SEASONAL DYNAMICS OF METHANE PATHWAYS ALONG A HYDROGEOMORPHIC PEATLAND GRADIENT

by

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A THESIS

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THESIS ABSTRACT

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Title: Seasonal Dynamics of Methane Pathways Along a Hydrogeomorphic Peatland Gradient

Northern peatlands occupy a very small proportion of the Earth's surface but contain nearly a third of the world's soil organic carbon. These wetland systems produce substantial fluxes of CH_4, a potent greenhouse gas, and are expected to experience particularly high annual mean temperature increases as global climate change proceeds, which could result in a significant positive feedback.

Using radioisotope labeling, we quantified the rates of two pathways of CH_4 production in six sites in northern Michigan along a physiochemical and ecological gradient that characterizes northern peatlands. We found that the rates of these two pathways displayed markedly different dynamics in space and time and that the hydrogenotrophic pathway, previously presumed to be less significant in these systems, can increase substantially and become dominant across the landscape when water tables are high. Quantifying these two pathways provides critical insight into understanding dynamics of CH_4 production in northern peatlands.

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To my hearthmate, Orange, who helped me make the change.

And to my wife, Jillian, who has been very patient.

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CHAPTER I

INTRODUCTION

Northern Peatlands: Climate Change Significance

Northern peatlands occupy approximately 3% of the terrestrial surface (3.8 x 10^6 km^2), but contain nearly a third of total terrestrial soil carbon (Jobbágy and Jackson 2000), conservatively estimated at 447 Pg (Joosten 2010). This is possible because peatland soils (histosols) are composed largely of organic material, and can range from 30cm to almost 10m in depth (Jobbágy and Jackson 2000).

These wetland soils are under largely anaerobic conditions, which contributes to the accumulation of such massive reserves of soil organic carbon; anaerobic carbon mineralization (the transformation of organic carbon to inorganic carbon) yields less metabolic energy for soil organisms than does aerobic respiration, and thus it tends to proceed more slowly, with a large portion of the organic carbon in the peat less accessible to these organisms (Köchy *et al.* 2015). However, a greater or lesser fraction of carbon mineralization in anaerobic system results in CH_4, a greenhouse gas ~45 times more potent than CO_2 per unit mass (Neubaurer and Megonigal 2015), as methanogenesis is an obligately anaerobic process (Conrad 1989).

These peatlands are located mostly north of the 45th parallel (Aselmann and Crutzen 1989), and are thus likely to be exposed to extraordinarily high annual maximum temperature anomalies (as high as 6°C) as global climate change proceeds (IPCC 2013, Kirtman *et al.* 2013). As higher soil temperatures tend to increase rates of methanogenesis, there is concern that large increases in mean annual temperature in peatlands could lead to greatly increased CH_4 flux, and thus potentially cause a strong positive feedback on global climate change (Limpens *et al.* 2008, Bridgham *et al.* 2013, Schuur *et al.* 2015). Therefore, a better understanding of how anaerobic CO_2 and CH_4 production varies across these systems is important for understanding their likely role in future global climate change.

The Anaerobic Carbon Cycle: Methanogenesis in Peatlands

In peatlands, as in other soil systems, heterotrophic microorganisms decompose organic carbon into increasingly oxidized forms to conserve metabolic energy to support their growth (Fig. 1.1). Microbes break down organic polymers (e.g. proteins and carbohydrates) into monomers (e.g. amino acids and sugars). In aerobic systems, these compounds are respired to CO_2, releasing significant metabolic energy through the reduction of molecular oxygen. In anaerobic systems, decomposition proceeds through more steps; organic monomers are first fermented into short chain fatty acids (chiefly acetate), releasing CO_2 and H_2 as byproducts, and the fate of those products depends on the presence or absence of alternative terminal electron acceptors (TEAs) which allow anaerobic respiration to occur.

In the presence of TEAs (e.g. ferrous iron, nitrate), decomposition continues in an analogous fashion to aerobic respiration; organic carbon is fully oxidized to CO_2, with the energy conserved through the reduction of the TEAs. It is in the absence of TEAs, however, that methanogenesis becomes a significant player in the process (Fig. 1.1).

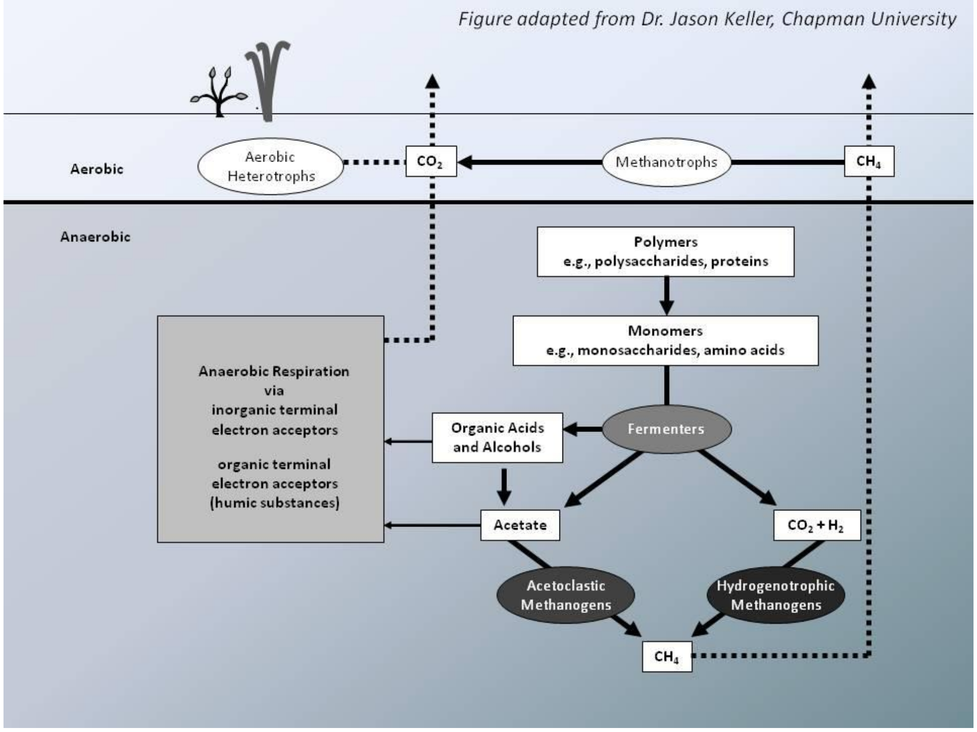


Figure 1.1. A schematic diagram of anaerobic carbon mineralization in wetland ecosystems.

CO_2 and H_2, byproducts of fermentation, are converted into CH_4 via hydrogenotrophic (aka autotrophic) methanogenesis, while acetate is transformed into CO_2 and CH_4 via acetoclastic methanogenesis (Conrad 1989).

CH_4 is produced exclusively by a polyphyletic group of archaea called methanogens (Liu 2010), with these two distinctly different pathways carried out by largely distinct groups. Of the six currently known orders of methanogens, five (the *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanopyrales*, and *Methanocellales*) are exclusively hydrogenotrophic, and one, the *Methanosarcinales*, are largely acetoclastic. This phylogenetic partitioning of methanogenesis pathways raises the possibility that the dynamics of the two pathways may be different in different ecosystems, and that the dynamics of CH_4 production generally may be more complex than one would presume if there was only a single pathway involved.

The Hydrogeomorphic Gradient

The term “northern peatlands” encompasses a variety of systems that exist on a wide spectrum of physiochemical and ecological contexts. This variation can be described as a gradient of groundwater influence (Fig. 1.2), from ombrotrophic systems (with hydrology dominated by precipitation) on one end, to minerotrophic systems (with hydrology dominated by groundwater) on the other (Bridgham *et al.* 1996). Ombrotrophic peatlands tend to have very poorly buffered porewater, resulting in low pH values, with values of 3.0-3.5 not at all uncommon in the most extreme examples, as well as relatively low dissolved nutrient availability resulting from consistent leaching with what amounts to distilled water. Minerotrophic peatlands, on the other hand, have much more neutral pH values (6.0-7.0 typical) in keeping with a strong groundwater influence, and generally have higher nutrient availability.

This gradient of hydrological influence has significant implications for many different ecosystem functions. In terms of anaerobic carbon mineralization, ombrotrophic peatlands have generally been found to produce less CH_4 than minerotrophic peatlands, and to have substantially higher ratios of anaerobic CO_2 to CH_4 production than minerotrophic sites (Bridgham *et al.* 1998, Keller and Bridgham 2007).

Also, ombrotrophic peatlands tend to produce more CH_4 through the hydrogenotrophic pathway, and minerotrophic peatlands more through the acetoclastic pathway (Kelly *et al.* 1992, Chasar *et al.* 2000, Avery *et al.* 2002), although there is evidence that this can change somewhat through time (Keller and Bridgham 2007). Therefore there appears to be an association between hydrogeomorphic position, dominant methanogenesis pathway, and CH_4 production, both total and as a proportion of total anaerobic carbon mineralization (McCalley *et al.* 2014), but this needs to be explored further.

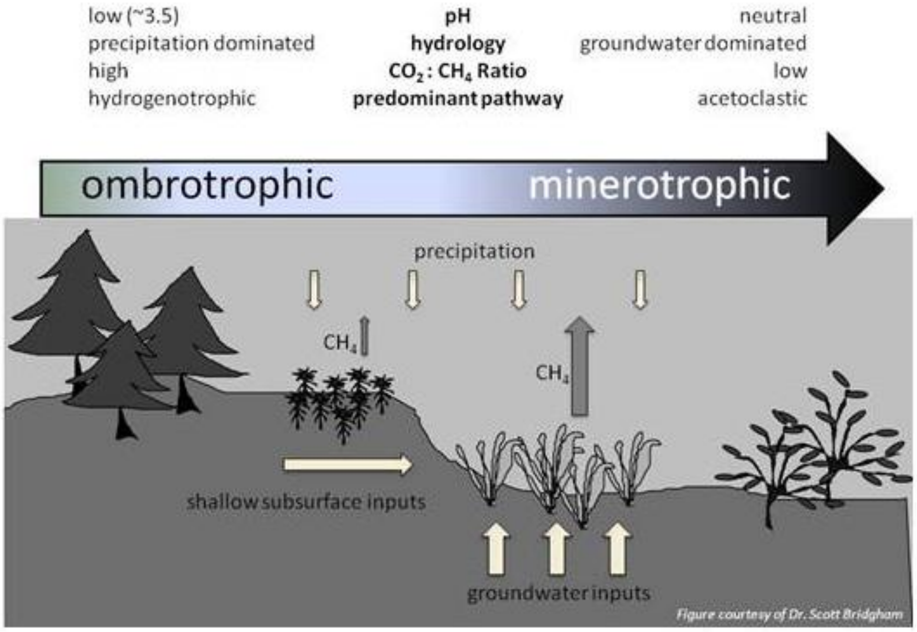


Figure courtesy of Dr. Scott Bridgham

Figure 1.2. The hydrogeomorphic gradient.

In this study, we quantified anaerobic CO_2 production and both pathways of methanogenesis in six sites distributed across the hydrogeomorphic gradient in the Upper Peninsula of Michigan over the course of two growing seasons. We hypothesized that total CH_4 production, as well as the ratio of CH_4 to CO_2 produced, would generally increase with increasing minerotrophy, as would the relative contribution of acetoclastic methanogenesis, and sought to determine whether this would manifest continuously or discontinuously along the gradient. We also examined the intra- and interannual consistency of these trends across the gradient.

CHAPTER II

SEASONAL DYNAMICS OF METHANE PATHWAYS ALONG A HYDROGEOMORPHIC PEATLAND GRADIENT

Introduction

Peatlands occupy only 3% of Earth's surface, but contain approximately 31% of terrestrial soil carbon (Bridgham *et al.* 2006, Jobbágy and Jackson 2000). Peatlands also produce a significant fraction of the global methane (CH_4) flux (Kirschke *et al.* 2013, Bridgham *et al.* 2013). Concentrated at northern latitudes, peatlands will be exposed to particularly large temperature increases as global climate change proceeds (Kirtman *et al.* 2013), and if their large soil carbon reserves were mineralized as CH_4 or carbon dioxide (CO_2), could cause a significant positive feedback to anthropogenic climate change (Limpens *et al.* 2008, Bridgham *et al.* 2013, Schuur *et al.* 2015). Anaerobic carbon cycling processes and the resultant relative production of CO_2 and CH_4 are particularly important because sustained fluxes of CH_4 have 45 times the global warming potential of CO_2 (Neubaurer and Megonigal 2015). Better understanding controls over anaerobic carbon mineralization in these ecosystems is an important step in predicting their response to climate change.

In all known freshwater systems, methanogenesis occurs through one of two pathways: acetoclastic (also called fermentative) methanogenesis, which utilizes acetate as a substrate and also produces CO_2 , and hydrogenotrophic (or autotrophic) methanogenesis, which utilizes H_2 and CO_2 as substrates (Conrad 1989). Methanogens do not compete well with microbes that utilize alternative terminal electron acceptors

(TEAs) for respiration; as a result, methanogenesis tends to predominate only where these TEAs (such as ferrous iron and sulfate) are not available (Megonigal *et al.* 2004). The stoichiometry of methanogenesis is such that the acetoclastic pathway should comprise approximately two-thirds of methanogenesis, and in fully methanogenic systems (those lacking alternative TEAs), the ratio CO_2 and CH_4 produced should be 1:1 through either pathway (Conrad 1999).

Peatlands, however, display a large range of CH4 production rates and efficiencies (Bridgham *et al.* 1998, Turetsky *et al.* 2014). They exist in a variety of forms along a hydrogeomorphic gradient (Bridgham *et al.* 1996)—from ombrotrophic bogs, which receive all water inputs from precipitation, and thus have low alkalinity and pH, to minerotrophic fens, which are influenced by groundwater and/or runoff and thus have higher alkalinity and pH. Nutrient availability, carbon quality, and basic cation concentrations also vary greatly across this gradient, as does plant community structure. CO_2 :CH_4 ratios also vary along this gradient, from minerotrophic sites that produce significant CH_4, to ombrotrophic sites that may scarcely produce CH_4 at all, with CO_2 :CH_4 ratios spanning several orders of magnitude (Bridgham *et al.* 1998, Keller and Bridgham 2007). While concentrations of inorganic TEAs are generally low in peatlands, substantial recent evidence indicates that organic TEAs are very important in surface peat (reviewed in Bridgham *et al.* 2013). However, even when organic TEAs are exhausted, high CO2:CH4 ratios persist, particularly in bog peat (Keller and Takagi 2013). In addition, a quinone compound was found to act primarily as a TEA in a fen peat but to have broad inhibitory effects in a bog peat, with particularly severe inhibition of methanogenesis (Ye *et al.* 2016). Thus, a mechanistic understanding of controls over CH_4 production in peatlands remains elusive.

The relative importance of the two dominant methanogenesis has received relatively little study in wetlands, particularly using methods that provide quantitative rates. Perhaps this is because many natural anaerobic habitats display the predicted 2:1 acetoclastic:hydrogenotrophic ratio of the pathways, and theoretically total CH_4 production should not depend on the pathway (Conrad 1999). However, peatlands along the hydrogeomorphic gradient display different ratios of the two pathways, with ombrotrophic sites more dominated by hydrogenotrophic methanogenesis, and minerotrophic sites more by acetoclastic methanogenesis (Kelly *et al.* 1992, Chasar *et al.* 2000, Keller and Bridgham 2007, Galand *et al.* 2010). Thus a substantial body of literature indicates that both total CH_4 production and the proportion of acetoclastic methanogenesis increase with increasing minerotrophy, suggesting a causative linkage between the relative dominance of the methanogenesis pathways and total CH_4 production. In support of this supposition, melting of permafrost in peatlands with a corresponding switch from ombrotrophic to minerotrophic conditions was recently found to cause a large increase in CH_4 fluxes coincident with a greater proportion of acetoclastic methanogenesis (McCalley *et al.* 2014).

These patterns are further complicated by fluctuations across time. The ratio of acetoclastic to hydrogenotrophic methanogenesis changes seasonally in some peatlands (Avery *et al.* 1999, Keller and Bridgham 2007), sometimes to the extent that which pathway is predominant changes during a growing season (Keller and Bridgham 2007). Hydrogenotrophic methanogenesis also often increases with peat depth, even as total methanogenesis decreases (Chanton *et al.* 1995, Lin *et al.* 2012); it is possible that there is a link between changes in water-table depth (and thus the carbon quality of the peat exposed to anaerobic conditions) and temporal shifts in the methanogenic pathways.

It is clear, then, that there are significant differences in CH_4 production, the ratio of acetoclastic to hydrogenotrophic methanogenesis, and the anaerobic CO_2:CH_4 mineralization ratio amongst peatlands along the ombrotrophic-minerotrophic gradien. Further, these patterns can change during a growing season, and with depth in the peat column, and the interannual variations have been little explored. Moreover, most studies of anaerobic carbon mineralization along the ombrotrophic-minerotrophic gradient in peatlands have sampled only a few sites at most (Chanton *et al.* 1995, Keller and Bridgham 2007; Galand *et al.* 2010, McCalley *et al.* 2014), leaving it unclear whether variations between ombrotrophic and minerotrophic sites occur gradually as one moves across the gradient. While some factors that affect anaerobic carbon cycling, such as pH, often change in a somewhat linear fashion across the gradient, other factors change in a discontinuous manner. For example, plant species can have broad or narrow distributions relative to pH, as a surrogate for degree of minerotrophy (Janssens 1992). Highly minerotrophic peatlands can be dominated by graminoids (i.e., rich fens) or trees (i.e., swamp forests), with corresponding very different physical peat characteristics (Bridgham *et al.* 1998, Kolka *et al.* 2016). In this study, we quantified anaerobic CO_2 production and both pathways of methanogenesis in six sites distributed across the hydrogeomorphic gradient over the course of two growing seasons. We hypothesized that total CH_4 production, as well as the ratio of CH_4 to CO_2 produced, would generally increase with increasing minerotrophy, as would the relative contribution of acetoclastic methanogenesis, and sought to determine whether this would manifest continuously or discontinuously along the gradient. We also examined the intra- and interannual consistency of these trends across the gradient.

Methods

Sites

We selected six sites in the Upper Peninsula of Michigan that are broadly representative of a hydrogeomorphic gradient from ombrotrophic to minerotrophic peatlands in this region based upon our extensive past research in the Upper Midwest. Position along the gradient was determined by soil pH and dominant plant communities. Sites are described in detail in Ye et al. (2012). Soil pH increases from below 4 in the two ombrotrophic bogs (B1, B2) to approximately 6 in the rich fen (RF) and cedar swamp sites (CS) (Table 2.1). All sites except for the rich fen and cedar swamp have extensive cover of *Sphagnum* spp. mosses, but Bog 1 is dominated by hummock-forming species (e.g., *Sphagnum fuscum* (Schimp)) and thus typically has a water table much further from the surface than the other sites (Table 2.1). The poor fen (PF) and intermediate fen (IF) sites have greater cover and diversity of graminoid and forb species than the bog sites. The nitrogen-fixing shrub speckled alder (*Alnus incana* (L.) Moench, ssp. *rugosa* (Du Roi) Clausen) is present in the intermediate fen and cedar swamp sites. The rich fen site is dominated by upright sedge (*Carex stricta* Lam.) tussosks and borders a lake, with 20 to 40 cm of standing water during all sampling times. The cedar swamp has an overstory of northern white cedar (*Thuja occidentalis* L.), and much more physically decomposed peat (hemic to sapric) compared to the other sites (fibric to hemic) reflecting its woody vegetation. All sites have extensive cover of ericaceous shrubs. Maximum measured peat depth was greater than 3 m in all sites (3.6 to 7.3 m) except the cedar swamp, where it was 2.9 m. Five of the sites are on the University of Notre Dame Environmental Research Center (UNDERC), whereas Bog 1 is located 100 km east in Crystal Falls, Michigan, USA.

Table 2.1. Site and sampling event characteristics.

		2009		2010		
	Site	Aug	Sep	May	Aug	Sep
Incubation Temperature (°C)	ALL	17	12	9	14	19
pH	B1	3.78 (0.45)	3.58 (0.05)	3.67 (0.11)	3.46 (0.18)	3.25 (0.2)
	B2	3.88 (0.41)	3.74 (0.12)	3.83 (0.23)	3.19 (0.38)	3.32 (0.46)
	PF	3.96 (0.48)	4.21 (0.07)	3.8 (0)	3.78 (0.1)	5.27 (0.17)
	IF	4.63 (0.08)	4.61 (0.32)	4.79 (0.17)	4.39 (0.2)	5.51 (0.23)
	RF	6.18 (0.17)	5.72 (1.48)	6.35 (0.2)	5.71 (0.3)	6.26 (1.33)
	CS	6.04 (0.11)	6.05 (0.79)	6.16 (0.32)	5.88 (0.26)	4.1 (0.45)
Water Table (cm)	B1	-31	-38	-22	-2	-1
	B2	-22	-18	-11	-1	-2
	PF	-1	-12	-8	-1	-4
	IF	-31	-21	-5	2	6
	RF	28	27	18	38	23
	CS	-8	-8	-1	4	4

Sampling

Sites were sampled five times in August and late September 2009 (Summer and Fall), and May, July, and September 2010 (Spring, Summer, and Fall). Five replicate peat cores were taken to a depth of 25 cm below the water table at the time of sampling from randomly chosen hollows at each site, for a total of 150 cores. The location of the water table varied within sites between sampling events (Table 2.1), and thus the portion of the peat column sampled varied as well; this location was chosen as being the likely location of maximum methanogenesis at each time (Updegraff et al. 1995; Tfaily et al. 2014). Cores were collected in 10 cm diameter polyvinyl chloride tubes, immediately topped off with porewater from the bore hole, and sealed on both ends with rubber plumbing caps to maintain anoxic condition. Cores were then transported on ice and processed within 60 hours.

Slurry Preparation and Incubation

Cores were processed in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI, USA), with a 5:95 atmosphere of H_2 and N_2 gas. Large wood fragments and living roots were removed before peat was thoroughly homogenized. Peat slurries (one per core) were prepared by transferring approximately 40 g of homogenized peat to 500 ml Mason jars (with lids equipped with rubber septa), and mixing 1:1 with porewater collected along with each core after bubbling with N_2. Pre-incubation pH was measured in the chamber before the jars were sealed. Subsamples of root-free peat were dried for 48 hours at 60°C in a drying oven for bulk density measurements. Immediately after preparation, the headspace of each Mason jar was purged with N_2 gas for 30 minutes. Slurries were regularly agitated during this process to release dissolved CO_2 and CH_4 from slurry porewater.

Slurries were then spiked with 0.6 μCi of $^{14}C–HCO_3$ (PerkinElmer, Waltham, Massachusetts, USA), stabilized in 150 μL of 0.01 M NaOH to prevent outgassing. The tracer changed ΣCO_2 concentration in the slurries by < 2μM, a small change unlikely to affect mineralization rates. Slurries were then incubated at *in situ* soil temperature (averaged across the six peatlands) for the time sampled (Table 2.1). Headspace concentrations of CO_2 and CH_4 were monitored at experiment start, 24, and 48 hours using a gas chromatograph (Model 8610C; SRI Instruments, Torrance, CA, USA) equipped with a methanizer and flame ionization detector, as well as a gas proportional radioactivity counter (GC-RAM; LabLogic Systems, Brandon, FL, USA). This setup allows the simultaneous quantification of both total and radioactive CO_2 and CH_4 in a single 1 ml headspace sample. Total CO_2 and CH_4 in each slurry were determined at each time point using Henry's Law to calculate dissolved CO_2 and CH_4 from headspace concentrations accounting for temperature, pH, and volumetric ratio between headspace and porewater (Bridgham and Ye 2013). Production of CH_4 and CO_2 were calculated by gas accumulation through time.

Of 150 separate peat core slurries, ten suffered failed seals during incubation or showed nonlinear accumulation of CO_2 and/or CH_4 over 24 hours. These samples were excluded, and in subsequent analysis, their anaerobic CO_2 , total CH_4 , and methanogenic pathway rates were taken as the average of the remaining slurries for that site at that sampling time.

Methanogenesis Pathways

Hydrogenotrophic methanogenesis was calculated as $R = aC\alpha/Atg$, where R is the rate of hydrogenotrophic methanogenesis, a is the amount of $^{14}CH_4$ produced, C is the average ΣCO_2 pool during the incubation, A is the average $^{14}CO_2$ pool during the incubation, t is the time elapsed, g is the dry mass of peat in the slurry, and α is the fractionation factor for hydrogenotrophic methanogenesis from $^{14}CO_2$ relative to $^{12}CO_2$

(i.e., 1.12). This method is adapted from Keller and Bridgham (2007). In that study, *C* and *A* the end of the experiment were used in the equation; given that these pools are not constant during the incubation, we assumed they changed linearly and used the average of the starting and ending values.

Acetoclastic methanogenesis was calculated by the difference between total CH_4 production and hydrogenotrophic methanogenesis, based on the generally accepted assumption that the hydrogenotrophic and acetoclastic pathways are the only significant sources of CH_4 in freshwater systems (Conrad 1989).

Statistical Analyses

Differences in anaerobic CO_2 production, total, hydrogenotrophic, and acetoclastic methanogenesis, and proportion of methanogenesis through the acetoclastic pathway were all analyzed by two-way ANOVA with season and site as the independent variables (Table 2.2). Since cores were chosen from separate random locations within a site during each sampling event, a repeated-measures ANOVA was inappropriate. The main effects of site and season, as well as their interaction, were significant for all dependent variables (with the exception of absolute acetoclastic methanogenesis), so the main effects were further analyzed with one-way ANOVAs (Supplementary Tables 1 and 2; see the Appendix for all supplementary figures and tables), with significant differences by season within sites and by sites within seasons identified by Tukey's post hoc comparisons. All statistics were performed in SPSS (Version 22.0, IBM Corp). All dependent variables were tested for normality and either square root or log-transformed where such significantly improved the distribution.

The effect of pH, water-table depth, and incubation temperature on the dependent variables was explored using stepwise multiple regressions, both forward and reverse. In all cases, both forward and reverse regressions converged on the same best-fit models, as summarized in Table 2.3.

Results

Water Table and pH Variation Through Time

pH generally increased along the gradient, and was fairly stable within sites through the first four sampling events (Table 2.1). In Fall 2010, the Poor Fen and Intermediate Fen becoming notably more alkaline, and the Cedar Swamp more acidic.

Water table depth varied substantially both between and within sites. While all sites experienced their highest water table levels in Summer or Fall of 2010, this change was much more pronounced in Bog 1, Bog 2 and the Intermediate Fen than in the Poor Fen or Cedar Swamp. The Rich Fen always had relatively deep standing water.

Anaerobic CO_2 Production and Methanogenesis Pathways

Anaerobic CO_2 production (Fig. 2.1) was generally highest in Summer and Fall 2010, and always varied significantly among sites (Supplementary Table 1). While sites with the highest or lowest rates were not always consistent, generally speaking the Cedar Swamp had the lowest CO_2 production and the Intermediate Fen the highest production. Only water-table depth was a significant predictor of CO_2 production but had low explanatory power ($r^2 = 0.05$).

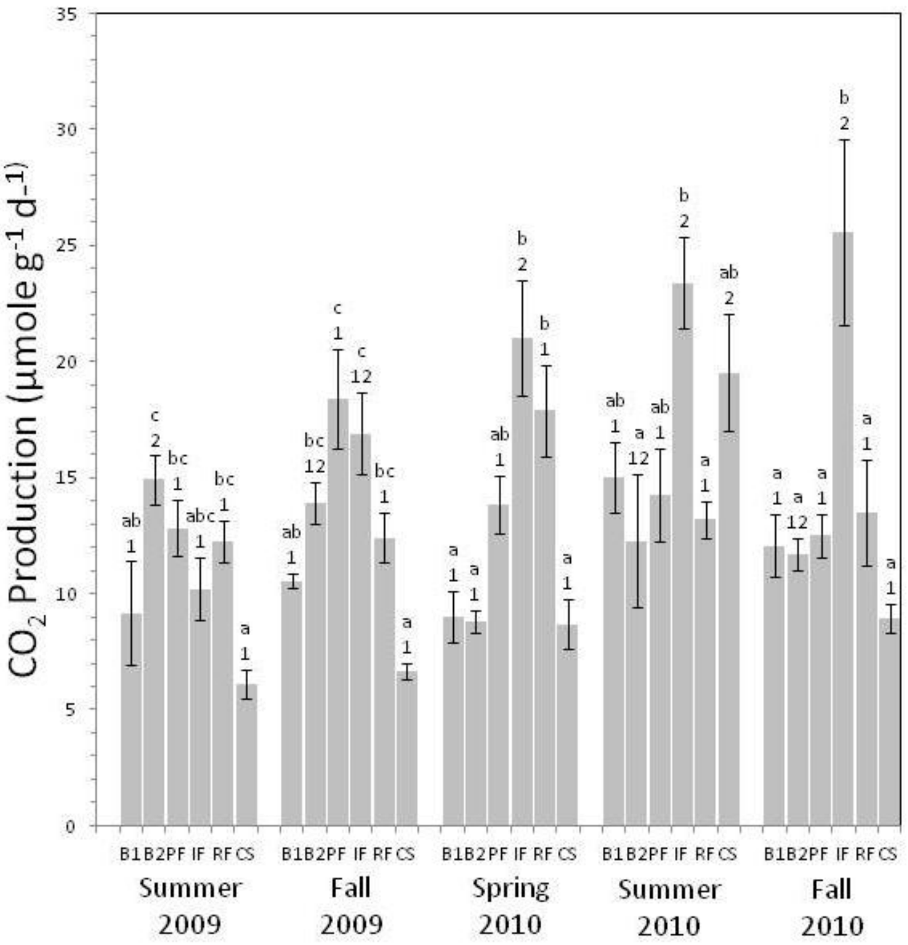


Figure 2.1. Anaerobic CO$_2$ production by site and season. In this and all subsequent figures, B1 = Bog 1, B2 = Bog 2, PF = poor fen, IF = intermediate fen, RF = rich fen, CS = cedar swamp. Letters represent significantly different rates across sites within seasons; numbers represent significantly different rates across seasons within sites.

Total methanogenesis generally increased towards the minerotrophic end of the gradient, with the exception of the cedar swamp (Fig. 2.2). It was generally greatest in Summer and Fall 2010, with the highest rate in the rich fen throughout 2009 and Spring

2010, but the intermediate fen had the highest rates in summer and fall 2010. pH and water table depth were the only significant predictors ($p<0.001$), with partial r^2 of 0.24 and 0.09, respectively.

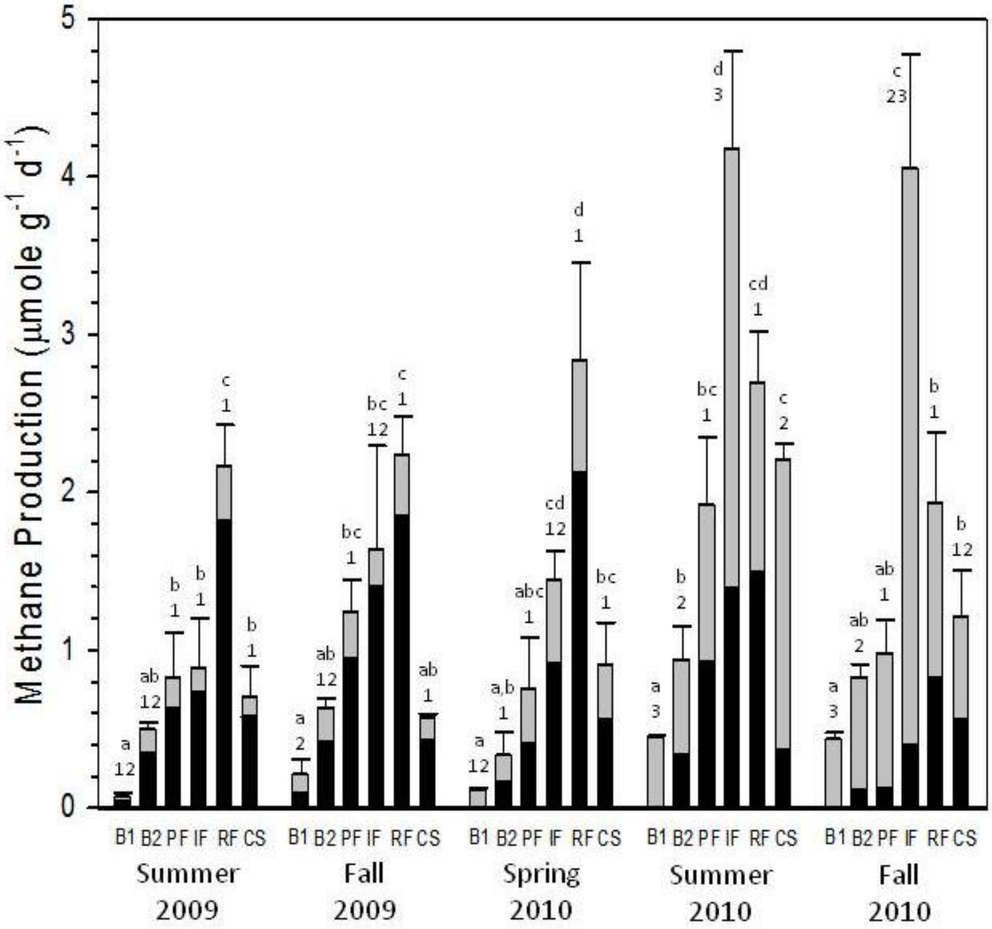


Figure 2.2. Methanogenesis rates by site and season. Acetoclastic in black, hydrogenotrophic in grey.

The acetoclastic and hydrogenotrophic methanogenesis pathways displayed strikingly different dynamics across sites and seasons. Absolute rates of acetoclastic methanogenesis increased towards the minerotrophic end of the gradient, with the exception of the cedar swamp, and this pattern was very consistent through time (Supplementary Fig. 1, ANOVA Site x Event p = 0.207). Stepwise regressions showed pH (partial $r^2 = 0.20$) and temperature (partial $r^2 = 0.05$) as the most significant predictors, with no effect of water-table.

Table 2.2. Two-way ANOVAs.

Dependent Variable		Season	Site	Interaction	Transform
Anaerobic CO_2 Production	DF	4,110	5,110	20,110	Square Root
	F	8.99	20.35	5.02	
	P	**<0.001**	**<0.001**	**<0.001**	
Total Methanogenesis	DF	4,110	5,110	20,110	Square Root
	F	10.77	57.11	3.32	
	P	**<0.001**	**<0.001**	**<0.001**	
Acetoclastic Methanogenesis	DF	4,110	5,110	20,110	Log 10
	F	7.26	33.63	1.28	
	P	**<0.001**	**<0.001**	**0.207**	
Hydrogenotrophic Methanogenesis	DF	4,110	5,110	20,110	Log 10
	F	48.87	46.45	4.73	
	P	**<0.001**	**<0.001**	**<0.001**	
Proportion Acetoclastic	DF	4,110	5,110	20,110	None
	F	65.96	23.58	1.96	
	P	**<0.001**	**<0.001**	**<0.001**	
CO_2:CH_4 Ratio	DF	4,110	5,110	20,110	None
	F	3.51	23.97	5.00	
	P	**<0.001**	**<0.001**	**<0.001**	

In contrast to acetoclastic methanogenesis, hydrogenotrophic methanogenesis showed inconsistent trends across the gradient across different sampling events. Thus, hydrogenotrophic CH_4 production had a significant interaction between space and season not seen in acetoclastic production (Table 2.2). Hydrogenotrophic rates were uniformly low in Summer and Fall 2009, and there were few significant differences among sites

(Supplementary Fig. 2). In Spring 2010, hydrogenotrophic CH_4 production remained low but a similar increase across the gradient was evident as with acetoclastic and total CH_4 production. Hydrogenotrophic rates increased substantially in Summer and Fall 2010, especially in the Poor Fen. Rates were highest in the Poor Fen, and in Fall 2010 there were few other significant differences across the gradient. Water-table was the strongest predictor of hydrogenotrophic methanogenesis (partial $r^2 = 031$), with pH adding modest additional predictive power (partial $r^2 = 0.05$).

The proportion of total CH_4 from acetate (Fig. 2.3) increased with the degree of minerotrophy in a fairly predictable manner in the first three sampling dates, and all sites were dominated by this pathway. However, Bog 1 went from approximately 50% acetoclastic methanogenesis in Summer 2009 and Fall 2009 to 0% acetoclastic methanogenesis in Spring 2010. Very different relative pathway dynamics were observed in Summer 2010 and Fall 2010. Bog 1 continued to have 0% acetoclastic methanogenesis, but now the other sites were also generally dominated by the hydrogenotrophic pathway. The proportion of the pathways also did not follow the gradient as closely, with only Bog 1 being lower than the Poor Fen and Cedar Swamp being similar in Summer 2010 and the two most minerotrophic sites (Rich Fen and Cedar Swamp) having dramatically higher acetoclastic methanogenesis in Fall 2010. The stepwise regression results were a blend of those predictor variables most significant for the two pathways individually: water table (most significant for the hydrogenotrophic pathway) explained the most variance (partial $r^2 = 0.17$), followed by pH (most significant for the acetoclastic pathway; partial $r^2 = 0.14$), and a mild effect of temperature (partial $r^2 = 0.03$).

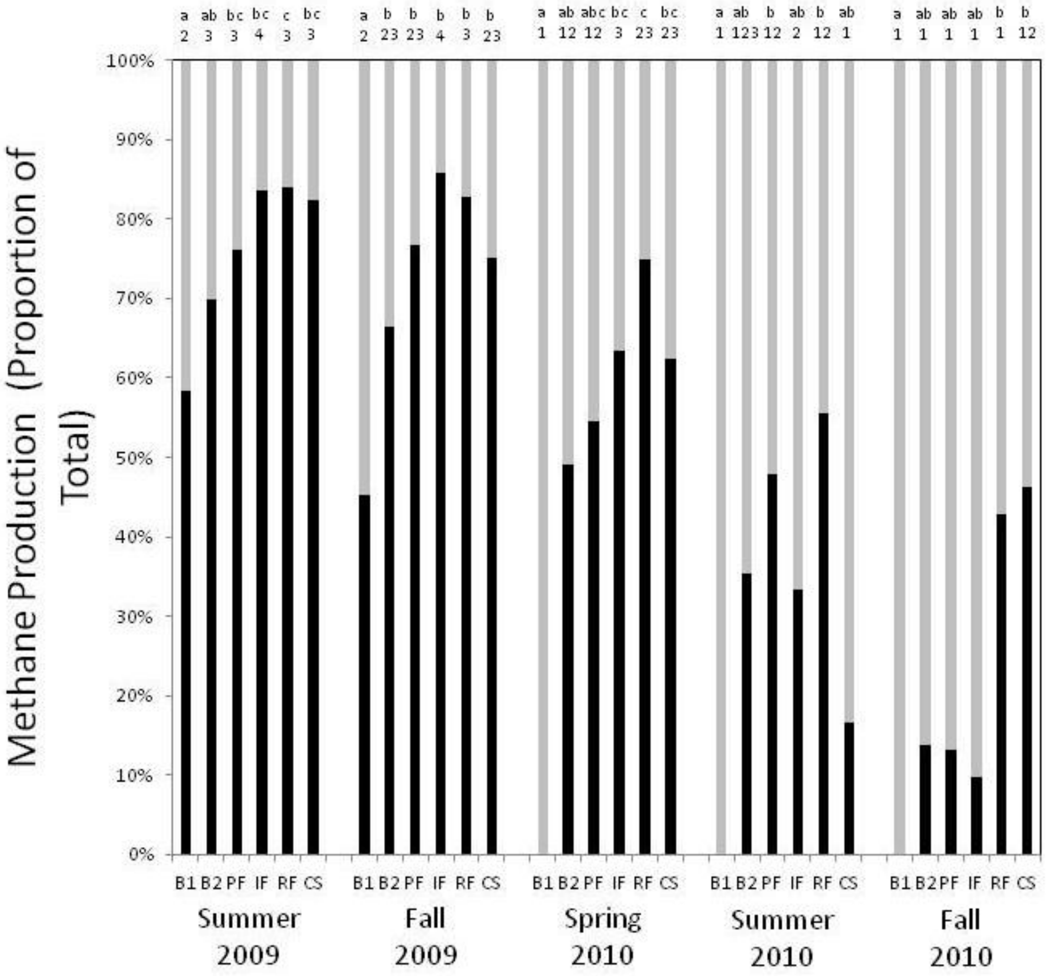


Figure 2.3: The proportion of methane by the acetoclastic (black) and hydrogenotrophic (grey) pathways, by site and season.

The ratio of CH_4 to CO_2 production (Fig. 2.4) showed a generally decreasing trend towards the minerotrophic end of the gradient, although only Bog 1 was significantly higher than the other sites, and was also the only site that experienced significant changes through time. A small portion of variance in this ratio was explainable by stepwise regression, with only pH having a discernible effect ($r^2 = 0.08$).

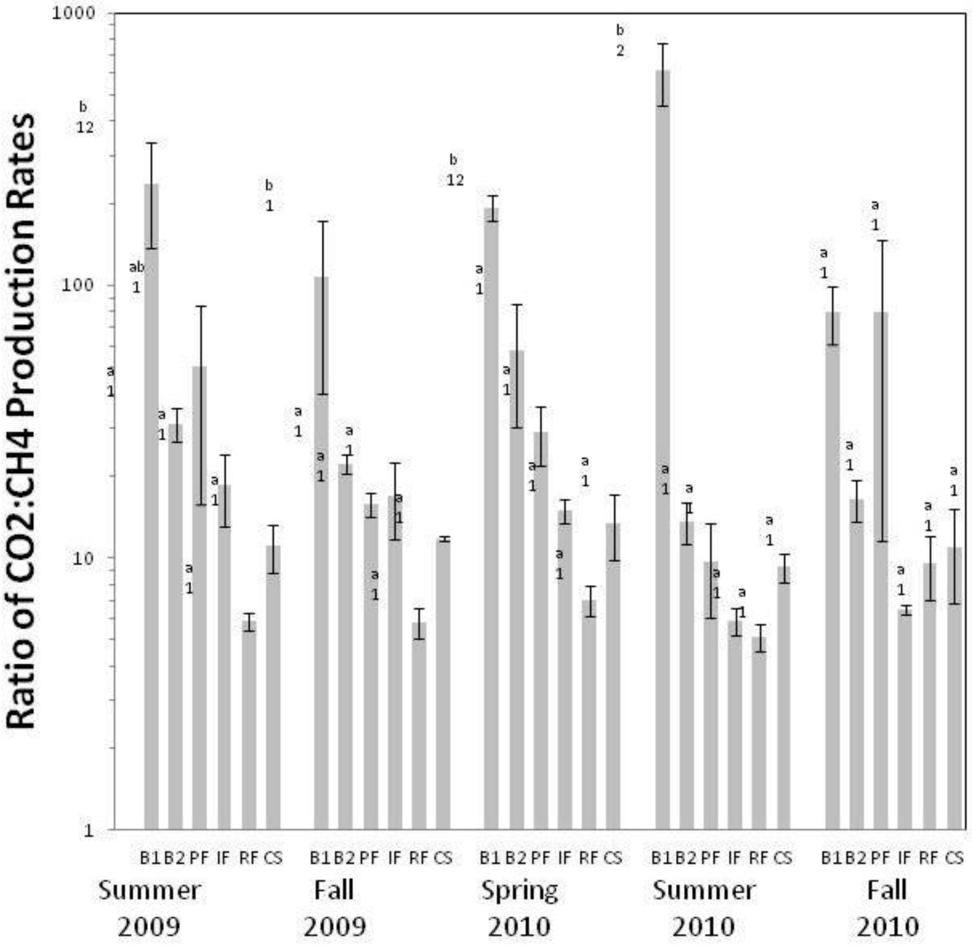


Figure 2.4: Ratio of total methanogenesis to anaerobic CO_2 production by site and season. Note the logarithmic scale.

Table 2.3. Step-wise regressions.

Dependent Variable		**r^2**	DF	F	p
Anaerobic CO2 Production	WT	**0.05**	1,128	7.06	0.009
Total Methanogenesis	pH	**0.24**	1,128	41.21	<0.001
	WT	**0.33**	1,127	15.57	<0.001
Acetoclastic Methanogenesis	pH	**0.20**	1,128	32.49	<0.001
	Temp	**0.25**	1,127	7.09	0.009
Hydrogenotrophic Methanogenesis	WT	**0.31**	1,128	56.42	<0.001
	pH	**0.36**	1,127	11.19	0.001
Proportion Acetoclastic	WT	**0.17**	1,128	25.68	<0.001
	pH	**0.31**	1,127	26.47	<0.001
	Temp	**0.34**	1,126	4.55	0.035
CO2:CH4 Ratio	pH	**0.08**	1,128	10.45	0.002

Discussion

This study quantified hydrogenotrophic and acetoclastic methanogenesis at relatively fine resolution across a hydrogeomorphic gradient through two growing seasons. We hypothesized that total methanogenesis would increase along the gradient, and that this increase would be driven largely by increases in the acetoclastic pathway, with relatively little contribution from the hydrogenotrophic pathway. These predictions were generally not borne out because of complicated temporal dynamics in the control of the two methanogenesis pathways. We are not aware of any previous studies that have shown such complicated and dramatic temporal CH_4 dynamics across the ombrotrophic-minerotrophic gradient of peatlands.

Total methanogenesis did increase across the gradient (with the exception of the Cedar Swamp), with a corresponding increase in acetoclastic methanogenesis responsible for this trend in the first three sampling events (Figs. 2.2, 2.3). However in the Summer and Fall of 2010, the large increase in total CH_4 production observed in many sites was largely driven by increases in hydrogenotrophic methanogenesis. While rates of acetoclastic methanogenesis varied through time, they did so in a relatively uniform manner across the gradient. In contrast, changes in hydrogenotrophic methanogenesis over time were much more idiosyncratic. Particularly large changes in hydrogenotrophic methanogenesis over time were observed in the Intermediate Fen and Cedar Swamp.

This gradient-wide shift in pathway dominance, from acetoclastic to hydrogenotrophic, has not to our knowledge been found before. Generally speaking, hydrogenotrophic methanogenesis has been found to predominate in ombrotrophic systems, which also produce relatively little CH_4 when compared to more minerotrophic sites, which are typically much more methanogenic. Seasonal shifts in the relative contributions of these two pathways have also been noted previously (Keller and Bridgham, 2007), but the degree of interannual change across the entire gradient that we found is surprising.

This large increase in the hydrogenotrophic pathway is interesting in the context of what is known about the community structure of methanogens in peatlands. Several studies (Lin *et al.* 2012, Yavitt *et al.* 2012, McAllister *et al.* unpublished data) have found that while methanogen community structure does differ between ombrotrophic and minerotrophic systems, communities across the gradient tend to be dominated by putative hydrogenotrophs, even where methanogenesis in the system is predominately acetoclastic. This decoupling of methanogen functional groups and realized pathways has been difficult to account for. In this study, our finding of a rapid increase in hydrogenotrophic methanogenesis, with no attendant shift in methanogen community structure (McAllister et al. unpublished data), indicates that in some systems hydrogenotrophic methanogens may be metabolizing at less than their full potential, and are capable of rapidly scaling up their activity when conditions are appropriate.

Cores were taken 25 cm below the water table at the time of sampling in the acrotelm, the surface zone of peat that experiences fluctuating water tables and which is also the area of greatest CH_4 production. Water table level was significantly positively correlated with rates of hydrogenotrophic methanogenesis, reflecting the large increase in rates when the water table rose in Summer and Fall 2010 (Table 2.1). Previous work strongly indicates that the proportion of methanogenesis through the hydrogenotrophic pathway increases with depth in the peat column, and this is generally presumed to be due to the decreasing lability of carbon in deeper, older peat (Chanton *et al.* 2005). However, the increasing dominance of hydrogenotrophic methanogenesis with increasing depth has previously been noted in individual soil cores taken at a single time point. In contrast, in this study we found greatly increased hydrogenotrophic methanogenesis without a corresponding increase in acetoclastic methanogenesis in very shallow peat upon a rise in the water table during the growing season. This shallow peat would presumably be more labile because it is younger, and in many of the sites anaerobic CO_2

production was higher in Summer and Fall 2010 (Fig. 2.1), supporting this suggestion. The Cedar Swamp typically had relatively low rates of CO_2, total CH_4, acetoclastic CH_4, and hydrogenotrophic CH_4 production, presumably due its more woody, sapric peat, but the proportion of the two CH_4 pathways was generally similar to the minerotrophic Rich Fen (Fig. 2.3). Thus, the relationship between the relative importance of the two methanogenesis pathways and carbon quality may not be as straight forward as previously thought. Oxidized humic substances have been recently shown to be important terminal electron acceptors in surface peat, which suppresses overall CH_4 production (Keller and Takagi 2013); however it is unclear if and how this would affect the two CH_4 pathways differentially.

We found that the acetoclastic and hydrogenotrophic pathways of methanogenesis are regulated very differently along the hydrogeomorphic gradient. The rate of acetoclastic methanogenesis was strongly correlated with gradient position, and remained so at all times. In contrast, the hydrogenotrophic pathway varied strongly through time, potentially responding to large changes in water-table depth and thus peat depth, age, carbon lability, and oxidation state of humic substances. The rate of total methanogenesis, the sum of these two pathways, and thus the ratio of methanogenesis to anaerobic CO_2 production, therefore varied significantly across the gradient, but in a complex, time-dependent manner. Given the potential importance of wetland CH_4 emissions as a positive feedback mechanism to anthropogenic climate change, we suggest that much further research needs to focus on controls over CH_4 pathways and how they affect total CH_4 production in wetlands. Furthermore, our results suggest that these studies need to include a diversity of wetland types and temporal hydrological conditions.

Such dynamics are not currently incorporated into large-scale current CH_4 models (Melton *et al.* 2013, Wania *et al.* 2013, Bridgham *et al.* 2013), and if our findings are generalizable to other peatlands, then these models will not be predictive of how future climatic conditions will affect wetland CH_4 emissions.

CHAPTER III

CONCLUSION

We found that the hydrogenotrophic and acetoclastic pathways of methanogenesis differ significantly in the spatial and temporal dynamics. Our finding that acetoclastic methanogenesis tended to increase towards the minerotrophic end of the hydrogeomorphic gradient is consistent with previous research, but the large increase in hydrogenotrophic methanogenesis in late 2010 is, to our knowledge, an unprecedented finding. A finding made all the more remarkable by the fact that this study sampled the hydrogeomorphic gradient extensively, and found that result throughout.

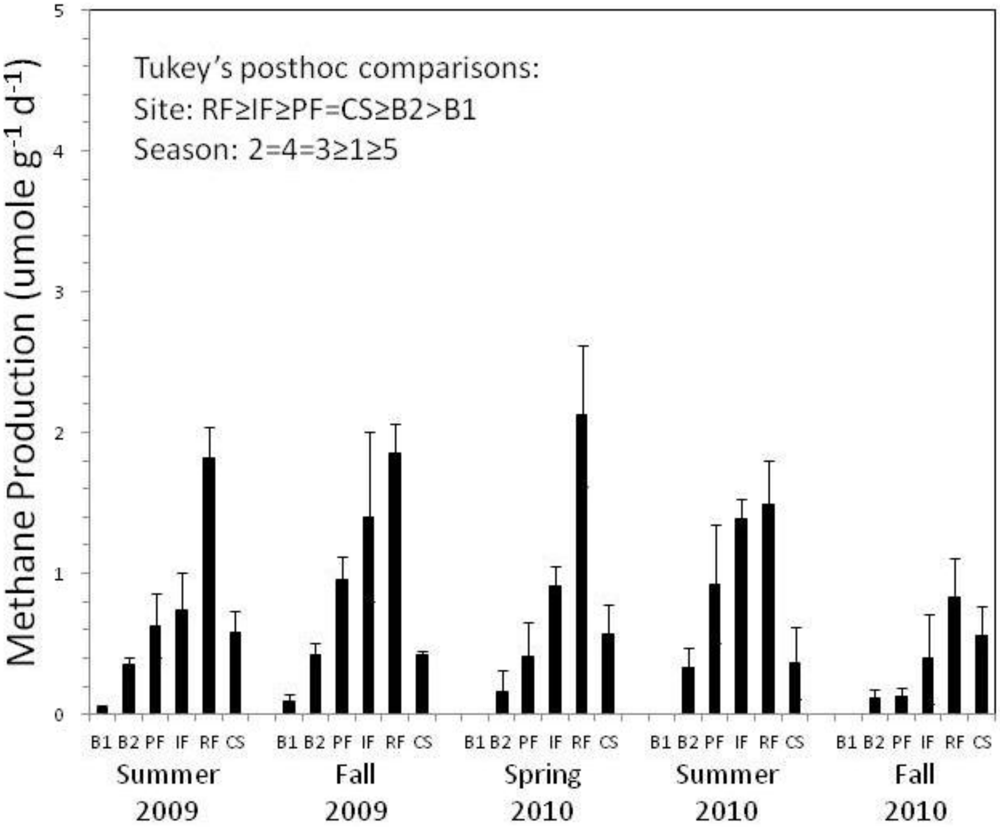
Because we sampled the top 25cm of inundated peat (and thus our sampling tracked the position of the water table), the depth, and thus age, of methanogenic peat varied between sampling events. As a result, the very high rates of hydrogenotrophic methanogenesis in late 2010 are associated with comparatively young peat. At first glance, this appears to clash with prior research, which showed that hydrogenotrophic methanogenesis tends to increase with depth, possibly due to depletion of labile organic carbon and increased dependence on inorganic carbon for substrate (e.g. Chanton *et al.* 2005). However, such work has analyzed the variation of methanogenesis pathways across the depth profile of cores within sampling times; in this research, we never measured pathways at different depths within times, but instead homogenized peat from a 25cm deep sample. Our depth variation is *between* times, and he great increase we found in hydrogenotrophic methanogenesis was not in shallow peat within a static core (which would be a counter-intuitive result), but was, instead, found in relatively shallow peat that was *recently inundated* by water table fluctuations.

This raises the possibility that the redox state of the solid phase of the peat could play some as-yet unclear role in controlling hydrogenotrophic methanogenesis, given recent research that indicates that humic substances can act as TEAs for anaerobic respiration, in portions of the peat column that are periodically submerged and drained (Keller and Takagi 2013, Bridgham *et al.* 2013). While both pathways of methanogenesis tend to be suppressed by anaerobic respiration, as methanogens are outcompeted for substrate (Conrad 1989, Shannon and White 1996), another recent study has found a surprisingly rapid return of methanogenesis in freshly inundated minerotrophic peat, even in the presence of TEAs (Estop-Arogonés *et al.* 2013).

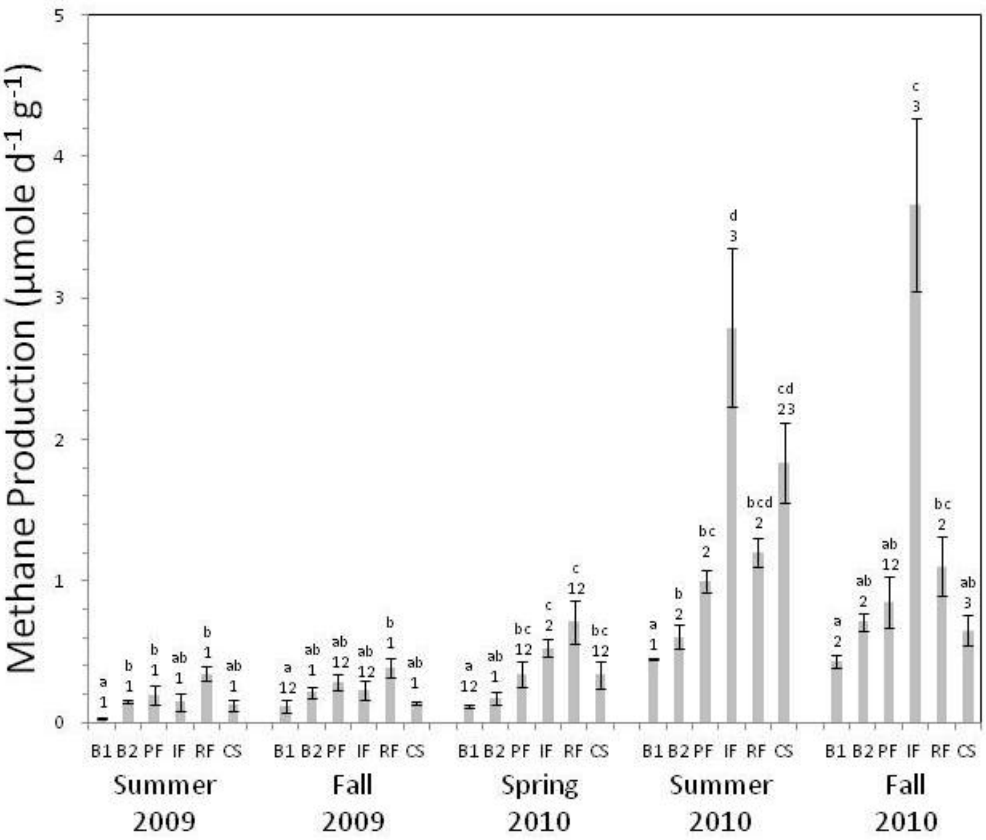
Altogether, these results indicate that attempts to quantify CH_4 production at the landscape and seasonal scale may be incomplete if they do not take into account seasonal fluctuations in water table depth, and attendant shifts in the dynamics of one or both pathways of methanogenesis. Radioisotope labeling of methanogenesis substrates allowed for the quantification of the two pathways, and revealed strikingly different dynamics that would not have been clearly discernible from total CH_4 production alone. Given the significance of CH_4 production in anaerobic carbon cycling, it’s implication for global climate change, and the complex interplay of the two pathways in both time and hydrogeomorphic space, it seems clear that more studies that specifically quantify the contributions of the two pathways in northern peatlands are needed.

APPENDIX

SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure 1. Acetoclastic methanogenesis rates by site and sampling event. Simple Tukey's posthoc comparisons are noted, as there was no significant interaction between site and season.



Supplementary Figure 2. Hydrogenotrophic methanogenesis rates by site and sampling event.

Supplementary Table 1: One-Way ANOVAs, Effect of Site Within Time

		Season				
		2009		**2010**		
Dependent Variable		**Summer**	**Fall**	**Spring**	**Summer**	**Fall**
Anaerobic CO_2 Production	DF	5,24	5,20	5,21	5,21	5,24
	F	5.978	11.391	12.762	3.985	8.943
	P	**0.001**	**<.001**	**<.001**	**0.011**	**<.001**
Total Methanogenesis	DF	5,24	5,20	5,21	5,21	5,24
	F	10.31	7.209	12.253	41.731	13.188
	P	**<.001**	**0.001**	**<.001**	**<.001**	**<.001**
Hydrogenotrophic Methanogenesis	DF	5,24	5,20	5,21	5,21	5,24
	F	6.358	3.461	14.041	68.377	9.47
	P	**0.001**	**0.02**	**<.001**	**<.001**	**<.001**
Proportion Acetoclastic	DF	5,24	5,20	5,21	5,21	5,24
	F	11.118	6.923	6.565	5.052	4.693
	P	**<.001**	**0.001**	**0.001**	**0.003**	**0.004**
CO_2:CH_4 Ratio	DF	5,24	5,20	5,21	5,21	5,24
	F	4.223	3.423	27.466	12.621	1.534
	P	**0.007**	**0.021**	**<.001**	**<.001**	**0.271**

Supplementary Table 2. One-Way ANOVAs, Effect of Time Within Site

		Site					
Dependent Variable		**B1**	**B2**	**PF**	**IF**	**RF**	**CS**
Anaerobic CO_2 Production	DF	4,17	4,17	4,19	4,18	4,20	4,19
	F	2.48	3.22	2.38	7.54	2.34	17.10
	P	0.08	0.04	0.09	0.00	0.09	0.00
Total Methanogenesis	DF	4,17	4,17	4,19	4,18	4,20	4,19
	F	5.19	4.54	1.90	8.10	1.03	6.06
	P	**0.01**	**0.01**	**0.15**	**0.00**	**0.42**	**0.00**
Hydrogenotrophic Methanogenesis	DF	4,17	4,17	4,19	4,18	4,20	4,19
	F	7.50	20.99	2.89	28.24	8.74	17.96
	P	**0.00**	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**
Proportion Acetoclastic	DF	4,17	4,17	4,19	4,18	4,20	4,19
	F	19.17	6.49	11.29	54.05	12.81	8.73
	P	**0.00**	**0.02**	**0.00**	**0.00**	**0.00**	**0.00**
CO_2:CH_4 Ratio	DF	4,17	4,17	4,19	4,18	4,20	4,19
	F	5.14	2.27	0.63	2.47	1.89	0.31
	P	**0.01**	**0.11**	**0.65**	**0.08**	**0.15**	**0.87**

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