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Soil incubations reproduce field methane dynamics in a subarctic wetland

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Abstract A major challenge in peatland carbon cycle modeling is the estimation of subsurface methane (CH_4) and carbon dioxide (CO_2) production and consumption rates and pathways. The most common methods for modeling these processes are soil incubations and stable isotope modeling, both of which may involve departures from field conditions. To explore the impacts of these departures, we measured CH_4/CO_2 concentration ratios and ^{13}C fractionation factors (α_C, indicating CH_4 production pathways) in field pore water from a thawing subarctic peatland, and compared these values to those observed in incubations of corresponding peat samples. Incubation CH_4/CO_2 production ratios were significantly and positively correlated with observed field CH_4/CO_2 concentration ratios, though observed field ratios were ~20 % of those in incubations due to CH_4's lower solubility in pore water. After correcting the field ratios for CH_4 loss with an isotope mass balance model, the incubation CH_4/CO_2 ratios and α_C were both significantly positively correlated with field ratios and α_C (respectively), both with slopes indistinguishable from 1. Although CH_4/CO_2 ratios and α_C were slightly higher in the incubations, these shifts were consistent along the thaw progression, indicating that ex situ incubations can replicate trends in in situ CH_4 production.

Keywords Peatlands · Methane · Geochemistry · Soil incubations · Stable isotopes

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Introduction

Accurately modeling methane (CH_4) and carbon dioxide (CO_2) production associated with anaerobic decomposition is essential for predicting carbon balances in northern peatlands. Although surface fluxes of CH_4 and CO_2 can be readily measured in situ, this is not the case for subsurface CH_4 and CO_2 production and consumption, so these rates must be determined by indirect methods such as soil incubations (reviewed by Nilsson and Öquist (2009) and Treat et al. (2015)) and isotope modeling (Knorr et al. 2008; Shoemaker and Schrag 2010; Corbett et al. 2013, 2015; Broder et al. 2015). Incubations often involve disturbances to in situ conditions, most notably initial transient oxygen exposure, higher temperatures, and isolation from the surrounding peat environment and vegetation, all of which may impact CH_4 production (Nilsson and Öquist 2009; Treat et al. 2015). Isotope models cannot easily incorporate all of the factors that influence peat decomposition, which include moisture content, electron acceptor availability, organic matter degradation potential (also known as lability), microbial activities, and complex interactions of all of these factors (Turetsky 2004). It is thus essential that the limitations of these methods be further evaluated.

CH_4 is produced from a limited number of substrates, with acetoclastic or hydrogenotrophic pathways predominant in northern peatlands (Balch et al. 1979; Lovley and Klug 1983; Yavitt and Lang 1990; Zinder 1993; Conrad 1999). Acetoclastic methanogenesis splits acetate into CH_4 and CO_2, while hydrogenotrophic methanogenesis reduces CO_2 with hydrogen (H_2) (both derived from organic matter fermentation) to produce CH_4. Only members of two genera (*Methanosarcina* and *Methanosaeta*) in the order Methanosarcinales can carry out acetoclastic methanogenesis (Kendall and Boone 2006), whereas a broad range of methanogenic archaea utilize the hydrogenotrophic pathway. Perhaps due to this greater genetic diversity, hydrogenotrophy can better withstand various conditions that often inhibit overall methanogenesis (Hines et al. 2008). Such conditions include winter temperatures (Sugimoto and Wada 1993; Hines and Duddleston 2001), nutrient limitation (Hines et al. 2008), more recalcitrant organic matter (Hornibrook et al. 1997, 2000; Kotsyurbenko et al. 2004; Hodgkins et al. 2014), and acidic conditions (Hines and Duddleston 2001; Kotsyurbenko et al. 2007; Hodgkins et al. 2014), with several hydrogenotrophic species that produce CH_4 at pH < 5 (Horn et al. 2003; Bräuer et al. 2006; Kotsyurbenko et al. 2007; Mondav et al. 2014). In the context of soil incubations, mechanical shaking may also inhibit acetoclastic methanogenesis (Dannenberg et al. 1997). Consistent with the predominance of hydrogenotrophy under less suitable conditions for methanogenesis, the proportion of methanogenesis by hydrogenotrophy has been found to increase as CH_4/CO_2 production ratios decrease (McCalley et al. 2014).

Methanogenesis pathways are commonly distinguished by the $\delta^{13}C$ values of the resulting CH_4 $\left(\delta^{13}C_{CH_4}\right)$, which are less negative for acetoclasty ($\delta^{13}C_{CH_4} = -65$ to -50 ‰) and more negative for hydrogenotrophy ($\delta^{13}C_{CH_4} = -110$ to -60 ‰) (Whiticar et al. 1986; Hornibrook et al. 1997, 2000; Whiticar 1999). The precision of this distinction is weakened by the coupling of CH_4 production to CO_2 cycling, which propagates ^{13}C isotopic fractionation such that $\delta^{13}C_{CH_4}$ is also influenced by $\delta^{13}C_{CO_2}$. To account for this, the apparent fractionation factor α_C (Whiticar et al. 1986) is used to more precisely characterize CH_4 production pathways. α_C is defined as:

$$\alpha_C = \frac{\delta^{13}C_{CO_2} + 1000}{\delta^{13}C_{CH_4} + 1000} \quad (1)$$

Acetoclastic methanogenesis typically results in α_C between 1.04 and 1.06, while α_C from hydrogenotrophic methanogenesis typically ranges from 1.05 to 1.09 (Whiticar et al. 1986). Note that both pathways often co-occur in the same system (e.g., McCalley et al. 2014), and that α_C can also be influenced by oxidation (often resulting in $\alpha_C < 1.03$; Whiticar and Faber 1986; Whiticar 1999). Thus it is most useful to interpret α_C in terms of relative pathway rates, rather than as evidence of one pathway to the exclusion of the other.

The goal of this study was to evaluate the ability of soil incubations (published in Hodgkins et al. (2014) and in Fig. 2b of McCalley et al. (2014)) to approximate previously unreported field pore water CH_4/CO_2 ratios and α_C in a subarctic peatland. Many of the incubation conditions, including peat type, anaerobicity, and pH, were similar to field conditions. However, the incubated peat experienced several disturbances, including (1) exposure to ambient oxygen during

sampling and incubation preparation, and to lower oxygen during storage; (2) storage for 6 months at 4 °C; (3) 25 days of pre-incubation plus 62 days of incubation at 22 °C, which was ~15 °C higher than the typical in situ temperatures of 3–12 °C; (4) isolation from the aboveground plant community; and (5) vigorous shaking for 30 s prior to each measurement. We compared the incubation CH_4/CO_2 production ratios both to the measured concentration ratios in field pore water, and to the estimated field production ratios determined with an isotope mass balance model (Corbett et al. 2013, 2015). Our study thus simultaneously evaluates three different methods (incubations, raw pore water measurements, and isotope modeling) for approximating in situ CH_4 and CO_2 production.

Methods

Study site

Stordalen Mire (68.35°N, 19.05°E) is a thawing peatland complex located ~10 km east of the Abisko Scientific Research Station in northern Sweden. The site can be divided into four terrestrial habitat types based on vegetation and hydrology, which are in turn determined by permafrost status: (i) dry ombrotrophic palsas with intact permafrost and dominated by ericaceous and woody vegetation; (ii) recently thawed, waterlogged, ombrotrophic thermokarst sinkholes (collapsed palsas) with transitional plant species including *Eriophorum vaginatum* and floating *Sphagnum* spp.; (iii) partially thawed, ombro-minerotrophic, *Sphagnum*-dominated bogs with a water table perched above deeper permafrost; and (iv) fully thawed, hydrologically connected minerotrophic fens dominated by *Eriophorum angustifolium*, *Carex rostrata*, and other sedges (Johansson et al. 2006; Hodgkins et al. 2014). Between 1970 and 2000, permafrost thaw caused shifts along this habitat succession towards wetter sites that produce more CH_4 (Malmer et al. 2005; Johansson et al. 2006; Bäckstrand et al. 2010), leading to an ~47 % increase in the greenhouse gas balance (in terms of CO_2-equivalent radiative forcing) of the entire mire (Johansson et al. 2006).

Sampling

From June 13–16, 2011, peat and pore water were collected from 9 sites at Stordalen (Table 1) spanning the known post-collapse stages of permafrost thaw and

Table 1 Sites and depths sampled, listed in order of thaw stage (Hodgkins et al. 2014)

Site name[a]	Habitat type	Dominant vegetation	Water table depth (cm)[b]	Thaw depth (cm)	Peat sample depth (cm)	Pore water sample depth (cm)	pH	In situ T (°C)
PHS	Collapsed palsa	*Eriophorum vaginatum*, woody species	0	< −90	−9 to −12	−8 to −12	4.0	8.3
PHB	Collapsed palsa	*E. vaginatum*, floating *Sphagnum*	>0	< −90	−10 to −15	−28 to −32	4.1	9.5
Bog1	Bog	*Sphagnum* spp.	−17	−25	−18 to −22	−21 to −25	4.2	3.8
SOS	Bog	*Sphagnum* spp.	−12	−34	−12 to −17	−8 to −12	4.0	3.5
S (triplicate cores)	Bog	*Sphagnum* spp.	−12 to −13	−30 to −31	−12 to −16	−12 to −16	4.2	2.9–3.3
EOS	Fen	*E. angustifolium*, *Sphagnum* spp.	5	−44 to < −90	−5 to −10	−8 to −12	4.9	9.5
E (triplicate cores)	Fen	*E. angustifolium*	0–4.5	−27 to < −90	−5 to −8	−5 to −8	5.8	3.0–12.3
Fen1	Fen	*Carex rostrata*	6–8	< −90	−5 to −8	−5 to −9	6.1	11.5
Fen2	Fen	*E. angustifolium*	12	< −90	0 to −10	−13 to −17	5.8	9

[a] Pore water sample depths, pH, and in situ temperature (T) are from this study. Site names, habitat types, dominant vegetation, water table depths, thaw depths, and peat sample depths are from Table S1 of Hodgkins et al. (2014)

[b] For all depths, negative values indicate depth below the peat surface, while positive values indicate height above the peat surface (most common sign convention for water table depth)

plant succession (types ii–iv above; Hodgkins et al. 2014). All samples were gathered from beneath the water table and 0–22 cm below the peat surface, with depths varied to account for differing water table positions (Table 1). Prior to sampling, in situ peat temperature was measured 13 cm below the peat or water table surface (whichever was shallower) with an EBRO Thermometer TFX 392 L.

Pore water was collected by suction with a 60-mL plastic syringe connected to a 1/4″ stainless steel tube with holes drilled along the bottom 3 cm. The pH was measured on site with an Oakton Waterproof pHTestr 10 (Eutech Instruments, precision ~0.2 units). The water was then filtered through Whatman GF/D glass microfiber filters (2.7-µm particle retention) and injected into 20- or 30-mL evacuated borosilicate glass serum vials sealed with butyl rubber septa until the vials were ~2/3 full. Duplicate vials were filled for each depth in each pore water profile. One pore water profile was gathered at each site except for site S and E (see Table 1), at which triplicate profiles were gathered. Samples were frozen within 8 h of collection and stored frozen until analysis.

Peat was collected with an 11-cm diameter circular push corer at all sites except PHS and Fen1, where peat was collected with a 10 × 10-cm square Wardenaar corer (Eijkelkamp, product code 05.09). Triplicate cores were collected from sites S and E, and single cores were collected from all other sites. Near-surface core sections from beneath the water table, and between 3 and −10 cm thick (Table 1; Hodgkins et al. 2014) were placed in plastic bags and stored at 4 °C for 6 months until incubation.

Pore water gas concentrations and $\delta^{13}C$

Pore water samples were thawed, acidified with 0.5 mL of degassed 21 % phosphoric acid (H_3PO_4) (excess), and brought to atmospheric pressure with helium. Headspace concentrations (% volume) and $\delta^{13}C$ (relative to the Pee Dee Belemnite [VPDB] standard, where $\delta^{13}C = [R_{sample}/R_{standard} - 1] \times 1000$, and R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios in the sample and standard) of CH_4 and CO_2 were analyzed on a continuous-flow Hewlett-Packard 5890 gas chromatograph at 40 °C (Agilent Technologies) coupled to a Finnigan MAT Delta S isotope ratio mass spectrometer via a Conflo IV interface system (Thermo Scientific, Bremen, Germany) (GC-IRMS).

Headspace concentrations of CH_4 and CO_2 were converted into dissolved CH_4 and DIC concentrations based on the extraction efficiencies of each gas, defined as the proportion of formerly-dissolved gas in the headspace after acidification. An extraction efficiency of 0.95 was used for CH_4 (Corbett et al. 2013, 2015), and the extraction efficiency of DIC was determined by acidification of dissolved bicarbonate standards (Corbett et al. 2013, 2015; Hodgkins et al. 2014). From here on, the term "dissolved CO_2" refers to DIC (ΣCO_2).

Incubations

The incubations presented here are from Hodgkins et al. (2014), who have described the procedures in detail. Briefly, three incubation replicates were prepared for each site except Fen1, which had only two replicates. These replicates represent triplicate cores for sites S and E, and subsamples of single cores for the other sites. For each replicate, 12–20 g of non-homogenized wet peat was placed into a 120-mL glass serum vial with 40 mL of degassed deionized water, then sealed with a rubber septum. Each vial headspace was flushed with N_2 for 30 s, shaken for 30 s, then flushed again for 30 s to remove oxygen. The vials were then pre-incubated in the dark for 25 days at 22 °C to consume any remaining oxygen or other electron acceptors. Following the pre-incubation, accumulated CH_4 and CO_2 were removed by alternately shaking and flushing the vials with N_2 five times, leaving an N_2 headspace 35–50 kPa above atmospheric pressure. After this final flushing (defined as the incubation start time), initial concentrations of CH_4 and CO_2 were low compared to those that later accumulated in the incubations (Hodgkins et al. 2014).

The vials were incubated in the dark at 22 °C for 62 days, during which headspace CH_4 and CO_2 concentrations and $\delta^{13}C$ were measured by GC-IRMS every 1–14 days using methods analogous to the pore water measurements. Prior to each measurement, the vials were shaken for 30 s, and the headspace pressures were measured. Total amounts of CH_4 and CO_2 in each vial (mol), including both headspace and dissolved gases, were then calculated for each time point based on their concentrations, the headspace pressures, and any changes in CO_2 concentrations and $\delta^{13}C$ following acidification with excess H_3PO_4 on the final day of incubation (Hodgkins et al. 2014).

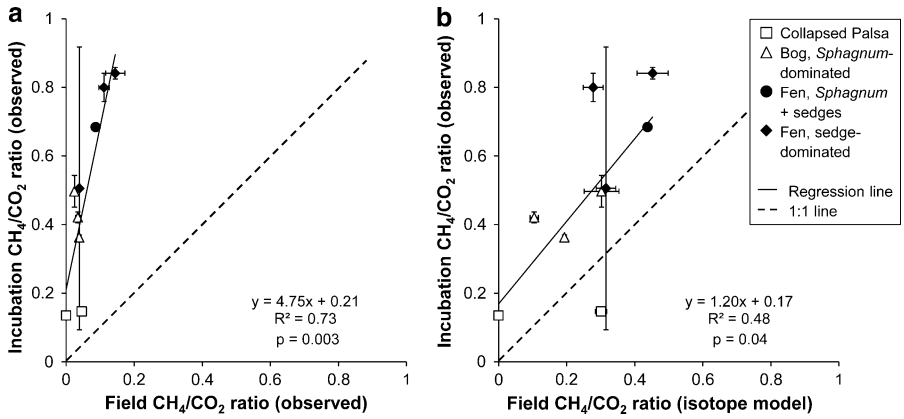

Fig. 1 Correlation of CH_4/CO_2 ratios in the incubations with ratios in field pore water. Each point with error bars represents the average and standard error of incubation (y axis) and pore water (x axis) replicates. **a** Observed ratios in the incubations versus observed ratios in the field, which are lower than the incubation ratios due to preferential CH_4 loss from pore water. **b** Observed ratios in the incubations versus calculated ratios in the field, corrected for CH_4 loss using the isotope mass balance model (Corbett et al. 2013, 2015). If the incubations perfectly replicated field CH_4/CO_2 production ratios, these points would plot along the 1:1 line

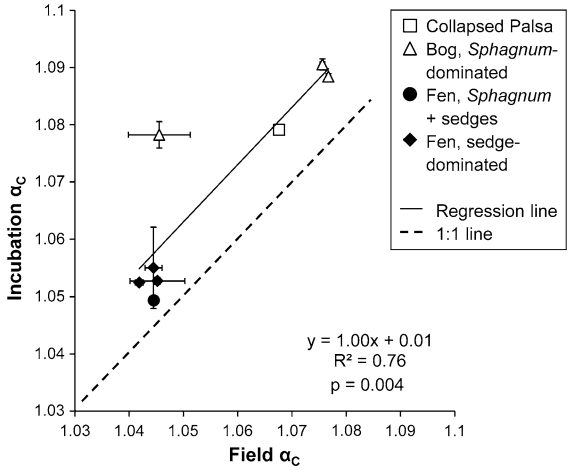

Fig. 2 Correlation of α_C in the incubations with α_C in field pore water. Each point with error bars represents the average and standard error of incubation (y axis) and pore water (x axis) replicates. The 1:1 line indicates the scenario of perfect agreement between incubation and field α_C

Statistical analyses

For the pore water, gas concentrations and $\delta^{13}C$ were measured for each vial, and the CH_4/CO_2 ratio and α_C (Eq. 1) were calculated based on the average concentrations and $\delta^{13}C$ across duplicate vials. Standard errors of CH_4/CO_2 ratios and α_C across duplicate vials were propagated based on the standard errors of gas concentrations and $\delta^{13}C$ values, respectively. For sites S and E, the averages and standard errors of CH_4/CO_2 ratios and α_C at each depth were calculated as the averages and standard errors of these values across all three profiles.

For each incubation replicate, CH_4 and CO_2 production rates were calculated with linear regressions of accumulated gas amounts (total moles in headspace plus liquid phase) vs. time, and overall $\delta^{13}C_{CH4}$ and $\delta^{13}C_{CO2}$ were defined as the acidification-corrected $\delta^{13}C$ values on day 62 (which represent integrated values for the entire incubation period). These production rates and overall $\delta^{13}C$ values from each replicate were used to calculate the CH_4/CO_2 production ratio and α_C (based on Eq. 1) for each incubation replicate (as shown in Supplementary Material Fig. 1). The CH_4/CO_2 ratios and α_C values in the individual replicates were then averaged and standard errors calculated to obtain values for each entire site (as shown in Figs. 1, 2).

Incubation and field data were compared by ordinary least squares linear regressions. One-sample t-tests, using the values and standard errors of the slope and intercept of each regression, were used to test whether the slope and intercept differed from their expected values of 1 and 0, respectively. This approach was also used to compare observed vs. modeled CH_4/CO_2 ratios in the incubations (Supplementary Material, *Model validation*).

Results and discussion

CH_4/CO_2 ratios

Incubation CH_4/CO_2 production ratios showed a significant positive relationship with raw field pore water CH_4/CO_2 concentration ratios ($p = 0.003$; Fig. 1a), though the overall range of CH_4/CO_2 ratios was much higher in the incubations. This difference is presumably due to CH_4's lower solubility compared to CO_2 and consequent preferential loss from field pore water, whereas the closed incubation system prevents this loss.

To examine whether the pore water CH_4/CO_2 ratios would better approximate incubation ratios if adjusted for CH_4 loss, we used an isotope mass balance model (Corbett et al. 2013, 2015) to calculate the theoretical in situ CH_4/CO_2 production ratios. A detailed description and evaluation of this model is provided in the Supplementary Material. Briefly, this model uses the $\delta^{13}C$ values of CO_2, CH_4, and organic matter to calculate the ratio of CO_2 produced during methanogenesis (CO_2-meth) to total CO_2, which also includes CO_2 produced from respiration and fermentation. Since methanogenesis from CH_2O-like organic matter (regardless of pathway) results in net equal production of CH_4 and CO_2 (Tarvin and Buswell 1934; Barker 1936; Conrad 1999; Corbett et al. 2013, 2015), this ratio of (CO_2-meth)/(CO_2-total) equals the theoretical CH_4/CO_2 concentration ratio if both species had similar solubility. This ratio is thus a proxy for the in situ CH_4/CO_2 production ratio. This corrected pore water CH_4/CO_2 ratio showed a significant positive relationship with the incubation CH_4/CO_2 production ratio ($p = 0.04$; Fig. 1b), with a slope not significantly different from 1 ($t(7) = 0.43$, $p = 0.7$) and an intercept not significantly different from 0 ($t(7) = 1.18$, $p = 0.3$). This somewhat surprising result (given the many assumptions in the isotope mass balance model, and the many disturbances induced in the incubations) indicates that measuring apparent CH_4/CO_2 production ratios by incubations, or by pore water concentrations corrected for CH_4 loss, reveals equivalent trends along the thaw gradient.

The disturbances induced during peat sampling, storage, and incubation could be expected to impact CH_4/CO_2 ratios (Nilsson and Öquist 2009). The slightly higher CH_4/CO_2 ratios in the incubations (Fig. 1b) may be related to the higher temperature sensitivity of CH_4 relative to CO_2 production (Treat et al. 2014), more stable redox conditions in the incubations following initial disturbance (Blodau and Moore 2003), and/or CH_4 oxidation in the field, which was demonstrated by variations in field pore water $\delta^{13}C_{CH4}$ and δD_{CH4} (McCalley et al. 2014). Based on the water table positions and vegetation at each of our sites (Table 1), oxygen may enter field pore water via water table fluctuation in bogs and collapsed palsas (Roslev and King 1996) and sedge transport in fens (Schütz et al. 1989; Chanton 2005). Notably, the overestimation of CH_4/CO_2 ratios by the incubations was unrelated to the sites' positions in the thaw sequence (Fig. 1b), suggesting that these processes have similar effects in different peatland types.

CH_4 production pathways

Similar to CH_4/CO_2 ratios, α_C values in the incubations showed a significant positive relationship with those in pore water ($p = 0.004$; Fig. 2). This correlation again had a slope not significantly different from 1 ($t(6) = 0.007$, $p = 1$) and an intercept not significantly different from 0 ($t(6) = 0.047$, $p = 1$). The strength of this relationship, with $R^2 = 0.76$, was also similar to Hines et al.'s (2008) correlation of incubation vs. field α_C (which had $R^2 = 0.63$ and $p < 0.001$). (For this analysis, no corrections for CH_4 loss were applied, and site PHS was not included because its pore water CH_4 concentration was too low to measure $\delta^{13}C_{CH_4}$.)

As with CH_4/CO_2 ratios, the changes induced before and during incubation might be expected to impact α_C. The higher α_C in the incubations was likely caused by one of the following: (1) higher rates of CH_4 oxidation in the field, which tends to increase $\delta^{13}C_{CH_4}$ and thus lower α_C (Whiticar and Faber 1986); (2) acetoclasts' greater sensitivity to disturbance (Hines et al. 2008), which would have allowed hydrogenotrophy to dominate in the incubations; or (3) substrate depletion and end-product inhibition by accumulated CH_4 in the incubations, which would have lowered thermodynamic energy yields and thus increased isotopic fractionation for both pathways. The lower CH_4/CO_2 ratios in the field (Fig. 1) suggest oxidation in the field as the most likely cause, as the other two mechanisms would likely lead to lower CH_4/CO_2 ratios in the incubations. CH_4 oxidation is likely responsible for the apparent outlier in Fig. 2

(representing site S), as this site had a very low pore water α_C of $\sim$1.04 even though microbiological analyses at this site (Mondav et al. 2014; McCalley et al. 2014) show strong evidence of predominately hydrogenotrophic methanogenesis.

The difference in α_C between the incubations and the field was much smaller than the differences between sites (Fig. 2), suggesting that the relative rates of acetoclastic versus hydrogenotrophic methanogenesis were similar in both settings. This lack of a strong difference, despite temporary aeration prior to incubation, is consistent with Knorr et al.'s (2008) finding that methanogenesis pathways are not affected by drying and rewetting. The slight overestimation of α_C by the incubations was also unrelated to the sites' positions in the thaw sequence, suggesting that any changes in relative pathway rates were consistent across habitat types. Combined with the consistent responses of CH_4/CO_2 ratios to the incubation conditions along the thaw sequence (Fig. 1b), these results suggest that the responses of CH_4/CO_2 ratios to incubation conditions are similar for different CH_4 production pathways.

Interpretations and directions for future research

Our results suggest that CH_4/CO_2 ratios and α_C measured in incubations are only minimally affected by the disturbances induced by sampling, storage, and incubation. Our incubations' apparent insensitivity to disturbance may be related to the spatial and ecological complexity of soil relative to pure cultures, where such sensitivities have been shown. Particularly when the soil structure is non-homogenized (as was the case in our study), soil heterogeneity may preserve methanogen populations in isolated microenvironments even as the bulk soil is disturbed (Mayer and Conrad 1990; Knorr et al. 2008). Facultatively anaerobic heterotrophs, which produce acetate and H_2 during fermentation, may also help to replenish methanogenic substrates following soil oxidation (Mayer and Conrad 1990). Even if the microbial community changed due to extinction of some species and introduction of others by contamination, broad CH_4 production patterns could have remained unaltered if the new community had a similar functionality to the original community. Similarity in community functionality for similar soil types has been demonstrated for CO_2 production in Alaskan peat samples, which produced similar amounts of CO_2 even after sterilization and inoculation with new microbial communities (Treat et al. 2014). Since different methanogen species that use the same substrates can co-exist in the same soil (Basiliko et al. 2003; Kotsyurbenko et al. 2004; Tveit et al. 2013), altered methanogen communities may be able to regain their original functionality following transient geochemical disturbance.

To further explore these possibilities, our group is repeating the incubation experiment of Hodgkins et al. (2014) with a thorough analysis of microbial community composition and activity under in situ, post-storage, and incubation conditions. If this and other future studies can successfully quantify the responses of CH_4 production to incubation conditions, it may eventually be possible to develop models that could directly extrapolate incubation results to predict trends in in situ CH_4 production rates and pathways across peatland types under changing conditions. Although such models are not yet reliable enough to be used quantitatively, the close relationship demonstrated here between in situ field and ex situ incubation CH_4 production strongly supports further development of this modeling approach.

Additional research is also needed to determine whether the agreement between incubation and field results holds across a broader range of environmental conditions. For instance, our methodology should be repeated at other sites with different climates, soil types (e.g. mineral soils), landscape-scale hydrology (e.g. floodplains), and other factors not covered by the variability within Stordalen. Our approach should also be extended throughout the growing season, as the effects of plant functional types on litter inputs and gas transport are known to vary seasonally (Treat et al. 2007; Noyce et al. 2014). The effect of sampling depth also merits further research, as CH_4/CO_2 ratios have been known to vary with depth due to a combination of water table fluctuation and changing organic matter quality with depth (Treat et al. 2015). All of these factors may have differing effects on incubation and field CH_4 production.

Conclusions

Despite disturbances to the incubated peat relative to field conditions, our incubations successfully replicated trends in CH_4/CO_2 ratios and α_C measured in

field-collected pore water, as well as isotopically-modeled CH_4/CO_2 ratios. Although the disturbances induced by peat sampling, storage, and incubation caused slight increases in CH_4/CO_2 ratios and α_C relative to field pore water values, these changes were consistent across different peatland types. This result demonstrates that the differences in in situ CH_4 production between different peatland subhabitats (e.g., bogs vs. fens) can be reliably replicated in the lab, supporting the applicability of soil incubations for analyzing in situ CH_4 production in peatlands. To further validate these results and generalize them to a broader range of environments, future incubation studies should include parallel measurements of CH_4 and CO_2 concentrations and isotopes in field pore water. Such measurements provide a simple, low-cost method for evaluating CH_4 and CO_2 production measurements obtained from soil incubations.

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