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Peatland vascular plant functional types affect methane dynamics by altering microbial community structure

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Summary

1. Peatlands are natural sources of atmospheric methane (CH_4), an important greenhouse gas. It is established that peatland methane dynamics are controlled by both biotic and abiotic conditions, yet the interactive effect of these drivers is less studied and consequently poorly understood.

2. Climate change affects the distribution of vascular plant functional types (PFTs) in peatlands. By removing specific PFTs, we assessed their effects on peat organic matter chemistry, microbial community composition and on potential methane production (PMP) and oxidation (PMO) in two microhabitats (lawns and hummocks).

3. Whilst PFT removal only marginally altered the peat organic matter chemistry, we observed considerable changes in microbial community structure. This resulted in altered PMP and PMO. PMP was slightly lower when graminoids were removed, whilst PMO was highest in the absence of both vascular PFTs (graminoids and ericoids), but only in the hummocks.

4. Path analyses demonstrate that different plant–soil interactions drive PMP and PMO in peatlands and that changes in biotic and abiotic factors can have auto-amplifying effects on current CH_4 dynamics.

5. *Synthesis.* Changing environmental conditions will, both directly and indirectly, affect peatland processes, causing unforeseen changes in CH_4 dynamics. The resilience of peatland CH_4 dynamics to environmental change therefore depends on the interaction between plant community composition and microbial communities.

Key-words: methane, methanogenesis, methanotrophic communities, mid-infrared spectroscopy, pathway analysis, phospholipid fatty acid, plant–soil (below-ground) interactions, *pmoA*, *Sphagnum*-dominated peatlands

Introduction

Due to prevailing waterlogged conditions and slow decomposition rates, peatlands are generally net sinks for carbon dioxide (CO_2). Annually, peatland C uptake is estimated to be between 0.07 (Clymo, Turunen & Tolonen 1998) and 0.10 (Gorham 1991) Gt C. As a consequence, Northern peatlands in particular act as large repositories for terrestrial carbon (C), containing up to 500 Gt of C (Yu 2012). However, these same conditions that constrain decomposition also lead to the

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production of methane (CH_4), an important greenhouse gas (Vasander & Kettunen 2006). As CO_2 uptake generally surpasses CH_4 emission, in the long term, peatlands exert a net cooling effect on the global climate (Dise 2009). Nevertheless, the balance between peatland C uptake and release is delicate, and variations in environmental and biological conditions can be expected to define the ability of peatlands to act as C sinks.

Methane fluxes from peatlands depend on the net balance of CH_4 production by methanogenic archaea and CH_4 oxidation by methane oxidizing bacteria (MOB). Methane production is the final step in anaerobic organic matter breakdown, whilst CH_4 oxidation occurs preferentially under aerobic conditions (Le Mer & Roger 2001). The effects of abiotic factors on CH_4 oxidation and production in wetlands have been well documented (Segers 1998; Lai 2009; Chowdhury & Dick 2013). Nevertheless, variation in CH_4 fluxes is large, and currently cannot be adequately explained by environmental conditions alone (Moore & Dalva 1997; Levy *et al.* 2012).

Peatlands are remarkably stable in terms of plant species composition (Backéus 1972; Rydin & Barber 2001); however, the relative abundance of these species varies naturally over time, or in response to environmental conditions (Bragazza 2006; Breeuwer *et al.* 2009; Jassey *et al.* 2013). Vegetation composition can have a strong influence on peatland C dynamics, as different plant functional types (PFTs) have been shown to have a varying effect on ecosystem CO_2 exchange (Ward *et al.* 2013; Kuiper *et al.* 2014). This is likely to be driven to a large extent by differences in the composition and quantity of root exudates from contrasting PFTs (De Deyn, Cornelissen & Bardgett 2008; Bridgham *et al.* 2013), as root exudates serve as a substrate for microbial respiration (Hütsch, Augustin & Merbach 2002; Berendsen, Pieterse & Bakker 2012) and therefore shape rhizosphere microbial communities (Bais *et al.* 2006; Haichar *et al.* 2008). Additionally, PFTs differ in their ability to aerate the rhizosphere through aerenchymatous tissue, which will strongly influence microbial decomposition and methane production. Despite the knowledge that changes in plant community composition and environmental conditions can both affect CH_4 dynamics, their interactive effect is less understood.

Here, we studied the differential effects of peatland vascular PFTs on potential methane oxidation (PMO) and production, and whether these effects are mediated by changes in peat organic matter properties and microbial community structure. We used two distinct microhabitats, wet lawns and relatively dry hummocks, to mimic the combined effects of water-table drawdown and changes in the vascular PFT composition. We hypothesized that distinct microbial communities would be associated with different PFTs and that plant removal would lower the peat organic matter quality. Furthermore, we expected that links among PFT composition, microbial community structure and the peat organic matter chemistry would describe potential methane production (PMP) and oxidation. Specific to these interactions, we hypothesized that the absence of vascular plants would lead to a reduction in both methane production (via substrate limitation) and oxidation (due to lower oxygenation of the peat). Using causal pathway analyses, the relationships among PFT composition, peat organic chemistry, microbial community structure and their influence on PMP and oxidation were examined.

Materials and methods

EXPERIMENTAL SITE AND SAMPLE COLLECTION

In early June 2011, 48 experimental plots (50 × 50 cm) were established in lawn (n = 24) and hummock microhabitats (n = 24), with homogenous vegetation (Table 1), in a *Sphagnum*-dominated ombrotrophic peatland in the Store Mosse National Park, Sweden (57°17′54 N, 14°00′39 E). In both microhabitats, the vascular PFT composition was manipulated by removing (clipping) ericoids (−Eric), graminoids (−Gram) or both. A set of control plots remained intact. All treatments were replicated six times, resulting in a total of 48 plots (4 treatments × 2 microhabitats × 6 replicates). Vascular plant regrowth was removed every third month, apart from during winter, by pulling out whole seedlings (incl. roots). To minimize the effects of root decomposition on subsequent measurements, plots were established 1 year before measurements started. Remaining differences in root decomposition were elucidated by comparing respiration rates, measured on 10 cm diameter collars using an automated soil CO_2 flux system (LI-8100; LI-COR Biosciences, Lincoln, NE, USA), from the removal plots with respiration rates from comparable areas in the control plots with little or no vascular plant cover (VPC).

In July 2012, single peat samples (*c.* 20 g. f. wt) were collected from all plots (n = 48) at the aerobic/anaerobic boundary (10–30 cm below the peat surface, microhabitat dependent). This layer was chosen as it is the most important for methane cycling and contains both methanotrophic and methanogenic communities (Krause *et al.* 2013; Generó *et al.* 2015). Samples were kept anoxic after sampling using *Anaerocult® A mini* (Merck Millipore, Merck KGaA, Darmstadt, Germany) and stored at 4 °C until analysis. Each sample was mixed to ensure homogenization of organic matter and microbial communities and divided into several subsamples for further analyses. One subsample was weighed, dried at 70 °C for 48 h and reweighed to determine water content (WC). Pore water samples were taken using Rhizon soil moisture samplers (type MOM, pore size 0.2 μm; Eijkelkamp, Giesbeek, the Netherlands) and pH was measured, after which the water samples were analysed spectrophotometrically for dissolved organic carbon (DOC) using a segmented flow analyser (SAN^{PLUS}; Skalar analytical, Breda, the Netherlands).

Table 1. Composition of the plant community in the Store Mosse National Park, the largest untouched peatland expanse south of Lapland, can be considered representative for Nemo-boreal peatlands

Dominant vegetation	Lawn	Hummock
Mosses	*Sphagnum cuspidatum*	*Sphagnum magellanicum* *Sphagnum rubellum*
Ericoids	*Erica tetralix* *Vaccinium oxycoccus* *Andromeda polifolia*	*Erica tetralix* *Vaccinium oxycoccus* *Andromeda polifolia* *Empetrum nigrum* *Calluna vulgaris*
Graminoids	*Eriophorum angustifolium* *Rhynchospora alba*	*Eriophorum vaginatum*

MICROBIAL COMMUNITY STRUCTURE: PLFA AND QPCR

Phospholipid fatty acids (PLFA) were extracted from 2.5 g fresh (moist) peat over a 2-h period in a solvent phase comprising 3.0 mL 50 mM phosphate (P) buffer (pH 7.0), 3.8 mL chloroform and 7.6 mL methanol (cf. Börjesson *et al.* 1998). PLFA 19:0 (Larodan, Malmö, Sweden) was added as an internal standard to the phospholipid fraction. PLFAs were methylated to form fatty acid methyl esters using 1 mL 0.2 M *methanolic* KOH (Sundh, Nilsson & Borga 1997; Chowdhury & Dick 2012) and analysed on a gas chromatograph according to Steger *et al.* (2003). Specific PLFA biomarkers were used to estimate biomasses of actinomycetes, gram-positive and gram-negative bacteria, and fungi (Denef *et al.* 2009).

We extracted DNA from 0.02 to 0.04 g lyophilized peat, as described by Steenbergh *et al.* (2010). Pelleted and cleaned DNA was resuspended in 50 μL sterilized water. The number of *pmoA* gene copies of type Ia, Ib and II methanotrophic bacteria was quantified as described by Kolb *et al.* (2003), using modified primer concentrations and temperatures for fluorescence data acquisition. Q-PCR amplification was performed on a Rotor Gene 6000 thermal cycling system (Corbett Research, Sydney, NSW, Australia). All reaction mixtures (20 μL) contained 2 μL of sample DNA (2 ng), 10 μL 2× SensiFastTM (BIOLINE Inc., Taunton, MA, USA) real-time PCR mix, 3.5 μL forward and reverse primers in optimized concentrations (3 pmol μL^{-1}) and 1 μL bovine serum albumin. Reactions were performed in duplicate. For type Ia and II specific Q-PCR, the thermal cycle consisted of an initial denaturation (3 min at 95 °C), followed by 45 cycles of denaturation (10 s at 94 °C), annealing (10 s at 60 °C) and extension (20 s at 72 °C). For type Ib, the procedure was similar but for a 64 °C annealing temperature. Fluorescence data were acquired at the extension to denaturation transition by holding temperatures at 82 °C (type Ia), 85 °C (type Ib) and 87 °C (type II) for 8 s. Reaction products were verified by DNA melting curve analysis at 70–99 °C, with 1 °C step increase and by 1.0% (wt vol^{-1}) agarose gel electrophoresis (data not shown). Only data from Q-PCRs of which product of expected size was visible on the gel were used for further analysis. Type Ia assays were calibrated using a dilution series of a known amount of PCR product from a *pmoA* harbouring plasmid (clone WPAN42, accession FN395340, 588 bp) previously amplified with a M13 primer set. Type Ib and II series were similarly calibrated using PCR product of clone WPBN2 (accession KF395333, 455 bp) and clone WP AD62 (accession FN395333, 599 bp), respectively.

PEAT ORGANIC MATTER BULK CHEMISTRY

Peat organic matter chemistry was characterized by diamond attenuated total reflectance (ATR)–Fourier transform infrared spectroscopy (Thermo iS10 FTIR spectrometer; Thermo Scientific, Waltham, MA, USA). Air-dried peat samples were ball-milled and further dried at 50 °C. Mid-infrared (MIR) spectra were obtained for each sample in triplicate by averaging 16 scans per replicate at 4 cm^{-1} resolution over the spectral range 4000–650 cm^{-1}. MIR spectra were corrected for interferences by H_2O and CO_2, processed and analysed using the *hyperSpec* (Beleites & Sergo 2012), *ptw* (Bloemberg *et al.* 2010) and *StreamMetabolism* (Sefick 2013) R packages. The contribution of minerals (e.g. silicates) to the MIR spectra was considered negligible (Pengerud *et al.* 2013).

Five waveband regions corresponding to specific C functional groups were selected for integration and peak ratio comparisons among treatments (Smith 1998; Pengerud *et al.* 2013): the SAT region (saturated hydrocarbons, Alkyl–CH_2/CH_3, 2985–2820 cm^{-1}), the C=O peak (1730–1720 cm^{-1}), the aromatic C=C peak (1605–1595 cm^{-1}), the POLY region (polysaccharide, O-Alkyl C, 1185–915 cm^{-1}) and the AROCH region (aromatic CH, 855–740 cm^{-1}). Indices for peat organic matter chemistry were calculated as ratios between absorbance at the SAT, C=O, C=C, POLY region and the soil organic carbon (SOC) content and between the AROCH region and the C=C peak (cf. Pengerud *et al.* 2013). This resulted in five indices: the peat hydrophobicity (SAT::SOC), degree of oxidation (C=O::SOC), the aromaticity (C=C::SOC), the peat polysaccharide content (POLY::SOC) and the degree of aromatic condensation (AROCH::C=C).

POTENTIAL METHANE PRODUCTION AND OXIDATION

Fresh peat, *c.* 10 g for PMP and *c.* 5 g for PMO, was incubated in 120-mL bottles containing 10 mL demineralized water. PMP measurements were performed in the dark, in bottles containing only N_2 gas. PMO measurements were performed in bottles with 1.1 mL CH_4 (10 000 ppmv), added to the headspace, to prevent methane limitation throughout the experiment. The bottles were then incubated on a shaker (150 rpm) at 20 °C, and headspace CH_4 concentrations were measured 2–3 times per day over an eleven-day period. In short, 200 μL headspace gas samples were directly injected into an Ultra GC gas chromatograph (Interscience, Breda, the Netherlands) equipped with a flame ionization detector and Rt-Q-Bond (30 m, 0.32 mm, ID) capillary column, with helium as a carrier gas, and an 80 °C oven temperature. PMP and PMO were calculated from the linear increase (PMP) or decrease (PMO) in CH_4 headspace concentration, using 30 and 24 h (PMP and PMO, respectively). This time lag was determined empirically and enabled calculation of processes in the linear parts of the process rates. We present positive flux values for both PMP and PMO.

STATISTICAL ANALYSES

Redundancy analyses (RDA) were applied to microbial PLFA (Hellinger transformed) using microhabitat (lawn/hummock), ericoid removal and graminoid removal as explanatory variables, all coded as binary factors. The significance of our models and the explanatory variables was tested using 999 permutations. As lawn and hummock PLFA communities differed significantly ($F_{1,34} = 5.3$, $P \leq 0.001$), partial RDAs were computed after removing the effect of microhabitat. Partial RDA site scores were used to perform hierarchical cluster analysis (Ward method). The resulting dendrogram was projected in the RDA ordination space, facilitating the detection of main discontinuities in PLFA community structure among treatments (Carlson *et al.* 2010). We used variance partitioning to identify the contribution of each separate factor, and their interactions, to the model. To elucidate the effects of vascular PFT composition on microbial PLFA composition in each microhabitat, we performed separate RDA for the lawn and hummock communities. RDA were performed using the *vegan* package for R (Oksanen *et al.* 2013).

Generalized linear models (GLMs) were used to test the effects of microhabitat (MH), ericoid removal (−Eric) and graminoid removal (−Gram) on PMP, PMO, and substrate quality variables. Data on PMP, PMO and the substrate quality variables C=C::SOC, POLY::SOC, and AROCH::C=C were log-transformed to meet the assumptions of normality of the data. Initially, we tested the treatment effects on PMP and PMO using a variety of models: one which only included the factorial treatments, and a set of models that included VPC, WC and pH, and all possible combinations, as random factors. Next, we performed stepwise GLM selection, and based on corrected Akaike information criterion (AIC), the final models (apart from the

PFT treatments) included VPC and WC, or VPC only, in the PMP and PMO models, respectively (Table S1 in Supporting Information). These analyses were performed with the software R 3.1.1 (R Core Team 2012).

STRUCTURAL EQUATION MODELLING

An initial conceptual path analysis model (causal model) was used to identify potential plant–soil links affecting PMP and PMO (Fig. S3, Table S4). Using structural equation modelling (SEM), we then excluded non-significant variables using a stepwise approach until a minimal adequate model remained (Yu 2012; Milcu *et al.* 2013). We built separate models for the lawn and hummock microhabitats, and all pathways constructed were represented using single-indicator variables. Ericoid or graminoid cover was used for the PFT treatment. Polysaccharide content (POLY::SOC) entered the final models as a proxy for organic matter quality. Fungal PLFA biomass and the gram-positive to gram-negative bacterial biomass ratio were used as metrics for variation in the microbial communities. Gene copies of *pmoA* type Ib and II were selected as an indicator for the size of the methanotrophic community in the PMO model in lawns and hummocks, respectively. All SEM analyses were conducted using the *sem* package in R (Fox 2006). Maximum likelihood procedures were used for model evaluation and parameter estimation. Adequate model fits were identified by non-significant chi-square tests ($P \geq 0.05$), low AIC, low root-mean-square error of approximation index (≤ 0.1), low standardized root-mean-square residual index (≤ 0.1) and high comparative fit index (≥ 0.90) (Grace *et al.* 2014).

Results

PLANT COMMUNITY COMPOSITION

Overall, VPC was greater in the hummocks than in the lawns ($F_{1,40} = 12.4$, $P \leq 0.01$). In the lawns, only graminoid removal significantly reduced total VPC ($F_{1,20} = 22.9$, $P \leq 0.001$), whist in the hummocks, both graminoid ($F_{1,20} = 14.6$, $P \leq 0.01$) and ericoid ($F_{1,20} = 61.8$, $P \leq 0.001$) removal decreased VPC (Fig. S1a). In comparison with the control plots, CO_2 respiration rates were lower in the plant removal plots, but this was not related to the amount of removed above-ground biomass (Fig. S1b). This observation implies that any potential effect of respiration of root litter in the treatment plots is smaller than autotrophic root respiration in the control plots, by which an apparent effect of plant removal on root decomposition seems unlikely.

ORGANIC MATTER PROPERTIES

Peat hydrophobicity (SAT::SOC), aromaticity (C=C::SOC), polysaccharide content (POLY::SOC) and the degree of condensation of aromatics (AROCH::C=C) were higher in the lawns compared to the hummock microhabitats (Table S2). One year after plant removal, clear effects of vascular PFTs on peat organic matter properties were not observed, although there is marginal evidence that the removal of graminoids decreased the polysaccharide content in both microhabitats ($F_{1,37} = 3.0$, $P = 0.09$; Table S2), reduced the oxidation index in the lawns (C=O::SOC; $F_{1,37} = 4.3$, $P \leq 0.05$) and caused an increase in the dissolved organic carbon content in the hummocks ($F_{1,40} = 5.3$, $P \leq 0.05$; Table S2).

MICROBIAL COMMUNITY STRUCTURE: PLFA AND QPCR

Microhabitat and PFT removal (both ericoids and graminoids) exerted a large control on the microbial community PLFA structure (Table 2a). After removing the microhabitat effect on the ordination space (Table 2b, Fig 1a), we conceptualized a division of the plots based on distinct microbial community PFLA profiles, which were highly related to the removal treatments (Fig. 1a). When the two microhabitats were analysed separately, the apparent PFT control on the microbial PLFA community structure remained (Table 2c,d; Fig. 1b,c). In comparison with the hummocks, this effect was almost twice as large in the lawns (Table 2c,d), indicating a stronger plant control on microbial community structure in lawn microhabitats. Variance partitioning suggested the effect of graminoid removal on the microbial community PLFA structure to be larger than the effect of ericoid removal, irrespective of microhabitat (Table 2c,d; Fig 1b,c).

Total PLFA biomass (data not shown) was not affected by vascular plant removal in the lawns (−Eric: $F_{1,14} = 2.3$, $P = 0.16$; −Gram: $F_{1,14} = 3.0$, $P = 0.11$), whilst in the hummocks, it was lower without ericoids ($F_{1,16} = 10.0$, $P \leq 0.01$). In the lawns, biomass of unspecific bacterial and gram-negative bacterial PLFA was lower without graminoids and ericoids, respectively. These effects were, however, alleviated by the parallel removal of the other PFT (−Eric × −Gram: $P \leq 0.05$; Fig. S2). Actinobacterial biomass was lower with both PFTs removed (−Eric: $F_{1,14} = 5.6$, $P \leq 0.05$; −Gram: $F_{1,14} = 15.4$, $P \leq 0.001$), whilst gram-positive (excl. actinobacteria) PLFA biomass was not affected by PFT removal (Fig. S2). In the hummocks, ericoid removal resulted in lower biomass of unspecific ($F_{1,16} = 5.2$, $P \leq 0.05$) and gram-negative bacterial ($F_{1,16} = 19.6$, $P \leq 0.001$) PLFA biomass, whilst the other groups were unaffected (Fig. S2).

In comparison with the lawns, the number of gene copies for the methanotrophic community (*pmoA*) was higher in the hummock microhabitats (Fig. 2; $F_{1,40} = 9.6$, $P \leq 0.01$). This pattern was observed across all methanotrophic types (type Ia: $F_{1,40} = 4.0$, $P = 0.05$; type Ib: $F_{1,40} = 8.9$, $P \leq 0.01$; type II: $F_{1,40} = 8.7$, $P \leq 0.01$; Fig. 2). Despite the observation that PFT removal did not significantly affect the overall number of *pmoA* genes in the lawns, graminoid removal did result in lower type II *pmoA* gene copies. In the hummocks, the combined removal of ericoids and graminoids resulted in lower numbers of type Ib and II methanotroph *pmoA* gene copies (Fig. 2).

POTENTIAL METHANE PRODUCTION AND OXIDATION

Overall, PMP was significantly higher in the lawns (Fig. 3, $F_{1,30} = 37.3$, $P \leq 0.001$), and when taking into account both microhabitats, PMP was on average 32% lower when graminoids were removed (Fig. 3a, $F_{1,30} = 3.0$, $P = 0.09$; Table S3). Considering PMO, rates were higher in the hummocks

Table 2. Results from the redundancy analyses (RDA) testing the influence of (a; Model 1) microhabitat and the removal of ericoids and graminoids (and all interactions) on the composition of the microbial PLFA community. Model 2 (b) show RDA results after the effect of microhabitat was partialled out. Models 3 and 4 (c,d) show the RDA results of the effects of plant functional type removal on the microbial PLFA communities in the lawn and hummock microhabitats, respectively

	df	F	*P*-value	r^2	RDA	
a						
Model 1 (full model)				0.57	RDA 1	19.3**
Microhabitat (MH)	1	9.9	≤ 0.001	0.09	RDA 2	18.3**
–Eric	1	8.0	≤ 0.001	0.07		
–Gram	1	4.6	≤ 0.001	0.03		
MH × –Eric	1	3.2	≤ 0.01	0.18		
MH × –Gram	1	11.6	≤ 0.001	0.25		
–Eric × –Gram	1	12.3	≤ 0.001	0.23		
MH × –Eric × –Gram	1	5.7	≤ 0.001	0.57		
Residual	30					
b						
Model 2 (Microhabitat effect partialled out)				0.44	RDA 1	19.2**
–Eric	1	8.0	≤ 0.001	0.07	RDA 2	17.7**
–Gram	1	4.6	≤ 0.001	0.03		
MH × –Eric	1	3.2	≤ 0.01	0.18		
MH × –Gram	1	11.6	≤ 0.001	0.25		
–Eric × –Gram	1	12.3	≤ 0.001	0.23		
MH × –Eric × –Gram	1	5.7	≤ 0.001	0.57		
Residual	30					
c						
Model 3 (lawn model)				0.65	RDA 1	30.0**
–Eric	1	9.2	≤ 0.001	0.14	RDA 2	24.7**
–Gram	1	12.0	≤ 0.001	0.20		
–Eric × –Gram	1	13.3	≤ 0.001	0.65		
Residual	14					
d						
Model 4 (hummock model)				0.33	RDA 1	22.6**
–Eric	1	2.5	≤ 0.05	0.04	RDA 2	15.9**
–Gram	1	4.7	≤ 0.001	0.12		
–Eric × –Gram	1	5.3	≤ 0.001	0.33		
Residual						

F and *p*-values were obtained from *Monte Carlo* permutations (n = 999). Variation partitioning using RDA and adjusted r^2 were applied to compare the respective effect of each individual (or interacting) factor(s). Additionally, the percentage explained by the first two RDA axes is given.
$^{**}P \leq 0.01$.

(Fig. 3b, $F_{1,36} = 20.2$, $P \leq 0.001$) and affected by VPC ($F_{1,36} = 9.5$, $P \leq 0.01$) and peat WC ($F_{1,36} = 4.8$, $P = 0.034$; Table S3). In the lawns, vascular PFT removal did not affect PMO, whilst in the hummocks, PMO was higher when both PFTs were removed (Fig. 3b, Table S3). Regression analysis showed that both overall, and in the lawns, PMO and total number of *pmoA* gene copies were significantly correlated (overall: $R^2 = 0.14$, $F_{1,44} = 6.03$, $P = 0.02$; lawns: $R^2 = 0.37$, $F_{1,20} = 9.41$, $P = 0.007$), whilst a non-significant relationship was found in the hummocks ($R^2 = 0.05$, $F_{1,20} = 0.97$, $P = 0.34$).

PLANT–SOIL PATHWAYS IN DRIVING PMP AND PMO

Path analyses highlighted a variety of above-ground–below-ground links that drive PMP and PMO, depending on microhabitat and cover and identity of the PFTs (Fig. 4). The lawn PMP models indicated a positive link between fungal PLFA biomass and PMP (Fig. 4a). Generally, fungal biomass increased with decreasing ericoid cover, but decreased with reduced graminoid cover (Fig. 4a). Graminoid removal, however, was also shown to enhance fungi indirectly through a negative, yet non-significant, effect on the peat polysaccharide content, which on in turn increased the fungal PLFA biomass (Fig. 4a). In the hummocks, increased fungal biomass and increased gram-positive to gram-negative bacterial ratio (G+/G−) stimulated PMP (Fig. 4b). As a reduction in either PFT led to enhanced PMP via stimulatory effects on either fungal (graminoids) or G+/G− (ericoids), their removal had the potential to increase PMP, albeit by differing mechanisms. We further identified a negative indirect effect of graminoid removal on PMP, through a reduction in G+/G− linked to a decrease in the peat polysaccharide content (Fig. 4b).

The lawn PMO models highlight a significantly positive impact of the number of type II *pmoA* gene copies on PMO, this being positively associated to a decrease in ericoids (Fig. 4c). In the hummocks, PMO was positively linked to the number of type Ib *pmoA* gene copies and higher G+/G− (Fig. 4d). The latter, however, can also negatively affect PMO through a reduction in the number of type Ib *pmoA* gene copies. As reduced ericoid cover is positively linked to G+/G− (Fig. 4d), our results indicate

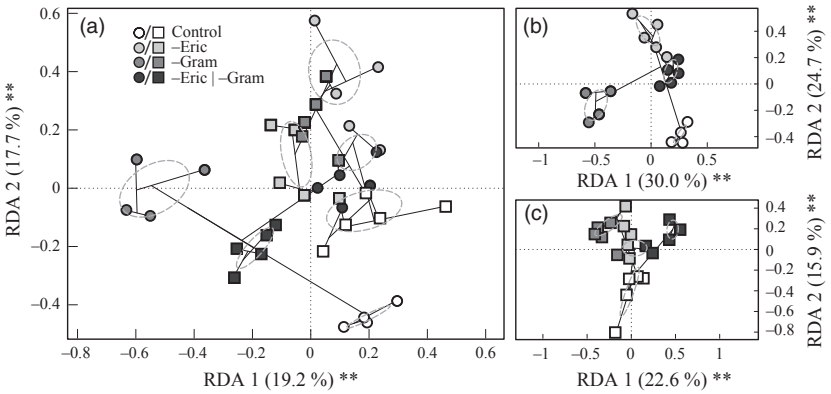

Fig. 1. Ordination biplot (partial redundancy analysis, pRDA) illustrating the effect of plant functional type (PFT) removal treatments on the microbial (phospholipid fatty acid, PLFA) communities ((a) lawn: circles; hummock: squares). pRDA ordination plots showing the effects of PFT removal on lawn (b) and hummock (c) microbial communities separately. pRDAs were performed on the Hellinger-transformed microbial PLFA community matrix, partialling out the effects of microhabitat (lawn, hummock). Biplot axes 1 and 2 (both significant; $P \leq 0.001$) and 'plot scores' (types 2 scaling) are shown.

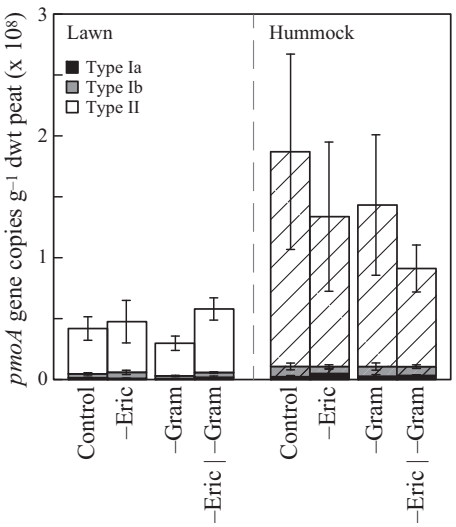

Fig. 2. Number of *pmoA* gene copies (±SEM) – coding for the particulate methane monooxygenase – for the type Ia, Ib and II methanotrophic subgroups (see Materials and methods) in the peat soil from plots with different plant functional type communities; intact (Control), ericoids removed (−Eric), graminoids removed (−Gram), and ericoids and graminoids removed (−Eric | −Gram).

that, in the hummocks, ericoid removal could simultaneously enhance or decrease PMO through two alternative pathways. Likewise, graminoid removal has ambiguous affects on PMO through an indirect negative effect on G+/G− (Fig. 4d).

Discussion

Whilst it has been noted that plant controls on methane fluxes go beyond differences in aerenchymatous transport (Saarnio *et al.* 1998; Green & Baird 2011; Levy *et al.* 2012), the mechanisms are still poorly understood. With this experiment, we took a multifaceted approach to study the effects of two important peatland vascular PFTs on below-ground microbial community structure and organic matter quality, and how these affect PMO and PMP in the peat. Our results demonstrate that microbial community structure is affected by microhabitat (Jaatinen *et al.* 2007; Kotiaho *et al.* 2013; Robroek *et al.* 2014) and plant community composition. The effect of plant community composition on microbial community structure can be direct, through established plant–microbe associations (Opelt *et al.* 2007), or indirect, through specific processes such as rhizodeposition and rhizosphere oxygenation (Fritz *et al.* 2011), both ultimately affecting ecosystem processes (Wardle *et al.* 2004). Changes in the influx of easily degradable root-derived C compounds due to changed plant community composition, for example, could alter microbial community structure and activity and change organic matter decomposition (Haichar *et al.* 2008; Schmidt *et al.* 2011). Reflecting current knowledge on ecohydrological controls on decomposition process (Belyea 1996; Laiho 2006), we show contrasting values for most proxies of organic matter bulk chemistry in both microhabitats (cf. Turetsky *et al.* 2007; Pengerud *et al.* 2013), for which PFT community composition was only marginally responsible. This is surprising, as recently vascular plant community composition has been shown to be associated to the peat organic matter fingerprint with further impacts on peatland methane production along a permafrost thaw gradient (Hodgkins *et al.* 2014). The apparent absence of a clear PFT control in our short-term study and the knowledge that long-term plant community changes affect organic matter composition and associated processes, highlights that a clearer, long-term perspective on these relationships may be crucial in understanding the current and future dynamics of terrestrial methane cycling.

Values for PMP in our experiment were comparable to other studies (see Bridgham & Richardson 1992). Nevertheless, overall they were higher than values found from Canadian peat (2.0–62.7 nmol g^{-1} h^{-1}; Brown, Mathur & Kushner 1989), but lower than those from *Sphagnum*-dominated bogs in Northern Sweden (Hodgkins *et al.* 2014). Indeed, variation in peatland PMP is large and dependent on incubation temperature and substrate quality (Moore & Dalva

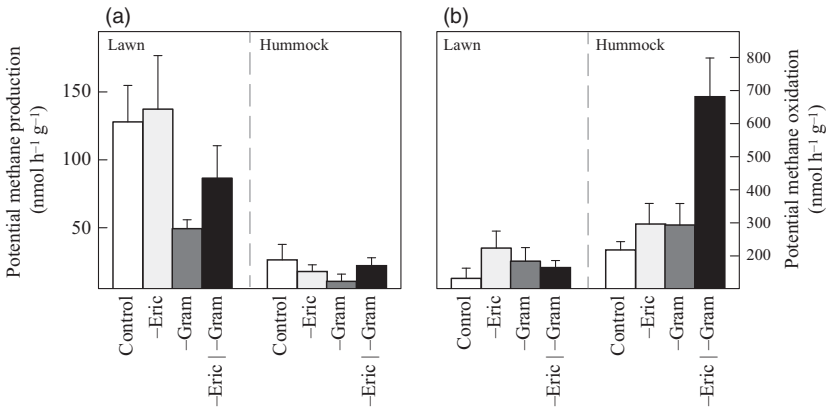

Fig. 3. Potential methane production (a) and oxidation (b) (±SEM) from peat soils from plots with different plant functional type communities: intact (Control), ericoids removed (−Eric), graminoids removed (−Gram) and ericoids and graminoids removed (−Eric | −Gram).

1997). The slightly reduced PMP values following graminoid removal in the lawns coincided with a reduction in peat polysaccharide content, indicating a role of the latter in controlling PMP. Indeed, path analysis does not exclude an indirect role for plant-mediated changes in peat organic matter quality on PMP. Intriguingly, PMP reduction following graminoid removal seems to be brought about by a different series of interactions in lawn and in hummock microhabitats, complicating generalizations on peatland above-ground–below-ground relationships. Reduced PMP was associated with reduced fungal biomass, remarkable given that fungi have recently been strongly linked to methane production (Lenhart *et al.* 2012). Such a pathway, however, is unlikely to explain our results for two reasons. First, fungal methane production seems to occur only in aerobic conditions (Lenhart *et al.* 2012). Secondly, graminoid removal enhanced fungal biomass in the hummock habitats, whilst PMP was reduced. Although the nature of our results does not allow for general conclusions, they support our hypothesis that plant removal reduces PMP, and highlight that this depression is most likely mediated by changes in microbial community structure, which itself is probably the result of a change in substrate for microbial communities (Galand *et al.* 2005; Ström *et al.* 2012).

As with PMP, PMO was strongly influenced by microhabitat, with higher values in the hummocks, most likely driven by the combined effect of differences in microbial communities, the abundance of methanotrophs, and contrasting abiotic conditions (Yrjälä *et al.* 2011). PMO is generally related to the number of *pmoA* gene copies (Freitag *et al.* 2010; Yrjälä *et al.* 2011), which encode for the membrane-bound (or particulate) methane monooxygenase (pMMO) enzyme. Although we found positive relationships between the number of *pmoA* gene copies and PMO, these relationships were far from one-to-one. Bodelier *et al.* (2013) showed an exclusive link between the abundance of a numerical minor subgroup of MOB (type Ia) and methane consumption, yet our study highlights a link between PMO and the number of gene copies for type Ib MOB in hummocks, and with the number of gene copies for type II MOB in lawns. The apparent weak relationship between *pmoA* gene abundance and PMO in the hummocks may be explained by several factors including mixotrophic growth of methanotrophs (Semrau, DiSpirito & Vuilleumier 2011), changed activity of the methanotrophic community or the activity of other novel methanotrophic microbes only possessing soluble methane monooxygenase (Vorobev *et al.* 2011). Indeed, the presence of methanotrophs, which are expected to thrive under substrate limitation (Dunfield & Dedysh 2014), may explain the high levels of PMO when all plants, and their associated rhizodeposits, were absent. Path analyses demonstrate that two PFT specific pathways effectuated induced hummock PMO: ericoid removal directly affected the structure of the microbial community, whilst such effect by graminoid removal was mediated by the peat polysaccharide content. More specifically, whilst positively affecting PMO, ericoid and graminoid removal have an antagonistic effect on the gram-positive to gram-negative bacterial ratio, supporting the presence of specific links between PFT and microbial community structure.

IMPLICATIONS

With changing environmental conditions, peatland PFT composition is expected to change (Dieleman *et al.* 2015). Our results highlight that changes in the peatland plant community composition may act as a major modulator of changes in methane dynamics. We provide evidence that apparent PFT controls on methane dynamics are mediated by alterations in soil organic matter bulk chemistry and the microbial community structure. Safeguarding the current functions of peatlands regarding methane dynamics therefore not only depends on maintaining abiotic conditions, but due to its strong controls on below-ground communities and below-ground processes, also depends on the stability of the plant functional community.

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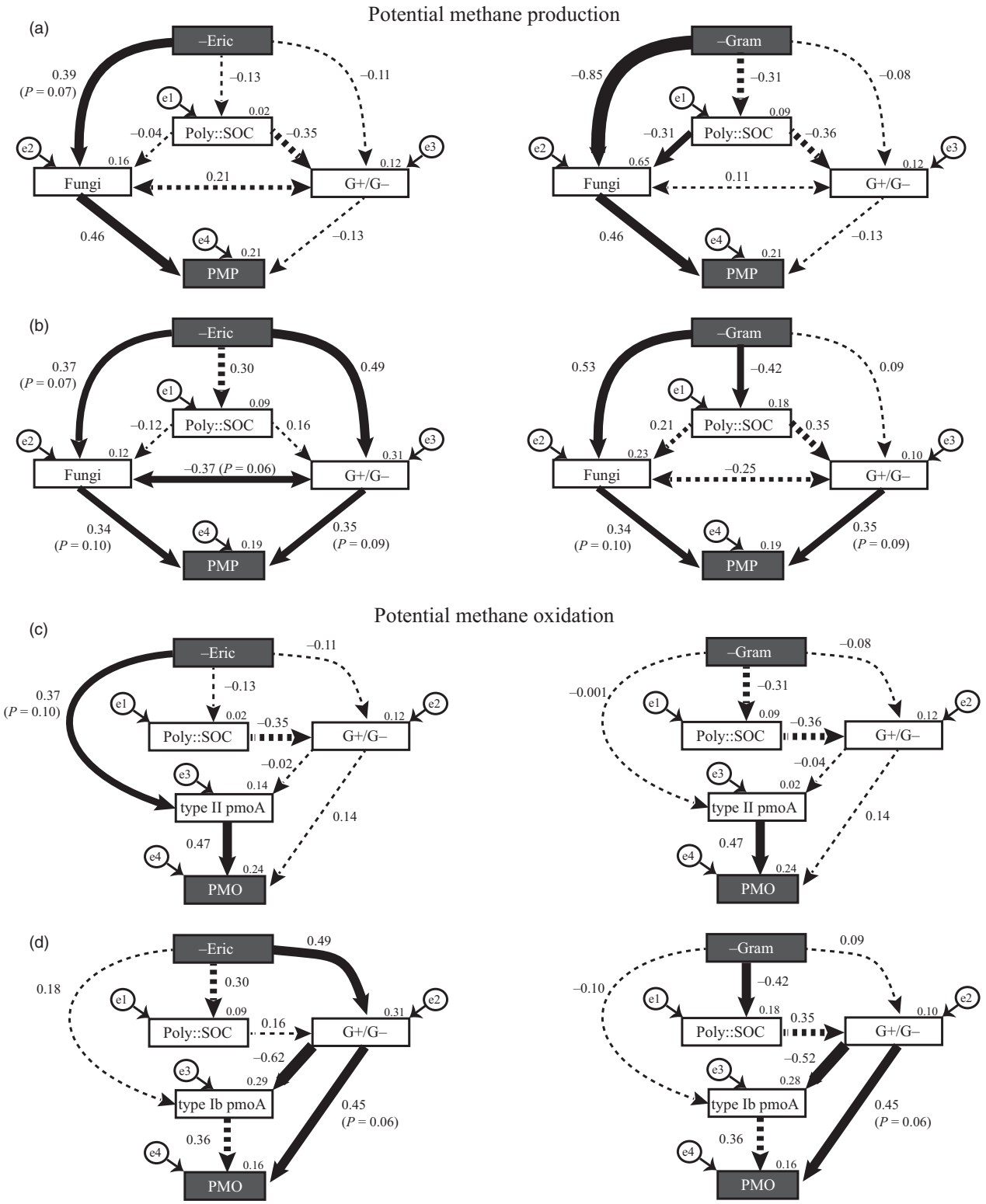

Fig. 4. Structural equation models for the effects of decreased ericoid and graminoid cover on organic matter properties, microbial community structure (including the number of gene copies coding for the particulate methane mono-oxygenase), as well their direct and indirect on potential methane production (PMP; a,b) and potential methane oxidation (PMO; c,d) in the lawn (a,c) and hummock (b,d) microhabitats (see Fig. S3 for the *a priori* meta model). Solid arrows show significant relationships (pathways) between variables, dashed arrows indicate non-significant paths. Numbers show standardized parameter estimates (i.e. standardized regression weights). Circles (e1–e4) indicate error terms. Squared multiple correlations (R^2) for the predicted/dependent factor are given on the box of the dependent variable. See Table S4 for model fits.

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Data accessibility

Data underlying the statistical analyses presented in this study can be accessed through the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.3216c (Robroek *et al.* 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (a) Remaining vascular plant cover after vascular plant functional type removal treatments; (b) Relationship between the response in CO_2 respiration rates after plant removal and the amount of removed biomass.

Figure S2. Functional group PLFA biomass.

Figure S3. Structural equation meta-model and its accompanying hypotheses.

Table S1. Results from statistical model testing.

Table S2. Organic matter quality properties as influenced by the plant removal treatments.

Table S3. Statistical information on the effects of microhabitat and plant removal treatment on methane production and oxidation.

Table S4. Structural equation meta-model hypotheses and model fits.