significantly higher CH_4 production than hummocks. The top soil layer of hollow and the 50 cm depth of hummocks were the main locations for CH_4 production (Fig. 1a). The CH_4 production was 10.6 fold lower at 50 cm depth (3.9 ng CH_4 g d.w.$^{-1}$ h^{-1}) as compared to the top 15 cm (41.7) in hollow, whereas in hummocks, it was 64 fold lower at 15 cm (0.3) than at 50 cm (19.2) (Fig. 1a). Below 50 cm, CH_4 production substantially decreased to a minimum of <0.1 ng CH_4 g d.w.$^{-1}$ h^{-1} and there were no differences either between microforms or between depths.

CO_2 production did not differ between the two microforms at each depth, and the surfacesoil of both microforms contributed 40-51% to the overall CO_2 production (Fig. 1b). The rate of CO_2 production substantially decreased under both microforms by ca. 77% from the top (15 cm: 4153-4997 ng CO_2 g d.w.$^{-1}$ h^{-1}) to the bottom soil layer (200 cm: 923-1216 ng CO_2 g d.w.$^{-1}$ h^{-1}). A significant decrease was observed from the top soil layer to a depth of 50 cm. The contribution of deeper soil layers (50-200 cm) to the overall CO_2 production varied between 9 and 20% (Fig. 1b).

3.2 Effects of BES on CH_4 and CO_2 production

Soil layers which had the highest CH_4 production prior adding the inhibitor BES, e.g. hollows 15, 50 cm and hummocks 50, 100 cm, generally showed an increasing trend of CH_4 production over time (Fig. 2). The addition of BES substantially suppressed CH_4 production (Fig. 2; arrow: addition date).

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

Among all depths and microforms, the suppressing effect of BES varied from 0 up to 68% (Fig. 3). Remarkably, adding milli-Q water in control treatments yielded a decrease of CH_4 production similar to BES (Fig. 2). This is probably due to trace amounts of dissolved oxygen left after N_2-bubbling. Therefore, the decrease of CH_4 production after (versus before) BES treatment was statistically significant solely at 50 cm depth in hummocks and at 15 cm depth in hollows (Fig. 3). In contrast to the control treatments, however, BES effectively suppressed methanogenesis until the end of the incubation period (Fig. 2). Contrary to CH_4, the CO_2 production did not change after adding BES (data not shown).

3.3 $\delta^{13}C$ of CH_4, CO_2 and SOM

Generally, $\delta^{13}C$ of CO_2 substantially varied between depths, but the difference was less pronounced between microforms (Fig. 4a). CO_2 from the top soil layer was the most depleted in ^{13}C ($\delta^{13}C$-CO_2 = -24‰ for hummocks and -29‰ for hollows), whereas at 50 cm depth the $\delta^{13}C$-CO_2 values were the highest (ca. -17‰). From 50 to 200 cm, a gradual depletion down to -21 to -24‰ occurred (Fig. 4a). Among microforms, $\delta^{13}C$-CO_2 values were lower in hummocks than in hollows, although the pattern of $\delta^{13}C$-CO_2 change with depth was similar in both microforms. Differences between microforms were significant at depths of 15 and 150 cm (Fig. 4a).

In both microforms, $\delta^{13}C$-CH_4 values strongly decreased with depth, ranging from -59‰ at 15 cm to -91‰ at 100 cm (Fig. 4b). The available data (for 50 and 100 cm) indicated significantly more depleted ^{13}C-CH_4 in hollows than in hummocks.

The stable C composition of peat SOM ($\delta^{13}C$-peat values) was measured at the 15, 50 and 200 cm layers of the two microforms (Fig. 4c). The $\delta^{13}C$-peat values in the surface soil were higher in hollows (-24.5‰) than in hummocks (-26.9‰). At 50 cm, there was either a decrease (in hollows) or increase (in hummocks) to ca. -26‰. In the deepest (200 cm) layer, $\delta^{13}C$-peat values further decreased (to -26.1‰ in hummocks and -28.0‰ in hollows) (Fig. 4c).

3.4 Total extractable nitrogen, ammonium and nitrate in soil

In general, both microforms showed an increasing trend of total extractable nitrogen (N_{extr}) and ammonium (NH_4^+) concentrations in peat-water extracts with depth (Fig. 5). N_{extr} in hollows ranged from 0.21±0.01 to 3.08±0.03 mg L^{-1} and in hummocks from "0" (measurement below the detection limit) to 2.55±0.02 mg L^{-1} from the top to the bottom soil layer,

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

respectively. The measured NH_4^+ concentration was approximately half of N_{extr} within each depth and microform (Fig. 5). The nitrate (NO_3^-) concentration was below the detection limit in all microforms and depths. Therefore, the difference between N_{tot} and NH_4^+ presumably corresponds to disolved organic N (DON).

220

4 Discussion

4.1 CH_4 and CO_2 dynamics in microforms and with peat depth

4.1.1 CO_2 production potential

The CO_2 production potential under anaerobic conditions was similar between hummocks and hollows at each of the depth
225 layer (Fig. 1b). This finding contradicts the hypothesized lower CO_2 production from hummocks vs. hollows under anaerobic conditions due to the overall *in situ* lower watertable level in the former (hence better aeration and adaptation of microbial communities to the O_2-rich environment). Nonetheless, similar non-significant differences in CO_2 production between hummocks and hollows, albeit under aerobic conditions, were reported for the same soil (Lozanovska, personal communication). This and Lozanovska´s incubation studies contradict *in situ* measurements reporting more than 3-times-
230 higher CO_2 production from hummocks as compared to hollows (Becker et al., 2008). Such inconsistency may reflect either lower *in situ* soil respiration of hollows due to the higher watertable level (decreased aeration) than in hummocks and/or an onsite higher contribution of root or rhizosphere respiration to the total soil CO_2 flux (Kuzyakov, 2006) in hummocks. In contrast, under controlled conditions, the lack of the regulatory effect of microform-specific plant communities on native soil CO_2 flux compensated the differences in SOM properties between the two microforms, resulting in similar CO_2 production
235 rates.

Another mechanism is related to the properties of soil microbial communities developing below microforms. The naturally greater seasonal variations due to watertable fluctuations in hummocks vs. permanently water-logged hollows promote the presence of aerobe and facultative anaerobe microbial species which can switch between fermentation and aerobic SOM decomposition (Cord-Ruwisch et al., 1988). Therefore, incubation of hummock surface soil under anaerobic conditions showed

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

similar CO_2 production rates as from the respective hollow samples. This interpretation must be tested by applying molecular biology methods to the community structure in soils of the two microforms.

Peat soil from both microtopographic positions showed decreasing rates of CO_2 production with increasing depth. CO_2 production from the top soil strongly decreased to a depth of 50 cm, followed by a further slow decrease to 200 cm (Fig. 1b). The decreasing CO_2 production rates with depth were similar to those reported in other peat soils studies under *in vitro* anaerobic and aerobic conditions (Moore and Dalva, 1997; Glatzel et al., 2004). These authors explained depth-dependent CO_2 patterns by a decreasing availability of fresh SOM and by the degree of decomposition (according to the Von Post Humification Index). Although the SOM decomposition of the deep peat is retarded, it is sustained year round in northern peatlands, in contrast to the surface soil, which freezes during the cold season (Maljanen et al., 2010). Considering the low hydraulic conductivity, porosity and higher soil density (Quinton et al., 2008; Morris and Waddington, 2011; Branham and Strack, 2014) of deep peat, there is a continuous accumulation of CO_2 belowground in peatlands (Beer et al., 2008). Therefore, this C stock should be considered in studies on GHG turnover in peatland ecosystems and when modelling regional to global C balances.

4.1.2 Methanogenic potential

The overall higher CH_4 production from hollows vs. hummocks (Fig. 1a) depends on SOM quality, which in turn is affected by aboveground plant communities. Greater rates of CH_4 production in peat soil from hollows as compared to hummocks were also found in a labeling study of plant-soil cores from the same peatland (Dorodnikov et al., 2011). Thus, the hollows-dominating *Scheuchzeria palustris* contributed 2-4 times more to methanogenesis than the hummocks-dominating *Eriophoprum vaginatum*. This mainly reflected differences in rhizodeposition. CH_4 emission rates from closed chamber experiments at the surface of the same peatland revealed a similar pattern of higher emissions from hollows (Becker et al., 2008; Dorodnikov et al., 2013). The trend of a decreasing CH_4 production rate from the top soil layer to a depth of 100 cm (Fig. 1a) agrees with the hypothesized higher CH_4 production rates in upper vs. deeper layers. Similar to CO_2 production, this highlights the importance of specific depth-dependent biochemical and physical parameters, such as peat quality and nutrient availability, which influence microbial composition and activity, driving methanogenesis (Lai, 2009).

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

The surface soil of hollows was responsible for the overall highest CH_4 production (ca. 91%) between all depths, whereas the surface soil of hummocks surprisingly contributed almost "0" to the total CH_4 production (Fig. 1a). The *in situ* high O_2 availability in the hummocks surface soil, among other factors, controls the abundance of methanogens. Oxygen acts as an inhibitor or toxic agent for strictly anaerobic microorganisms such as methanogens (Shen and Guiot, 1996). Hence, hummocks surface soil may not contain a sufficient amount of obligatory anaerobic methanogens, resulting in low CH_4 production even under controlled anaerobic conditions. This assumption, in turn, is supported by high net CH_4 production in the surface soil of hollows, which naturally provides mostly anoxic and therefore more suitable conditions. Also a greater frequency and duration of anaerobic conditions are responsible for a larger active biomass of methanogens in hollows than in hummocks (Yavitt and Seidman-Zager, 2006).

According to another mechanism, methanogens could be outcompeted by microorganisms, which primarily perform more energetically favorable reactions with higher Free Gibbs Energy (ΔG) (Schink, 1997; Beer et al., 2008). Thus, reactions such as denitrification (NO_3^-) (Rubol et al., 2012; Schlesinger and Bernhart, 2013), sulfate (SO_4^{2-}) reduction (Lovley and Klug, 1983; Pester et al., 2012) or iron (Fe) transformation (Lovley et al., 1996; Cervantes et al., 2002) provide higher ΔG than methanogenesis, when oxygen is not available. We therefore correlated the content of macro- and microelements from the same soil samples to the CH_4 production rates (Fig. 6). In hollows, the depletion of sulfur (S), Fe and NH_4^+ was strongly accompanied by an increasing CH_4 production rate, whereas in hummocks a surprisingly weak correlation was observed. As no other anaerobic processes except of methanogenesis were followed in the study, the mentioned mechanism should be tested in additional experiments by measuring the anion and cation concentrations as well as gaseos products (e.g. N_2O for nitrification/denitrification) in the dynamics.

4.2 Estimation of methanogenic pathways based on $\delta^{13}C$ and by inhibition with BES

The use of specific inhibitors in combination with stable isotopes is a reliable method for the determination of CH_4 sources (Conrad, 2005). Among inhibitors for methanogenesis, 2-bromo-ethane sulfonate (BES) at a concentration of 1 mM was proposed to distinguish between two pathways – hydrogenotrophic (CO_2 reduction with H_2) and acetoclastic (acetate splitting) (Zinder et al., 1984). It was hypothesized that the adding BES inhibits the acetoclastic pathway (Whiticar et al., 1986).

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

The CH_4 production was strongly inhibited in the two microforms and at all depths by BES (Fig. 3). Unfortunately, very low CH_4 concentrations hindered the $\delta^{13}C$-CH_4 analyses in samples with BES. Thus, the hypothesis about partitioning between methanogenic pathways could not be rigorously proven. However, ^{13}C-CH_4 depletion with depth in a treatment without BES (Fig. 4b) reflected an increasing contribution of the hydrogenotrophic pathway to total methanogenesis (Whiticar et al., 1986). Since the suppression of CH_4 production with BES was substantial in all samples with the hydrogenotrophic pathway dominating before the addition, its inhibition was not selective, i.e. both hydrogenotrophic and acetoclastic pathways were blocked. Therefore, the hypothesized selective inhibition of the acetoclastic pathway by BES was not supported in the studied soil. Importantly, the reported concentration (1 mM) was tested in pure culturs of microorganisms and thus may vary in soils.

In general, the acetoclastic methanogenesis corresponds to $\delta^{13}C$-CH_4 values between -65 and -50‰ (Whiticar et al., 1986). The current experiment showed that the most intensive CH_4 production took place in the hollows surface soil (Fig. 1a), where the value was -59‰ (Fig. 4b). Simultaneously, the released $\delta^{13}C$-CO_2 was close to native peat organic matter (Fig. 4a,c). This indicated both the restricted CH_4 oxidation (in this case $\delta^{13}C$-CO_2 should be closer to the $\delta^{13}C$-CH_4 source) and relatively low CO_2-reduction pathway of methanogenesis. In the latter case, ^{13}C-CO_2 becomes more enriched due to discrimation by methanogens against heavier $^{13}CO_2$, while $^{12}CO_2$ is consumed during the methanogenesis (Popp et al., 1999). Therefore, we conclude that methanogenesis in the surface soil of hollows was dominated by the acetoclastic pathway (Fig. 7). Significantly lower $\delta^{13}C$-CO_2 values in hummocks vs. hollows (Fig. 4a) reflected the difference in C isotopic characteristics between the respective peat-SOM of the two microforms (Fig. 4c). This, in turn, is connected with the $\delta^{13}C$ signature of initial plant residues because different species dominated the two microforms (Becker et al., 2008; Dorodnikov et al., 2011).

The strongly negative $\delta^{13}C$-CO_2 values in the deeper soil layers may indicate the occurrence of so-called anaerobic oxidation of methane – AOM (Smemo and Yavitt, 2011). The C source for microorganisms conducting AOM is a strongly ^{13}C-depleted CH_4, and its utilization should dilute the total ^{13}C-CO_2, resulting in an overall decrease of $\delta^{13}C$-CO_2 values. This issue must be tested in separate experiments using ^{13}C-labeled CH_4 and analyzing the ^{13}C in released CO_2 as an end-product of oxidation under strictly anaerobic conditions.

In contrast to the surface soil, $\delta^{13}C$ values of SOM at 50 cm depth were similar for both microforms but the released CO_2 was 30-40% more enriched than SOM (Fig. 4a,c). As described above, the CO_2 enrichment occurs during the hydrogenothrophic

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

pathway of methanogenesis (Popp et al., 1999). However, δ^{13}C-CH_4 at 50 cm was ca. 23% higher in hummocks than in hollows (Fig. 4b), indicating that the acetoclastic pathway may co-exist with the hydrogenotrophic one. Both, the low δ^{13}C-CH_4 and the high δ^{13}C-CO_2 values in hollows, provided evidence for the increased contribution of the hydrogenotrophic pathway to total methanogenesis (Fig. 7).

At the deepest soil layers (100, 150 and 200 cm) the CH_4 production was very low (Fig. 1a). Where measurable, δ^{13}C-CH_4 values ranged from -79 to -91‰ (Fig. 4b), coinciding with the reported range of δ^{13}C-CH_4 due to hydrogenotrophic methanogenesis (Whiticar et al., 1986) (Fig. 7). This finding corroborates the *in situ* domination of the hydrogenotrophic pathway at deep peat layers of the same peatland (Dorodnikov et al., 2013). Interestingly, the measured "0" production under anaerobic conditions along with δ^{13}C-CO_2 depletion with depth (Fig. 4a) may reflect the AOM.

5 Conclusions

The CH_4, CO_2 production and δ^{13}C of CH_4, CO_2 and SOM before and after the addition of BES to peat soil at five depths (15-200 cm) below two contrasting microforms – naturally dry hummocks and wet hollows – revealed the following: (i) CH_4 production was significantly higher at hollows compared to hummocks but CO_2 production did not differ between microform types (Hypothesis I conditionally supported); (ii) production of CH_4 and CO_2 was significantly higher in the surface peat soil compared to deeper soil layers (Hypothesis II supported); (iii) overall higher contribution of hydrogenotrophic vs. acetoclastic methanogenesis corresponded to hollows as compared to hummocks (Hypothesis III supported).

Acknowledgements

Authors' are thankful to technical staff of the Department of Soil Science of Temperate Ecosystems - Anita Kriegel, the Department of Agricultural Science - Ingrid Ostermeyer, Karin Schmidt, Susann Enzmann and the Centre for Stable Isotope Research and Analysis (KOSI) - Dr. Jens Dyckmans and Reinhard Langel from the Georg-August University of Göttingen for invaluable help in laboratory work and instrumental measurements. The study was done with the support of the project "Stable

carbon composition of methane in Eurasian peatlands: CH_4 production, transport and oxidation" sponsored by German Research Foundation (Deutsche Forschung Gemeinschaft, DFG). Project number DFG DO 1533/1-1.

References

Alm, J., Saarnio, S., Nykänen, H., Silvola, J., Martikainen, P. J.: Winter CO_2, CH_4 and N_2O fluxes on some natural and drained boreal peatlands, Biogeochemistry, 44, 163-186, 1999.

Avery, G. B. Jr., Shannon, R. D., White, J. R., Martens, C. S., Alperin, M. J.: Effect of seasonal changes in the pathways of methane production on the $\delta^{13}C$ values of pore water methane in a Michigan peatland, Global Biogeochemical Cycles, 13, 475-484, 1999.

Baird, A. J., Belyea, L.R., Morris, P. J.: Up scaling of peatland-atmosphere fluxes of methane: small scale heterogeneity in process rates and the pitfalls of 'Bucket-and-Slab' models, Geophysical Monograph, 184, 37-53, 2009.

Becker, T., Kutzbach, L., Forbrich, I., Schneider, J., Jager, D., Thees, B., Wilmking, M.: Do we miss hotspots? The use of very high- resolution aerial photographs to quantify carbon fluxes in peatlands, Biogeosciences, 5, 1387-1393, 2008.

Beer, J., Lee, K., Whiticar, M., Blodau, C.: Geochemical controls on anaerobic organic matter decomposition in a northern peatland, Limnology and Oceanography, 53, 1393-1407, 2008.

Bodegom, P.M., Stams, A.J,M.: Effect of alternative electron acceptors and temperature on methanogenesis in rice paddy soils, Chemosphere, 39, 167-182, 1999.

Branham, J. E., Strack, M.: Saturated hydraulic conductivity in Sphagnum-dominated peatlands: do microforms matter? Hydrological Processes, 27, 4352-4362, 2014.

Bubier, J. L., Costello, A., Moore, T. R.: Microtopography and methane flux in boreal peatlands, northern Ontario, Canada, Canadian Journal of Botany, 71, 1056-1063, 1993.

Bubier, J. L., Moore, T. R., Juggings, S.: Predicting methane emission from bryophyte distribution in northern Canadian peatlands, Ecology, 76, 677-693, 1995.

Cervantes, F. J., de Bok, F. A. M., Duong-Dac, T., Stams, A. J. M., Lettinga, G., Field, J. A.: Reduction of humic substances by halorespiring, sulphate-reducing and methanogenic microorganisms, Environmental Microbiology, 4, 51-57, 2002.

Conrad, R.: Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and proposal, Organic Geochemistry, 36, 739-752, 2005.

Cord-Ruwisch, R., Seitz, H. J., Conrad, R.: The capacity of hydrogenothrophic anaerobic- bacteria to compete for traces ofhydrogen depends on the redox potential of the terminal elector acceptor, Archives of Microbiology, 149, 350-357, 1988.

Crill, P., Bartlett, K., Roulet, N.: Methane flux from boreal peatlands, Suo, 43, 173-182, 1993.

Dalva, M., Moore, T. R.: Methane and soil and plant community respiration from wetlands, Kejimkujik National Park, Nova Scotia: Measurements, predictions, and climatic change, 2001, Journal of Geophysical Research, 106, 2955-2962, 2001.

Dise, P., Verry, E.S.: Suppression of peatland methane emission by cumulative sulfate deposition in simulated acid rain, Biogeochemistry, 53, 143-160, 2001.

Dorodnikov, M., Knorr, K.-H., Kuzyakov, Y., Wilmking, M.: Plant-mediated CH_4 transport and contribution of photosynthates to methanogenesis at a boreal mire: a ^{14}C pulse-labeling study, Biogeosciences, 8, 2365-2375, 2011.

Dorodnikov, M., Marushchak, M., Biasi, C., Wilmking, M.: Effect of microtopography on isotopic composition of methane in porewater and efflux at a boreal peatland, Boreal Environmental Research, 18, 269-279, 2013.

Eriksson, T., Oquist, M.G., Nilsson, M.B.: Production and oxidation of methane in a boreal mire after a decade of increased temperature and nitrogen and sulfur deposition, Global Change Biology, 16, 2130-2144, 2010.

Gauci, V., Dise, N., Fowler, D.: Controls on suppression of methane flux from a peat bog subjected to simulated acid rain sulfate deposition, Global Biogeochemistry Cycles, 16, 1-12, 2002.

Glatzel, S., Basiliko, N., Moore, T.: Carbon dioxide and methane production potentials of peats from natural harvested and restored sites, Eastern Quebec – Canada, Wetlands, 24, 261-267, 2004.

Granberg, G., Sundh, I., Svensson, B. H., Nilsson, M.: Effects of temperature, nitrogen and sulfur deposition on methane emission from a boreal mire, Ecology, 82, 1982-1998, 2001.

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

Hornibrook, E. R. C., Longstaffe, F. J., Fyfe, W. S.: Spatial distribution of microbial methane production pathways in temperate zone wetland soils: Stable carbon and hydrogen isotope evidence, Geochimica et Cosmochimica acta, 61, 745-753, 1997.

IPCC: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp., 2014.

Jager, D. F., Wilmking M., Kukkonen, J. V. K.: The influence of summer seasonal extremes on dissolved organic carbon export from a boreal peatland catchment: Evidence from one dry and one wet growing season, Science of the total Environment, 407, 1373-1382, 2009.

Jarrel, K. F., Kalmokoff, M. L.: Nutritional requirements of the methanogenic archaebacterial, Canadian Journal of Microbiology, 34, 557-576, 1988.

Kettunen, A.: Connecting methane fluxes to vegetation cover and water table fluctuations at microsite level: A modeling study, Global Biochemical Cycles, 17, 1051, 2003.

Kluber, H. D., Conrad, R.: Effects of nitrate, nitrite, NO and N_2O on methanogenesis and other redox processes in anoxic rice field soil, Microbiology Ecology, 25, 301-318, 1998.

Kuzyakov, Y.: Sources of CO_2 efflux from soil and review of partitioning methods, Soil Biology & Biochemistry, 38, 425-448, 2006.

Lai, D. Y. F.: Methane dynamics in northern peatlands: A review, Pedosphere, 19, 409-421, 2009.

Limpens, J., Berendse, F., Blodau, F., Canadell, J.G., Freeman, C., Holden, J., Roulet, N., Rydin, H., Schaepman-Strub, G.: Peatlands and the carbon cycle: from local processes to global implications – a synthesis, Biogeosciences, 5, 1475-1491, 2008.

Lovley, D. R., Klug, M. J.: Sulfate reducers can out-compete methanogens at fresh-water sulfate concentrations, Applied and Environmental Microbiology, 45, 187-192, 1983.

Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, J.P., Woodward, J. C.: Humic substances as electron acceptors for microbial respiration, Nature, 382, 445-448, 1996.

Loftfield, N., Flessa, H., Augustin, J., Beese, F.: Automated gas chromatographic system for rapid analysis of the atmospheric trace gases methane, carbon dioxide and nitrous oxide, Journal of Environmental Quality, 26, 560-564, 1997.

Maljanen, M., Sigurdsson, B. D., Guðmundsson, J., Óskarsson, H., Huttunen, J. T., Martikainen, P. J.: Greenhouse gas balances of managed peatlands in the Nordic countries – present knowledge and gaps, Biogeosciences, 7, 2711–2738, 2010.

Moore, T. R., Knowles, R.: The influence of water table levels on methane and carbon dioxide emissions from peatland soils, Canadian Journal of Microbiology, 69, 33-38, 1989.

Moore, T. R., Roulet, N. T.: Methane flux: water table relations in northern wetlands, Geophysical Research Letters, 20, 587-590, 1993.

Moore, T. R., Dalva, M.: Methane and carbon dioxide exchange potentials of peat soils in aerobic and anaerobic laboratory incubations, Soil Biology and Biochemistry, 29, 1157-1164, 1997.

Morris, O. J., Waddington, J. M.: Groundwater residence time distributions in peatlands: Implications for peat decomposition and accumulation, Water Ressources Research, 47, W02511, 2011.

Müller, V., Blaut, M., Gottschalk, G.: Bioenergetics of methanogenesis, In: Ferry JG, editor, Methanogenesis: Ecology, Physiology, Biochemistry and Genetics, New York: Chapman & Hall, 360-406, 1993.

Pester, M., Knorr, K.-H., Friedrich, M. W., Wagner, M., Loy, A.: Sulfate-reducing microorganisms in wetlands – fameless actors in carbon cycling and climate change, Frontiers in Microbiology, 3, 72, 2012.

Popp, T. J., Chanton, J. P., Whiting, G. J., Grant, N.: Methane stable isotope distribution at a Carex dominated fen in north central Alberta, Global Biogeochemical Cycles, 13, 1063-1077, 1999.

Quinton, W. L., Hayashi, M., Carey, S. K.: Peat hydraulic conductivity in cold regions and its relation to pore size and geometry, Hydrological Processes, 22, 2829-2837, 2008.

Rubol, S., Silver, L., Bellin, A.: Hydrologic control on redox and nitrogen dynamics in a peatland soil, Science of the Total Environment, 432, 37-46, 2012.

Saarnio, S., Alm, J., Silvola, J., Lohila, A., Nykänen, H., Martikainen, P.J.: Seasonal variation in CH_4 emissions and production and oxidation potentials at microsites on an oligotrophic pine fen, Oecologica, 110, 414-422, 1997.

Schink, B.: Energetics of Syntrophic Cooperation in Methanogenic Degradation, Microbiology and Molecular Biology, 61, 262-280, 1997.

Schlesinger, W. H., Bernhart, E. S.: Biogeochemistry: an analysis of global change, 3rd edition, Wetland ecosystems, 233-274, Academic Press, USA, Oxford, UK, 2013.

Shen, C. F., Guiot, S. R.: Long term impact of dissolved O_2 on the activity of anaerobic granules, Biotechnology and Bioengineering, 49, 611-620, 1995.

Smemo, K. A., Yavitt, J. B.: Evidence for anaerobic CH_4 oxidation in freshwater peatlands, Geomicrobiology Journal, 24, 583-597, 2007.

Smemo, K. A., Yavitt, J. B.: Anaerobic oxidation of methane: an underappreciated aspect of methane cycling in peatland ecosystems?, Biogeosciences, 8, 779-793, 2011.

Steinmann, P., Eilrich, B., Leuenberger, M., Burns, S. J.: Stable carbon isotope composition and concentrations of CO_2 and CH_4 in deep catotelm of a peat bog, Geochimica et Cosmochimica acta, 72, 6015-6026, 2008.

Ström, L., Mastepanov, M., Christensen, R.: Species-specific effects of vascular plants on carbon turnover and methane emissions from wetlands, Biogeochemistry, 75, 65-82, 2005.

Sutton-Grier, A. E., Keller, J. K., Koch, R., Gilmour, C., Megonigal, J. K.: Electron donors and acceptors influence anaerobic soil organic matter mineralization in tidal marshe, Soil Biology & Biochemistry, 43, 1576-1583, 2011.

Svensson, B. H., Sundh, I.: Factors affecting methane production in peat soils, Suo, 43, 183-190, 1992.

Waddington, J.M., Roulet, N.T.: Groundwater flow and dissolved carbon movement in a boreal peatland, Journal of Hydrology, 191, 122-138, 1997.

Whiticar, M. J., Faber, E., Schoell, M.: Biogenic methane formation in marine and freshwater environments: CO_2 reduction vs. acetate fermentation - isotopic evidence, Geochimica et Cosmochimica acta, 50, 693-709, 1986.

Whiticar, M. J.: Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane, Chemical Geology, 161, 291-314, 1999.

Whiting, G. J., Chanton, J. P.: Primary production control of methane emission from wetlands, Nature, 364, 794-795, 1993.

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

Yavitt, J. B., Williams, C. J., Wieder, R. K.: Controls on microbial production of methane and carbon dioxide in three sphagnum-dominated peatland ecosystems as revealed by a reciprocal filed peat transplant study, Geomicrobiology Journal, 39, 194-204, 2000.

Yavitt, J. B., Seidman-Zager, M.: Methanogenic Conditions in Northern Peat Soils, Geomicrobiology Journal, 23, 119-127, 2006.

Zinder, S. H., Anguish, T., Cardwell, S. C.: Selective-inhibition by 2-Bromoethane-sulfonate of methanogenesis from acetate in a thermophilic anaerobic digester, Applied and Environmental Microbiology, 47, 1343-1345, 1984.

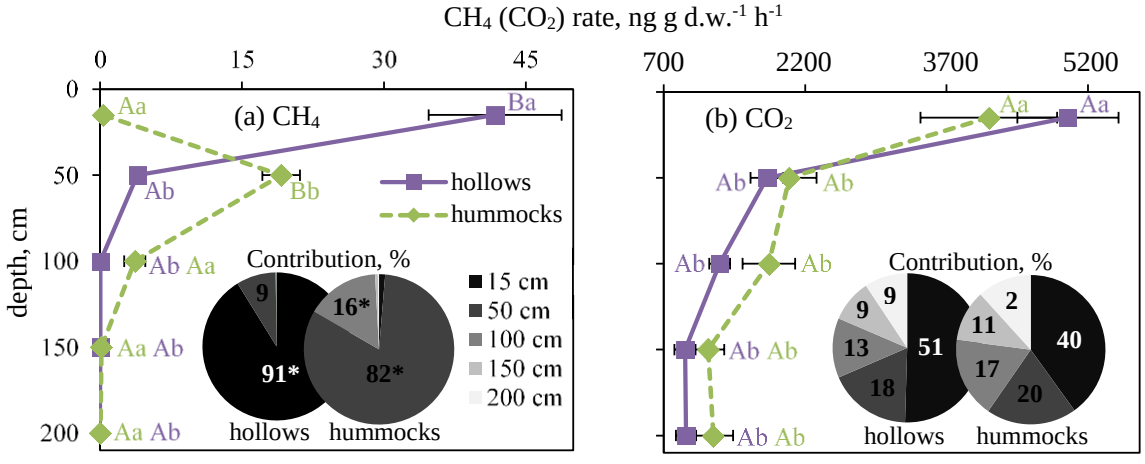

Figure 1: Mean CH_4 (A) and CO_2 (B) production rate for hollows (purple) and hummocks (green) at depths of 15, 50, 100, 150 and 200 cm without addition of the methanogenesis inhibitor (BES). The same letters show absence of significant differences ($P<0.05$) between microforms of the same depth (uppercase letters) and between five depths within the same microform (lowercase letters). The integrated pie charts show the distribution (in %) of the overall CH_4 and CO_2 production from all depths of hollows and hummocks. The contribution of depths below 100 cm to overall CH_4 production in hollows was < 0.3%. In hummocks, depths 15, 150 and 200 cm comprised 1.3, 0.6 and 0.2% of overall CH_4, respectively. Asterisk: significant difference ($P<0.05$) between the two microforms within the same depth.

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

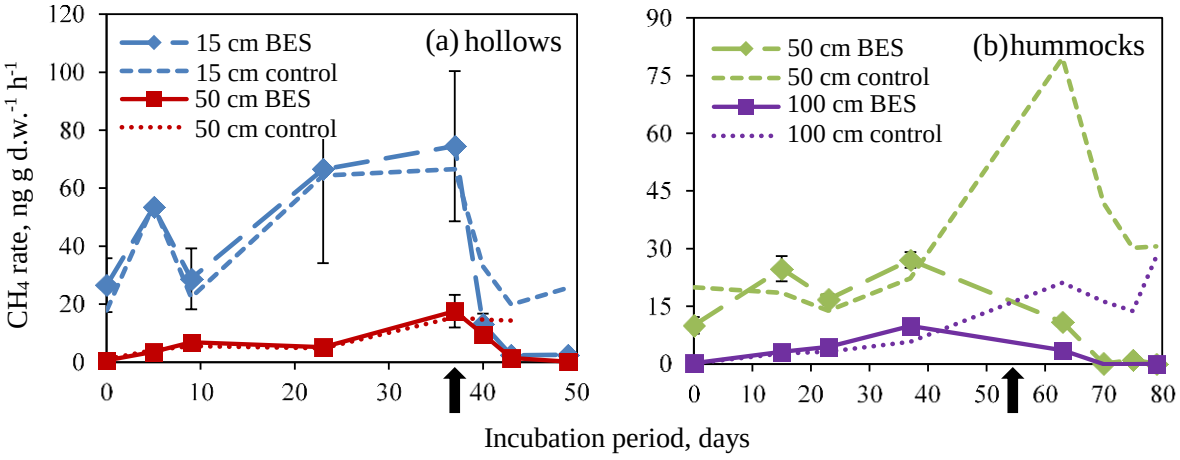

Figure 2: CH_4 production rate at two depths in (A) hollows (15 and 50 cm) and two depths in (B) hummocks (50 and 100 cm), where the effect of the methanogenic inhibitor BES was the most pronounced. Black arrow: date of BES addition. Dashed and dotted lines correspond to control soil with milli-Q water addition.

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

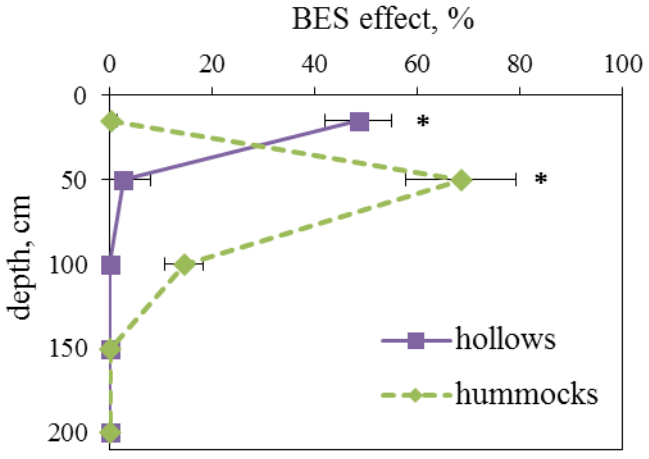

Figure 3: BES suppression (in %) of the CH_4 production rate for hollows (purple) and hummocks (green) down to 200 cm depth. The effect was calculated as the difference of the mean CH_4 production rate before and after adding BES. Changes in respective control treatments before and after the addition of milli-Q water were subtracted from the treatment effect. BES treatments of each microform were "weighted" against each depth according to their contribution to overall CH_4 production. Asterisk: significant effects ($P<0.05$).

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

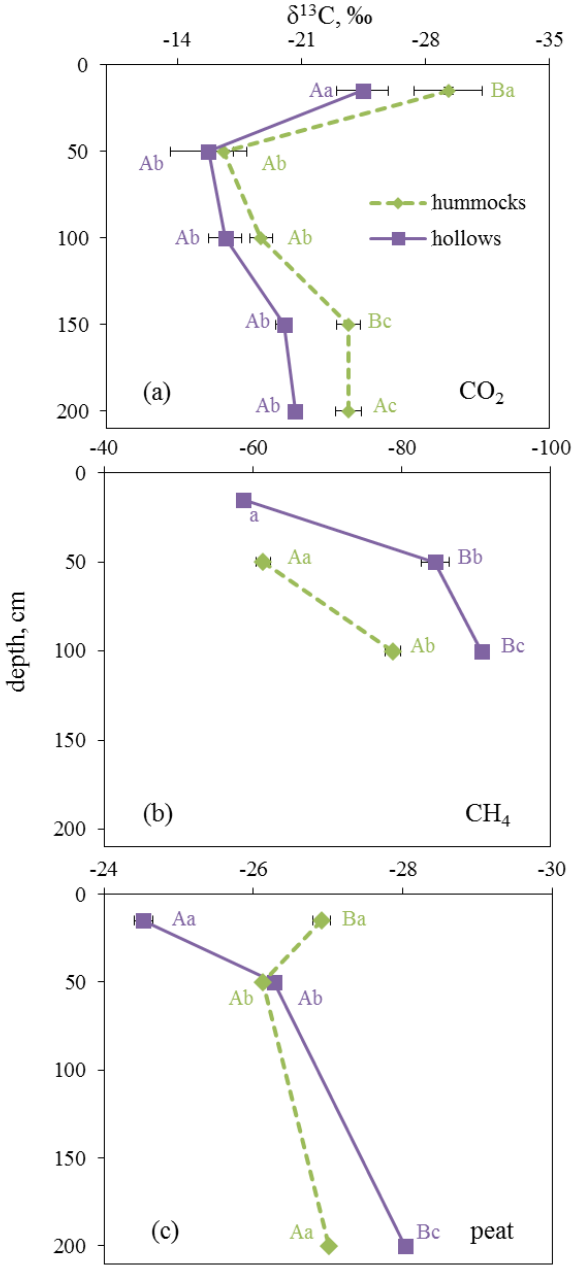

Figure 4: Delta (δ) ^{13}C of CO_2 (A), CH_4 (B) and peat soil organic matter (C) depending on depths of hollows and hummocks. Same letters: no significant differences ($P<0.05$) between microforms of the same depth (uppercase letters) and between five depths within the same microform (lowercase letters).

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

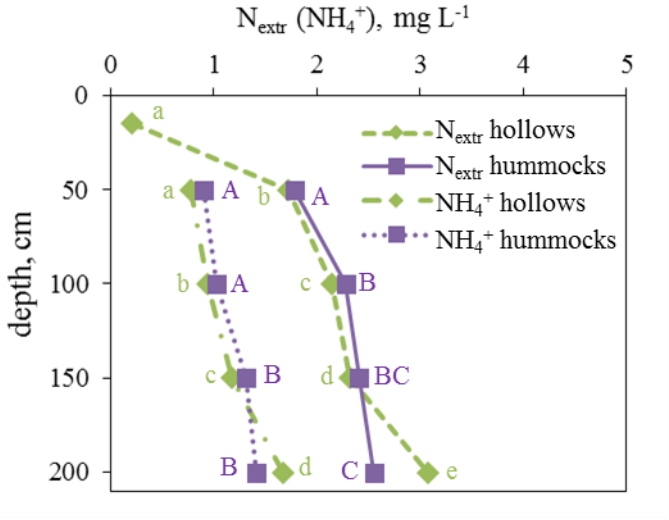

Figure 5: Concentration of total extractable nitrogen (N_{extr}) and ammonium (NH_4^+) for hollows (purple) and hummocks (green) down to 200 cm. Same letters: no significant differences ($P<0.05$) between depths of hummocks (uppercase letters) and hollows (lowercase letters) at $P<0.05$. No significant difference was observed between microforms at each single depth layer.

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

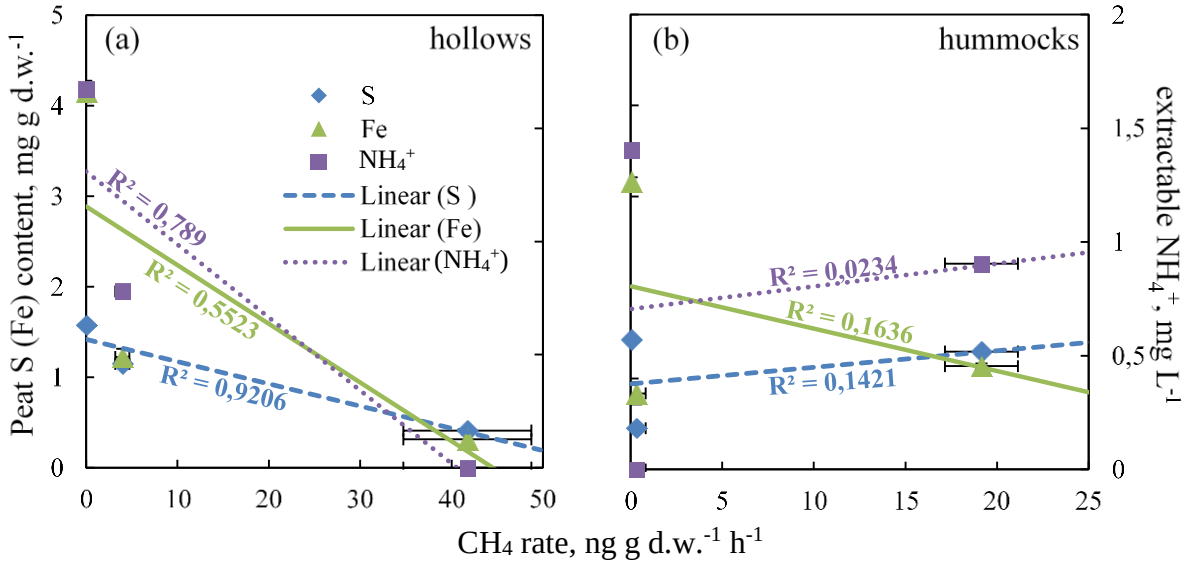

Figure 6: Relationship between mean CH_4 production rate (without inhibitor BES) and concentrations of total peat sulfur (S, blue), iron (Fe, green) in mg g d.w.$^{-1}$ (left y-axis) and extractable ammonium (purple) in mg L^{-1} (right y-axis) for 15, 50 and 200 cm depths below hollows (A) and hummocks (B).

Biogeosciences Discuss., doi:10.5194/bg-2016-162, 2016
Manuscript under review for journal Biogeosciences
Published: 30 May 2016

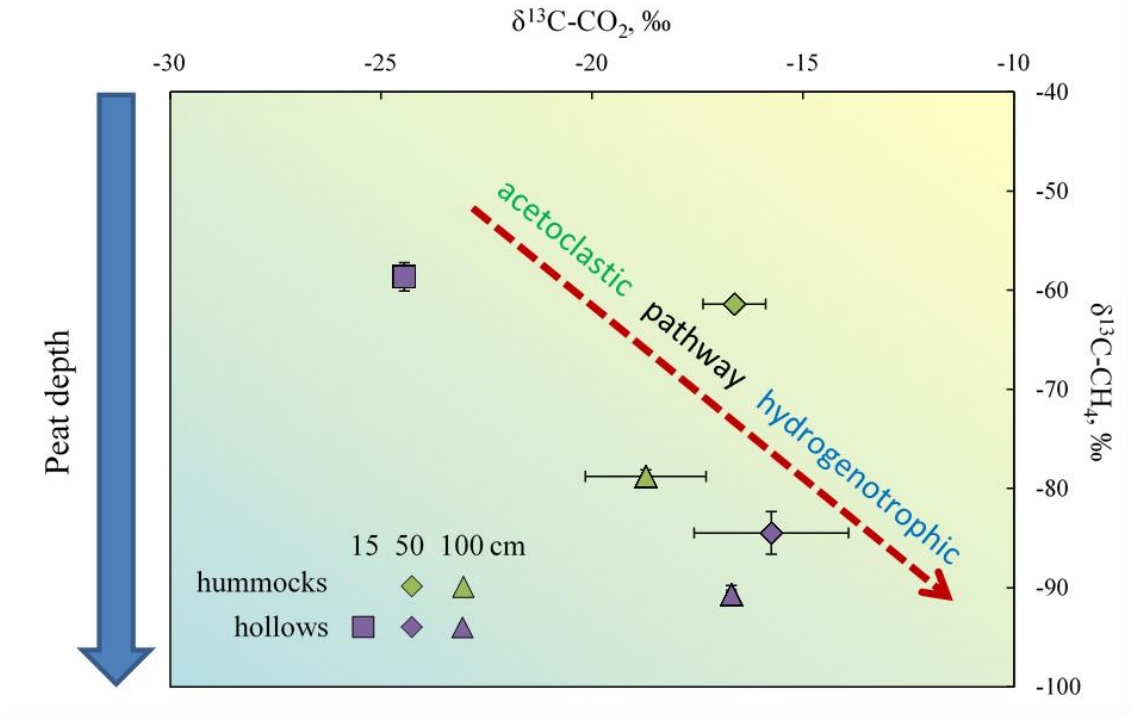

Figure 7: Cross-plot of $\delta^{13}C$ of CH_4 and CO_2 (+- SE) demonstrating the shift in methanogenesis (red dashed arrow) from acetoclastic to hydrogenotrophic pathway with peat depth below hummocks (green) and hollows (purple). Depths where both parameters were measurable are shown (15, 50 and 100 cm). Background color reflects the gradient in $\delta^{13}C$ from the lowest (blueish, left bottom corner) to the highest (yellowish, upper right corner) values.