Microform-related community patterns of methane-cycling microbes in boreal Sphagnum bogs are site specific

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One sentence summary: Methane producing and consuming microbes in four northern acidic Sphagnum moss bogs showed site-specific variation with small-scale topographic gradients of hummocks, lawns and hollows.

Editor: Gary King

ABSTRACT

Vegetation and water table are important regulators of methane emission in peatlands. Microform variation encompasses these factors in small-scale topographic gradients of dry hummocks, intermediate lawns and wet hollows. We examined methane production and oxidation among microforms in four boreal bogs that showed more variation of vegetation within a bog with microform than between the bogs. Potential methane production was low and differed among bogs but not consistently with microform. Methane oxidation followed water table position with microform, showing higher rates closer to surface in lawns and hollows than in hummocks. Methanogen community, analysed by mcrA terminal restriction fragment length polymorphism and dominated by Methanoregulaceae or ‘Methanoflorentaceae’, varied strongly with bog. The extent of microform-related variation of methanogens depended on the bog. Methanotrophs identified as Methylocystis spp. in pmoA denaturing gradient gel electrophoresis similarly showed effect of bog, and microform patterns were stronger within individual bogs. Our results suggest that methane-cycling microbes in boreal Sphagnum bogs with seemingly uniform environmental conditions may show strong site-dependent variation. The bog-intrinsic factor may be related to carbon availability but contrary to expectations appears to be unrelated to current surface vegetation, calling attention to the origin of carbon substrates for microbes in bogs.

Keywords: methanogen; methanotroph; peatland; mcrA; pmoA; microtopography
INTRODUCTION

Boreal peatlands comprise a large carbon sink and at the same time, due to their high water table and large volumes of anoxic peat, are important sources of methane (CH$_4$, Limpens et al. 2008). Methane emissions from northern peatlands are proposed to have large potential for positive feedbacks with climate warming (Bridgham et al. 2013). On a broad scale, CH$_4$ production and oxidation processes determining the emission are controlled by water table, vegetation, nutrient status and temperature (Whalen 2005). These factors are intertwined in ecological gradients describing variation between peatlands, such as the minerotrophy–ombrotrophy gradient from groundwater-fed fens with higher pH and vascular plant cover to precipitation-fed, nutrient-poor and acidic Sphagnum-dominated bogs.

The main factors regulating CH$_4$ cycling vary along smaller scale spatial gradients within peatlands. Due to microform variation of water table level and vegetation, closely situated areas of the same peatland can become either carbon sinks or sources (Waddington and Roulet 2000). In dry hummocks characterized by shrubs, peat surface rises above the water level, creating a large aerobic peat layer which allows CH$_4$ consumption by aerobic methanotrophic bacteria. Consequently, hummocks show low CH$_4$ emissions or can even be sinks of CH$_4$ (Bubier et al. 1993; Saarnio et al. 1997; Renzel and Karofeld 2000; Moore et al. 2011). Lawns, where the dominant vegetation shifts from shrubs to sedges, have intermediate moisture conditions and emissions. The highest CH$_4$ emissions and production rates are often observed in wet hollows, where water table level is close to or above the peat surface (Bubier et al. 1993; Saarnio et al. 1997). The high emission has been attributed to the lack of an aerobic peat layer and thus reduced CH$_4$ oxidation, but also to higher decomposability of organic matter, providing more readily available substrates for methanogenesis (Svensson and Sundh 1992; Belya 1996). Although the general patterns of microform-dependent variation of CH$_4$ emissions and cycling are fairly well established, information on the effect of microform on microbial community is still quite fragmented. Water table level and vegetation type, the main factors changing with microform, have been shown to affect the CH$_4$-cycling microbial community (Jaatinen et al. 2005; Rooney-Varga et al. 2007; Chen et al. 2008; Kotiaho et al. 2010; Yrjälä et al. 2011; Tian et al. 2012). Accordingly, in a boreal fen, the methanogenic archaeal community differed between dry hummocks and sedge lawns in surface peat but not in deeper peat (Galand, Fritze and Yrjälä 2003). In sedge-dominated high altitude wetlands in the Tibetan plateau, methanogen, methanotroph and bacterial communities showed differences between hummocks and hollows, although main separation was according to wetland site (Deng et al. 2014). Similarly, in comparison of microforms in three bogs with different nitrogen inputs, the overall microbial community and methanogen community showed mostly variation with bog (Robroek et al. 2014; Marti et al. 2015). An effect of microform on the overall microbial community was detected in surface peat when site-related variation was partialled out. However, in these studies showing stronger site-related than microform variation, either vegetation, pH or both varied between the sites.

Ombrotrophic (i.e. rain-fed) bogs are characterized by acidic (pH < 4) and nutrient-poor conditions, resulting from their detachment from groundwater as a consequence of peat accumulation (Rydin and Jeglum 2006; Soudzilovskaia et al. 2010). These conditions favour a specific type of vegetation able to thrive in the harsh environment. Bog vegetation is strongly dominated by Sphagnum mosses and highly specialized in microforms (hummocks, lawns and hollows). Consequently, vegetation may vary more among microforms within a bog than among bogs even across different latitudes (Gignac 1993; Rydin, Sjörs and Löfroth 1999). It was shown previously that the actinobacterial community in four bogs along a latitude transect from middle boreal to hemiboreal region varied more with microform within a bog than between the four bogs (Kotiaho et al. 2013). These bogs have similar water table levels and similar vegetation composition with microform. Considering this result and the effects of microform on CH$_4$ emission, production and oxidation, we hypothesized that CH$_4$ cycling microbes in these boreal bogs will vary more between the three microforms than between the four sites. The variation with bog site, microform and vegetation of both contributors of CH$_4$ cycling, CH$_4$ production and oxidation, was compared at three depths in the peat profile. We expected any microform-dependent vegetation effects to be stronger in surface peat than in deeper peat.

MATERIALS AND METHODS

Study sites and sampling

We sampled four near-natural ombrotrophic bogs in the boreal vegetation zone along a ca. 700-km latitudinal transect in September 2009 (Table 1, Kotiaho et al. 2013). The sampling sites were Honkanere (HN, middle boreal), Lakkasuo (LS, in transition between southern and middle boreal), Kontolanrahka (KR, southern boreal) in Finland, and Männikjärve (MJ, hemiboreal) in Estonia. The bogs have differences in their peat formation history (Korhola 1992; Tuittila et al. 2007; Sillasoo, Väliranta and Tuittila 2011; Mellais 2013), but at present they all have a clear microtopographical variation of dry hummocks, intermediate lawns and wet hollows. KR and MJ also have open pools. Vegetation in hummocks is dominated by shrubs Calluna vulgaris, Ledum palustre and Empetrum nigrum and by a thick moss layer of Sphagnum fuscum. Lawns are characterized by sedge Eriophorum vaginatum and S. magellanicum, S. balticum and S. rubellum. Hollows are more sparsely vegetated, typically by herb Scheuchzeria palustris, sedge Rhynchospora alba and S. cuspidatum. The vegetation composition has been analysed in more detail in Kotiaho et al. (2013). pH and water table depth at the time of sampling are given in Table 1.

In each bog, we collected three replicate peat profiles with a box sampler (8 × 8 × 100 cm) from each microform habitat, resulting in nine peat cores per bog. At each sampling point, vegetation structure was determined as a projection cover of each plant species (Kotiaho et al. 2013). Water table level was determined from the sampling hole. The upper 60-cm segment of each peat core was cut to three 20-cm sections (0–20, 20–40 and 40–60 cm). These intervals were chosen to cover the typical root depth of bog plants and the range of water table fluctuation. This yielded 108 samples representing four bogs, three replicate cores from three microforms and three sampling depths from each core. The material for molecular and CH$_4$ production and oxidation analyses was collected from the inner part of the peat sections in order to minimize the exposure to air. Peat was evenly picked throughout the whole length of the section and transferred to flasks for gas analyses, or frozen (−20°C) until DNA extraction.
Analyses of potential CH₄ production, CH₄ oxidation and basal respiration

Rate measurements were carried out in 120-ml flasks with 15 ml of peat as described in Merilä et al. (2006; CH₄ production), Jaatinen et al. (2005; CH₄ oxidation) and Perkiömäki and Fritze (2002; basal respiration) with incubation in the dark at 15 °C. Production of CH₄ was monitored for 14–16 days, CH₄ oxidation 1–3 days and CO₂ production for 2 days (measured in the same flasks as CH₄ oxidation after the oxidation measurements were finished). The flasks for CH₄ production contained 30 ml of H₂O and were flushed with N₂ before and after sample addition to remove oxygen. Flasks were stored at 4 °C for 4–8 days until the gas chromatographic (GC) analyses and flushed with N₂ (CH₄ production) or air (CO₂ production and CH₄ oxidation) before the first measurement to remove accumulated gases. Flasks for CH₄ oxidation received 100 μl of CH₄ to an initial concentration of 833 ppm. The production and oxidation rates were calculated from the slope of linear regression of the head space gas concentration increase or decrease over time. The production and oxidation rates were calculated based on previous sequence data from 11 peatlands (Mart et al. 2013). Negative values indicate depth below peat surface.

DNA extraction

Total DNA was extracted from the peat samples with the Power Soil DNA extraction kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer’s instructions.

PCR, T-RFLP fingerprinting and cloning of mcrA for methanotrophs

The primer pair mlas and mcrA-rev (Steinberg and Regan 2008) was used to amplify a partial fragment of mcrA gene (approximately 470 bp), coding for the α subunit of methyl-coenzyme M reductase, for terminal restriction fragment length polymorphism (T-RFLP) analysis of methanogens. The forward primer mlas had the fluorescent label D4 (Sigma) in the 5′ end. Each 50-μl PCR reaction contained 0.5 μM of each primer, 200 μM of dNTPs and 1 U of DNA polymerase (Biotools B & M Labs S.A., Madrid, Spain) in 1 × reaction buffer and 1 μl of template DNA. Thermal cycling was started by initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, extension at 72 °C for 30 s and a final extension at 72 °C for 5 min. Products were digested for T-RFLP analysis with MboI (3 U, Promega) and HhaI (3 U, Promega) in a volume of 20 μl for 6 h. The enzymes were selected for optimal separation of methanogens common in northern peatlands based on previous sequence data from 11 peatlands (Martí et al. 2015). Digested products were ethanol precipitated, dissolved in 10 μl of Hi-Di formamide (Applied Biosystems) and 4 μl of the sample was mixed with 0.2 μl of 600-bp standard (Beckmann Coulter) and Sample Loading Solution (Beckmann Coulter) to a total volume of 30 μl. The samples were analysed by capillary electrophoresis in a Beckmann CEQ 8000 Genetic Analysis System. The fragment data was analysed in a CEQ System Fragment Analysis module. Further analysis was based on relative peak areas, and peaks representing less than 1% of the total peak area of a T-RFLP profile were removed. Fragments of 80–493 bp were included in the analyses.

To identify terminal restriction fragments (T-RFs), mcrA PCR was carried out for cloning as above but without the fluorescent label for a pool of three samples from each bog (HN, KR, LS, MJ) selected based on the T-RFLP results to best cover the detected T-RFs. The products were cloned with TOPO TA cloning kit (Invitrogen) following the manufacturer’s instructions. Clone PCR products were screened by T-RFLP with the enzymes used for T-RFLP (MboI and HhaI). Based on 23–28 clones from each library, 21 clones chosen to cover the observed RFLP groups and when possible the different bogs were sequenced using vector primer T7 (Macrogen Inc., Seoul, South Korea). Terminal fragments were identified by determining the in silico T-RFs of the sequences and by identifying the sequences by Blast searches and phylogenetic analysis (Juottonen et al. 2012). The mcrA sequence data was submitted to the EMBL database under accession numbers LN812921–LN812940.

PCR and DGGE fingerprinting of pmoA for methanotrophs

The primer pair A189f and A621r (Tuomivirta, Yrjälä and Fritze 2009) was used to amplify partial pmoA (approximately 500 bp) coding for particulate methane monoxygenase. The reverse primer has been designed to target peatland methanotrophs that are not amplified with more commonly used primers (Tuomivirta, Yrjälä and Fritze 2009). The 50-μl PCR reactions contained 0.5 μM of each primer, 200 μM of dNTPs and 0.5 U of DNA polymerase (Biotools B & M Labs S.A., Madrid, Spain) in 1 × reaction buffer. A GC-clamp sequence (Henckel, Friedrich and Conrad 1999) was attached to the primer A621r to promote efficient separation in denaturing gradient gel electrophoresis (DGGE). Thermal cycling was started by initial denaturation at 95 °C for 3 min, followed by 42 cycles of denaturation at 95 °C for 30 s, annealing including 1 °C touchdown from 70 to 59 °C (in 12 cycles) for 45 s, extension at 72 °C for 30 s and a final extension at 72 °C for 10 min.

The PCR products were analysed by DGGE in a denaturing urea gradient of 35–70% and acrylamide-bisacrylamide (37:5:1) (Biorad, Hercules, CA, USA) concentration of 6.5% (wt/vol). The gels were prepared and run in 1 × TAE at 61 °C with a constant voltage of 180 V for 5 h (D-Code, Biorad), stained with SYBR Gold II (Molecular Probes, Eugene, OR, USA) and visualized on a Dark Reader transilluminator (Clare Chemical Research, Dolores, CO, USA). For comparison of band positions between the runs, a

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**Table 1.** Location of studied bogs, water table (WT) level (n = 3) and pH (n = 9) at each microform (mean ±SD). Data from Kotiaho et al. (2013). Negative values indicate depth below peat surface.

<table>
<thead>
<tr>
<th>Site</th>
<th>Coordinates</th>
<th>Hummock WT (cm)</th>
<th>Hummock pH</th>
<th>Lawn WT (cm)</th>
<th>Lawn pH</th>
<th>Hollow WT (cm)</th>
<th>Hollow pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN</td>
<td>64°57′N, 25°34′E</td>
<td>−26 ± 4</td>
<td>4.2 ± 0.1</td>
<td>−9 ± 4</td>
<td>4.1 ± 0.2</td>
<td>−1 ± 2</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>LS</td>
<td>61°47′N, 24°18′E</td>
<td>−24 ± 2</td>
<td>3.9 ± 0.1</td>
<td>−8 ± 3</td>
<td>3.9 ± 0.1</td>
<td>0 ± 1</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>KR</td>
<td>60°47′N, 22°47′E</td>
<td>−22 ± 2</td>
<td>3.8 ± 0.2</td>
<td>−9 ± 2</td>
<td>3.8 ± 0.1</td>
<td>−5 ± 5</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>MJ</td>
<td>58°52′N, 26°15′E</td>
<td>−26 ± 7</td>
<td>3.9 ± 0.1</td>
<td>−11 ± 2</td>
<td>3.9 ± 0.1</td>
<td>−3 ± 2</td>
<td>4.0 ± 0.2</td>
</tr>
</tbody>
</table>
control ladder of several known DGGE bands was included in each run. Bands with different mobility were excised from the gel and purified by using the gel piece in water as template in subsequent PCR-DGGE. Some excised bands that persistently showed multiple bands, despite repeated purification by PCR-DGGE, were discarded from further analyses. Bands with unique mobility in DGGE were sequenced (Macrogen Inc, Seoul, South Korea). Sequences were identified by Blast searches and phylogenetic analysis (fjutten et al. 2012). A binary matrix based on the presence or absence of pmoA DGGE bands was used in statistical analyses. The pmoA sequence data was submitted to the EMBL database under accession numbers LN681134–LN681146.

Data analysis

Methane production and oxidation and basal respiration were analysed using linear mixed effects models and a split-plot design. Bog was a fixed blocking factor, the sampling spot was a random whole plot factor, and the microforms containing the sampling depths were fixed factors. Prior to analyses, CH₄ production was log transformed after adding half of the smallest non-zero value to account for zero production rates. Basal respiration values were log transformed. Pairwise comparison of significant effects was carried out by Tukey’s Honestly Significant Differences tests, considered significant at p < 0.05 or for a marginal effect at p < 0.1. The analyses were carried out in R v.3.2.0 (R Core Development Team 2014) using function lme in package nlme (Pinheiro et al. 2013).

Community composition among bogs, microforms, and peat depths was compared by non-metric multidimensional scaling (NMDS) plots and by Adonis as a multivariate analysis of variance, both based on Bray–Curtis distances (binary format for methanotrophs). Methanogen data was square root transformed. Significance testing in Adonis was based on 999 permutations with permuting within bogs. To visualize the community variation due to bog, microform and peat depth, we carried out redundancy analyses (RDAs). Methanogen and methanotroph community data were Hellinger transformed for RDA. Significance testing with function anova.cca was based on 999 permutations with permuting within bogs. Three samples from hummock 0–20 cm layer were excluded from pmoA analyses and two samples from the 0–20 cm layer from mcrA analyses, because no PCR products could be obtained. Effect of vegetation on communities overall and at each depth separately was tested by RDAs, where the axes of a vegetation ordination were used as explanatory variables. The vegetation ordination was carried out by detrended correspondence analysis (DCA), and the first and second DCA axes were used in RDAs. All multivariate analyses of methanogen and methanotroph communities and vegetation were carried out in R with the vegan package (v. 2.0, Oksanen et al. 2013).

RESULTS

Potential activities

Methane production potentials of the four bogs were in general low, ranging from 0 to 34 nmol gdw⁻¹ h⁻¹ with a mean of 1.5 nmol gdw⁻¹ h⁻¹ and a median of 0.1 nmol gdw⁻¹ h⁻¹ (Fig. 1). Production rates differed between the bogs (p < 0.001). Bogs HN and KR showed higher or marginally higher rates than bogs LS and MJ, which had rates often close to or at zero. When tested over the four bogs, peat depth had a marginal effect on CH₄ production (p = 0.087), with the highest rates generally measured in the 0–20 cm layer. No differences were observed between the three microforms (p = 0.708). When tested within each bog, production decreased with depth at bog MJ (from 2.8 ± 5.3 at 0–20 cm to 0.1 ± 0.3 nmol gdw⁻¹ h⁻¹ at 40–60 cm, mean and standard deviation, p = 0.004), but no differences between the microforms or depths were observed for HN and LS. At bog KR, the effect of depth on production varied with microform (interaction of microform and depth, p = 0.047), and production was highest in the 0–20 cm layer in hollows (1.9 ± 1.7 nmol gdw⁻¹ h⁻¹) but at 20–40 cm in lawns and hummocks (0.8 ± 0.7 nmol gdw⁻¹ h⁻¹ lawns, 2.9 ± 4.5 nmol gdw⁻¹ h⁻¹ hummocks).

Methane oxidation rates varied from 0 to 221 nmol gdw⁻¹ h⁻¹ with a mean of 66 nmol gdw⁻¹ h⁻¹ and a median of 47 nmol gdw⁻¹ h⁻¹. Oxidation potentials differed between the bogs (p = 0.025) with the lowest rates at bog KR (Fig. 1). Unlike CH₄ production, CH₄ oxidation differed clearly with depth (p < 0.001). Oxidation was higher at surface (0–20 cm, 20–40 cm) than in deeper peat (40–60 cm). Microform had no overall effect on CH₄ oxidation (p = 0.308), but the effect varied with depth (interaction of depth and microform p < 0.001). When the layers were considered separately, at 0–20 cm lawns and hollows showed higher rates than hummocks (102 ± 70 nmol gdw⁻¹ h⁻¹ in lawns, p = 0.02; 119 ± 44 nmol gdw⁻¹ h⁻¹ in hollows, p = 0.003, compared to 45 ± 45 nmol gdw⁻¹ h⁻¹ in hummocks). In turn, at 20–40 cm, hummocks had higher oxidation rates than lawns and hollows (139 ± 62 nmol gdw⁻¹ h⁻¹; in hollows 44 ± 23 nmol gdw⁻¹ h⁻¹, p < 0.001; in lawns 67 ± 65 nmol gdw⁻¹ h⁻¹, p = 0.003). No difference between the microforms was observed at the depth of 40–60 cm (p = 0.117).

Basal respiration, i.e. aerobic CO₂ production, which was measured as a general measure of microbial activity and substrate availability, ranged from 43 to 698 nmol gdw⁻¹ h⁻¹ with a mean of 66 nmol gdw⁻¹ h⁻¹ and a median of 47 nmol gdw⁻¹ h⁻¹. The rates differed between the bogs (p = 0.001) and with depth (p < 0.001) but not with microform (p = 0.520; Fig. 1). Following the pattern of CH₄ production, HN and KR showed higher respiration rates than MJ and LS. Rates were higher at the depth of 0–20 cm than in deeper peat. When the CO₂ production was compared for each depth separately, microforms did not differ at 0–20 cm, but at 20–40 cm hummocks had higher basal respiration than lawns (p = 0.034) and marginally higher rates than lawns at 40–60 cm (p = 0.067).

Methanogen community composition

In mcrA T-RFLP analysis of methanogens, the clearest separation was between the bogs (Fig. 2). Peat depth and microform also affected the community structure but with less explanatory power (Table 2). It should be noted that variation in multivariate spread between the bogs may contribute to the observed differences. Because significant interactions suggested that microform effect varied with bog and depth, we analysed the effects of microform separately for each bog and depth. In LS and MJ, microform explained 24 and 20% of methanogen community variation, which is more than the microform effect over all the bogs (2%). HN and KR showed no marked microform effect (Table 2). When tested separately for each depth, microform explained 11 and 7% of community variation at the layers 0–20 cm and 20–40 cm, but no effect was found in the deepest layer. RDA showed that the effect of site separated the communities of all four bogs from each other (Fig. 3A). The variation along the RDA axis 1 was related to variation in relative abundance of two
dominant taxa: Methanoregulaceae and ‘Candidatus Methanoflorentaceae’. Methanoregulaceae (T-RFs 405 and 470 bp) were most common in surface peat of LS and MJ and in hummocks of LS and MJ. In LS, the surface peat in lawns and hollows and the 20–40 cm layer in hummocks showed nearly exclusively Methanoregulaceae (Table S1, Supporting Information). ‘Methanoflorentaceae’ (Rice cluster II, Mondav et al. 2014, T-RFs 299 and 383 bp) were dominant in all samples from KR, representing 61–100% of total peak area, and in the deeper layers of all bogs (at 20–40 cm and 40–60 cm in HN and MJ, at 40–60 in LS). Surface peat (0–20 cm) in KR, HN and MJ also showed Methanosarcinaceae (T-RF 226 bp) and Methanobacteriaceae (T-RF 218 bp), most clearly in lawns and hollows (Fig. 3A). In HN, Methanobacteriaceae were also detected in deeper layers, unlike in the other bogs. The detected mcrA sequences were in most cases most similar to sequences from various northern peatlands, and in the case of Methanoregulaceae, ‘Methanoflorentaceae’ and Methanobacteriaceae clustered also with peatland isolates or genomes (Fig. S1, Supporting Information).

Separate RDAs were carried out to test the effect of vegetation on the community and if the surface peat was more affected by vegetation differences between the microforms than the deeper peat layers. Vegetation explained only ≤ 6.3% of methanogen community variation, and vegetation was a marginally significant explanatory factor at the depth of 0–20 cm but non-significant in deeper peat (Table 3).

**Methanotroph community composition**

In pmoA DGGE analysis of methanotrophs, 13 bands were identified. Three of these occurred in the majority of the samples: mob-1 in 52%, mob-6 in 82% and mob-8 in 78% of 108 samples analysed. These three bands were identified as type II methanotrophs grouping with Methylocystis spp. and showed 100% similarity to pmoA sequences from peatland environments (Fig. S2, Supporting Information; McDonald and Murrell 1997; Tuomivirta, Yrjäla and Fritz 2009; Liebner and Svenning 2013). The other less common bands were likewise identified as Methylocystis spp. with 95–100% similarity to those detected in acidic peat, Sphagnum mosses and forest soils (Dumont et al. 2006; Chen et al. 2008; Tuomivirta, Yrjäla and Fritz 2009; Kip et al. 2012; Danilova and Dedysh 2014). Sequences of bands mob-11 and mob-13 grouped with Methylocystis bryophila, a facultative methanotroph able to grow slowly on acetate (Belova et al. 2011).

No bands specific to a microform were observed. Bands mob-4 and mob-12 were specific to bog HN. When looking at bands occurring with peat depth, mob-10, mob-11, mob-12 and mob-13 were most common in the 0–20 cm layer and bands mob-2 and mob-9 in the 40–60 cm layer.

Bog had the strongest effect on methanotroph community, yet peat depth and microform showed comparable levels of effect (Fig. 4). These factors each explained 8–17% of methanotroph community variation (Table 2). Effects of microform were
Figure 2. NMDS ordination of methanogen community based on mcrA T-RFLP analysis in four boreal bogs. The same plot is shown coloured by bog (A), microform (B) and peat depth (C). Both the individual data points and the means and standard deviations of axis scores (highlighted points with error bars) are shown. Stress = 0.135.
also analysed separately for each bog and depth. Within all bogs except LS, microform explained 15–40% of methanotroph community variation, more than over all the bogs (Table 2). When peat layers were analysed separately, microform effect was stronger at 0–20 cm and 20–40 cm than in the deepest layer. In RDA, axis 1 separated bogs LS and HN from KR and MJ (Fig. 3B). This axis also separated methanotrophs in hollows from those in hummocks and lawns, which were more similar to each other. These separations appeared to be driven by occurrence of the common bands mob-1 and mob-8 versus mob-9 (Fig. 3B).

In RDAs addressing the effect of vegetation, ≤13.5% of methanotroph community variation was explained by vegetation (Table 3). The explanatory power of vegetation was highest in the two upmost peat layers, particularly at 20–40 cm (Table 3).

**DISCUSSION**

We expected CH4-cycling microbes to vary more with microform than between the four bogs with seemingly similar environmental conditions at different latitudes. Microforms comprise gradients in two central factors controlling CH4 production and oxidation: water table level and vegetation. The conditions in ombrotrophic bogs drive succession towards characteristic vegetation and dominance of Sphagnum mosses. As a result, variation of vegetation among microforms within a bog may be larger than overall variation among bogs, as in the bogs studied here (Kotiaho et al. 2013). Against expectations, bog site had a more pronounced effect on the methanogen and methanotroph community as well as on CH4 production and oxidation potentials than microform. Microform effect on methanogens and methanotrophs was apparent within the bogs, but the pattern differed between the four bogs, and some bogs showed weak or no microform effect (HN, KR for methanogens, LS, KR for methanotrophs).

In the studied bogs with similar vegetation, water table level and pH, none of these factors frequently shown to regulate CH4 cycling could explain the strong site-related variation of microbial community and activity. The bogs were located at different latitudes and differed in annual temperature sums (Kotiaho et al. 2013), but because only one bog per latitude was sampled, we are lacking sufficient data to draw reliable conclusions on the effects of latitude or climate. Previously, methanogen communities in three Scandinavian bogs at different latitudes and with some differences in vegetation showed no microform effect, but site-related differences correlated with DOC levels (Marti et al. 2015). Bogs HN and KR with the highest CH4 production had also higher basal respiration (aerobic CO2 production), which could reflect higher overall microbial activity or substrate availability (Yavitt, Williams and Wieder 1997). These bogs with higher CH4 production potential, and particularly their surface layers, showed a higher proportion of methanogen groups previously connected with increased CH4 production or high substrate availability: ‘Methanoflorentaceae’, Methanobacteriaceae and Methanosarcinaceae (Jetten, Stams and Zehnder 1992; Lu et al. 2005; Sakai et al. 2009; McCalley et al. 2014). This observation suggests that the bog-dependent variation of CH4 production and methanogens could originate from carbon availability or quality. Despite the currently similar surface vegetation, the bogs may differ in past vegetation and thus in peat composition and carbon compounds below the surface. Verifying this explanation would require a further study of peat chemistry for bog and depth. However, these results together with the previous study of Marti et al. (2015) suggest that bog-specific differences in carbon compounds due to factors other than current vegetation may regulate CH4 cycling and the strength of microform effect in ombrotrophic bogs.

The weak microform and particularly depth-related variation of CH4 production, which conflicts patterns repeatedly observed in peatlands, may partly be explained by the very low CH4 production rates. Low CH4 production is typical to bogs compared to

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**Table 2.** Permutational multivariate analysis of variance (Adonis) of methanogen and methanotroph communities based on Bray–Curtis distances in bogs HN, LS, KR and MJ with microform and depth.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Methanogens (mcrA)</th>
<th>Methanotrophs (pmoA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R^2</td>
<td>p</td>
</tr>
<tr>
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<td></td>
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<tr>
<td>Bog</td>
<td>0.36</td>
<td>0.001</td>
</tr>
<tr>
<td>Microform</td>
<td>0.02</td>
<td>0.042</td>
</tr>
<tr>
<td>Depth</td>
<td>0.08</td>
<td>0.001</td>
</tr>
<tr>
<td>Split by bog</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HN</td>
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<tr>
<td>Microform</td>
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<td>0.064</td>
</tr>
<tr>
<td>Depth</td>
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<td>0.002</td>
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<td>0.001</td>
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<tr>
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<td>0.001</td>
</tr>
<tr>
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<tr>
<td>Depth</td>
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<td>0.001</td>
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<td>Microform</td>
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<td>0.002</td>
</tr>
<tr>
<td>Depth</td>
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<td>0.004</td>
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<tr>
<td>0–20 cm</td>
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<tr>
<td>Bog</td>
<td>0.40</td>
<td>0.001</td>
</tr>
<tr>
<td>Microform</td>
<td>0.11</td>
<td>0.005</td>
</tr>
<tr>
<td>20–40 cm</td>
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<td></td>
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<tr>
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<td>0.001</td>
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<tr>
<td>Microform</td>
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<tr>
<td>40–60 cm</td>
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<tr>
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<td>0.001</td>
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<tr>
<td>Microform</td>
<td>0.06</td>
<td>0.099</td>
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Figure 3. RDA of methanogens (A) and methanotrophs (B) in four boreal ombrotrophic bogs (KR, HN, MJ, LS) with microform (hummock, lawn, hollow) and depth (0–20 cm, 20–40 cm, 40–60 cm). Species are shown in grey (mcrA T-RFs for methanogens, pmoA DGGE bands for methanotrophs). In A, only the T-RFs contributing markedly to the solution are shown. Mreg, Methanoregulaceae; Mbac, Methanobacteriaceae; Ms, Methanosarcinaceae; Mflo, 'Candidatus Methanoflorentaceae'. The convex hulls are provided for visualization to connect the levels of each variable (bog, microform, depth).

Table 3. RDA on the effect of vegetation on methanogen and methanotroph communities with depth.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Methanogens (mcrA)</th>
<th>Methanotrophs (pmoA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–20</td>
<td>6.3</td>
<td>9.6</td>
</tr>
<tr>
<td>20–40</td>
<td>4.2</td>
<td>13.5</td>
</tr>
<tr>
<td>40–60</td>
<td>2.2</td>
<td>6.2</td>
</tr>
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</table>

more nutrient-rich or higher pH peatlands (Moore and Knowles 1990; Valentine, Holland and Schimel 1994) and has been reported for bog LS previously (Galand et al. 2005; Juottonen et al. 2005). Microform effects on production have been observed to be weaker in bogs than in fens (Bubier et al. 1993). The low pH and the low nutrient levels of bogs limit microbial activity, and in addition, competing anaerobic microbial processes appear to direct carbon to anaerobic CO₂ production instead of methanogenesis in bogs (Yavitt and Seidman-Zager 2006; Galand, Yrjälä and Conrad 2010; Ye et al. 2012; Bridgham et al. 2013). The possible contribution of anaerobic CH₄ oxidation cannot be excluded either, as this process is currently poorly known in peatlands (Gupta et al. 2013). In addition, methanogenesis in ombrotrophic bogs can be considered homogenous with depth compared to more nutrient-rich peatlands in terms of methanogenic pathways and composition of dissolved organic carbon (Chasar et al.
Figure 4. NMDS ordination of methanotroph community based on pmoA DGGE analysis in four boreal bogs. The same plot is shown coloured by bog (A), microform (B) and peat depth (C). Both the individual data points and the means and standard deviations of axis scores (highlighted points with error bars) are shown. Stress = 0.066.
The patterns of CH\textsubscript{4} production that were detected despite the low rates match the expected: the highest rate values were measured in surface peat, rather than in the more decomposed peat of deeper layers, and in hollows, where water level is closest to peat surface. In bog KR, the depth of highest production followed the water table position and was lower in hummocks than in lawns and hollows. In most cases, the effect of depth was more obvious on the methanogen community than on CH\textsubscript{4} production.

All the methanogens detected in the bogs were either known or putative hydrogenotrophic methanogens, fitting with the notion that hydrogenotrophic methanogenesis rather than acetoclastic methanogenesis is the prevailing pathway in ombrotrophic bogs (Kelly, Díse and Martens 1992; Chasar et al. 2000a; Galand et al. 2005). Methanosarcinaceae, many of which can carry out both hydrogenotrophic and acetoclastic methanogenesis, were detected as minor members of the communities, mostly in surface peat. Methanosaetaceae, the obligate acetoclastic methanogens, were not detected, resembling the results of other studies on acidic Sphagnum bogs reporting either no Methanosetaeae (Kotsyurbenko et al. 2004) or only a low proportion of Methanosaetaceae in this type of ecosystem (Basiliko et al. 2003; Cadillo-Quiroz et al. 2006; Dettling et al. 2007; Yavitt et al. 2012). The methanogen communities were dominated by two groups: Methanoregulaceae and ‘Methanoflorentaceae’. Methanoregulaceae have been the prevalent methanogens in surface peat (0–20 cm below water level) in several low nutrient and low pH (ca. 4) Sphagnum bogs (Cadillo-Quiroz et al. 2006; Dettling et al. 2007; Sun et al. 2012; Yavitt et al. 2012), including the bog LS (Galand et al. 2005; Juutonen et al. 2005). ‘Methanoflorentaceae’, formerly known as Rice cluster II, occur in a wide range of peatlands including ombrotrophic bogs (Kotsyurbenko et al. 2004; Yavitt et al. 2012; Monav et al. 2014). Methanoregulaceae were found particularly in the bogs LS and MJ with extremely low CH\textsubscript{4} production rates, whereas ‘Methanoflorentaceae’ dominated in KR and HN with higher production. However, the increase of ‘Methanoflorentaceae’ in relative abundance with depth in all bogs does not allow linking the distribution of Methanoregulaceae and ‘Methanoflorentaceae’ with CH\textsubscript{4} production potential. Rather, the results showed the occurrence of several methanogen community types in the studied bogs, dominated either by Methanoregulaceae or ‘Methanoregulaceae’ or both, and complemented by Methanobacteriaceae and Methanospirillaceae. This variation of dominant group shows that despite similar vegetation, distinctive conditions and dominance of hydrogenotrophic methanogenesis, additional bog-intrinsic factors appear to drive methanogen community composition in acidic Sphagnum bogs (Yavitt et al. 2012; Marti et al. 2015).

Although CH\textsubscript{4} oxidation and methanotrophs showed strong bog-related variation similar to CH\textsubscript{4} producers, they also showed clear effects of microform, vegetation and depth. The depth variation of CH\textsubscript{4} oxidation followed the well-established observation showing the highest activity close to the water table level, where both CH\textsubscript{4} and oxygen are available (Sundh et al. 1995). The oxidation rates were highest at 0–20 cm in hollows and lawns, but at 20–40 cm in hummocks, where the 0–20 cm layer is above the water level. The depth variation of CH\textsubscript{4} oxidation activity and methanotroph community composition suggests that as aerobic microbes, methanotrophs are strongly affected by the water table gradient of microform variation. Accordingly, methanotroph community in the water-submerged hollows differed from the drier lawns and hummocks. The effect of vegetation and microform on the methanotroph community reached the depth of 20–40 cm, well below the water table level in lawns and hollows. This result may partly be explained by the presence of vascular plant roots providing oxygen to water-submerged peat at the depth of 20–40 cm (Stephen et al. 1998). Typical lawn and hollow plant species in these four bogs, E. vaginatum and Sc. palustris, have well developed air-conducting root tissues (Redin and Jeglum 2006). Considering that the actinobacterial community in our study bogs differed predominantly with microform rather than with bog (Kotiaho et al. 2013), it appears that microform affects aerobic bacteria in bogs more clearly than the anaerobic methanogenic archaea. Further support for this conclusion is provided by earlier mentioned studies carried out on three Scandinavian bogs, reporting some variation with microform for overall bacterial and fungal communities, but no effects for methanogens (Robroek et al. 2014; Marti et al. 2015).

To the best of our knowledge, this is the first report of microform effects on methanotroph communities across several boreal peatlands. Methanotrophs showed variation with bog, microform and depth. However, these factors explained a smaller proportion of community variation than with methanogens. The detected methanotroph sequences formed eight clusters grouping with Methylocystis spp., potentially representing different Methylocystis species (Fig. S2, Supporting Information). The resolution of variation is therefore on a smaller scale than in the case of methanogens. Methylocystis spp. are highly common in northern acidic peatlands (Dedysh et al. 2003, 2007; Chen et al. 2008; Dedysh 2009). The microform and depth effects on closely related methanotrophs suggest the presence of different types of Methylocystis spp. adapted to specific peatland conditions. It should be noted that the pmOA primers used in this study are optimized for detection of peatland Methylocystis missed by other primers and may therefore lack coverage of other groups (Tuomivirta, Yrjälä and Fritze 2009). Type I methanotrophs not detected here have previously been found at bog LS with a different primer set, but also in that study Methylocystis was the characteristic group in the bog (Jaatinen et al. 2005). Our approach should be considered as fingerprinting of the prevalent methanotrophs in the bogs, rather than a description of the total methanotroph diversity. The pmOA-based approach would miss Methylocella spp. and Methyloferula stellata, which are known to occur in peat and have only a soluble methane monooxygenase and thus no pmOA (Dedysh 2009; Vorobev et al. 2011). To insure best capturing the methanotroph diversity in peat, an approach including several primer pairs would be necessary.

As expected, vegetation and microform effects on the microbial community, when detected, were strongest in surface peat and weaker in the deepest layers. A similar pattern has been observed in a minerotrophic fen for archaea (Galand, Fritze and Yrjälä 2003), for overall microbial community in boreal bogs (Robroek et al. 2014), and for actinobacteria in the bogs studied here (Kotiaho et al. 2013). The deeper layers represent further decomposed, constantly anoxic peat outside the influence of water table variation or vascular plant roots and are thus fairly unaffected by microform. However, considering the strong bog-dependent variation, additional factors appear to influence CH\textsubscript{4}-cycling microbes in bogs more than current surface vegetation. If microbial activity in Sphagnum bogs relies more on solid-phase peat compared to dissolved organic carbon than in segedominated peatlands (Chanton et al. 2008), the past vegetation composition and its changes with depth could affect present-day microbial activity. The possible contribution of climate, differences in water chemistry in seemingly similar ombrotrophic
bogs, and differences in dispersal of microbes during peatland development cannot be excluded either.

In conclusion, methane-cycling microbes and their activity in the bogs did not consistently follow the vegetation and water level gradient of hummocks, lawns and hollows. Instead, microform effects on methane producers and oxidizers were bog specific or not detected. The results show that the characteristic conditions in ombrotrophic bogs with Sphagnum-dominated vegetation and low pH do not alone shape the communities of methanogens and methanotrophs. Bog-intrinsic factors possibly related to the origin of carbon compounds available for microbes may be more important than previously recognized.

**SUPPLEMENTARY DATA**

Supplementary data are available at FEMSEC online.

**ACKNOWLEDGEMENTS**

We thank Minna Väliaranta, Edgar Karofeld, Antti Miettinen, and Hannu Latvajärvi for their contribution to peat sampling, Juha Piispanen and Timo Mikkonen (Natural Resources Institute Finland, Oulu) for arranging facilities for peat sampling in the north, and Minna Oksanen and Hanna Aulanko for assistance with laboratory work.

**FUNDING**

This work was supported by the Academy of Finland (project 131409, project 218101, project 138041, additionally project 133743 to H.J.).

Conflict of interest. None declared.

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