

Methane dynamics regulated by microbial community response to permafrost thaw

Carmody K. McCalley^{1†‡}, Ben J. Woodcroft², Suzanne B. Hodgkins³, Richard A. Wehr¹, Eun-Hae Kim⁴, Rhiannon Mondav^{2†}, Patrick M. Crill⁵, Jeffrey P. Chanton³, Virginia I. Rich⁴, Gene W. Tyson², and Scott R. Saleska^{1‡}

¹Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, 85721, USA

²Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, 4072, Australia.

³Department of Earth, Ocean and Atmospheric Science, Florida State University, Tallahassee, FL, 32306, USA

⁴Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ, 85721, USA

⁵Department of Geological Sciences, Stockholm University, Stockholm 106 91, Sweden

[†]Present address: Earth Systems Research Center, University of New Hampshire, Durham, NH, 03824, USA (C.K.M). Department of Ecology and Genetics, Uppsala University, Uppsala 75 236, Sweden (R.M)

[‡]Corresponding authors. Email: carmody.mccalley@unh.edu (C.K.M), saleska@email.arizona.edu (S.R.S)

Permafrost contains approximately 50% of global soil carbon¹. It is thought that permafrost thaw can lead to soil carbon loss in the form of methane and carbon dioxide emissions^{2,3}. The magnitude of the resulting positive climate feedback of such greenhouse

gas emissions remains uncertain³ and may to a large extent depend on the poorly understood role of microbial community composition in regulating the metabolic processes that drive such ecosystem-scale greenhouse gas fluxes. Here we use a natural landscape gradient of permafrost thaw in northern Sweden^{4,5}, as a model to investigate the role of microbial communities in regulating methane cycling, and to test whether knowledge of community dynamics could improve predictions of carbon emissions under permafrost loss. We find that changing vegetation and increasing methane emissions with permafrost thaw are associated with a switch from hydrogenotrophic to partially acetoclastic methanogenesis, resulting in a large shift in the $\delta^{13}\text{C}$ signature (10-15‰) of emitted methane. Abundance of the methanogen, *Candidatus Methanoflorens stordalenmirens*⁶, is a key predictor of the methane isotope shifts, which in turn predicts the proportion of carbon emitted as methane versus carbon dioxide, an important factor for simulating the climate feedback associated with permafrost thaw in global models^{3,7}. By showing that the abundance of key microbial lineages can be used to predict atmospherically relevant patterns in methane isotopes and the proportion of carbon metabolized to methane during permafrost thaw, we establish a basis for scaling changing microbial communities to ecosystem isotope dynamics. Our findings suggest that microbial ecology can play an important role in the ecosystem scale responses to global change.

Multiple factors—including hydrology, vegetation, organic matter chemistry, pH, and soil microclimate—are affected by permafrost loss^{5,8,9}. Together these factors regulate microbial metabolisms that release carbon dioxide (CO_2) and methane (CH_4) from thawing permafrost^{10–12}, and are the basis for earth system model predictions of future CH_4 emissions^{7,13,14}. However, the

role of microbial community composition in regulating the metabolic processes that drive ecosystem-scale fluxes is unknown.

At our study site in Stordalen, as in other thawing permafrost peatlands^{8,15}, permafrost loss causes hydrologic and vegetation shifts: well-drained permafrost-supported palsas collapse into partially-thawed moss-dominated (*Sphagnum* spp.) bogs and fully-thawed sedge-dominated (e.g. *Eriophorum angustifolium*) fens⁴. Between 1970 and 2000, 10% of Stordalen's palsa habitat thawed into such wetlands⁴. This transition drives an appreciable global warming impact because CO₂-emitting palsa is converted to bogs and fens which take up CO₂ but emit CH₄ (a more potent greenhouse gas³)^{4,5,16}. The net effect is that the high methane-emitting fen contributes 7 times the greenhouse impact per unit area as the palsa. This thaw progression is also associated with an increase in overall organic matter lability, including a decrease in C:N and an increase in humification rates⁹. We hypothesized, consistent with previous studies of *in situ* bog and fen systems^{17–19}, that thaw progression also facilitates a shift from hydrogenotrophic to acetoclastic CH₄ production.

We used the distinct isotopic signatures of different microbial CH₄ production and consumption pathways to directly relate changes in CH₄ dynamics across the thaw gradient to underlying changes in the microbial community. Methane produced by hydrogenotrophic methanogens generally has lower $\delta^{13}\text{C}$ and higher δD ($\delta^{13}\text{C}$ = -110 to -60‰ and δD = -250 to -170‰) relative to that produced by acetoclastic methanogens ($\delta^{13}\text{C}$ = -60 to -50‰ and δD = -400 to -250‰)^{19,20}. If methanotrophic microbes then oxidize CH₄, lighter molecules are preferentially consumed, leaving the remaining CH₄ ¹³C- and D-enriched relative to the original CH₄ pool (see expected patterns in Extended Data Fig 1)^{19,20}.

High temporal-resolution measurements of the magnitude and isotopic composition of CH₄ emissions, using a quantum cascade laser spectrometer (QCLS, Aerodyne Research Inc.) connected to autochambers, showed that CH₄ emissions and their ¹³C content increased with thaw. Average CH₄ fluxes increased from effectively zero at the intact permafrost palsa site to 1.46 (±0.37) mg CH₄ m⁻² h⁻¹ at the thawing *Sphagnum* site, to 8.75 (±0.50) mg CH₄ m⁻² h⁻¹ at the fully thawed *Eriophorum* site (Fig. 1a, p < 0.001). The average δ¹³C of emitted CH₄ also increased significantly, from -79.6‰ (±0.9) in the *Sphagnum* site to -66.3‰ (±1.6) in the *Eriophorum* site (Fig. 1b, p= 0.03). This consistent 10-15‰ divergence between sites was maintained through the growing season but overlain by parallel fluctuations in δ¹³C-CH₄, suggesting that weather patterns exerted a common influence over the magnitude of isotopic fractionation. Porewater CH₄ isotopes showed a similar pattern, with *Eriophorum* site porewater δ¹³C ~10‰ higher than *Sphagnum* (July and August, Fig. 1b, Extended Data Table 1). Porewater CH₄ was ¹³C-enriched by 5-20‰ relative to emitted CH₄, as expected due to diffusive fractionation (Methods equation (2))^{18,21}.

The apparent fractionation factor for carbon in porewater CH₄ relative to CO₂, α_C (Methods equation (2), Extended Data Table 1), is a related index of changes in CH₄ production²². Greater fractionation is associated with hydrogenotrophic methanogenesis, and was found in the thawing *Sphagnum* site (average α_C = 1.053 ± 0.002). Significantly less fractionation (p=0.002) associated with more acetoclastic production or with consumption by oxidation was found in the fully thawed *Eriophorum* porewater (average α_C = 1.046 ± 0.001). Here, increases in acetoclastic production, not oxidation, best explain isotopic shifts because lower α_C and higher δ¹³C-CH₄ are accompanied by significantly lower δD-CH₄ (Extended Data Fig. 1, p< 0.001)¹⁹. This is consistent with the pattern of isotopes in CH₄ emissions as well as

incubations of Stordalen peat⁹ and studies showing bog-to-fen shifts from hydrogenotrophic to acetoclastic methanogenesis^{17–19}.

The CH₄ flux and isotope results provide compelling but indirect evidence for changes in CH₄-cycling microbial communities with permafrost thaw. These microbiological changes could be shifts in activity of particular community members or changes in community composition. We examined the role of community composition through 16S rRNA gene amplicon sequencing. All known methanogens belong to a small number of archaeal lineages within the Euryarchaeota²³. As expected, the shift from CH₄-neutral intact permafrost palsa to CH₄-emitting wetland corresponded to a substantial increase in the relative abundance of methanogenic archaeal lineages (Fig. 1c, Extended Data Table 2,3). In the aerobic palsa and surface *Sphagnum* habitats, methanogens were found in low relative abundance (average <0.6%), while the anaerobic environments of the *Eriophorum* and deeper (below the water table) *Sphagnum* habitats harbored communities with a substantially higher relative abundance of methanogens (20-30%).

More significantly, the abundance of specific methanogenic lineages varied across the thaw gradient (Fig. 1c, Extended Data Table 2) in a manner corresponding to shifts in CH₄ production mechanism inferred from the isotope data (Fig. 1b). At the partially thawed *Sphagnum* site, where CH₄ isotopes were more hydrogenotrophic, the methanogen community was dominated by hydrogenotrophic populations (>=57% of sequences). Members of the genus *Methanobacterium* and close relatives of the recently described hydrogenotroph, Candidatus ‘*Methanoflorens stordalenmirens*’⁶ (a partial genome of which has also been identified in incubations of Alaksan permafrost¹²), were the most abundant phylotypes. While present, the metabolically versatile *Methanosarcina* (capable of utilizing a wide range of substrates including acetate and hydrogen²⁴), was much less abundant, averaging ~15% of the methanogen sequences.

At the fully thawed *Eriophorum* site (where isotope signatures shifted towards acetoclastic), members of the obligately acetoclastic genus *Methanosaeta* increased in abundance, comprising approximately one-third of the methanogenic population. The remaining methanogenic community was taxonomically diverse, including lineages present at the *Sphagnum* site as well as an additional hydrogenotrophic genus, *Methanoregula* (Extended Data Table 2). Differences in the functional (hydrogenotrophic versus acetoclastic) composition of the methanogen community between the sites were smallest in October, coinciding with a convergence in $\delta^{13}\text{C}$ - CH_4 (Fig. 1a and Extended Data Table 2,3).

Together, the isotope and microbial sequence data suggest that microbial community shifts drive large, concordant variations in CH_4 isotope biogeochemistry both seasonally and during permafrost thaw, a novel observation at the ecosystem scale. The early successional hydrogenotroph '*M. stordalenmirens*'⁶ dominates methanogenic metabolism in the early stages of thaw, followed by the subsequent emergence of a more diverse methanogen community, including obligate acetoclastic methanogens. This microbial succession provides direct evidence for how changes in ecosystem structure during permafrost thaw (plant succession and increases in organic matter quality⁹) translate into altered CH_4 biogeochemistry.

To quantify the effect of this shifting microbial community composition for CH_4 isotopic patterns, we examined the relationships between isotope fractionation (α_{C}), environmental conditions known or expected to impact methanogenesis, and the relative abundance of specific methanogenic lineages (Extended Data Table 4). Surprisingly, rather than a functional group (such as hydrogenotrophic methanogens), a single organism -- the hydrogenotroph '*M. stordalenmirens*' -- was the best one-variable predictor of isotopic patterns in the field (Fig. 2a). Several variables that typically differentiate bogs and fens, including pH and water table

depth¹⁸, were significant predictors of α_C , however, it was the relative abundance of ‘*M. stordalenmirens*’ that explained both the large range of α_C observed at the *Sphagnum* site ($R^2 = 0.7$, $p < 0.001$) as well as patterns across sites ($R^2 = 0.6$, $p < 0.001$). This suggests, contrary to the current practice of focusing on community functional diversity, that an individual microbial lineage can have a disproportionate influence on ecosystem biogeochemistry.

Stepwise regression identified environmental variables (water table depth, peat C:N and peat $\delta^{13}C$) that improved model predictions of α_C (to $R^2 = 0.8$, $p < 0.001$). While confirming the central importance of ‘*M. stordalenmirens*’ in explaining variation in α_C (Extended Data Table 5) this model also supports the hypothesis that organic matter chemistry underlies shifts in CH_4 metabolism^{9,25}. Interestingly, the dependence on the abundance of this lineage was evident despite the relative rather than absolute nature of the community composition analysis, and measurement of abundance rather than activity. We hypothesize that direct measures of gene expression or metabolic activity (meta-transcriptomics and –proteomics) will have an even stronger association than community composition data with isotopic signatures.

Further analysis showed that α_C significantly correlates ($R^2 = 0.7$, $p = 0.004$) with the large range in $CH_4:CO_2$ production ratio (0.13-0.84) measured in anaerobic incubations of Stordalen peat (Fig. 2b). Thus it is likely that changes in the proportion of anaerobically mineralized C that ends up as CH_4 —a key, but poorly constrained parameter in global CH_4 models²⁶—tracks the abundance of ‘*M. stordalenmirens*’, which acts as an index of the concerted changes in microbial community and organic matter chemistry that together control the efficiency of carbon metabolism.

Incorporating this understanding of the imprint of microbial communities could be critical to (1) improved model prediction of future climate change CH_4 feedbacks and (2)

accurate attribution of the portion of global atmospheric CH₄ change that derives from permafrost thaw. First, in simulating CH₄ cycling, earth system models typically prescribe as fixed the fraction of anaerobically metabolized carbon that becomes CH₄²⁶. The lack of a basis for predicting this parameter across ecosystems and in response to climate change limits current modeling efforts (3). Our finding that the CH₄:CO₂ production ratio is highly variable and predictable from isotopic indicators of methanogenic community composition (Fig. 2b) supports improving representation of microbial ecology in models^{17,27}. While simulating microbial population dynamics is beyond the scope of current global models, identification of microbial lineages that predict key parameters, such as the CH₄:CO₂ ratio, provides insights that improve simulations of CH₄ biogeochemistry used to estimate global emissions.

Second, atmospheric inversion studies which use CH₄ mixing ratios and isotopes to infer global sources and sinks of atmospheric CH₄ assume that wetland microbial sources are dominated by acetate fermentation (-58 to -65‰), and, critically, that isotopic signatures from biological sources are constant over time^{28,29}. In contrast, we observed isotopic compositions that varied across a gradient of permafrost thaw, with hydrogenotrophic methanogenesis estimated to produce ~50–75% of total CH₄ emission at Stordalen (Extended Data Table 6), with $\delta^{13}\text{C}$ averaging -80‰ (Fig. 1b). The hydrogenotrophic $\delta^{13}\text{C}$ observed at Stordalen and other Arctic wetlands³⁰, may be a ubiquitous characteristic of thawing permafrost, particularly during thaw stages that generate recalcitrant organic matter^{9,25}, such as that observed here in the intermediate-thaw *Sphagnum* site.

To test whether these observed thaw-induced changes in microbial metabolism could be relevant for large-scale atmospheric methane dynamics, we used a simple box model of atmospheric mixing (Methods equation (3)) to quantify the effect of different methanogen

communities within recently constructed scenarios of CH₄ emission from thawing permafrost² (Extended Data Fig. 2a,b). We found that if hydrogenotrophic lineages regulate CH₄ isotope patterns in permafrost thaw generally, as at Stordalen, then projected CH₄ emissions (Fig. 3a) will produce larger reductions in $\delta^{13}\text{C}$ of atmospheric CH₄ than expected from current inversion model assumptions that acetoclasts dominate emissions (Fig. 3b, Extended Data Fig. 2c,d). This, in turn would constrain our simple box model to substantially overestimate the amount of CH₄ released from thawing permafrost and underestimate emissions from non-wetland sources, most notably fossil fuels (Fig. 3c). The greater the prevalence of hydrogenotrophic lineages in CH₄ emissions, the larger will be the overestimate of fluxes from thaw (Fig 3c). The numerical size of the mis-estimation error here is illustrative; state-of-the-art 3D inversion models have spatially resolved constraints that would likely force smaller flux mis-estimation. But the general implication is that microbial effects are sufficiently important that accurate global accounting of the different sources of CH₄ under future climate change can be improved by understanding the microbial community dynamics underlying biological feedbacks in natural systems.

By showing that the abundance of key microbial lineages can be used to predict atmospherically relevant patterns in CH₄ isotopes and the proportion of carbon metabolized to CH₄ during permafrost thaw, this work establishes a basis for scaling changing microbial communities to ecosystem and global-scale atmospheric isotope dynamics. It also highlights the central role that microbial ecology can play in ecosystem-scale responses to global change and the benefit of incorporating microbial dynamics into earth system models.

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286 **Supplementary Information** is linked to the online version of the paper at
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www.nature.com/reprints. Correspondence and requests for materials should be addressed to
C.K.M (carmody.mccalley@unh.edu) or S.R.S (saleska@email.arizona.edu).

Figure 1. Increases in the magnitude and $\delta^{13}\text{C}$ signature of CH_4 during permafrost thaw track shifts in methanogen communities. **a**, Average daily CH_4 emissions (error bars represent s.e.m, n = 2-3) **b**, $\delta^{13}\text{C}$ composition of emitted and porewater CH_4 (error bars represent s.e.m, flux n = 2-3, porewater n = 6-9) and **c**, relative abundance of methanogenic groups as inferred by taxonomic identity assigned from 16S rRNA amplicon sequencing, for a permafrost thaw sequence at Stordalen Mire. For the intermediate thaw *Sphagnum* site, aerobic communities were sampled above the water table, anaerobic communities were sampled below the water table.

Figure 2. Correlation between the effective isotopic fractionation of methanogenesis, and both the relative abundance of the methanogen Candidatus ‘*Methanoflorens stordalenmirens*’, and the anaerobic $\text{CH}_4:\text{CO}_2$ production ratio. **a**, The relative abundance of a single methanogen, Candidatus ‘*Methanoflorens stordalenmirens*’ was significantly correlated ($p < 0.001$) with porewater effective fractionation (α_C), an isotopic indicator of methanogenic production pathway. **b**, Anaerobic incubations of peat collected from a related thaw sequence at Stordalen Mire (see methods in ⁹) show a significant correlation between α_C

and the CH₄:CO₂ production ratio, suggesting that the abundance of '*M. stordalenmirens*' may be indicative of the proportion of organic matter metabolized to CH₄.

Figure 3. Simulated effect of CH₄ from different methanogen communities in thawing permafrost on atmospheric $\delta^{13}\text{C}$ -CH₄ in a box model of the atmosphere. **a**, Modeled CH₄ emissions under high (red bounding lines) and low (orange bounding lines) climate warming scenarios, and a range within each (in gray) spanning high and low C release scenarios². The red dashed line is an intermediate emissions scenario used to simulate **b**, consequent reductions in $\delta^{13}\text{C}$ of atmospheric CH₄ due to emissions dominated by hydrogenotrophic lineages, as in intermediate-thaw *Sphagnum* sites (green line, $\delta^{13}\text{C}$ = -80‰), or more by acetoclasts, as in fully-thawed "*Eriophorum*" sites (blue line, $\delta^{13}\text{C}$ = -65‰). Atmospheric inversion models typically assume emissions have $\delta^{13}\text{C}$ ranging from = -60 (black line) to -65 (blue line). (The dotted horizontal line indicates the current detection limit for atmospheric CH₄ isotopes²⁸). These imply an underestimate of the effect on atmospheric $\delta^{13}\text{C}$ for the given emissions scenario (blue or green). In order to match observed atmospheric isotopes, the box model would then require **c**, a corresponding overestimate of CH₄ flux attributed to permafrost thaw (vertical axis). The magnitude of the overestimate depends on the mismatch between model-assumed isotopic composition (upper line = -60‰, lower line = -65‰), and the actual isotopic composition produced by different communities, which ranges here along the horizontal axis from -80‰ (hydrogenotroph-dominated as in the partially-thawed "*Sphagnum*" sites), to -65‰ (acetoclastic, as in the fully thawed "*Eriophorum*" sites).

341 **Methods**

342 **Site Description and permafrost thaw**

343 Stordalen is a sub-arctic palsa mire located 10km east of Abisko in the discontinuous
344 permafrost zone of northern Sweden (68°21'N 18°49'E, altitude 363 m a.s.l.). This work focuses
345 on three distinct subhabitats, common to northern wetlands and together covering ~98% of the
346 Mire surface: (i) permafrost-dominated, well-drained palsas occupied by feather mosses and
347 ericaceous and woody plants, covering 49% of the mire (ii) intermediate permafrost sites with
348 variable water table depth, dominated by *Sphagnum* spp., covering 37% of the mire, and (iii) full
349 summer-thaw, wet sites with *Eriophorum angustifolium*, covering 12% of the mire. Between
350 1970 and 2000, as permafrost thawed and palsas collapsed, *Sphagnum* sites and *Eriophorum*
351 sites expanded by 3% and 54%, respectively⁴.

352 Formation of wetlands following permafrost thaw, as observed at Stordalen, is a
353 widespread characteristic of peatlands affected by permafrost loss^{8,31–33}. Thawing of ice-rich
354 features results in peatland collapse and the formation bogs and fens. At Stordalen, thaw is
355 associated with a progression from ombrotrophic bogs to minerotrophic fens due to thaw-
356 induced subsidence increasing hydrologic connectivity. A similar successional shift from bogs
357 dominated by *Sphagnum* spp. to tall graminoid fens has been observed in other northern
358 peatlands^{8,33–35}. More generally, landscape features and hydrologic conditions dictate the
359 characteristics and trajectory of wetland communities formed following permafrost thaw³⁶. For
360 example, rapid fen development is observed at the subsiding margins of permafrost plateaus³⁷,
361 whereas collapse bogs and thermokarst lakes often form within large, thawing peatland
362 complexes³². Large uncertainty in model predictions of the extent and characteristics of wetland

formation arising from permafrost thaw is a critical limitation to current understanding of carbon-climate feedbacks^{7,14}. As demonstrated in this study, improved characterization and modeling of peatland transformation during thaw will be essential for accurately predicting post-thaw microbial communities and the resultant magnitude and isotopic composition of CH₄ emissions under climate change.

Methane Isotope systematics

We use standard δ notation for quantifying the isotopic compositions of CH₄ and CO₂: the ratio R of ¹³C to ¹²C (or D to H) in the measured sample is expressed as a relative difference (denoted $\delta^{13}\text{C}$ or δD) from the Vienna Pee Dee Belemnite (VPDB) international standard material. For example, for C:

$$d^{13}\text{C} = \frac{R - R_{\text{VPDB}}}{R_{\text{VPDB}}} = \frac{R}{R_{\text{VPDB}}} - 1 \quad (1)$$

$\delta^{13}\text{C}$ is often expressed in parts-per-thousand (per mil, ‰).

Isotopic fractionation in chemical reactions (including methanogenesis or methanotrophy) or due to diffusion may be quantified as (Farquhar et al., 1989):

$$a = \frac{R_{\text{source}}}{R_{\text{product}}} = \frac{d_{\text{source}} + 1}{d_{\text{product}} + 1} \quad (2)$$

For diffusive fractionation, R_{source} is taken to be the isotopic ratio in the concentrations of the gradient and R_{product} the ratio in the resultant net flux. Because diffusion discriminates against the heavy isotope, $R_{\text{product}} < R_{\text{source}}$, which implies, for example, that the isotopic ratio of porewater (the “source”) will be greater than that of the flux of gas diffusing out, as we see here (Fig. 1a). Methanogenesis and methanotrophy also discriminate against the heavier isotopes, so

that $R_{product} < R_{source}$ (and hence $\alpha > 1$) for both C and H in methane. Note that $\alpha > 1$ for methanotrophy implies that the products of CH_4 oxidation (CO_2 and H_2O) are lighter (have lower R) in both C and H relative to the source CH_4 ; but mass balance then requires the residual methane not oxidized to become heavier in both C and H, relative to the starting composition of the CH_4 pool before oxidation.

The degree of C isotopic fractionation between CO_2 and CH_4 differs between the two main biochemical pathways of methanogenesis, *acetoclastic* ($\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$) and *hydrogenotrophic* ($\text{CO}_2 + 4\text{H}_2 \rightarrow 2\text{H}_2\text{O} + \text{CH}_4$). Carbon isotope fractionation (α_C) is greater for hydrogenotrophic than for acetoclastic methanogenesis, but α_H (hydrogen isotope fractionation) follows the opposite pattern: α_H (hydrogenotrophic) $<$ α_H (acetoclastic) (Extended Data Fig. 1; Chanton et al¹⁹). Hence, variations in C and H isotopic compositions of CH_4 that arise from variations in methanogenic pathway will be anti-correlated: shifts from hydrogenotrophic to acetoclastic production will cause C isotope ratios to increase but H isotope ratios to decline, moving along a negatively-sloped “production line” in H vs C isotope space (Extended Data Fig. 1). Isotopic variations that arise from variations in the degree of methanotrophy, by contrast, will be positively correlated: shifts towards increasing methanotrophy will cause both C and H isotope ratios to increase along a positively sloped “oxidation line” (Extended Data Fig. 1).

In a field study like this one, it is difficult to directly estimate fractionation factors directly; hence, we follow standard practice in the methane biogeochemistry literature (eg. Whiticar et al.^{22,38}) and estimate the net or effective fractionation factor from *in situ* pore water data. For example, we estimate α_C , the effective fractionation factor for C in CH_4 , by applying equation (2), setting $\delta_{product} = \delta^{13}\text{C}_{\text{CH}_4}$ and $\delta_{source} = \delta^{13}\text{C}_{\text{CO}_2}$, where $\delta^{13}\text{C}_{\text{CH}_4}$ and $\delta^{13}\text{C}_{\text{CO}_2}$ are the

observed C compositions of CH₄ and CO₂, respectively³⁸. Using CO₂ isotope composition for δ_{source} follows directly for hydrogenotrophic methanogenesis (for which CO₂ is the source C substrate), and has been found to work also in practice for acetoclastic methanogenesis, as porewater CO₂ arises primarily from respiration of organic matter (a non-discriminatory reaction), and so is typically isotopically indistinguishable from organic matter^{20,39}.

Autochamber measurements

The autochamber system at Stordalen mire has previously been described in detail for measurements of CO₂ and total hydrocarbons^{16,40}. Briefly, a system of 8 automatic gas-sampling chambers made of transparent Lexan was installed in the three habitat types at Stordalen Mire in 2001 (n=3 each in the palsa and *Sphagnum* habitats, and n=2 in the *Eriophorum* habitat). Each chamber covers an area of 0.14 m² (38 cm x 38 cm), with a height of 25–45 cm, and is closed once every 3 hours for a period of 5 min. The chambers are connected to the gas analysis system, located in an adjacent temperature controlled cabin, by 3/8" Dekoron tubing through which air is circulated at approximately 2.5 L min⁻¹. During the 2011 season the system was updated with a new chamber design similar to that described by Bubier *et al*⁴¹. The new chambers each cover an area of 0.2 m² (45 cm x 45 cm), with a height ranging from 15–75 cm depending on habitat vegetation. At the Palsa and *Sphagnum* site the chamber base is flush with the ground and the chamber lid (15 cm in height) lifts clear of the base between closures. At the *Eriophorum* site the chamber base is raised 50–60 cm on lexon skirts to accommodate large stature vegetation. Additionally, each chamber is instrumented with thermocouples measuring air and surface ground temperature, and water table depth is measured manually 3–5 times per week. The Palsa site chambers are located within the palsa site in Mondav, Woodcroft *et al*⁶ and correspond to the

hummock site class (I) described in Johansson et al⁴. The *Sphagnum* site chambers are located within the bog site in Mondav, Woodcroft et al⁶ or site S in Hodgkins et al⁹ and correspond to the semi-wet and wet site class (II and III) described in Johansson et al⁴. The *Eriophorum* site chambers are located within the fen site in Mondav, Woodcroft et al⁶ or site E in Hodgkins et al⁹ and correspond to the tall graminoid site class (IV) described in Johansson et al⁴.

QCLS measurement and calibration

Methane fluxes and isotopes were measured using a Quantum Cascade Laser Spectrometer (QCLS, Aerodyne Research Inc), deployed to Stordalen Mire in June 2011. The QCLS instrument deployed at Stordalen is a modification of the technology described in detail by Santoni et al⁴². Briefly, the QCLS uses a room-temperature continuous wave mid-infrared laser whose frequency was tuned to rapidly (900 kHz) scan across ¹²CH₄ and ¹³CH₄ absorption lines in the 7.5 μm region. The laser light enters a multipass sample cell (effective path length of ~200m) containing sample air at low pressure (~5 kPa) and is detected by a thermoelectrically cooled detector (no cryogenics are needed). Aerodyne Research's custom TDL Wintel software averages high-frequency spectra to produce independent ¹²CH₄ and ¹³CH₄ mixing ratios in the sample airstream at 1 sec intervals. The ratio, *R*, of ¹³CH₄ to ¹²CH₄ and can then be expressed in standard notation as δ¹³C, the part-per-thousand (per mil, ‰) deviation of the measured ratio from the VPDB standard ¹³C/¹²C ratio *R*_{VPDB}, according to equation (1).

Instrument precision in the field at Stordalen Mire was assessed using time-series measurements of calibration tank air over 30–40 min. The precision of δ¹³C CH₄ measurements using a 1 second integration time was 1‰. The Allan variance technique (used to characterize the minimum possible measurement error and the averaging time required to achieve it⁴³),

showed that the minimum measurement error on $\delta^{13}\text{C-CH}_4$ was $<0.2\text{‰}$, achieved with 60 seconds of averaging time. This approaches the precision of comparable measurements made using GC-IRMS.

We connected the QCLS to the main autochamber circulation using $\frac{1}{4}$ " Dekoron tubing and a solenoid manifold that enables selection between the autochamber flow and an array of calibration tanks. During measurement periods, filtered ($0.45\text{ }\mu\text{m}$, Teflon filter) and dried (Perma Pure PD-100T-24MSA) sample-air flows at 1.4 slpm through the 2L QCLS sample cell volume at 5.6 kPa. A downstream solenoid controls the QCLS return flow so that air only recirculates during autochamber measurement periods, during calibration periods exhaust air is vented to the room.

Calibrations were done every 60 min using 3 calibration gases spanning the observed concentration range (1.5–10 ppm). The CH_4 concentration and $\delta^{13}\text{C}$ composition of each calibration tank was determined by inter-calibration with a set of 4 well-characterized primary standard tanks. The primary tanks (Scott Marin, Inc, Riverside CA) were calibrated to the VPDB scale by means of flask samples, which were analyzed by GC-IRMS at Florida State University (see porewater methods for GC-IRMS details). Each isotopologue, $^{12}\text{CH}_4$ and $^{13}\text{CH}_4$, was treated as an independent measurement and calibrated separately. For each calibration period a linear calibration curve was fitted for each isotopologue and the fit parameters were then linearly interpolated between calibration periods. The interpolated fit parameters were applied to the measured sample isotopologue mixing ratios to give calibrated measurements of $^{12}\text{CH}_4$, $^{13}\text{CH}_4$, and total CH_4 , from which $\delta^{13}\text{C-CH}_4$, was calculated.

Autochamber data processing

For each autochamber closure we calculated flux and $\delta^{13}\text{C}$ signature of emitted CH_4 . Fluxes were calculated using a method consistent with that detailed by Bäckstrand et al⁴⁴ for CO_2 and total hydrocarbons, using a linear regression of changing headspace CH_4 concentration over a period of 2.5 min. Eight 2.5 min regressions were calculated, staggered by 15 sec, and the most linear fit (highest r^2) was then used to calculate flux. Keeling plots^{45–47} using the entire closure period were used to estimate the isotopic composition of the emitted CH_4 . As demonstrated by Santoni *et al*⁴², negligible error in measurement of CH_4 relative to that of $\delta^{13}\text{CH}_4$ for this instrumentation meant that Type I regression was sufficient for the Keeling plot analysis. When the total change in headspace CH_4 was low⁴⁵, there was high error in the Keeling intercept. We used a threshold of 3‰ uncertainty in the Keeling intercept as a cut-off for including isotopic values in the calculation of daily and annual averages, resulting in a total of 1569 observations at the *Sphagnum* site and 1168 at the *Eriophorum* site. No Palsa chamber closures had sufficient CH_4 flux to calculate $\delta^{13}\text{CH}_4$. Daily and whole-season average flux and isotopic composition for each habitat were calculated based on individual chambers as the unit of replication (n=3 for Palsa and *Sphagnum*, n=2 for *Eriophorum*). Significant differences in the magnitude and isotopic composition of CH_4 emissions were determined using a Student's t-test (isotopic composition) and ANOVA (flux magnitude) in R⁴⁸, with seasonal averages for each auto-chamber as the unit of replication. Statistical significance was determined at $\alpha = 0.05$.

Porewater sampling and analysis

Porewater samples were collected on July 12, 2011, August 15, 2011 and October 15, 2011 at three locations adjacent to the *Sphagnum* and *Eriophorum* auto-chamber sites (Extended Data Table 1). Samples were collected by suction with a syringe through a stainless steel tube and filtered through 25-mm diameter Whatman Grade GF/D glass microfiber filters (2- μm

particle retention). Porewater pH was measured in the field (Oakton Waterproof pHTestr 10, Eutech Instruments). Samples for the analysis of the concentration and $\delta^{13}\text{C}$ of CH_4 and CO_2 were injected into 30-mL evacuated vials sealed with butyl rubber septa and frozen within 8 hours of collection. The samples for $\delta\text{D-CH}_4$ were injected into 120-mL evacuated vials sealed with butyl rubber septa and containing 0.5 g of KOH. For $\delta\text{D-H}_2\text{O}$, water was filtered directly into 2-mL plastic screw cap vials so that the vials were completely filled, then frozen within 8 hours of collection. All samples were shipped frozen to Florida State University for analysis.

Samples collected for analysis of CH_4 and CO_2 concentrations and $\delta^{13}\text{C}$ were thawed, acidified with 0.5 mL of 21% H_3PO_4 , and brought to atmospheric pressure with helium. The sample headspace was analyzed for concentrations and $\delta^{13}\text{C}$ of CH_4 and CO_2 on a continuous-flow Hewlett-Packard 5890 gas chromatograph (Agilent Technologies) at 40°C coupled to a Finnigan MAT Delta S isotope ratio mass spectrometer via a Conflo IV interface system (Thermo Scientific, Bremen, Germany). The headspace gas concentrations were converted to porewater concentrations based on their known extraction efficiencies, defined as the proportion of formerly-dissolved gas in the headspace. An extraction efficiency of 0.95 (based on repeated extractions) was used for CH_4 , and the extraction efficiency for CO_2 relative to DIC was determined based on CO_2 extraction from dissolved bicarbonate standards⁴⁹.

Samples collected for analysis of $\delta\text{D-CH}_4$ were brought to atmospheric pressure with helium and measured on a gas chromatograph connected to a ThermoFinnigan Delta Plus continuous flow isotope ratio mass spectrometer at the National High Magnetic Field Laboratory (Tallahassee, FL). δD of CH_4 is affected by δD of H_2O because CH_4 exchanges H atoms with water during methanogenesis^{20,38,50}, so measurement of $\delta\text{D-H}_2\text{O}$ is necessary for correct

assignment of CH₄ production mechanisms and oxidation based on δD and $\delta^{13}C$ of CH₄. Samples collected for δD -H₂O were measured on an LGR DT-100 liquid water stable isotope analyzer at Florida Agricultural and Mechanical University (Tallahassee, FL). Data analysis for these samples was performed using an MS Excel template from the IAEA Water Resources Programme (<http://www.iaea.org/water>).

Significant differences in α_C and δD and $\delta^{13}C$ of porewater CH₄ between the *Sphagnum* and *Eriophorum* sites were determined using a Student's t-test (α_C , δD -CH₄, $\delta^{13}C$ -CH₄) and Hotelling's t-test (multivariate δD and $\delta^{13}C$ of CH₄) in R⁴⁸. Statistical significance was determined at $\alpha = 0.05$.

Peat sampling

Peat samples were collected on July 12, 2011, August 16, 2011 and October 16, 2011 at three locations adjacent to the Palsa, *Sphagnum* and *Eriophorum* auto-chamber sites. For the *Sphagnum* and *Eriophorum* sites samples were collected at the same depths and locations used for porewater sampling (Extended Data Table 1), sample depths for the Palsa site are detailed in Mondav et al⁶. Peat cores were collected using a 11 cm diameter push corer (Palsa and *Sphagnum* sites) or a 10 cm x 10 cm Wardenaar corer (*Eriophorum* site). Cores were subsampled by depth and were subdivided in the field for microbial and chemical analysis, avoiding the outer 1cm of the core. Samples for microbial analysis were placed in cryotubes, saturated with ~3 volumes LifeGuard solution (MoBio Laboratories, Carlsbad, CA, USA) and stored at -80°C until processing. Samples for chemical analysis were placed in plastic bags and frozen until processing.

Peat chemical analysis

For peat %C, %N, C:N ratio, and $\delta^{13}\text{C}$ measurements, 5–10 g of peat was dried at 60 °C until completely dry (3–10 days) and ground to a fine powder. Subsamples of ground peat (80–100 µg for %C and $\delta^{13}\text{C}$ analysis, and 5000–6000 µg for %N analysis) were wrapped in tin capsules and analyzed by combustion to CO_2 and N_2 at 1020 °C in an automated CHN elemental analyzer coupled with a ThermoFinnegan Delta XP isotope ratio mass spectrometer at the National High Magnetic Field Laboratory (Tallahassee, FL). Samples were run in non-dilution mode for carbon analysis and dilution mode ($\times 10$) for nitrogen analysis. C:N was calculated as the ratio of (%C)/(%N) (by weight) for corresponding pairs of subsamples.

SSU rRNA gene amplicon analysis

Sampling and extraction was carried out as per Mondav et al.⁶. Several additional samples were analyzed for this paper, multiplex identifiers for those runs not reported in Mondav et al.⁶ are provided in Extended Data Table 7. SSU rRNA gene sequences were processed using APP 3.0.3 (<https://github.com/Ecogenomics/APP>). Homopolymer errors were corrected using Acacia⁵¹ and the resulting reads were processed using the CD-HIT-OTU 0.0.2 pipeline with minor adjustments⁵². All reads were trimmed to 250bp and reads <250bp were discarded. Sequences were clustered at 97% identity and each cluster assigned a taxonomy using BLASTN 2.2.22⁵³ through the QIIME script `assign_taxonomy.py`⁵⁴ against the GreenGenes Oct 2012 database clustered at 99% identity (Supplementary Data 1). Each abundant methanogenic cluster's taxonomy was confirmed using parsimony insertion with ARB⁵⁵. Amplicon sequence clusters were identified as potential hydrogenotrophic or acetoclastic methanogens based on taxonomic relationship to known methanogenic lineages (Extended Data Table 2)^{23,24,56}. Within

the order *Methanosarcinales*, lineages most closely related to *Methanosaeta* were classified as obligate acetoclasts, whereas those most closely related to *Methanosarcina* were considered facultative acetoclasts, having the potential for both acetoclastic or hydrogenotrophic production²³.

Regression analysis

A step-wise regression approach using Akaike's information criterion (AIC) as the model selection criterion was used to identify a sub-set of microbial and environmental predictor variables that best explained CH₄ metabolism patterns quantified as porewater α_C (Extended Data Table 5). Model selection was done using the stepAIC package in R and the relative importance of the predictor variables in the selected model was then calculated using the relaimpo R package⁴⁸. Variables included in the model selection process included the relative abundance of the 6 most abundant methanogen operational taxonomic units (OTUs) (comprising >93% of total methanogen sequences; see Extended Data Table 2) plus soil temperature, water table depth, pH, porewater CH₄ and DIC concentration, and peat C:N, % C, % N and $\delta^{13}C$ (Extended Data Table 1). Strong correlation between pH and both water table depth and peat $\delta^{13}C$ as well as peat %N and both %C and C:N meant that pH and %N were excluded from the regression analysis. Removing non-significant predictor variables (DIC and relative abundance of an unidentified *Methanobacterium* spp. (otu-3636, Extended Data Table 2)) had a minimal effect on the model AIC value (<1) and this simplified version was therefore selected as the optimal model (Model 2 in Extended Data Table 5). Stepwise regression was also done with $\delta^{13}C$ -CH₄ as the dependent variable. This analysis resulted in a similar model outcome, but with a lower R² (Model 1 in Extended Data Table 8). Stepwise regression analysis with environmental predictor variables and

the relative abundance of the influential methanogen '*M stordalenmirens*' (otu-10747) as the dependent variable showed that patterns in this methanogen's abundance were influenced by environmental conditions, particularly water table depth and peat chemistry (Model 2 in Extended Data Table 8). These environmental variables alone, however, cannot fully replace microbial data when modeling α_C . Stepwise regression analysis using only environmental variables to predict α_C yielded a model with a lower AIC and R^2 (Model 3 in Extended Data Table 8). It is the combination of methanogen and environmental variables that yields a model that explains the most variability in α_C (Extended Data Table 5).

Box Model of atmospheric methane

The model used here was a 1-box model simplified from the 2-box model of Tans⁵⁷ (and also used in the Kai et al.²⁸ methane inversion study):

$$\begin{aligned} \frac{dM}{dt} &= F_{CH_4} - \lambda M \\ \frac{d(RM)}{dt} &= R_{CH_4} F_{CH_4} - \alpha_{OH} \lambda (RM) \end{aligned} \quad (3)$$

where M is the mixing ratio (in ppbv) of CH_4 in the atmosphere, F_{CH_4} is the source flux of CH_4 to the atmosphere, λ is atmospheric removal rate ($1/9 \text{ yr}^{-1}$, assumed for this illustration to be fixed), the R terms are the ratio of $^{13}CH_4$ to $^{12}CH_4$, as defined for equation (1), and α_{OH} is the isotopic fractionation ($= 0.994$, or about -6‰) for atmospheric oxidation of CH_4 by OH ²⁸. Baseline flux to the atmosphere (F_{CH_4}) was set to 559 Tg CH_4 the 1980 value²⁸. The isotopic composition of CH_4 inputs to the atmosphere (R_{CH_4}) were set to the equivalent of -53‰ to allow steady-state modern atmospheric CH_4 to have the observed value of $\sim -47\text{‰}$.

We implemented this model numerically in the R software package⁴⁸, simulating the effect on the atmosphere of CH₄ emission due to permafrost thaw and partial decomposition of the 1,700 PgC stock of permafrost C anticipated over the next 300 years, as summarized in Schurr et al² and Tarnocai et al¹. High and low permafrost carbon release scenarios for both high (IPCC scenario RCP8.5, leading to release of 120 to 195 PgC) and low (IPCC scenario RCP2.6, approximated as one-third the C release of the high scenario) climate change scenarios (Extended Data Fig. 2a) generated CH₄ emissions (Fig. 3a) (based on 2.3% of released permafrost carbon emerging as CH₄, Schurr et al²) and corresponding impacts on the atmospheric concentrations of CH₄ (Extended Data Fig. 3b). We simulated the impacts of these emissions on the isotopic composition of atmospheric CH₄ by assuming the $\delta^{13}\text{C}$ of CH₄ emitted was in the range of what we report here for Stordalen Mire, from very light (-80‰, like that measured in the *Sphagnum* site) to only moderately light (-65‰, like that measured in the *Eriophorum* site), giving a range of isotopic perturbations to atmospheric CH₄ under high climate change (Extended Data Fig. 2c) and under low climate change (Extended Data Fig. 2d). In all scenarios, the induced change in atmospheric $\delta^{13}\text{C}$ is significantly larger than the atmospheric detection limit of 0.1‰ (reported in Kai et al²⁸ and shown as a dotted horizontal line in Extended Data Fig. 3c,d).

For the analysis shown in Fig. 3, we focused on a mid-range value of permafrost C release (high climate change scenario with low C release, 120 Pg total C by 2100), corresponding to emissions of 2.8 PgC as CH₄ by 2100 (the dashed black-and-red line in Fig. 3a). (By comparison, the IPCC estimates that up to 5 PgC may be released as CH₄ by 2100.³) We explored the mis-attribution of C release that would occur by (mistakenly) assuming that isotopic composition of emitted CH₄ was in the range of assumptions used in previous

atmospheric inversions, from -60‰ to -65‰²⁸, instead of the range measured at Stordalen Mire (-65‰ to -80‰). We estimated the magnitude of mis-attribution (or error flux, Fig. 3c) by simulating the amount of additional carbon that would need to be released (at nominally assumed isotopic composition values of -60 or -65) in order to have the same effect on atmospheric composition as the carbon released under scenarios with isotopic compositions like those observed in the field.

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Extended Data Figure and Table Legends:

Extended Data Figure 1. Expected and observed relationships between δD and $\delta^{13}\text{C}$ content of porewater CH_4 . The thick gray arrow shows the expected pattern in H and C isotopes of CH_4 when variations are caused by shifts between acetoclastic (lower right) and hydrogenotrophic (upper left) production. The thin black arrows pointing to the upper right indicate the expected pattern in H and C isotopes of CH_4 when variations are caused by changes in CH_4 oxidation¹⁹. The points are observed isotopic compositions of samples collected July–October 2011 at the partly thawed *Sphagnum* and fully thawed *Eriophorum* sites, with site averages shown with error bars (error bars represent s.e.m, $n = 13$ (*Sphagnum*) and 20 (*Eriophorum*)). Although the scatter allows for some variation in both production and oxidation, the average *Eriophorum* porewater CH_4 had significantly more ^{13}C and less D relative to *Sphagnum* porewater (Hotelling's T^2 Test, $p = 0.0001$), indicating that overall inter-site isotopic differences were due mostly to differences in CH_4 production pathway rather than to differences in CH_4 oxidation. Additionally, in August there was also a significant, negative relationship between $\delta^{13}\text{C}\text{-CH}_4$ and $\delta\text{D}\text{-CH}_4$ of porewater samples collected across sites (dashed line, $R^2 = 0.5$, $p < 0.02$). Note that on the vertical axis,

$\delta\text{D-H}_2\text{O}$ has been subtracted from $\delta\text{D-CH}_4$ to correct for the effect of δD exchange between H_2O and CH_4 ^{20,38,50}.

Extended Data Figure 2. Simulations, using high and low temperature and C release scenarios, of the effect of CH_4 release from thawing permafrost on atmospheric $\delta^{13}\text{C-CH}_4$.

a, Scenarios of permafrost C release due to thaw (high temperature, red bounding lines; low temperature, orange bounding lines; with the range in each case defined by high and low C-release scenarios); **b**, Impact on atmospheric methane mixing ratios (assuming 2.3% of released C is emitted as methane); **c**, Impact of high climate change scenario on atmospheric methane isotopes, assuming “*Eriophorum*-like” emissions ($\delta^{13}\text{C} \approx -65\text{‰}$, blue bounding lines), or assuming “*Sphagnum*-like” emissions ($\delta^{13}\text{C} \approx -80\text{‰}$, green bounding lines); and **d**, Same as (c), except for low climate change scenario. In (c) and (d) dotted horizontal lines indicate the detection limit for CH_4 isotopes²⁸.

Extended Data Table 1. Summary of porewater chemistry, average (standard error), n=3.

Extended Data Table 2. Relative abundance, taxonomic classification and predicted methanogenic pathway of the dominant methanogen operational taxonomic units (OTUs).

Extended Data Table 3. Relative abundance of methanogen functional groups within the Archaea

*Above the water table

†Below the water table

739

740 **Extended Data Table 4. Results of linear regression analysis for predicting α_c from**
741 **relative abundances of methanogenic pathways, dominant methanogenic lineages**
742 **and environmental variables (n = 41)**

743 *see Extended Data Table 2 for taxonomic details

744

745 **Extended Data Table 5. Results of stepwise multiple regression analysis for**
746 **predicting α_c from relative abundances of methanogenic lineages and environmental**
747 **variables**

748 *see Extended Data Table 2 for taxonomic details

749

750 **Extended Data Table 6. Estimate of the relative contribution of hydrogenotrophic**
751 **production to annual CH₄ emission at Stordalen mire**

752 *Based on Johansson et al. ⁴, the *Sphagnum* site in this study is representative of the
753 Semiwet and Wet vegetation classes.

754 † Annual total hydrocarbon emissions from Bäckstrand et al. ¹⁶ corrected for non-methane
755 volatile organic compound (NMVOC) flux using the reported proportions (25% NMVOC for
756 the *Eriophorum* site, 15% for the *Sphagnum* site). The magnitude of growing season CH₄
757 emissions measured in this study is comparable to the growing season CH₄ flux used in the
758 Bäckstrand et al. estimate of annual flux.

759 ‡Two approaches: isotopic, using mixing of acetoclastic (-60‰) and hydrogenotrophic (-
760 80‰) sources to yield mean emitted $\delta^{13}\text{C}$ -CH₄, and molecular, using proportion of the
761 methanogen community identified as hydrogenotrophic.

^s Molecular approach: on average 86% of methanogen community in the anoxic CH₄-producing peat was identified as hydrogenotrophic, all of the acetoclasts were facultative so this is likely an underestimation of potential hydrogenotrophic production.

^{||} Isotopic approach: $-79.6\text{‰} \sim -80\text{‰} * 0.98 + -60\text{‰} * 0.02$

[¶] Isotopic approach: $-66.3\text{‰} \sim -80\text{‰} * 0.32 + -60\text{‰} * 0.68$

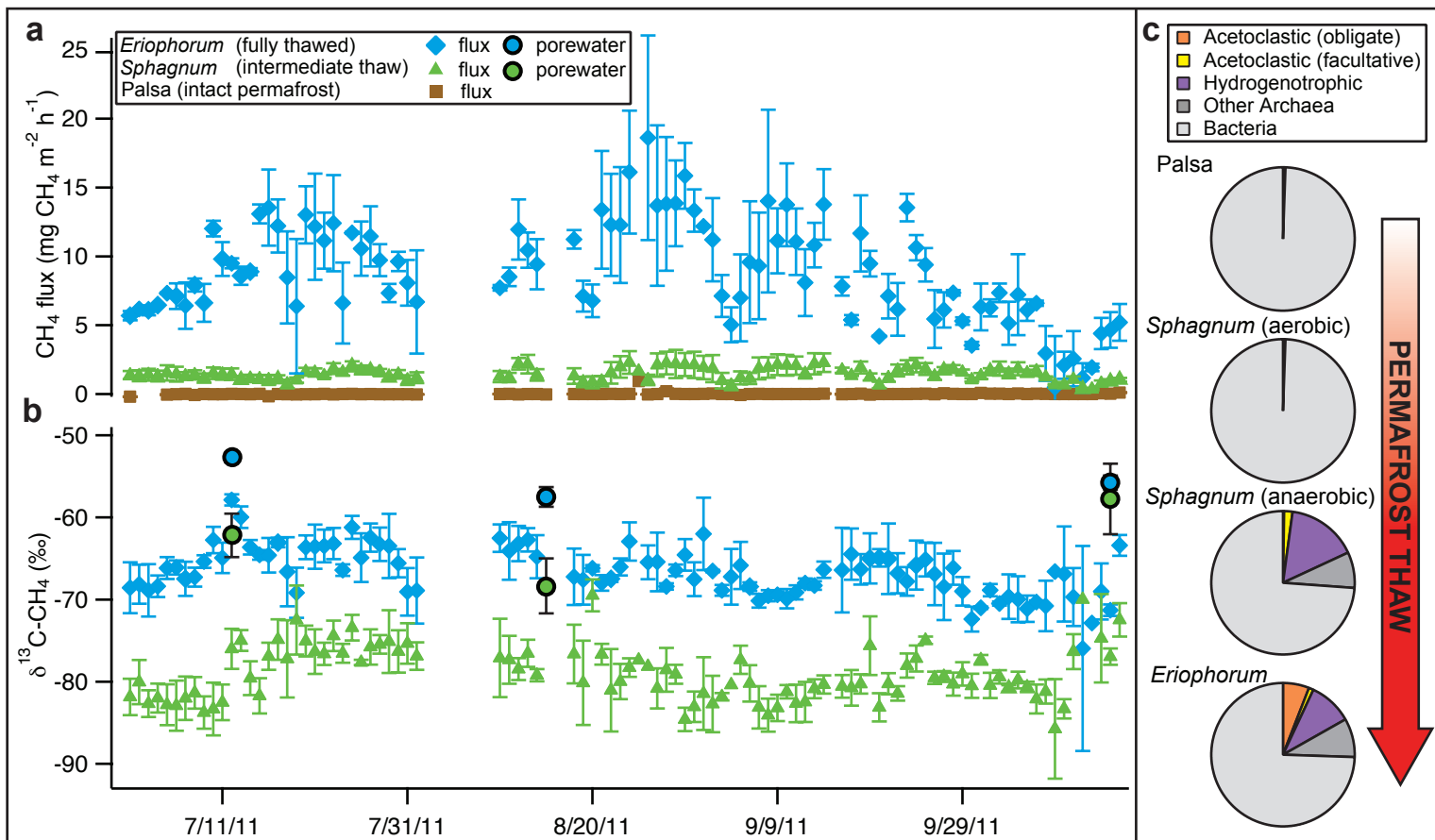
[#] Molecular approach: on average 62% of the methanogen community was identified as hydrogenotrophic.

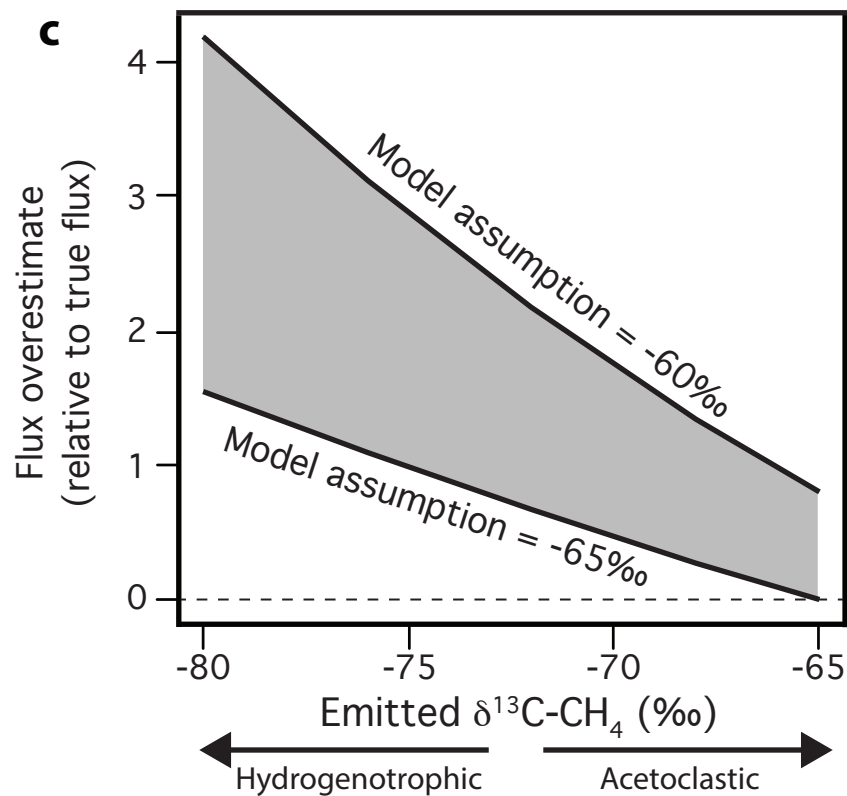
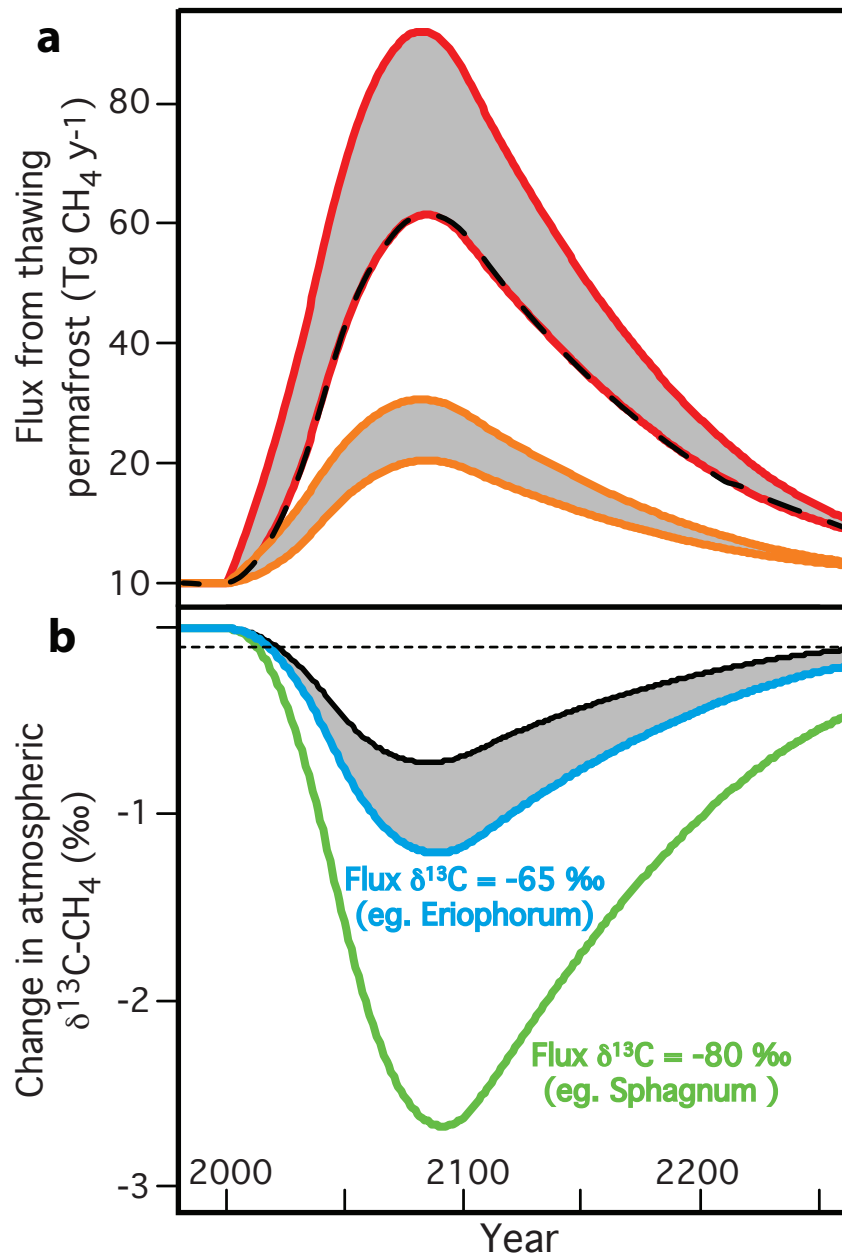
Extended Data Table 7. SSU rRNA gene amplicon multiplex identifiers (MIDs) used for each sample

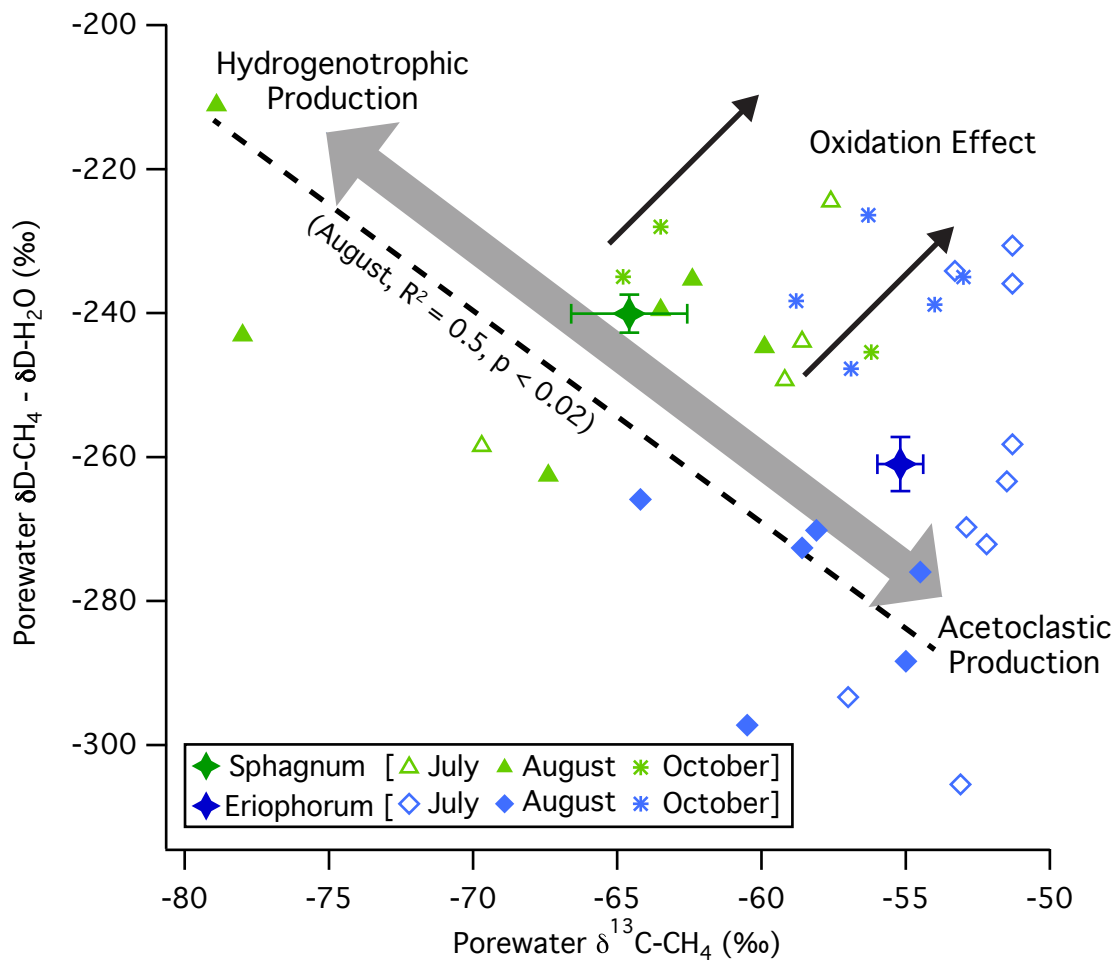
* Sample names are comprised of the date of sampling, followed by P, S or E for Palsa, *Sphagnum*, or *Eriophorum* sites, respectively, the number indicates the core within the site, and S, M or D indicates surface, middle or deep sampling within the core, respectively.

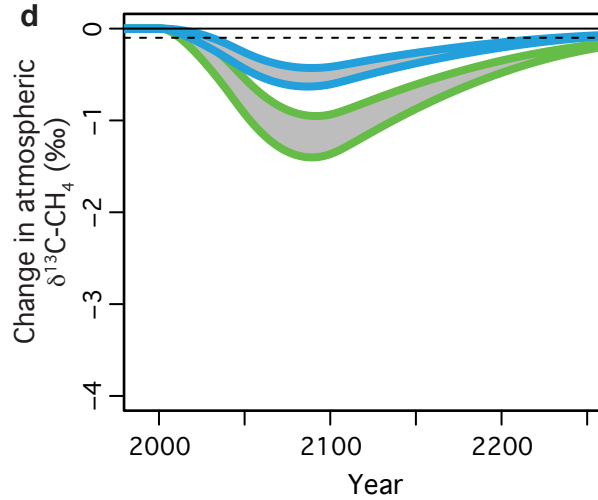
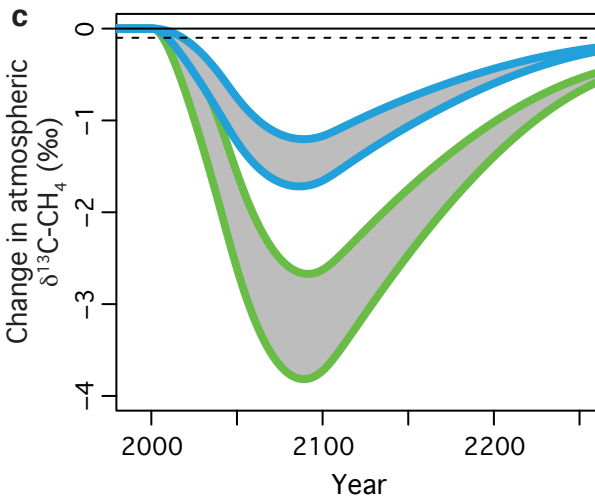
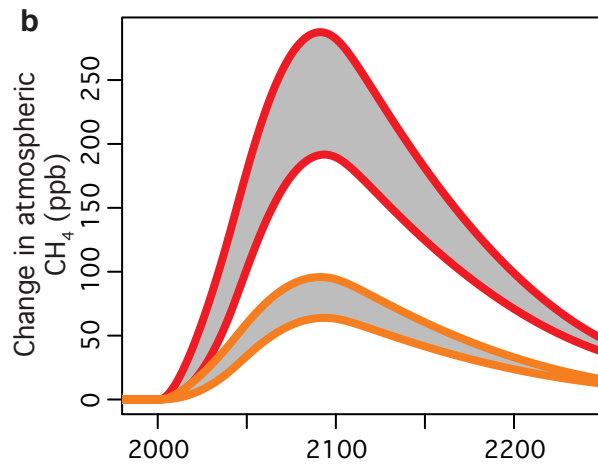
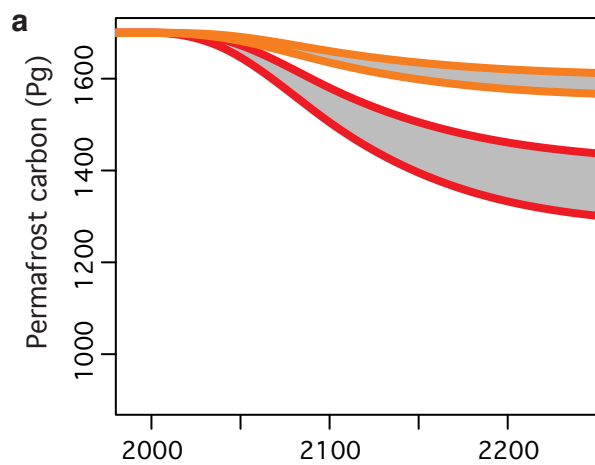
[†] Samples were multiplexed in six separate runs, each time with samples not related to this study. The multiplex identifiers of the first five runs are given in Mondav et al ⁶.

Extended Data Table 8. Results of stepwise multiple regression analysis for predicting $\delta^{13}\text{C-CH}_4$ from relative abundances of methanogenic lineages and environmental variables (Model 1), the relative abundance of '*M. stordalenmirens*' from environmental variables (Model 2), and α_{C} from environmental variables (Model 3)









ED Table 1

Sample	Depth (cm)	pH	mM CO ₂	mM CH ₄	$\delta^{13}\text{CO}_2$ ‰	$\delta^{13}\text{CH}_4$ ‰	α_C
July, 2011							
<i>Sphagnum</i> - M	13	4.1 (0.06)	3.02 (0.78)	0.09 (0.04)	-15.7 (1.6)	-62.2 (3.8)	1.050 (0.005)
<i>Sphagnum</i> - D	19	4.2 (0.09)	3.50 (0.57)	0.15 (0.05)	-14.1 (0.6)	-62.2 (4.5)	1.051 (0.005)
<i>Eriophorum</i> - S	3	5.8 (0.09)	2.29 (0.92)	0.18 (0.12)	-14.1 (1.1)	-52.1 (0.5)	1.040 (0.001)
<i>Eriophorum</i> -M	7	5.6 (0.06)	3.06 (0.77)	0.28 (0.07)	-12.9 (1.0)	-52.6 (0.6)	1.042 (0.001)
<i>Eriophorum</i> - D	24	5.6 (0.03)	3.56 (0.80)	0.36 (0.07)	-11.6 (1.7)	-53.3 (1.9)	1.044 (0.004)
August, 2011							
<i>Sphagnum</i> - M	21	4.2 (0.10)	4.89 (0.37)	0.23 (0.04)	-12.0 (1.5)	-66.7 (5.7)	1.059 (0.008)
<i>Sphagnum</i> - D	26	4.1 (0.13)	4.80 (0.48)	0.23 (0.04)	-10.7 (1.6)	-69.9 (4.6)	1.064 (0.007)
<i>Eriophorum</i> - S	3	5.7 (0.19)	1.62 (0.28)	0.06 (0.04)	-13.5 (0.5)	-60.0 (2.6)	1.049 (0.003)
<i>Eriophorum</i> -M	7	5.7 (0.10)	1.93 (0.25)	0.10 (0.02)	-13.9 (0.4)	-56.6 (2.1)	1.045 (0.002)
<i>Eriophorum</i> - D	26	5.6 (0.15)	3.58 (0.62)	0.31 (0.11)	-11.1 (2.4)	-55.9 (1.1)	1.047 (0.001)
October, 2011							
<i>Sphagnum</i> - M	10	4.3 (0.06)	1.24 (0.42)	0.03 (0.02)	-16.4 (1.6)	-59.2 (6.5)	1.046 (0.006)
<i>Sphagnum</i> - D	15	4.5 (0.10)	3.21 (0.90)	0.10 (0.04)	-13.8 (2.4)	-61.5 (2.7)	1.051 (0.0004)
<i>Eriophorum</i> - S	3	5.9 (0.15)	2.15 (1.43)	0.19 (0.13)	-14.1 (1.0)	-56.4 (2.4)	1.045 (0.001)
<i>Eriophorum</i> -M	7	5.9 (0.15)	2.71 (1.25)	0.29 (0.14)	-13.7 (1.6)	-57.8 (3.1)	1.047 (0.002)
<i>Eriophorum</i> - D	26	5.7 (0.12)	3.84 (1.64)	0.53 (0.27)	-11.3 (3.1)	-58.1 (2.2)	1.050 (0.001)

ED Table 2

Sample	Candidatus <i>Methanoflorens</i> (otu-10747) Hydrogenotrophic	<i>Methanobacterium</i> (otu-3636) Hydrogenotrophic	Candidatus <i>Methanoregula</i> (otu-20819) Hydrogenotrophic	<i>Methanosarcina</i> (otu-7308) Acetoclastic (facultative)	<i>Methanosaeta</i> (otu-10220) Acetoclastic (obligate)	<i>Methanosaeta</i> (otu-15150) Acetoclastic (obligate)
July, 2011						
Palsa – S	0.0	0.0	0.0	0.0	0.0	0.0
Palsa – M	0.0	0.0	0.0	0.0	0.0	0.0
Palsa – D	0.0	0.4	0.0	0.0	0.0	0.0
<i>Sphagnum</i> – S	0.3	0.4	0.0	0.1	0.0	0.0
<i>Sphagnum</i> – M	4.0	12.9	0.0	3.4	0.0	0.0
<i>Sphagnum</i> – D	16.4	5.8	0.0	3.3	0.0	0.0
<i>Eriophorum</i> – S	1.0	2.7	5.8	0.7	4.5	1.8
<i>Eriophorum</i> – M	5.3	3.7	4.0	2.2	5.0	2.7
<i>Eriophorum</i> – D	8.3	1.6	1.9	0.6	4.2	1.2
August, 2011						
Palsa – S	0.0	0.0	0.0	0.0	0.0	0.0
Palsa – M	0.0	0.0	0.0	0.0	0.0	0.0
Palsa – D	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphagnum</i> – S	0.1	0.4	0.0	0.2	0.0	0.0
<i>Sphagnum</i> – M	11.6	4.0	0.0	1.9	0.0	0.0
<i>Sphagnum</i> – D	32.1	3.1	0.0	1.4	0.0	0.0
<i>Eriophorum</i> – S	0.6	2.1	3.6	0.4	3.3	1.0
<i>Eriophorum</i> – M	6.3	6.1	5.1	2.6	9.0	3.9
<i>Eriophorum</i> – D	6.5	0.3	3.4	1.2	1.7	0.6
October, 2011						
Palsa – S	0.0	0.0	0.0	0.0	0.0	0.0
Palsa – M	0.1	1.1	0.0	0.1	0.0	0.0
Palsa – D	0.1	0.7	0.0	0.0	0.0	0.0
<i>Sphagnum</i> – S	0.0	0.1	0.0	0.0	0.0	0.0
<i>Sphagnum</i> – M	0.0	3.4	0.0	1.1	0.0	0.0
<i>Sphagnum</i> – D	0.6	8.4	0.0	1.2	0.0	0.0
<i>Eriophorum</i> – S	2.5	1.7	1.7	0.6	1.4	0.6
<i>Eriophorum</i> – M	2.1	1.9	1.0	0.8	2.5	2.2
<i>Eriophorum</i> – D	6.0	1.1	3.7	0.1	5.1	5.8

ED Table 3

Site	Hydrogenotrophic	Acetoclastic (facultative)	Acetoclastic (obligate)	Other Archaea
July, 2011				
Palsa	35.9	2.9	0.0	61.2
<i>Sphagnum</i> (aerobic) [†]	83.1	15.5	0.0	1.4
<i>Sphagnum</i> (anaerobic) [†]	82.1	14.2	0.0	3.8
<i>Eriophorum</i>	39.5	4.2	21.4	34.9
August, 2011				
Palsa	0.0	8.7	0.0	91.3
<i>Sphagnum</i> (aerobic) [†]	68.2	30.7	0.0	1.1
<i>Sphagnum</i> (anaerobic) [†]	91.2	6.1	0.0	2.8
<i>Eriophorum</i>	39.5	5.1	21.9	33.5
October, 2011				
Palsa	56.5	2.6	0.4	40.5
<i>Sphagnum</i> (aerobic) [†]	65.7	24.0	0.7	9.6
<i>Sphagnum</i> (anaerobic) [†]	15.6	2.8	2.6	79.0
<i>Eriophorum</i>	35.8	2.4	27.6	34.2

ED Table 4

Variable	R ²	F-statistic	p-value
' <i>M. stordalenmirens</i> '	0.58	54.09	<0.001
otu-3636*	0.00	0.01	0.926
otu-10220*	0.12	5.36	0.026
otu-20819 *	0.15	6.82	0.013
otu-15150 *	0.06	2.27	0.140
otu-7308 *	0.01	0.32	0.576
Hydrogenotrophic	0.44	30.63	<0.001
Acetoclastic (obligate)	0.12	5.23	0.028
Water table depth	0.44	31.1	<0.001
pH	0.19	8.97	0.005
Porewater CH ₄ (mM)	0.00	0.07	0.796
Porewater DIC (mM)	0.25	13.33	0.001
Peat C:N	0.00	0.17	0.682
Peat %C	0.02	0.75	0.393
Peat %N	0.00	0.14	0.709
Peat $\delta^{13}\text{C}$	0.13	5.99	0.019

ED Table 5

Variable	Coefficient	Std Error	t value	p value	Cumulative AIC
Model 1 - stepwise regression, direction = both ($R^2 = 0.81$, $F = 23.71$ on 6 and 34 df, $p < 0.001$)					
Water table depth	-0.0004	0.0001	-5.398	<0.001	-422.33
' <i>M. stordalenmirens</i> '	0.0271	0.0084	3.221	0.002	-436.79
C:N	-0.0002	0.0001	-2.872	0.007	-438.80
Peat $\delta^{13}\text{C}$	0.0014	0.0006	2.516	0.017	-440.71
DIC (mM)	0.0007	0.0005	1.396	0.171	-445.42
otu-3636*	-0.0271	0.0161	-1.345	0.188	-445.58
Intercept	1.089	0.0167	65.193	<0.001	-445.71
Model 2 – significant predictor variables from model 1 ($R^2 = 0.79$, $F = 33.71$ on 4 and 36 df, $p < 0.001$)					
Water table depth	-0.0004	0.0001	-5.202	<0.001	-425.11
' <i>M. stordalenmirens</i> '	0.0351	0.0072	4.867	<0.001	-427.36
C:N	-0.0002	0.0001	-2.613	0.013	-440.97
Peat $\delta^{13}\text{C}$	0.0014	0.0006	2.470	0.018	-441.67
Intercept	1.089	0.0164	66.583	<0.001	-446.09

ED Table 6

Habitat	Area (ha)*	Annual Flux (g CH ₄ m ⁻²) [†]	Annual Emission (kg CH ₄) ^{*,†}	Estimated Emission from Hydrogenotrophy (kg CH ₄ yr ⁻¹) [‡]
<i>Sphagnum</i>	6.2	6.2	288.3	247.9 [§] - 282.5
<i>Eriophorum</i>	2.0	36.0	540.6	172.8 [¶] - 335.2 [#]
Total			828.9	420.7(51%) – 617.7 (75%)

ED Table 7

Sample name	Run #	Multiplex identifier (MID)
20110712_E_3_M	6	CGAGC
20110712_S_1_M	6	CGCAT
20110712_S_3_M	6	CGTAC
20110712_P_1_S	6	CGTGT
20110712_P_2_S	6	CTAGT
20110712_P_3_S	6	CTGAC
20110816_S_2_S	6	TACGC
20110816_S_1_D	6	TATGT
20110816_P_1_M	6	TCAGT
20111016_P_1_S	6	TCGAT

ED Table 8

Variable	Coefficient	Std Error	t value	p value	Cumulative AIC
Model 1 - stepwise regression, dependent variable = $\delta^{13}\text{C-CH}_4$, direction = both ($R^2 = 0.75$, $F = 21.25$ on 5 and 35 df, $p < 0.001$)					
Water table depth	0.299	0.07	4.512	<0.001	130.95
' <i>M. stordalenmirensis</i> '	-23.25	6.79	-3.426	0.002	124.01
Peat $\delta^{13}\text{C}$	-1.51	0.54	-2.779	0.009	120.33
CH ₄ (mM)	10.60	4.12	2.576	0.014	119.28
C:N	0.12	0.05	2.149	0.039	117.24
Intercept	-102.14	15.23	-6.705	<0.001	114.16
Model 2 - stepwise regression, dependent variable = ' <i>M. stordalenmirensis</i> ', direction = both ($R^2 = 0.53$, $F = 7.77$ on 5 and 35 df, $p < 0.001$)					
Water table depth	-0.0053	0.0015	-3.634	<0.001	-188.03
C:N	-0.0035	0.0010	-3.495	0.001	-188.88
DIC (mM)	0.0214	0.0106	2.025	0.050	-196.61
% C	0.0033	0.0018	1.799	0.081	-197.53
Soil temperature	0.0059	0.0040	1.483	0.147	-198.66
Intercept	-0.0558	0.0805	-0.692	0.493	-199.15
Model 3 - stepwise regression, dependent variable = α_c , direction = both ($R^2 = 0.71$, $F = 21.71$ on 4 and 36 df, $p < 0.001$)					
Water table depth	-0.0005	0.0001	-6.465	<0.001	-402.97
C:N	-0.0003	0.0001	-4.514	<0.001	-416.18
DIC (mM)	0.0015	0.0006	2.629	0.013	-427.36
Peat $\delta^{13}\text{C}$	0.0017	0.0007	2.574	0.014	-427.63
Intercept	1.0990	0.0192	57.396	<0.001	-432.56