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Methanogen Biomarkers in the Discontinuous Permafrost Zone of Stordalen, Sweden

M. Lupascu,[1]*,† *J. L. Wadham,*[1] *E. R. C. Hornibrook*[2] *and R. D. Pancost*[3]

[1] Bristol Glaciology Centre, University of Bristol, Bristol, UK
[2] Bristol Biogeochemistry Research Centre and Cabot Institute, School of Earth Sciences, University of Bristol, Bristol, UK
[3] Organic Geochemistry Unit, Bristol Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Bristol, UK

ABSTRACT

Permafrost peatlands are both an important source of atmospheric CH_4 and a substantial sink for atmospheric CO_2. Climate change can affect this balance, with higher temperatures resulting in the conversion of permafrost soils to wetlands and associated accelerated mineralisation and increased CH_4 emission. To better understand the impact of such processes on methanogen populations, we investigated the anaerobic decay of soil carbon in a low Arctic, discontinuous permafrost peatland. Cores were collected monthly from sedge and *Sphagnum* mires in north Sweden during the summer of 2006. We determined CH_4 concentrations and production potentials, together with variations in the size of the methanogenic community as indicated by concentrations of archaeal lipid biomarkers (phosphorylated archaeol, archaeol and hydroxyarchaeol). Concentrations of methanogen biomarkers generally were higher at the sedge site, increased with depth for all sites and months, and were usually below the detection limits in shallow (<10 cm) *Sphagnum* peat. The distribution of biomarkers reflects the strong influence of water table depth on anaerobic conditions and methanogen populations, while differences in biomarker concentrations can be explained by differences in vegetation cover and pH. However, methanogen populations inferred from biomarker data show a decoupling from *in-situ* CH_4 production over the season and from CH_4 production potential, suggesting that other factors such as the availability of labile organic substrates can influence methanogen abundance. Archaeal lipid biomarkers appear to offer a potential new means to investigate permafrost biogeochemical processes but the interpretation of signals remains complex. Copyright © 2014 John Wiley & Sons, Ltd.

KEY WORDS: methanogenesis; permafrost; lipid biomarkers; archaeol; *sn2*-hydroxyarchaeol

INTRODUCTION

Permafrost in northern peatlands is undergoing substantial changes due to increasing global air temperatures (IPCC, 2014). In some Arctic regions, the active layer, which thaws during summer, has thickened since 1970 (Christensen *et al.,* 2004), and complete thawing of permafrost has been reported in many locations (Turetsky *et al.,* 2002; Johansson *et al.,* 2006; Wu and Zhang, 2010; Callaghan *et al.,* 2010). The results of permafrost degradation are spatially and regionally variable, with impacts including subsidence, lowering or raising of the soil water table (Brown *et al.,* 2000; Hinzman *et al.,* 2003), thermokarst erosion (Hinzman *et al.,* 1997; Plug *et al.,* 2008; Pohl *et al.,* 2009), increased or decreased soil organic matter decomposition rates (Oberbauer *et al.,* 1996; Hobbie *et al.,* 2000; Scanlon and Moore, 2000) and changes in plant communities (Svensson *et al.,* 1999; Christensen *et al.,* 2004; Schuur *et al.,* 2007). In northern peatlands, plant composition, temperature and water table level are the main factors affecting CH_4 emissions (Valentine *et al.,* 1994; Schimel *et al*., 1996; Waddington *et al.,* 1996; Bellisario *et al.,* 1999; Frenzel and Karofeld, 2000). A detailed study at a site in Abisko, northern Sweden, has shown that the thaw of permafrost and subsequent vegetation change has increased landscape-scale CH_4 emissions by 22 to 66 per cent over the period 1970–2000 (Christensen *et al.,* 2004).

Many studies of northern wetlands have considered ecosystem-scale differences in CH_4 production (Valentine *et al.,* 1994; Bergman *et al.,* 1998, 2000; Whalen and Reeburgh, 2000; Yavitt *et al.,* 2005) and CH_4 oxidation

*Correspondence to: M. Lupascu, Bristol Glaciology Centre, University of Bristol, Bristol, UK. E-mail: mlupascu@uci.edu
†Present address: Department of Earth System Science, University of California, Irvine, CA 92697, USA

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(Whalen and Reeburgh, 2000; Wagner *et al.,* 2005; Liebner and Wagner, 2007). Key processes in the CH_4 cycle are largely carried out by highly specialised methanogenic Archaea and CH_4-oxidising bacteria, although other microorganisms are essential for mediating substrate supply to the methanogens (Winter and Knoll, 1989). Recent work has shown that methanogenic Archaea and CH_4-oxidising bacteria exist in permafrost soils in numbers comparable to those in temperate soil environments (Kobabe *et al.,* 2004; Liebner and Wagner, 2007; Barbier *et al.,* 2012). In this context, it is worthwhile to evaluate methanogen abundance and understand its relationship with CH_4 concentration, production or active layer geochemistry. Although some studies have examined the methanogen and bacterial populations in northern peatlands using microbiological techniques (Metje *et al.,* 2005, 2007; Rivkina *et al.,* 2007; Barbier *et al.,* 2012), there have been relatively few biomarker-based investigations (Wagner *et al.,* 2005, 2007; Pancost *et al.,* 2011; Bischoff *et al*., 2013).

In this study, the permafrost methanogen population was investigated in sites with different plant compositions through the use of the archaeal diether lipid biomarkers, phosphorylated archaeol, archaeol and hydroxyarchaeol. These archaeal lipids, or their intact polar lipid (IPL) analogues, have been identified in soils (Asakawa *et al.,* 1998; Bai *et al.,* 2000; Fritze *et al.,* 1999), wetlands (Fritze *et al.,* 1999), lakes (Franzmann *et al.,* 1992; Schouten *et al.,* 2001) and permafrost (Wagner *et al.,* 2005, 2007; Bischoff *et al*., 2013). Thus, the primary aims of this study are: (1) to determine if archaeal lipids, both intact or partly degraded forms, are present in the discontinuous permafrost zone of two contrasting peatlands (minerotrophic sedge and *Sphagnum* mires) of the Swedish Arctic; (2) to examine how their concentrations vary among sites, with depth at individual sites, and during early, mid- and late summer; and (3) to compare archaeal lipid profiles to soil CH_4 concentration and production potential.

MATERIAL AND METHODS

Field Site Description

The study area is located in the Stordalen sub-Arctic mire complex near Abisko on the south shore of Lake Torneträsk, northern Sweden (68°21'N, 18°49'E), approximately 200 km north of the Arctic Circle and 385 m asl (Figure 1). The average annual air temperature during the period 2004–06 was 1.1 °C and the average total annual precipitation was 612 mm. Winter precipitation is mainly snow, with a mean snow depth of 18 cm for the period 2004–06. The Stordalen mire covers 25 ha and is a typical sub-Arctic tundra environment characterised by discontinuous permafrost with small-scale (~2 m) variations in topography consisting of wet minerotrophic depressions, elevated areas with a micro-relief pattern of hummocks and small ombrotrophic depressions, and streams carrying water to and from the complex. The sub-habitats differ in their nutrients, moisture conditions and plant assemblages. We studied a sedge and a *Sphagnum* mire site, which constitute 2 (12%) and 8.3 (49%) ha, respectively, of the mire complex, with the remaining area consisting of ombrotrophic bogs, open water and bare rock (Figure 1; Johansson *et al.,* 2006). The ombrotrophic bog was excluded in this study because of its lack of a water table and negligible CH_4 emissions.

Climate records from the Abisko Research Station indicate that the annual mean air temperature in the region increased by 2.5 °C from 1913 to 2006 (Bäckstrand *et al.,* 2008; Callaghan *et al.,* 2010), which led to a deeper active layer and permafrost disappearance in some areas of Stordalen (Åkerman and Johansson, 2008).

The dominant plant species in the sedge mire site is the cottongrass *Eriophorum angustifolium*. The site was underlain by seasonally frozen ground at the time of the first sampling in June 2006 (at 20 cm depth) and fully thawed conditions generally prevailed by mid-June. Vegetation at the second study mire was dominated by *Sphagnum* spp. The site was underlain by permafrost at the time of sample collection. The soil thaw depth was 22 cm in June and up to 90 cm in August and September 2006, when it was considered to represent the active layer depth.

Sampling and In-Situ Measurements

The field campaign was conducted during mid-June, early August and the end of September 2006. At each site, three soil cores were collected using a 60 cm long × 15 cm diameter metal peat corer that had been rinsed with ethanol and flame treated. All the cores collected were composed entirely of peat. The seasonally frozen ground and soil thaw depths and water table level were measured prior to sampling using a graduated metal rod. One core was wrapped in aluminium foil (pre-combusted at 450 °C for 4.5 h) and then placed in a −20 °C cold room at the Abisko Research Station. On site and immediately after collection, temperature and moisture content were measured along the profile of a second core every ~5 cm using a Hanna Check Temperature probe (Woonsocket, Rhode Island, USA) (resolution: 0.1 °C; accuracy: ±0.3 °C) and a soil moisture Delta T HH2 Theta Probe (Cambridge, UK) (accuracy: 1–5%). The pH of wet peat was measured on core subsamples using an Orion 250 A pH meter (Vernon Hills, Illinois, USA) (resolution: 0.01/0.1 pH; accuracy: ±0.02). The third core was subsampled in the Abisko Research Station for CH_4 concentrations and CH_4 production potential analysis.

Biomarker Analyses

Sample Preparation and Lipid Extraction.

The frozen cores were transported to the UK for biomarker analysis and stored in a −20 °C cold room. Before subsampling, the cores were defrosted in an incubator at 5 °C. Each core was subsequently cut into four to six sections along the soil profile, corresponding to the same

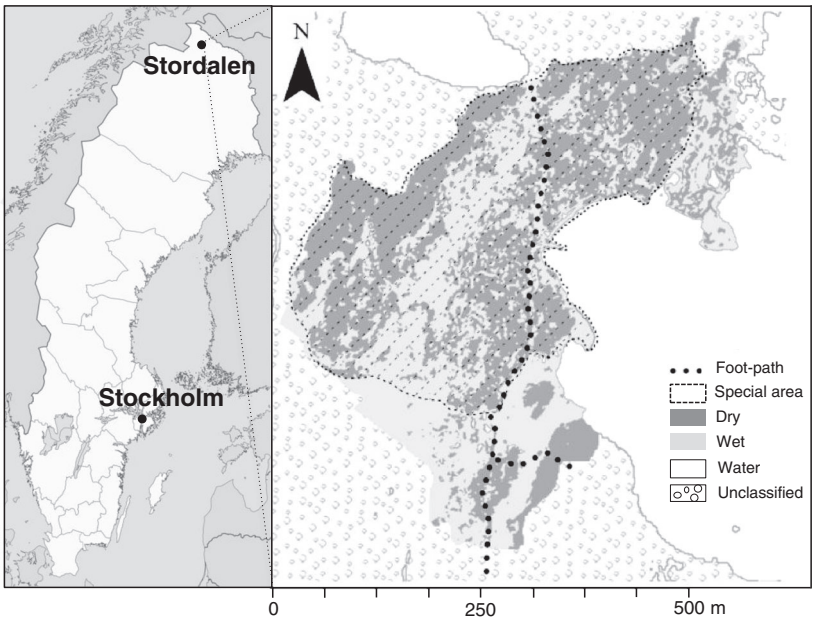

Figure 1 Map of the Stordalen mire and its surroundings (modified from Malmer *et al.,* 2005; wet areas are prevalently covered by sedge and *Sphagnum* mires; dry areas by palsas).

depth intervals used in CH_4 production potential experiments. The subsampling interval depended on the core length, which ranged from 22 to 50 cm. Each core section was ~10 cm in diameter and ~4 cm long for the chosen depths to analyse. Each sample was freeze-dried, typically for 3 days, and ground to a fine powder in liquid nitrogen using a mortar and pestle.

Approximately 1 g dry weight of the ground soil was extracted using a modified Bligh and Dyer (1959) method. Buffered water was prepared by adding 2 g Potassium dihydrogen phosphate to 300 ml of double-distilled water to yield a 0.05 M solution. The pH was then adjusted to 7.2 by the addition of NaOH pellets and the mixture was extracted three times with 50 ml of dichloromethane (DCM). The monophasic Bligh-Dyer solvent mixture was prepared in the following proportions by volume: 4:5:10 buffered water:chloroform:methanol. After the addition of 8 ml of Bligh-Dyer solvent to the sample, the mixture was sonicated for 15 min and then centrifuged at 3200 rpm for 5 min. The supernatant was decanted into a large vial and the extraction was repeated four times. Addition of 2 ml of buffered water and 2 ml of chloroform separated the organic and aqueous phases and the lower organic layer was removed. The aqueous phase was subsequently extracted three times with 2 ml of chloroform. The extracts were combined and activated Cu turnings were added to remove sulphur. After 24 h, the extracts were filtered through glass wool and 4 $\mu g\,\mu l^{-1}$ of androstane and hexadecan-2-ol were added as standards.

The total lipid extract was separated into three fractions (neutral, acid and highly polar (nominally phospholipids)) using solid-phase extraction with aminopropyl cartridges (Uppsala, Sweden) (Isolute; NH_2, 500 mg). Fractions were eluted sequentially with a 2:1 (vol:vol) DCM:isopropanol solution, 2 per cent acetic acid in diethyl ether and methanol. The neutral fraction was further separated into apolar (hydrocarbons) and polar (alcohols and ketones) fractions using alumina flash column chromatography, eluted using three to four column volumes each of *n*-hexane:DCM (9:1, vol:vol) and DCM:methanol (1:2, vol:vol), respectively. The neutral polar lipid fractions contained the already partly degraded (loss of head group) archaeal cell membrane components, archaeol and hydroxyarchaeol. The phosphorylated head groups of the intact archaeol phospholipids (PL-Ar) in the highly polar fraction were cleaved using alkaline hydrolysis (heating with 1 ml 0.5 M methanolic NaOH for 1 h at 70 °C), leading to the release of the archaeol core lipids. Alcohol moieties in the neutral polar and saponified phospholipid fractions, such as archaeol and hydroxyarchaeol, were converted to trimethylsilyl derivatives by heating with 25 µl each of *N, O*-bis(trimethylsilyl)trifluoroacetamide and pyridine at 70 °C for 1 h.

Instrumental Analyses and Data Processing.

The neutral polar and saponified phospholipid fractions were analysed on a Carlo Erba 5300 (Hofheim, Taunus, Germany) series gas chromatograph (GC) equipped with a flame ionisation detector and fitted with a fused capillary column (50 m x 0.32 mm i.d.) coated with a CP Sil5-CB (Agilent Technologies, Stockport, Cheshire, UK) (dimethylpolysiloxane) equivalent. The carrier gas was H_2 and the oven temperature was programmed from 70 to 130 °C at 20 °C min^{-1}, from 130 to 300 °C at 4 °C min^{-1} and finally held at 300 °C for 25 min. GC-mass spectrometry (GC-MS) was performed using a Finnigan Trace GC-MS (Hemel Hempstead, UK) with electron ionisation at 70 eV operating in full-scan mode 40–650 amu, 1 scan s^{-1}. GC conditions were the same as

described above except that He was used as a carrier gas. The processing of spectra acquired from the GC-MS was conducted using Xcalibur 2.0 software (thermoscientific, Hemel Hempstead, UK).

Dissolved CH_4 Concentrations and CH_4 Production

After removal of large root fragments, smaller peat samples (3 replicates each) from six depths were taken from the soil cores for both sites using a metal cylinder (2 cm diameter). The samples were analysed for dissolved CH_4 concentration and for CH_4 production potential. For the former, each sample was placed in a 35 ml glass bottle fitted with a butyl rubber septum and screw cap. The bottle contained 20 ml of 10 wt per cent KCl solution that had been acidified to pH ~1 using 6 M HCl. The vial headspace gas, representing total dissolved CH_4 in sediment porewater, was analysed as described in Lupascu *et al.* (2012) and subsequently normalised to sample volume and porosity.

CH_4 production potential was measured by placing each subsample in a 35 ml glass bottle that was flushed with oxygen-free nitrogen. The samples were stored in the dark at 4 °C at the Abisko Research Station before being transported under cool conditions to the UK. The samples were then incubated at 4, 14 and 24 °C and headspace gas from the vials was analysed for CH_4 content as described in Lupascu *et al.* (2012).

RESULTS

In-situ Measurements

The sedge and *Sphagnum* mires were waterlogged during all sampling periods, with water table levels at ~2 cm above the ground surface. In June, only the upper 20 cm of soil was thawed at both sites and by August, the sedge mire site was fully thawed while the *Sphagnum* site had a permafrost table at 90 cm depth. Mean surface soil temperatures for the field sites on the days of core collection were 3.4, 11.2, 5.7 °C in June, August and September, respectively (Figures 2 and 3). Soil pH was relatively constant with depth at each site, with lower values at the *Sphagnum* mire (pH ~4.1 ± 0.2) compared to the sedge mire (pH ~5.7 ± 0.2).

Intact PL-Ar, Archaeol and Hydroxyarchaeol Concentrations

Sedge Mire.

Archaeol obtained by saponification of intact PL-Ar but also from already degraded (loss of head group) archaeal (Ar) membrane lipids were present in all three cores (June, August and September) collected from the sedge mire (Figure 2). The *sn2*- and *sn3*- isomers of hydroxyarchaeol also were present in all cores but were only identified in their free forms (without head group). Although there are some variations among sampling periods, archaeal diether lipid concentrations in both the intact and non-intact forms increase with depth in the three cores, with notable increases in concentration occurring approximately 15, 35 and 27 cm below the water table level for June, August and September, respectively. In the core collected in June, phosphorylated archaeol, *sn2*- and *sn3*-hydroxyarchaeol concentrations (Figure 2; A1 and A3) were very low or below detection limits in the upper 10 cm and concentrations increased at 15 cm depth to 3600 and 160 ng g^{-1}, respectively. Similarly, in the core collected in August (Figure 2; B1 and B3), both phosphorylated archaeol and *sn2*-hydroxyarchaeol concentrations were low at shallow depths (0 and 74 ng g^{-1}, respectively) and increased with depth (4000 and 780 ng g^{-1}, respectively). In contrast, *sn3*-hydroxyarchaeol was detected only in the upper part of the August core at a maximum concentration of 190 ng g^{-1} at 18 cm depth. The phosphorylated archaeol and hydroxyarchaeol concentrations in the September core (Figure 2; C1 and C3) exhibited less depth-dependent variations: archaeol and *sn2*- and *sn3*-hydroxyarchaeol concentrations increased to maxima of 6400, 160 and 120 ng g^{-1}, respectively, at 26 cm depth and then decreased slightly. The concentration of non-intact phospholipid archaeol (Ar) was lower than that of archaeol occurring in the phosphorylated form (<1000 ng g^{-1}) but exhibited similar depth profiles with the exception of June.

Sphagnum Mire.

Phosphorylated archaeol, non-intact PL archaeol (Ar) and *sn2*-hydroxyarchaeol were present only in cores collected in August and September. *Sn3*-hydroxyarchaeol was not detected in the cores. In the June core (Figure 3; A1 and A3), the shallow thawed layer extended only to approximately 20 cm depth and no diether lipids were detected in the shallow peat. In the August core (Figure 3; B1 and B3), concentrations of the two archaeal biomarkers were low in the top 26 cm (<160 ng g^{-1} for phosphorylated archaeol and *sn2*- hydroxyarchaeol was absent) but increased significantly at 35 cm depth (~2200 and 170 ng g^{-1}, respectively). Phosphorylated archaeol and *sn2*-hydroxyarchaeol concentrations in the September core (Figure 3; C1 and C3) again were low in the upper 18 and 30 cm, respectively, but increased at depth to concentrations greater than maximum values measured in August. The highest concentrations of archaeol and *sn2*-hydroxyarchaeol occurred at 38 cm depth (3500 and 400 ng g^{-1}, respectively). Similar to the sedge mire, concentrations of diether lipids detected (especially phosphorylated archaeol) increased over the summer; however, the increase was largely confined to horizons below 20 cm depth. Non-intact PL archaeol (Ar) was present in very low concentrations compared to the intact form (<300 ng g^{-1}) and was observed only in samples collected from depths >30 cm.

CH_4 Concentrations and Production Potential

Concentrations of dissolved CH_4 were similar between the sedge (~50–215 μmol l^{-1}; Figure 2; A2, B2 and C2) and the *Sphagnum* (~25–265 μmol l^{-1}; Figure 3; A2, B2 and C2) mires. Depth profiles for both sites and all months generally

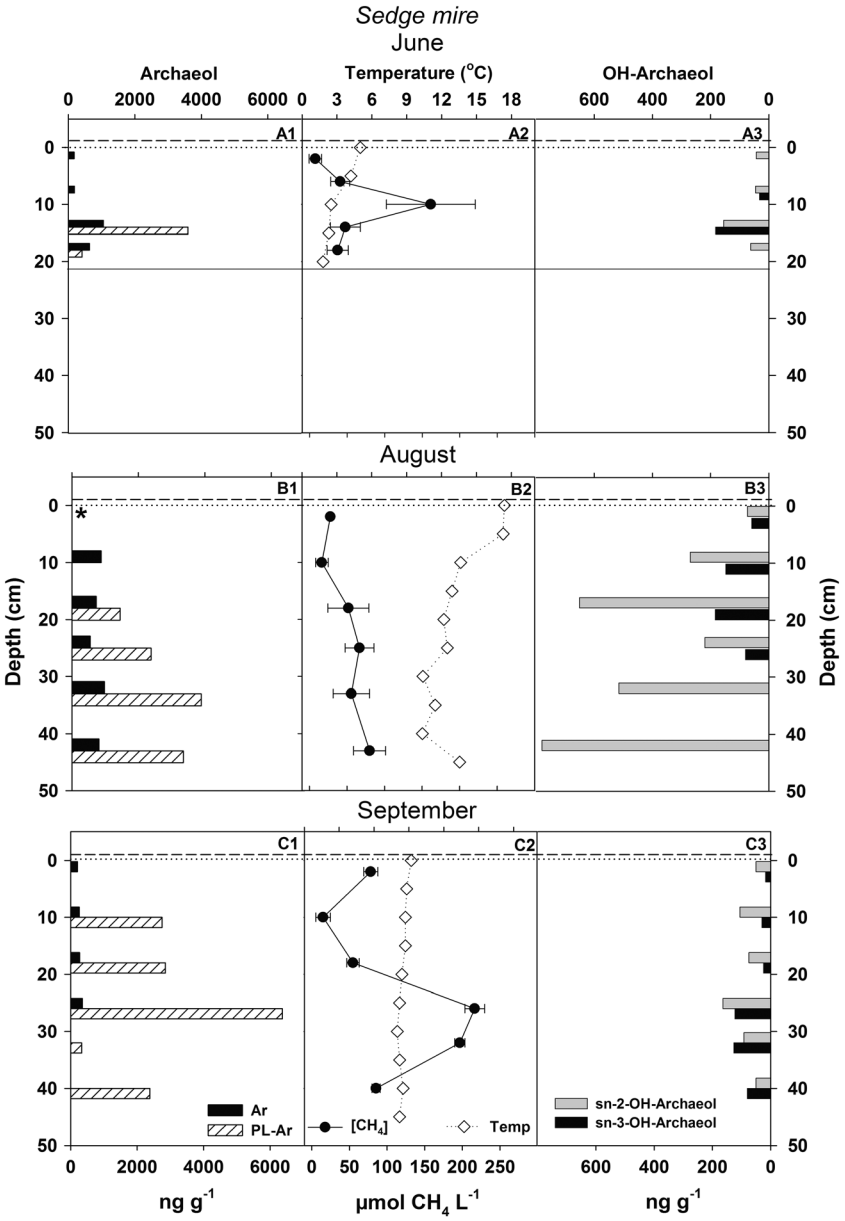

Figure 2 Archaeol from intact (PL-Ar) and from already partly degraded (Ar; core lipids after loss of head group) phospholipid archaeols (A1, B1, C1) and *sn2*- and *sn3*-hydroxyarchaeol (A3, B3, C3) concentration depth profiles for the sedge site in the months of June, August and September. Also shown are CH_4 concentration and temperatures (A2, B2, C2) for the same months (dashed lines represent the water table at the time of sampling, dotted lines represent the ground surface and the solid line represents the frozen ground table; * shallow sample at 5 cm depth in August is absent owing to a data error).

were characterised by an increase in CH_4 concentration with depth (Figures 2 and 3; A2, B2 and C2). During the summer, CH_4 concentrations decreased from June to August and then increased in September.

Full details of the CH_4 production potential experiments are reported in Lupascu *et al.* (2012) and only select aspects of that data-set are selected here in the context of biomarker data. Incubation of core subsamples in the absence of oxygen demonstrated that CH_4 production potential varied with temperature, peatland trophic status and soil depth. In general, the increase in CH_4 production in response to increased temperature was greater in the sedge mire (Figure 4a; maximum rate 142.3 µg CH_4 d^{-1} g^{-1}) compared to the *Sphagnum* mire (Figure 4b; maximum rate 44.4 µg CH_4 d^{-1} g^{-1}).

The CH_4 production potential in the sedge mire decreased markedly with increasing depth in all sampling months (Figure 4a). The *Sphagnum* mire displayed similar profiles between months, but CH_4 production rates were always lower than in the sedge mire for the same incubation temperatures (Figure 4b) and rate variations with depth were less pronounced (mean differences of 7.4 µg CH_4 d^{-1} g^{-1} at 14 °C and 30.3 µg CH_4 d^{-1} g^{-1} at 24 °C).

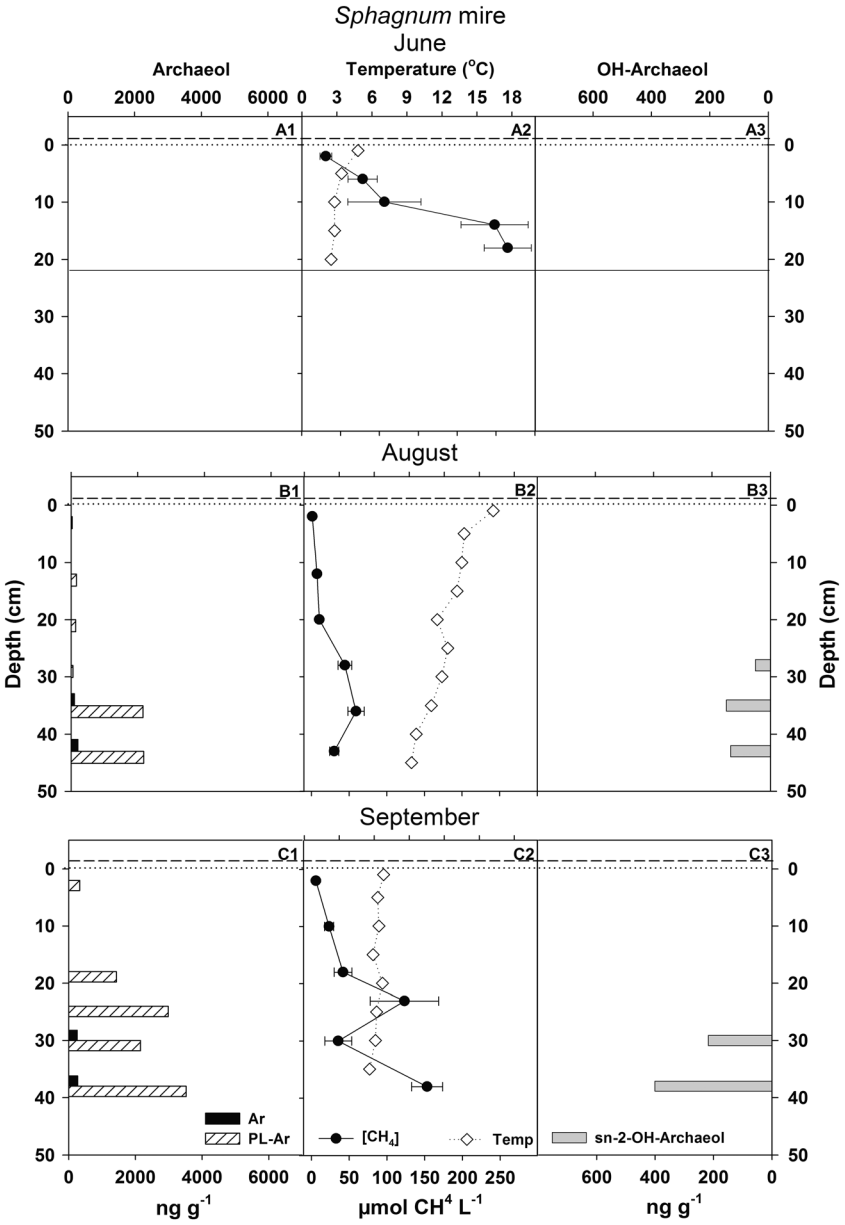

Figure 3 Archaeol from intact (PL-Ar) and from already partly degraded (Ar; core lipids after loss of head group) phospholipid archaeols (A1, B1, C1) and *sn2*- and *sn3*-hydroxyarchaeol (A3, B3, C3) concentration depth profiles for the *Sphagnum* site in the months of June, August and September. Also shown are CH_4 concentration and temperatures (A2, B2, C2) for the same months (dashed lines represent the water table at the time of sampling, dotted lines represent the ground surface and the solid line represents the permafrost table).

DISCUSSION

Quantification of archaeal lipids in peat provides information about labile lipids derived from presumably living biomass (phospholipids) or a combination of living organisms and fossil biomass (core lipids). However, glycosidically bound diethers (glycolipids) were not quantified, which can represent an important fraction of archaeal lipids (e.g. Koga *et al.,* 1998a, 1998b; Lim *et al.,* 2012). Thus, we focus primarily on variations in phosphorylated archaeol concentrations as an indicator of living methanogenic Archaea and we do not attempt to directly calculate methanogen biomass. Furthermore, owing to the long-term stability of core (or simple) archaeal lipids (Pease *et al.,* 1998), the interpretation of seasonal patterns also focuses on intact phosphorylated archaeal lipids. For all lipid forms, we examined depth- and site-related variations, specifically the differences in archaeal biomarker concentrations in shallow CH_4-free soil versus deeper peat, and differences in methanogen biomarker abundances between sites which may result from variations in plant assemblages.

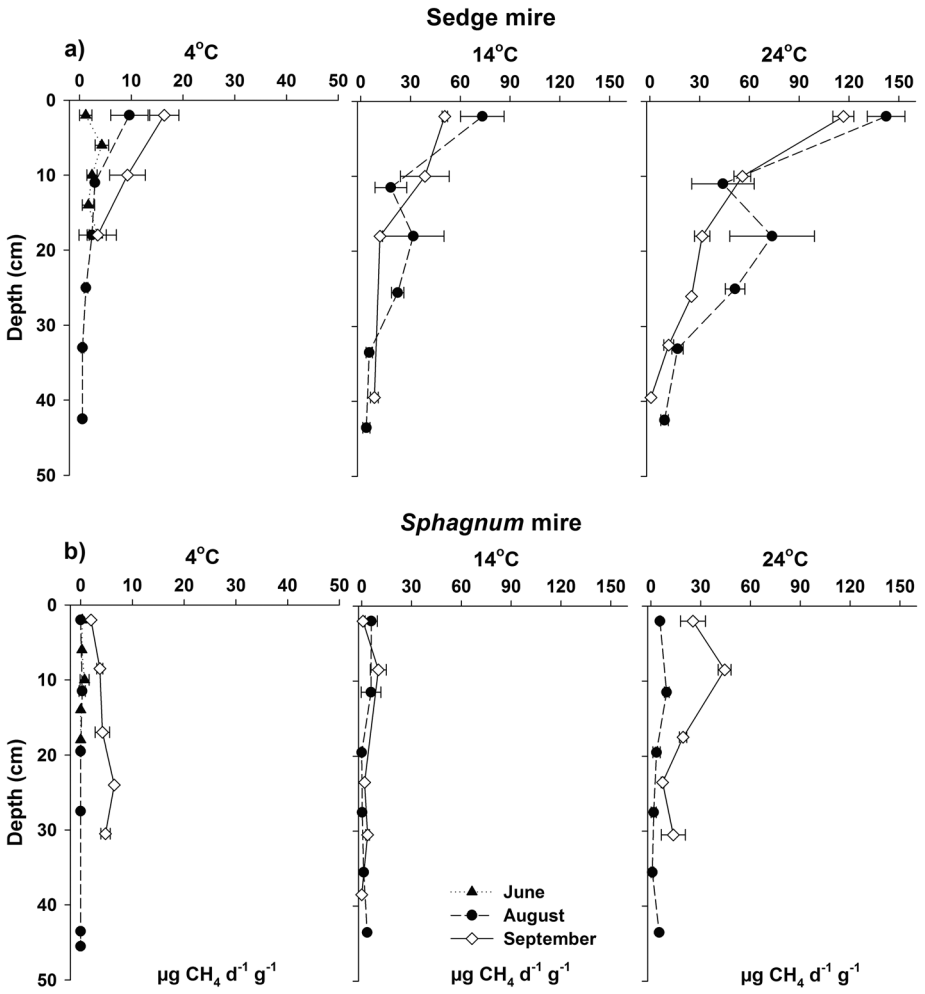

Figure 4 CH_4 production potential of dry weight at 4, 14 and 24 °C in the (a) sedge and (b) *Sphagnum* mires (modified from Lupascu *et al.*, 2012).

The Presence of Archaeal Lipids in Low Arctic Soils

Phosphorylated archaeol, archaeol core lipid and *sn2*-hydroxyarchaeol were detected in both peatland types and all cores, except for shallow cores collected in June from the *Sphagnum* sites. In contrast, the less common isomer *sn3*-hydroxyarchaeol was detected only at the sedge site. Although archaeal diethers are common in a range of environments (Asakawa *et al.,* 1998; DeLong *et al.,* 1998; Hinrichs *et al.,* 1999; Pancost *et al.,* 2000, 2001), we interpret their source to be primarily methanogens in this setting, which is consistent with the widespread occurrence of archaeal diethers, especially hydroxyarchaeol, in cultured methanogens (Sprott *et al.,* 1990; Koga *et al.,* 1993). Archaeol has been reported in halophiles, thermophiles and methanogens and is the most common and ubiquitous compound among the archaeal lipids (Koga *et al.,* 1998a, 1998b). The biomarker *sn2*-hydroxyarchaeol occurs predominantly in methanogenic Archaea, and especially those of the order *Methanosarcinales* (Sprott *et al.,* 1993; Koga *et al.,* 1998a), and anaerobic methanotrophic Archaea (e.g. Hinrichs *et al.,* 1999). Organisms known to contain *sn3*-hydroxyarchaeol include *Methanococcus voltae* (Sprott *et al.,* 1993) and *Methanolobus bombayensis* (S. Schouten, personal communication), both of which also produce *sn2*-hydroxyarchaeol, and *Methanosaeta concilii*, which predominantly produces *sn3*-hydroxyarchaeol (Ferrante *et al.,* 1988).

The inference that archaeal diethers derive from methanogens is consistent with previous microbiological work in which fluorescence *in-situ* hybridisation (Kobabe *et al*., 2004) or 16S rRNA and methyl coenzyme M reductase gene sequences have been used to profile the archaeal community structure in northern permafrost peatlands (Basiliko *et al.,* 2003; Kotsyurbenko *et al.,* 2004; Høj *et al.,* 2006; Ganzert *et al.,* 2007; Barbier *et al.,* 2012). Each of these investigations reported that methanogens were the main population of Archaea present in permafrost peatlands. Therefore, it is likely that the archaeol, both in its phosphorylated and non-intact PL form, and hydroxyarchaeol present in the Abisko cores also derive from methanogens.

Although all of the diethers likely represent a combination of living and dead biomass, we suggest that the phosphorylated components reflect the former. By analogy with the polar acyl ester lipids of bacteria, which lose polar head groups via enzymatic hydrolysis upon cell death or cell lysis (White *et al.,* 1979), intact polar ether lipids have been argued to indicate the presence of living or potentially viable biomass rather than fossil archaeal biomass (Sturt *et al.,* 2004; Biddle *et al.,* 2006). However, we do note that ether-bound archaeal IPLs are more stable than ester-bound bacterial IPLs as observed in an experiment over a period of 100 days (Logemann *et al.,* 2011). Intriguingly, phosphorylated archaeol and *sn2*-hydroxyarchaeol exhibit a similar pattern along the soil profiles and between the different months (with the exception of the sedge mire in September), suggesting a similar source and behaviour. This could arise from the labile nature of the *sn2*-hydroxyarchaeol (Nichols *et al.,* 1993), such that it also can be utilised as a marker for living methanogen biomass.

Archaeal Biomarker Concentrations – Variations among Sites and Depths

The sedge and *Sphagnum* mires, which were both characterised by relatively shallow water tables, exhibit significantly different phosphorylated archaeol abundances ($p < 0.05$, student t-test) and depth profiles. It is likely that this reflects differences in plant cover because plant species differ both in litter chemistry and root exudates (Crow and Wieder, 2005; Meier and Bowman, 2008), and thus affect below-ground biota. This result is further corroborated by the CH_4 production profiles (Figure 4) and emissions (Bäckstrand *et al.,* 2008), which were higher at the sedge than the *Sphagnum* site.

The large difference in archaeol concentrations between the shallow and deeper samples for both sites likely reveals a difference between permanent (deep) and intermittent (shallow) anoxic conditions related to oxygen diffusion (Kiener and Leisinger, 1983). Although the water table level was near the ground surface for all three sampling months, the increase in methanogen biomarker concentrations occurred at ~10 cm (or deeper), suggesting that biomass is highest under more persistent anoxic conditions. Archaeol concentrations bear some resemblance to dissolved non-purgeable organic carbon levels rather than the organic carbon content of peat (Lupascu *et al.,* 2012), which highlights the influence of labile organic carbon sources on methanogen populations (Liu *et al.,* 2011).

These distributions of methanogens are similar in general to those documented in other studies, although we note that previous investigations utilising archaeol as a biomarker proxy for methanogenic communities have employed a diversity of approaches. Most have focused on analysis of either free or phospholipid archaeol (Fritze *et al.,* 1999; Pancost *et al.,* 2011; Bischoff *et al*., 2013) and only a few studies have examined archaeol bound by glycosidic headgroups (Lim *et al.,* 2012). Other investigations of archaeal lipid biomarkers in high-latitude permafrost areas have focused on the collective quantification of intact phospolipid ether lipids (PLEL: isoprenoidal di- and tetraethers from glycerol backbones (archaeols and caldarchaeols, respectively) or more complex polyols) to track active archaeal populations (Wagner *et al.,* 2005, 2007).

In an acidic mire near Umea (Sweden), where the water table level was near the surface, Fritze *et al.* (1999) observed a lower concentration of phosphorylated archaeol and archaeol core lipids (~10.2 ± 3.1 ng g^{-1}) than reported here. Similar to our sites, the peak in archaeol concentration occurred below the surface, at 20 cm depth. Pancost *et al.* (2011) did not detect archaeol (phosphorylated archaeol and archaeol core lipids) in surface sediments (<10 cm) of several European peatlands where the peak concentration of archaeol occurred 20 to 50 cm below the water table level. Concentrations of phosphorylated archaeol and archaeol core lipids at two sites in Ireland and the UK were greater than at Abisko, whereas concentrations at mires in Germany and Finland were similar. In other terrestrial settings that were not water saturated, Lim *et al.* (2012) reported lower concentrations than the ones that we measured in Abisko peat, with values of 300 ± 200 ng g^{-1} (phosphorylated archaeol, archaeol core lipids and glycolipids) in a moist grassland soil in the UK. Bischoff *et al.* (2013) reported archaeol concentrations (phosphorylated archaeol and archaeol core lipids) ranging from 0.3 to 67 ng g^{-1} in permafrost soil of the Lena Delta, Siberia.

Studies from other terrestrial settings also report that ether lipid concentrations, including both diether and tetraether lipids, increase with depth at or below the water table (Weijers *et al.,* 2004; Wagner *et al.,* 2005, 2007). In the majority of the studies, water table depth, and therefore O_2 concentration, likely represents one of the main factors influencing the distribution of archaeol in the subsurface. The increase in methanogen biomarker concentrations several centimetres below the water table level is consistent with previous studies that have reported maxima in methanogen abundance (Fritze *et al.,* 1999; Pancost *et al.,* 2011) and activity (Daulat and Clymo, 1998) at that depth in peat soil. However, an extensive root system also may inhibit growth of a methanogen population in the surface layer because of rhizosphere input of O_2.

Seasonal Trend

Phosphorylated archaeol concentrations increased markedly during the summer months and peaked in September. A similar trend occurred for hydroxyarchaeol concentrations in the *Sphagnum* mire but not the sedge mire, where the highest concentrations were measured in August. Collectively, these observations are consistent with an increase in methanogen population during summer. Previous work has shown that CH_4 emissions are highest at these sites in August (Bäckstrand *et al.,* 2008), coincident with the highest air and soil temperatures. Our data appear to document an expanding methanogen community that parallels

increased *in-situ* CH_4 production; however, community abundance continued to increase late in summer after the peak period of CH_4 flux. Although a greater methanogen population is present, lower CH_4 emissions in September probably are linked to plant senescence and a reduction in the ability of vascular plants to function as conduits for export of CH_4 from soil to the atmosphere (Joabsson *et al.,* 1999; Heikkinen *et al.,* 2002).

Methanogen Biomarker and CH_4 Production Potential

Methanogen biomarker abundance and dissolved CH_4 concentration (Figures 2 and 3) generally increase with depth in both the sedge and *Sphagnum* mires at Abisko, although over the depth range investigated we could not determine whether methanogen biomarker and CH_4 concentrations eventually decoupled. In contrast, CH_4 production potential (Figure 4) decreased with depth in the sedge and *Sphagnum* mires, indicating that factors controlling production potential are more complex than methanogen abundance alone. However, it is important to note that CH_4 production potential in our short-term incubations represents the capacity of *in-situ* microbial populations collectively to metabolise available labile substrates under induced anoxic conditions at different temperatures (C. Treat *et al.,* 2014). As short-term substrate pools are depleted, the lack of living plants and thus absence of new root exudates limit the capacity for significant growth of microbial communities. In contrast, different controls regulate *in-situ* dissolved CH_4 concentration (e.g. the collective effects of production, diffusion, consumption and transport) and methanogen biomarker abundance (e.g. substrate availability, temperature, water content and oxygen availability).

The high CH_4 production potential in shallow peat, despite a low initial abundance of methanogens, suggests that high concentrations of labile organic matter and induced anoxic conditions drive a rapid increase in methanogenesis under experimental conditions. This result is opposite to *in-situ* conditions where despite the presence of labile organic matter, porewater CH_4 concentrations are low and the inferred methanogen population is small. This difference likely results from higher levels of dissolved oxygen in surface water (Elberling *et al.,* 2011; Estop-Aragonés *et al.,* 2012) inhibiting methanogen growth (Knorr *et al.,* 2009).

CH_4 production potential was much lower in peat collected from deeper horizons (Figure 4). The presence of abundant archaeal biomarkers for living methanogens in deeper soils suggests that production at depth was not limited by a lack of methanogens but rather by organic matter recalcitrance and possibly lower temperatures, which collectively inhibit metabolic activities of methanogens and other anaerobic microorganisms. In contrast to the incubation experiments, CH_4 production *in situ* is maintained by the secretion of labile organic carbon especially at the sedge site where the dominant vascular plant, *E. angustifolium*, is associated with the release of significant quantities of root exudates in many types of mire (Joabsson and Christensen, 2001; Christensen *et al.,* 2004). On the other hand in the *Sphagnum* site, the lack of a well-developed rhizoid system results in little labile organic carbon being released to anaerobic peat layers (Galand *et al.,* 2005), leading to a smaller methanogen population and lower CH_4 production potential compared to the sedge site. The lower pH (~4.1 ± 0.2) at the *Sphagnum* site compared to the sedge site (~5.7 ± 0.2) can further explain these differences because experimental manipulation of soil pH has demonstrated that excess acidity diminishes CH_4 production in peatlands (Williams and Crawford, 1984; Dunfield *et al.,* 1993; Kotsyurbenko *et al.,* 2004).

Our results are consistent with the findings of Wagner *et al.* (2005), who reported the absence of a relationship between archaeal PLEL concentrations and CH_4 production under *in-situ* conditions. However, a stronger relationship was observed between archaeal PLEL concentration and CH_4 production potential when acetate or hydrogen was added, suggesting that methanogenesis was substrate limited (Wagner *et al.,* 2005). Although organic C is abundant in permafrost soils, the implication of these observations is that much of the organic matter is resistant to biological degradation under *in-situ* conditions.

CONCLUSIONS

This study shows that a constant high water table level and differences in vegetation cover and pH are important factors influencing the depth distribution of the methanogenic community in the Low Arctic peatlands at Abisko (Sweden), as monitored by phosphorylated archaeol, core archaeol and hydroxyarchaeol concentrations and profiles. Decoupling of methanogen abundance from CH_4 production potential during the summer months suggests that the availability of labile organic carbon also exerts a key control on the rates of methanogenesis and distribution.

Future work should also consider glycosidically bound archaeol as well as phosphorylated archaeol and *sn2*-hydroxyarchaeol as proxies for the characterisation of methanogen biomass within permafrost soils. This can lead to a better understanding of the incidence and prevalence of methanogenesis as a result of future changes in environmental conditions and their potential for CH_4 production.

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