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Methane and methanogen community dynamics across a boreal peatland nutrient gradient

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ABSTRACT

Field and lab-based methane (CH_4) fluxes and methanogen community structure were characterized across three peatlands in central Ontario (Canada) representing a successional and nutrient gradient from rich to poor fens. Air temperature was a strong and significant predictor of both CH_4 and carbon dioxide (CO_2) fluxes among the three sites. Net CH_4 efflux and *in vitro* CH_4 production potential were significantly greater in the rich and intermediate than the poor fen site. Although the poor fen site had the lowest water table position, this was not a significant predictor of CH_4 emissions and in general the 3 sites were relatively wet compared to many northern peatlands. Consistently, during spring and fall, ethanol stimulated *in vitro* CH_4 production potential from the poor fen, but not the rich and intermediate sites, indicating substrate limitation for CH_4 production in the poor fen. Lower rates of CH_4 production and emissions in the poor fen site were consistent with our hypotheses based on poorer substrate quality and a lack of sedges in that peatland type. However phylogeny of dominant methanogens inferred from terminal restriction fragment length polymorphism (T-RFLP) analyses of 16S rDNA illustrated inconsistencies with previous reports of methanogens in northern peatlands. For example members likely of the family *Methanosaetaceae* (obligate high-affinity acetate fermenters) comprised a substantial portion of total methanogen population in the poor fen. In contrast, members of the order *Methanomicrobiales* (obligate CO_2 reducers) were important methanogens in the rich and intermediate fens and not detected in the poor fen. Methanogen community structure based on T-RFLP across the 3 sites was distinct during spring, while during fall methanogen communities in the poor fen samples were still somewhat distinct from those in the rich and intermediate fens. Methanogen diversity (community richness and evenness) was not correlated with rates of CH_4 production in the spring when soil respiration, and presumably rhizosphere activity, was slow. However, diversity was a significant predictor of CH_4 production in the early fall (when both production and emissions rates were higher), indicating that methanogen diversity can potentially play a role in biogeochemical cycling and greenhouse gas emissions in northern peatlands.

1. Introduction

Peatlands in northern latitudes represent an important global reservoir of fixed carbon (C) that has accumulated throughout the Holocene due to a net imbalance between rates of primary production and microbial decomposition (Turunen et al., 2002). Flooded, anaerobic soils are one of the key reasons that decomposition rates are relatively slow in these wetlands, yet this condition also ultimately supports the production of methane (CH_4), a greenhouse gas considerably more potent per molecule than carbon dioxide (CO_2) (Forster et al., 2007; Moore and Basiliko, 2006). Methanogenesis is one of the potential terminal steps of several anaerobic decomposition pathways and is carried out by members of the phylum Euryarchaea living below the water table (Liu and Whitman, 2008). In wetland environments there are two major physiological groups of methanogens based on substrate utilization; acetoclastic methanogens convert acetate into CH_4 and

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CO_2, while hydrogenotrophic methanogens convert CO_2 and hydrogen (H_2) into CH_4 and water (Conrad, 2007). Hydrogenotrophic methanogenesis might be the dominant pathway for CH_4 production in most peatlands, as it is more energetically favorable than the acetoclastic pathway (Galand et al., 2005; Liu and Whitman 2008). However, acetoclastic methanogenesis has been shown to be an important pathway for CH_4 production in nutrient rich peatlands and those covered in sedges (Galand et al., 2005). Sedges can serve as conduits for CH_4 emissions and supply labile organic substrate in the form of acetate (and other organic acids) below the water table (Marinier et al., 2004). Acetate has also been shown to be the key terminal anaerobic decomposition product in oligotrophic *Sphagnum* moss dominated peatlands, while presence and increasing sedge cover seems to facilitate fermentation of acetate to CH_4 (Hines et al., 2008); an intriguing, but poorly understood issue. Some phylogenetic groups of methanogens utilize only one pathway, while others can use both, but the role of methanogen community structure in net CH_4 production and emissions is not yet clear.

Peatlands comprise a spectrum of rich fens receiving pH-buffered, nutrient rich groundwater to true bogs where hydrological input occurs only from precipitation (National Wetlands Working Group, 1997). In many North American sites, peatlands typically follow a successional pathway from fens to bogs. This results in part from surface peat accumulation isolating surface peat from groundwater (Siegel and Glazer, 1987). Because of this, the water table level in bogs or poor fens might be typically lower than that of young fens (Holden, 2006; Webster and McLaughlin, 2010). This may enhance CH_4 oxidation to CO_2 in the aerobic surface peat. However, it is not clear whether water table position, presence of sedges, or other physicochemical peat properties make fens, per unit area, larger emitters of CH_4 (Bridgham et al., 1998). For example Leppälä et al. (2011) illustrated that CH_4 emissions were faster from bogs than fens along a post-glacial peatland successional gradient in coastal Finland, except under abnormal periods of high precipitation. Competition for C substrate between methanogenesis and more energetically favorable anaerobic respiration pathways utilizing ferric iron, nitrate, or sulphate as alternative mineral electron acceptors may be more prominent in rich wetland sites and can suppress CH_4 production despite sites being well flooded (e.g. Frenzel et al., 1999; Roy and Conrad, 1999).

Molecular based techniques using archaeal 16S rRNA and *mcrA* (methyl coenzyme A reductase) gene sequences have revealed both a wide diversity of methanogens in peatland ecosystems (e.g. Basiliko et al., 2003; Cadillo-Quiroz et al., 2006; Dettling et al., 2007) or more constrained phylogenetic diversity in certain peatland sites/types lacking methanogens capable of acetoclastic methanogenesis (Galand et al., 2005; Rooney-Varga et al., 2007). Studies of methanogens in fens have been less common than bogs and it is still uncertain if and how methanogen alpha- or functional diversity influences CH_4 production and emissions, and how these relationships change both seasonally and over the longer term.

In this work we characterized methanogen community structure and CH_4 production and emissions, tested for redox substrate limitation, and explored linkages between diversity and activity across a peatland successional gradient (rich, intermediate and poor fens). We hypothesized that CH_4 emission rates would be slowest in the poor fen due to constrained rates of CH_4 production. We also hypothesized that there would be different methanogen communities between the rich and poor fens potentially due to prior known differences in soil pH and organic substrate characteristics and the lack of sedges in the poor fen that might exclude obligate acetoclastic methanogens.

2. Materials and methods

2.1. Study sites

Study sites were located in long-term experimental watersheds maintained by the Ontario Ministry of Natural Resources, Ontario Forest Research Institute, 10 to 20 km southwest of White River, Ontario, Canada (48°21′ N, 85°21′ W). Three fens representing stages along a successional gradient and aggrading peat deposits in separate headwater catchments were chosen for this study including a rich fen dominated by *Eriophorum vaginatum* L. and *Carex* sp. sedges and *Myrica gale* (a known N-fixer) shrubs and an acidic poor fen dominated by *Sphagnum* sp., *Vaccinium* sp. and other evergreen shrubs, and *Picea mariana* and *Larix laricina* trees. A third intermediate fen site had vegetation characteristics of both the rich and poor sites, with *Sphagnum* moss present at about one-tenth the mass (per area) of that in the poor fen, with similar sedge biomass as in the rich fen and similar shrub biomass as the poor fen, but lacking trees (Webster and McLaughlin, 2010). Vegetation, hydrology, peat chemistry and microbial characteristics of these three sites have been described in detail previously (Myers et al., 2012; McLaughlin and Webster, 2010; Webster and McLaughlin, 2010) and longer term pore water chemical constituent concentrations (monthly measurements through the growing seasons from 2005 to 2009, following sampling and measurement approaches of Webster and McLaughlin, 2010) are reported in this study (Table 1).

2.2. Environmental monitoring and field gas flux measurements

To record local meterological conditions, a weather station was installed in the intermediate fen. The station recorded air temperature using a HOBO H21-001 (Onset Computer Corp., Cape Cod, MA, USA) datalogger. Two water table wells (5-cm diameter PVC pipe) were installed to 100 cm and pressure transducers (Solonist 3001 LT Levelogger M5 (Solonist Canada LTD, Georgetown, ON, Canada) were fitted to each well to assess continuous water table depths. Both temperature and water table depth were measured at 1 min intervals and recorded as 15 min averages.

Once a month during May through October, 2009, gas sampling in the rich and poor fens was conducted at four locations (two transects × two sample locations per transect) within each peatland, while sampling in the intermediate fen was conducted at six locations (three transects × two sample locations per transect) using 30-cm diameter and 90.2-L volume opaque PVC static gas flux chambers equipped with battery-powered internal fans for air

Table 1
Mean (standard deviation) 2005 through 2009 porewater chemistry ($mg\,l^{-1}$) at 25 cm below peat surface based on monthly sampling during the growing seasons. Dissolved organic and inorganic C are abbreviated DOC and DIC respectively.

Constituent	Fen type		
	Rich	Intermediate	Poor
pH	6.31 (0.24)	6.15 (0.77)	4.77 (0.66)
DOC	19.0 (6.1)	17.8 (7.3)	41.1 (23.5)
DIC	7.70 (4.84)	2.13 (2.07)	0.98 (0.95)
Total N	0.89 (0.45)	0.70 (0.64)	1.32 (1.05)
NH_4^+	0.08 (0.17)	0.03 (0.06)	0.16 (0.30)
NO_3^-	0.11 (0.35)	0.12 (0.60)	0.12 (0.48)
Ca^{2+}	17.1 (8.1)	9.84 (5.61)	6.84 (5.19)
K^+	0.24 (0.33)	0.21 (0.23)	0.22 (0.15)
Mg^{2+}	2.56 (1.09)	1.17 (1.24)	1.27 (0.93)
SO_4^{2-}	10.9 (7.3)	7.58 (8.32)	11.2 (11.3)
Cl^-	0.16 (0.12)	0.36 (0.41)	0.50 (0.88)
Na^+	1.12 (0.71)	1.20 (0.56)	1.53 (0.46)