

Effect of microtopography on isotopic composition of methane in porewater and efflux at a boreal peatland

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The application of stable isotopes is an approach to identify pathways of methanogenesis, methane (CH₄) oxidation and transport in peatlands. We measured the stable C isotopic characteristics ($\delta^{13}\text{C}$) of CH₄ in peat profiles below hummocks, lawns and hollows of a Finnish mire to study the patterns of CH₄ turnover. Porewater CH₄ concentrations ([CH₄]; at 0.5–2 m) increased with depth below all microforms. Emissions of CH₄ from hummocks were the lowest, and increased with the increasing water-saturated zone, being ~10 times higher from hollows. Thus, the microtopography of the peatland did not affect the porewater [CH₄] in the water-saturated part of the peat profile, but the CH₄ emissions were affected due to differences in the oxidative potential of the microforms. There was a decrease in $\delta^{13}\text{C}\text{-CH}_4$ with depth below all microforms indicating dominance of CO₂-reduction over acetate cleavage pathway of methanogenesis at deep peat layers. However, estimated potential portions of transported CH₄ comprised 50%–70% of the $\delta^{13}\text{C}\text{-CH}_4$ enrichment on microforms at the 0.5-m depth, hereby masking the acetate cleavage pathway of methanogenesis. Stable C composition ($\delta^{13}\text{C}$) of CH₄ proved to be a suitable (but not sufficient) tool to differentiate between types of methanogenesis in continuously water-saturated layers below microforms of a peatland. Combined flux-based and multi-isotopic approaches are needed to better understand the CH₄ turnover process.

Introduction

Boreal peatlands represent about 15% of the total storage of terrestrial carbon (C) (Turunen *et al.* 2002) and are substantial contributor (30%) of methane (CH₄) — an important greenhouse gas (IPCC 2007) — to the atmosphere (Reeburgh *et al.* 1998). Understanding the processes of C cycling in boreal peatlands is thus critical

for estimating current and future global CH₄ budgets.

Generally, C cycling in peatlands is controlled by a number of natural parameters, which results in high heterogeneity of CH₄ fluxes. In addition to the well-studied controls of CH₄ fluxes in peatlands, such as water-table position, peat temperature and substrate quality (reviewed in Lai 2009), identification of the C substrates

for CH₄ production also plays a key role for understanding spatial and temporal variations of CH₄ fluxes.

Application of stable isotopes is an approach to identify the pathway by which CH₄ is formed (Whiticar 1999, Conrad 2005). Methane produced by acetate cleavage (acetoclastic pathway) is not as depleted in ¹³C as CH₄ produced from CO₂ reduction with H₂ (hydrogenotrophic pathway) (Whiticar *et al.* 1986). Based on vertical profiles of CH₄ stable isotope ratios in peat, it was shown that the upper peat profile of wetlands was dominated by acetoclastic and the lower profile by hydrogenotrophic methanogenesis (Hornibrook *et al.* 1997, Popp *et al.* 1999). Knowledge about the contribution of different methanogenic pathways to the total CH₄ production within a peat profile will help to identify the pattern of decomposition of fresh *vs.* old C, thus, the fate of C pools with rapid turnover time *vs.* long term C pools (Beer & Blodau 2007). Enrichment of ¹³C in CH₄ in the upper peat profile is also caused by methanotrophic activity, as methanotrophic microbes discriminate strongly against ¹³C (Whiticar 1999, De Visscher *et al.* 2004). Transport of CH₄ either mediated by plants or due to simple diffusion through the peat profile also preferentially removes ¹²C-CH₄ from the soil. This fractionation depends on a transport mechanism, water-table level, time of day, and season (Popp *et al.* 1999, De Visscher *et al.* 2004, Chanton *et al.* 2005).

Whereas some information exists about seasonal and vertical changes in isotopic composition of CH₄ in peat profiles (Avery *et al.* 1999, Steinmann *et al.* 2008), there is a lack of information about the effect of the peatland microtopography on the patterns of CH₄ isotopic signatures. The surface of a (boreal) peatland can be differentiated into microscale subunits, so called microforms (hummocks, lawns, hollows), according to hydrological characteristics (water table level) and main vegetation communities (Becker *et al.* 2008). In turn, plant communities, especially bryophytes, are good predictors of CH₄ flux, and vascular vegetation may play an active and passive role in promoting CH₄ emission (Bubier *et al.* 1995). Carbon compounds exuded from plant roots can act as labile substrates which enhance methanogen-

esis, and vascular plants may act as a conduit for CH₄ from anaerobic zone of a wetland to the atmosphere bypassing oxidation in the aerobic zone (reviewed by Lai 2009). Water-table levels vary between microforms increasing in the order hummocks–lawns–hollows, thus resulting in differences in thickness of the oxidative zone and, hence, in CH₄ fluxes. Studies utilizing chamber technique to measure CH₄ emissions generally show the lowest CH₄ fluxes at hummocks and the highest at hollows (Dalva *et al.* 2001, Johansson *et al.* 2006, Forbrich *et al.* 2010). However, the processes involved in methanogenesis in deeper layers below different microforms have not been well studied.

In the current study, we aimed to identify the CH₄ production pathways at hummocks, lawns and hollows of a minerogenic, oligotrophic low-sedge pine peatland in Finland. We used stable C isotopic characteristics ($\delta^{13}\text{C}$) of CH₄ to differentiate between types of methanogenesis in continuously water-saturated layers below microforms of the peatland and attempted to follow CH₄ throughout the peat profile up to the atmosphere. The research questions of the study were: (i) how do [CH₄] and $\delta^{13}\text{C}\text{-CH}_4$ at the three microform types change from the below-ground water-saturated peat layers to CH₄ emitted to the atmosphere and (ii) how does microtopography of the peatland affect the CH₄ turnover processes? Based on the research questions we put forward the following hypothesis: contribution of hydrogenotrophic *vs.* acetoclastic type of methanogenesis should increase with depth and differ between microforms due to differences in plant communities and water table depth at hummocks, lawns and hollows.

Material and methods

Experimental site

The study was conducted on a natural minerogenic, oligotrophic low-sedge pine fen Salmisuo in eastern Finland, located in the North Karelian Biosphere Reserve (62°47'N, 30°56'E). The site is described in detail elsewhere (Saarnio *et al.* 1997, Alm *et al.* 1999, Becker *et al.* 2008, Jager *et al.* 2009). The surface of the peatland was sub-

divided into three main microform types according to vegetation communities and moisture conditions: dry and elevated hummocks (dominating plants *Eriophorum vaginatum*, *Pinus sylvestris*, *Andromeda polifolia*, *Sphagnum fuscum*), intermediate lawns (*Eriophorum vaginatum*, *Sphagnum balticum*, *Sphagnum papillosum*), and wet hollows (*Scheuchzeria palustris*, *Sphagnum balticum*). During the study period, the depth of the water table was -23 ± 5 cm from the surface of hummocks, -5 ± 2 cm from the surface of lawns and 0 ± 2 cm on hollows.

Porewater CH_4 collection and CH_4 flux measurements

The CH_4 sampling campaign was carried out between 1 and 20 July 2009. During that time span weather conditions were moderately humid with 26 mm of precipitation and the average daily temperature of 18.1 °C. Because of these weather conditions, no substantial water-table-level fluctuations were observed at the experimental site.

Porewater CH_4 was sampled *in situ* using modified diffusion chambers (“peepers”; Steinmann and Shotyk 1996). A diffusion chamber consisted of a polypropylene centrifuge tube (Rotilabo Eco 50 ml, 30 × 115 mm) with a cutout window (20 × 65 mm) and a polyethersulfone membrane filter (Sterlitech Corp., WA, USA) tightly sealed over the window by melted polyethylene (Steinel Vertrieb GmbH, Germany). Chosen materials of the chamber were inert to chemical composition of peatland water and resistant to microbial activity, thus minimizing potential influence on porewater [CH_4] and its isotopic composition. Pore diameter of the membrane filter (0.2 μm) allowed ions and dissolved gases to enter the inner volume of a chamber, but prevented penetration of microorganisms and fine roots (Steinmann and Shotyk 1996). Prior to installation on the peatland, each chamber with closed cap was tested for watertightness. Diffusion chambers (76 units) pre-filled with deionized water were inserted below the microforms (8 hummocks, 7 lawns, 5 hollows) at depths of 0.5, 1.0, 1.5 and 2.0 m. All installed chambers were allowed to equilibrate for 20 days. Pre-

liminary tests showed that this amount of time was sufficient for equilibration of CH_4 in diffusion chambers with the surrounding environment (data not shown). To install chambers into the peat below the microforms, polypropylene tubes (diameter 40 mm, wall thickness 2 mm) were used. Separate tubes were used for each of the four depths studied. Each installation tube was closed with a cap from the bottom preventing peat from filling the tube during installation. A side of a tube was perforated about 15 cm from the bottom in order to allow free movement of water through the tube. Tubes were vertically inserted into the peat down to the depths studied and left for two days prior to installation of the diffusion chambers in order to allow the peat to recover from the disturbance. After 20 days of porewater CH_4 equilibration, ca. 30 ml aliquot of the water in diffusion chambers was transferred with a syringe to glass bottles (100 ml; flushed with N_2 and prevacuated) at the site, and transported to the laboratory for subsequent measurements. All the chambers including those at the shallowest 0.5-m depth under hummocks were permanently under water during the equilibration period.

Measurements of CH_4 efflux to the atmosphere were performed using the closed chamber technique (Forbrich *et al.* 2010). Namely, aluminium chambers with the size 600 × 600 × 320 mm were employed. The chambers were equipped with a vent tube and a fan to allow for air mixing inside the chamber. Chamber fluxes were measured from previously installed frames at the same sampling plots, where porewater CH_4 was collected. Five air samples were taken with 60-ml syringes for determination of CH_4 flux at even intervals during closure time of 20 min. The 5th sample was taken in duplicate for $^{13}\text{C}\text{-CH}_4$ measurements. During the 20 days porewater sampling period, chamber CH_4 fluxes were measured four times at each sampling plot.

Methane concentrations in chamber flux samples were analyzed within one day from sampling with a gas chromatograph (Shimadzu 14-A) equipped with a flame ionisation detector. Two repeated measurements were made from each gas sample. Porewater [CH_4] was measured from the headspace of 100 ml sample bottles (3 replicates of 1 ml) on the same chromatograph

but using a calibration standard with a higher CH₄ concentration (50 ppm instead of 3 ppm for chamber flux samples).

Stable isotopic analysis of C-CH₄

The samples of porewater and emitted CH₄ were injected with over pressure to 35 ml Wheaton glass vials equipped with rubber septa. The vials were further sealed with hot-melt glue for storage until the stable isotopes of C-CH₄ were analyzed at the Department of Environmental Science, University of Eastern Finland, Kuopio. Porewater samples with high [CH₄] were diluted with 99.999% N₂ to reach concentrations suitable for the analysis (no such dilution was needed for chamber CH₄ flux samples). The ¹³C/¹²C ratios were determined with an isotope ratio mass spectrometer (Delta plus XP; Thermo, Bremen, Germany) interfaced with a gas chromatograph (Trace GC Ultra, Finnigan) by a continuous flow system (Conflo III; Thermo Finnigan Germany; GC/C/IRMS) as described in Kankaala *et al.* (2007).

Calculations and statistics

Porewater [CH₄] were recalculated into μmol l⁻¹, and above-ground CH₄ flux is given in mg m⁻² h⁻¹. The natural ¹³C/¹²C ratio in CH₄ was expressed in δ¹³C per mil PDB (‰):

$$\delta^{13}\text{C} (\text{‰}) = [(R_{\text{sample}}/R_{\text{PDB}}) - 1] \times 1000, \quad (1)$$

where R_{sample} is the isotopic ratio ¹³C/¹²C of CH₄ in the sample, and R_{PDB} is the isotopic ratio of Pee Dee Belemnite as the standard for C.

Stable C isotopic composition (δ¹³C) of emitted CH₄ was calculated according to Krüger *et al.* (2002) by applying a correction for the contribution of the isotopic composition of atmospheric CH₄ present at the time of the chamber closure. An initial [CH₄] of 2.06 ± 0.2 ppm (atmospheric value from eddy measurements at the same site; I. Forbrich, unpubl. data) and an initial δ¹³C-CH₄ of -44.93‰ ± 1.98‰ (atmospheric value from a littoral wetland in the same region; N. Welti

unpubl. data) were used in the calculations. Because of very low emission rates of CH₄ at hummocks, it was not possible to estimate the respective δ¹³C-CH₄ values in the fluxes above hummocks.

The differences in porewater [CH₄], CH₄ fluxes and δ¹³C-CH₄ values between microforms and depths were evaluated with two-way ANOVA and Fischer's LSD test using STATISTICA 7.0 (StatSoft, USA). Prior to testing, all the data were checked for normality (Kholmogorov-Smirnov test) and homogeneity (Levene's test). The variables were treated as independent for all depths below a microform type and a certain depth between microforms.

A simple model for isotopic fractionation was used to assess the potential effect of CH₄ oxidation and transport on shifts in δ¹³C-CH₄ across the peat profile for current experimental data (adapted from Liptay *et al.* 1998). The larger the estimated portion of CH₄ transported and/or oxidized, the weaker evidence for methanogenic pathway is provided by measured δ¹³C-CH₄ values.

The portion of CH₄ transported in a peat profile was calculated using the following equation:

$$|f_{\text{tr}}| = (\delta_{n+1} - \delta_n) / [(\alpha_{\text{tr}} - 1) \times 10], \quad (2)$$

where f_{tr} is the portion (%) of transported CH₄, δ_n and δ_{n+1} are the δ¹³C values of CH₄ in lower- and upper-laying peat horizons, respectively, and α_{tr} is the isotopic fractionation associated with gas transport ($\alpha_{\text{tr}} = 1.0178$ from De Visscher *et al.* 2004). It has to be noted, that the gas diffusion, in theory, should result in the enrichment of CH₄ of the n layer as compared with the $n + 1$ layer (towards which the "lighter" CH₄ is diffused). Therefore, negative $|f_{\text{tr}}|$ values are acceptable, which, in turn, may indicate the direction of gas diffusion. For example, f_{tr} between the depths of 2.0 and 1.5 m under hollows is calculated as follows: $\delta_{2.0\text{ m}} = -69.2\text{‰}$ and $\delta_{1.5\text{ m}} = -70.1\text{‰}$, hence $|f_{\text{tr}}| = [(-70.1) - (-69.2)] / [(1.0178 - 1) \times 10] = 5\%$.

The portion of CH₄ oxidized in the aerobic surface layer (within 0.5–0 m) and in the water-saturated rooted zone of aerenchymatic plants (0.5–1.0 m) of peatland was calculated using the

following equation:

$$f_{\text{ox}} = (\delta_n - \delta_{n+1}) / [(\alpha_{\text{ox}} - 1) \times 10], \quad (3)$$

where f_{ox} is the portion (%) of oxidized CH_4 , δ_n and δ_{n+1} are the $\delta^{13}\text{C}$ values of CH_4 from the porewater horizon 0.5 m and CH_4 emission, and 0.5 m and 1.0 m peat horizons, respectively; α_{ox} is the fractionation factor accounting for the preference of methanotrophic microbes for CH_4 containing the lighter C isotope ($\alpha_{\text{ox}} = 1.022$ from Coleman *et al.* 1981, Liptay *et al.* 1998).

For the f_{tr} calculations we assumed that CH_4 transport but not oxidation ($\alpha_{\text{ox}} = 1$) had a predominant effect on $\delta^{13}\text{C}\text{-CH}_4$ in the deep water-saturated horizons (from 2 m to 1.0 m depth). Oxidation mostly affected $\delta^{13}\text{C}$ in the surface peat (0–0.5 m) and, hence, the emitted $\delta^{13}\text{C}\text{-CH}_4$ above the peat surface ($\alpha_{\text{tr}} = 1$), whereas between the depths of 0.5 and 1.0 m, both CH_4 transport and oxidation in a rooted zone of aerenchymatic plants are equally important for the $\delta^{13}\text{C}\text{-CH}_4$ values ($f_{\text{tr}} + f_{\text{ox}}$). The $\delta^{13}\text{C}\text{-CH}_4$ at 2-m depth was assumed to be unaffected by diffusion, since lateral advection was reported to have a negligible effect on $\delta^{13}\text{C}\text{-CH}_4$ (Chanton *et al.* 2002).

Results

Porewater $[\text{CH}_4]$ and above-ground CH_4 fluxes

Porewater $[\text{CH}_4]$ increased with depth down to 1.5 m below all microforms (Fig. 1). At the depth of 2.0 m, however, porewater $[\text{CH}_4]$ did not differ below hummocks and lawns, but was significantly lower below hollows and as compared with the 1.5-m depth (Fig. 1 and Appendix 1). Type of microform (hummocks vs. lawns vs. hollows) had no statistically significant effect on porewater $[\text{CH}_4]$ at any of the depths studied (Fig. 1 and Appendix 3).

Fluxes of CH_4 decreased in the order hollows \geq lawns $>$ hummocks and did not differ significantly among measurement days (Table 1). The lowest fluxes were $0.4 \text{ mg CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ at hummocks and the highest $6.0 \text{ mg CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ at hollows (Table 1). Because there were no sig-

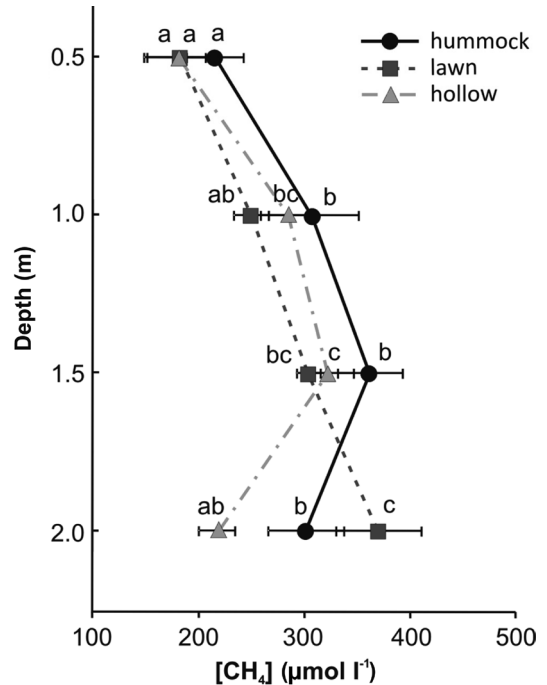


Fig. 1. Porewater CH_4 concentrations ($\mu\text{mol l}^{-1}$) at different depths below hummocks ($n = 8$), lawns ($n = 7$) and hollows ($n = 5$). Error bars show standard errors. Values followed by the same letters are not significantly different (at $p \leq 0.05$ according to two-way ANOVA and Fischer's LSD test) between depths below each type of a microform. There were no significant differences between types of a microform for any depth horizon.

nificant differences between measurement days, the average of four CH_4 flux values could be related to porewater $[\text{CH}_4]$ equilibrated during 20 days of the field campaign. This was especially important for comparison of stable $^{13}\text{C}/^{12}\text{C}$ isotope ratio in porewater and emitted CH_4 .

Stable $^{13}\text{C}/^{12}\text{C}$ isotope ratios in porewater and emitted CH_4

Generally, there was an overall decrease of porewater $\delta^{13}\text{C}\text{-CH}_4$ values with depth below all microforms (Fig. 2). However, no significant differences in $\delta^{13}\text{C}\text{-CH}_4$ were found below 1 m under any microform (Fig. 2). Porewater at the shallowest depth (0.5 m) was enriched in $\delta^{13}\text{C}\text{-CH}_4$ (-62.5‰ to -64‰) as compared

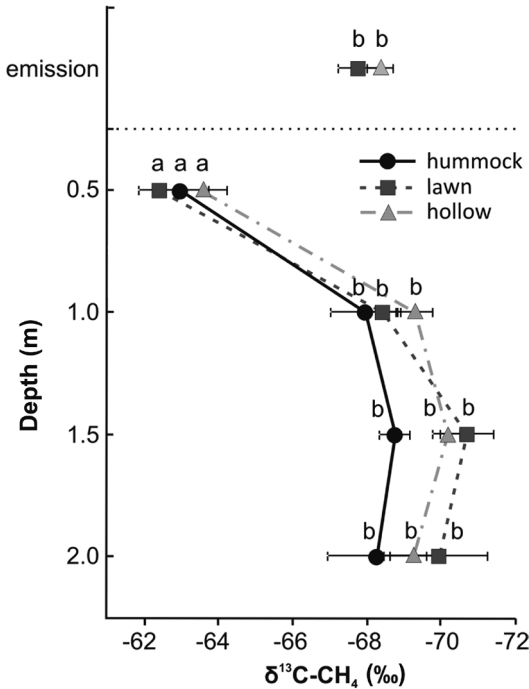


Fig. 2. Porewater $\delta^{13}\text{C-CH}_4$ values \pm SE (‰) at different depths below hummocks ($n = 8$), lawns ($n = 7$), hollows ($n = 5$) and $\delta^{13}\text{C-CH}_4$ values (‰) in emission from the surface of lawns ($n = 7$) and hollows ($n = 5$). The $\delta^{13}\text{C-CH}_4$ values in emission from hummocks could not be determined because of low CH_4 efflux. Values followed by the same letters are not significantly (at $p \leq 0.05$ according to two-way ANOVA and Fischer's LSD test) different between depths below each type of a microform and emission (where possible). There were no significant differences between types of a microform for a depth horizon and in emission.

with porewater the deeper peat layers (up to -71%). There were no significant differences in $\delta^{13}\text{C-CH}_4$ among microforms at any of the studied depths (Fig. 2 and Appendix 2)

CH_4 emitted from the lawns and hollows was significantly more depleted in ^{13}C (-68% to -69%) than porewater CH_4 at the 0.5 m depth (Appendix 2) but the values were in the same range as $\delta^{13}\text{C-CH}_4$ values at the other depths (Fig. 2). For hummocks, $\delta^{13}\text{C-CH}_4$ values in CH_4 emission are not shown due to low CH_4 fluxes, which made it impossible to differentiate between $\delta^{13}\text{C}$ in CH_4 efflux and ambient atmospheric $\delta^{13}\text{C-CH}_4$. Where measurable, $\delta^{13}\text{C}$ values in emitted CH_4 were not significantly affected by the type of microforms (Fig. 2).

Assessed portions of transported (f_{tr}) and oxidized (f_{ox}) CH_4

The calculated f_{tr} was the smallest at the deep peat horizons (1.0–2.0 m) comprising 3%–5% (Fig. 3). The intermediate peat horizon (0.5–1.0 m) was the most affected by the processes of CH_4 transport and oxidation, since $f_{tr} + f_{ox}$ ranged between 51% and 68% under hummocks and lawns-hollows, respectively (Fig. 3 and Appendix 4). In contrast to porewater CH_4 , the estimation of f_{ox} in CH_4 emitted from lawns and hollows provided negative (unreliable) values due to higher depletion of $\delta^{13}\text{C-CH}_4$ in efflux as compared with $\delta^{13}\text{C-CH}_4$ in porewater $\delta^{13}\text{C-CH}_4$ at 0.5 m (Fig. 3). At hummocks, it was not possible to estimate f_{ox} because of unreliable $\delta^{13}\text{C}$ values measured in emitted CH_4 .

Discussion

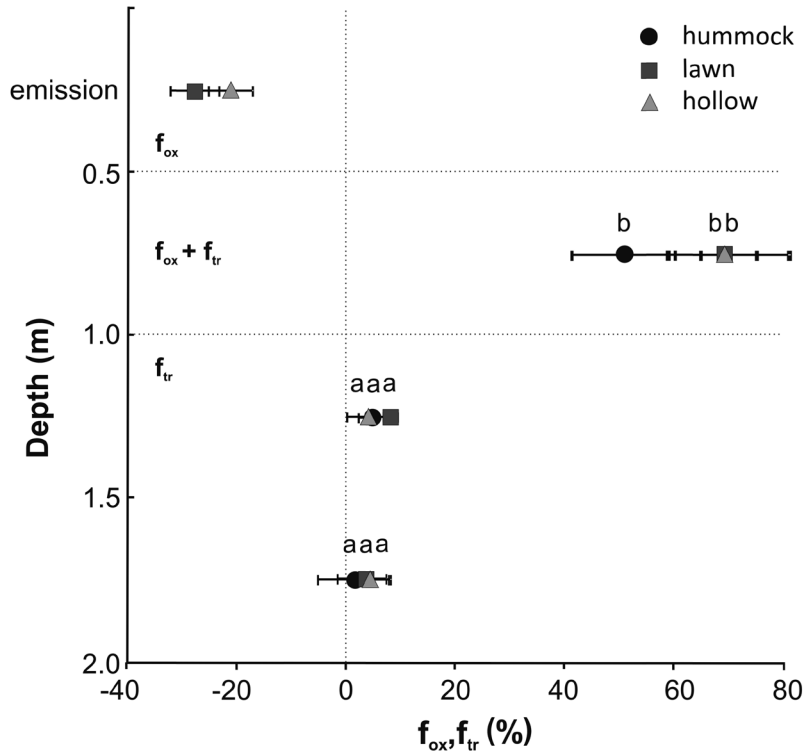
Porewater $[\text{CH}_4]$ and above-ground CH_4 fluxes: effect of microtopography

The overall increase in porewater CH_4 with depth at the studied boreal mire complex is in agreement with the results of many other stud-

Table 1. Above-ground CH_4 fluxes from hummocks ($n = 8$), lawns ($n = 7$) and hollows ($n = 5$) during 20 days of the field campaign (1–20 July 2009). Same letters indicate no significant differences (at $p \leq 0.05$ according to two-way ANOVA and Fischer's LSD test) between the types of microsites on one sampling date. There were no significant differences among dates of sampling for each type of microsite.

Microsite type	Day of sampling	Flux \pm SE (mg m ⁻² h ⁻¹)
Hummock	5	0.40 \pm 0.09 ^a
	9	0.45 \pm 0.15 ^a
	13	0.40 \pm 0.08 ^a
	17	0.42 \pm 0.07 ^a
Lawn	5	3.69 \pm 0.62 ^b
	9	3.43 \pm 0.59 ^b
	13	3.79 \pm 0.41 ^b
	17	4.53 \pm 0.64 ^b
Hollow	5	5.10 \pm 0.69 ^b
	9	4.95 \pm 0.87 ^b
	13	5.05 \pm 0.65 ^b
	17	5.97 \pm 0.87 ^b

Fig. 3. Transported (f_{tr}) and oxidized (f_{ox}) CH_4 below hummocks ($n = 8$), lawns ($n = 7$) and hollows ($n = 5$). f_{ox} is not available for hummocks because of low CH_4 efflux. Error bars show standard errors. Values followed by the same letters are not significantly different (at $p \leq 0.05$ according to two-way ANOVA and Fischer's LSD test) between depths below each type of a microform. There were no significant differences between microform types. Portions f_{tr} and f_{ox} were calculated based on respective $\delta^{13}\text{C}\text{-CH}_4$ values (Fig. 2) using Eqs. 2 and 3.



ies (Hornibrook *et al.* 1997, Chasar *et al.* 2000, Steinmann *et al.* 2008). Along with this, a threshold of 1.0 m existed, below which $[\text{CH}_4]$ was not significantly different (Fig. 1). Since the deeper *vs.* upper peat layers have lower hydraulic conductivity and less temperature fluctuations, and typically remain anoxic year round (Hornibrook *et al.* 1997), the CH_4 production there is sustained. Seemingly, such homogeneity of deeper peat layers revealed no effect of the type of a microform (hummocks, lawns, hollows) on the $[\text{CH}_4]$ (Fig. 1 and Appendix 1). However, the current results are preliminary and longer observations are required to reveal the possible effect of microtopography of a peatland on the below-ground $[\text{CH}_4]$ dynamics.

In contrast to porewater $[\text{CH}_4]$, CH_4 emitted from the surface was affected by microtopography: the lowest fluxes were found at the elevated hummocks and the highest at the water-saturated hollows, while intermediate fluxes of CH_4 were measured at the lawns (Table 1 and Appendix 3). The current results are consistent with those reported earlier for the same site (Saarnio *et al.* 1997, Forbrich *et al.* 2010) and those from other

observations (Dalva *et al.* 2001, Johansson *et al.* 2006) and can be explained by the increase of the oxidation layer in the order hollows < lawns < hummocks.

Isotopic evidence on CH_4 production, transport and oxidation

Porewater CH_4 below 1-m depth was significantly more depleted in ^{13}C as compared with the shallowest 0.5-m depth (Fig. 2 and Appendix 1). Still, similarly to $[\text{CH}_4]$, $\delta^{13}\text{C}\text{-CH}_4$ values did not differ significantly among microforms (and Appendix 2). Although rather few studies exist on $\delta^{13}\text{C}\text{-CH}_4$ in peat layers below 1 m depth, our data are in a good agreement with most of the reported results from *Typha*-dominated fen in Canada (Hornibrook *et al.* 1997), littoral wetlands in the United States (Chasar *et al.* 2000) and acidic *Sphagnum* bog in Switzerland (Steinmann *et al.* 2008).

Overall depletion of ^{13}C in CH_4 with depth suggests increased contribution of hydrogenotrophic or CO_2 reduction pathway to the total methanogenesis (Whiticar *et al.* 1986, Horni-

brook *et al.* 1997, Conrad 2005), as the discrimination of methanogenic microbes against heavier ^{13}C is stronger during the hydrogenotrophic pathway as compared to the acetoclastic pathway (methanogenesis due to splitting of fermentation-derived acetic acid/acetates). Thus, the hydrogenotrophic pathway of methanogenesis at the depths below 1.0 m was sustained for the entire experimental site and was not affected — at least in the short-term — by the microtopography of the studied peatland.

It is important to note, that $\delta^{13}\text{C}-\text{CH}_4$ values of the upper peat horizon (above 1.0 m) of the studied peatland could have been substantially affected by the processes of CH_4 transport and oxidation (Popp *et al.* 1999, Whiticar 1999, Chanton *et al.* 2005). This limits our possibilities to draw conclusions about the importance of the different methanogenic pathways for the total CH_4 production. In this study, we assumed that molecular diffusion, driven by the $[\text{CH}_4]$ gradient between the anaerobic peat layers and the atmosphere, was the dominant transport mechanism by which CH_4 was released from the peatland. Although ebullition is an important transport mechanism in some wetlands (Glaser *et al.* 2004, Lai 2009), it was not considered in this study, since no events of ebullition were detected during the 20-day field campaign (neither by chamber measurements, nor by eddy covariance). Molecular diffusion affects isotopic composition of CH_4 differently than the other transport mechanisms, plant-mediated CH_4 transport and ebullition (Chanton *et al.* 1992, 2005, Liptay *et al.* 1998, De Visscher *et al.* 2004). When CH_4 is released by ebullition or through plants, it bypasses oxidation in the aerobic zone of peatland and, thus, remains unaffected by the substantial isotopic fractionation that occurs when CH_4 is oxidized. Both microbial-culture studies (Coleman *et al.* 1981, Liptay *et al.* 1998) and field studies (Tyler *et al.* 1994, Chanton *et al.* 2005) have shown that methanotrophic organisms preferentially consume lighter isotopes, leaving residual CH_4 enriched in ^{13}C . Based on differences in $\delta^{13}\text{C}-\text{CH}_4$ among peat layers, and CH_4 efflux and isotopic fractionation factors associated with gas transport and oxidation, we could assess the potential portions of transported/oxidized CH_4 . This allowed understanding to which extent our data of $\delta^{13}\text{C}-\text{CH}_4$ could be affected by the men-

tioned processes. The larger the portion of CH_4 transported or oxidized, the less certainly we can determine the methanogenic pathway based on measured $\delta^{13}\text{C}-\text{CH}_4$ values, since changes in $\delta^{13}\text{C}-\text{CH}_4$ induced by the oxidation and/or diffusive transport would mask the isotopic signal from methanogenesis.

Our data suggest that CH_4 transport by diffusion had a relatively small effect on $\delta^{13}\text{C}-\text{CH}_4$ in deep peat horizons (> 1 m), as about 3% to 5% of CH_4 was potentially transported from 2-m through 1.5-m to 1-m depth during the 20-day measurement period. This was probably due to a relatively small $[\text{CH}_4]$ gradient between 1-m and 2-m depths, and is generally in agreement with the concept of CH_4 dynamic in deeper peat horizons discussed above. Hence, $\delta^{13}\text{C}-\text{CH}_4$ in deep peat layers below microforms were predominately affected by the methanogenic pathway (hydrogenotrophy) and to a lesser extent by CH_4 transport.

In contrast to the depths below 1 m, $\delta^{13}\text{C}-\text{CH}_4$ between 1 m and 0.5 m was much more affected by CH_4 transport (Appendix 4). Moreover, CH_4 oxidation processes related to the supply of oxygen by aerenchymatic plants to roots in water saturated peat horizons (Joabsson and Christensen 2001) may have additionally affected $\delta^{13}\text{C}-\text{CH}_4$ values measured at the 0.5–1.0-m depths. Thus, estimated portions of both transported and oxidized CH_4 at these depths reached about 70% under lawns and hollows. Thus, the relative enrichment of porewater CH_4 in ^{13}C at the shallowest depth (0.5 m) of the studied peatland as compared with the deeper peat horizons cannot be attributed solely to the production by acetoclastic methanogenesis but to a large extent to CH_4 transport (diffusion and/or plant-mediated transport) and oxidation in the rhizosphere of aerenchymatic plants.

The calculated portions of oxidized CH_4 (f_{ox}) within the top 0.5 m of peat showed a negligible effect of oxidation on $\delta^{13}\text{C}$ of CH_4 emission from the lawns and hollows, which is in agreement with higher water table at these microforms. For the hummocks with thicker aerobic peat layer, oxidation was probably substantial, but it was not possible to reliably calculate $\delta^{13}\text{C}$ values in CH_4 due to low rates of efflux and small $[\text{CH}_4]$. The relative depletion of ^{13}C in CH_4 emission from the lawns and hollows as compared with

$\delta^{13}\text{C}\text{-CH}_4$ at the 0.5-m depth could be attributed to the dilution with more ^{13}C -depleted CH_4 from water-saturated peat horizons (deeper than 0.5 m) by means of plant-mediated transport (Chanton *et al.* 1992, Whalen 2005, Lai 2009).

Outlook

As discussed above, the effect of CH_4 transport and oxidation is especially important for the upper peat layers, where these processes can obscure the isotopic signature of acetoclastic methanogenesis and probably also level down the effect of microtopography on $\delta^{13}\text{C}\text{-CH}_4$ values. Additional isotopic characteristics of CH_4 (D) and CO_2 (^{13}C) would help to reveal patterns of CH_4 turnover in peatlands, including the processes of CH_4 formation, transport and consumption (Bellisario *et al.* 1999, Clymo and Bryant 2008, Steinmann *et al.* 2008). Further, more information about $[\text{CH}_4]$ and $\delta^{13}\text{C}\text{-CH}_4$ in the uppermost peat horizon (0–0.5 m) would be required. On the other hand, the lack of effect of peatland microtopography on porewater $[\text{CH}_4]$ and its isotopic composition may be attributed to the relatively short time-span of the current study. Hence, studies extended in time and in space (including other peatlands with similar biogeochemical characteristics) may provide insights into the effects of microtopography onto the processes of CH_4 turnover in peatlands.

Conclusions

Stable C isotopic composition of porewater and emitted CH_4 proved to be a suitable (but not sufficient) tool to differentiate between types of methanogenesis in continuously water-saturated layers under microforms of a peatland. Combined flux-based and multi-isotopic approaches are needed to better understand the CH_4 turnover process. Based on $[\text{CH}_4]$ in porewater, CH_4 fluxes to the atmosphere and $\delta^{13}\text{C}\text{-CH}_4$ values we conclude:

- The CO_2 reduction pathway contributed more than the acetate cleavage to total methanogenesis *in situ* in deep peat layers (> 1 m),

whereas in the upper peat horizons (< 1 m) CH_4 transport and oxidation may substantially enrich $^{13}\text{C}\text{-CH}_4$ hence masking the $^{13}\text{C}\text{-CH}_4$ enrichment due to acetoclastic pathway of methanogenesis.

- The microtopography of the studied peatland had an effect on CH_4 emission but not on $[\text{CH}_4]$ in the water-saturated peat layers. The above-ground CH_4 fluxes increased in the order hummocks < lawns \leq hollows. This trend was most probably caused by the oxidative potential of the studied microforms.

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Appendix 1. Comparisons (ANOVA) of CH_4 concentrations [CH_4] among microsites and depths.

	SS	df	MS	F	p
[CH_4] among depths below					
hummocks	7662878	3,22	2554293	2.8	0.063
lawns	5219294	3,20	1739765	2.0	0.152
hollows	6760264	3,20	2253421	3.2	0.047
[CH_4] among microforms at					
0.5 m	506960	2,16	253480	0.3	0.726
1.0 m	2192443	2,15	1096221	1.7	0.221
1.5 m	1017656	2,16	508828	0.7	0.494
2.0 m	1381486	2,15	690743	0.6	0.586

Appendix 2. Comparisons (ANOVA) of $\delta^{13}\text{C}-\text{CH}_4$ among microsites and depths.

	SS	df	MS	F	p
$\delta^{13}\text{C}-\text{CH}_4$ among depths below					
hummocks	150	3,22	50	9.9	< 0.001
lawns	262	3,20	87	19.2	< 0.001
hollows	218	3,20	73	23.1	< 0.001
$\delta^{13}\text{C}-\text{CH}_4$ among microforms at					
0.5 m	1.2	2,16	0.6	0.1	0.865
1.0 m	6.0	2,15	3.0	1.0	0.402
1.5 m	12.8	2,16	6.4	3.8	0.046
2.0 m	8.3	2,15	4.1	0.5	0.619

Appendix 3. Comparisons (ANOVA) of above-ground CH_4 fluxes from peatland's microforms during the field campaign.

	SS	df	MS	F	p
CH_4 fluxes among sampling points from					
hummocks	0.1	3,28	0.04	0.1	0.959
lawns	15.7	3,24	5.2	2.2	0.110
hollows	23.3	3,16	7.8	0.9	0.451
CH_4 fluxes among different microforms of					
sampling point 1	47.2	2,17	23.6	12.8	< 0.001
sampling point 2	98.6	2,17	49.3	7.7	0.004
sampling point 3	66.9	2,17	33.5	32	< 0.001
sampling point 4	133.5	2,17	66.8	26.3	< 0.001

Appendix 4. Comparisons (ANOVA) of estimated portions of CH_4 oxidized (f_{ox}) and transported (f_{tr}) below peatland's microforms and among depths.

	SS	df	MS	F	p
$f_{\text{tr}}, f_{\text{ox}}$ among depths below					
hummocks	1.1	2,16	0.5	12	0.001
lawns	1.8	2,15	0.9	60.6	< 0.001
hollows	1.9	2,15	0.9	25.4	< 0.001
$f_{\text{tr}}, f_{\text{ox}}$ among microforms at					
0.5–1.0 m	0.118	2,16	0.059	0.9	0.443
1.0–1.5 m	0.007	2,15	0.004	0.6	0.554
1.5–2.0 m	0.004	2,15	0.002	0.1	0.916