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Limited effects of six years of fertilization on carbon mineralization dynamics in a Minnesota fen

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Abstract

Peatlands, including fens, are important ecosystems in the context of the global carbon cycle. Future climate change and other anthropogenic activities are likely to increase nutrient loading in many peatland ecosystems and a better understanding of the effects of these nutrients on peatland carbon cycling is necessary. We investigated the effects of six years of nitrogen and phosphorus fertilization, along with liming, on carbon mineralization dynamics in an intermediate fen in northern Minnesota. Specifically, we measured CO_2 and CH_4 emission from intact peat cores, as well as CH_4 production and CH_4 consumption at multiple depths in short-term laboratory incubations. Despite increased nitrogen and phosphorus availability in the upper 5 cm of peat, increased pH, and clear shifts in the vegetation community, fertilization and liming had limited effects on microbial carbon cycling in this fen. Liming reduced the net flux of CO_2 approximately 3-fold compared to the control treatment, but liming had no effect on CH_4 emissions from intact cores. There were no nutrient effects on CO_2 or CH_4 emissions from intact cores. In all treatments, rates of CH_4 production increased with depth and rates of CH_4 consumption were highest near the in situ water-table level. However, nutrient and liming had no effect on rates of CH_4 production or CH_4 consumption at any depth. Our results suggest that over at least the intermediate term, the microbial communities responsible for soil carbon cycling in this peatland are tolerant to wide ranges of nutrient concentrations and pH levels and may be relatively insensitive to future anthropogenic nutrient stress.

Keywords: Carbon dioxide; Methane; Nitrogen and phosphorus fertilization; Peatlands; pH

1. Introduction

Although they occupy less than 3% of the terrestrial land surface (Bridgham et al., 2001), peatlands are important ecosystems in the context of global climate change as they are currently responsible for approximately 8% of the global methane (CH_4) emissions (Bartlett and Harriss, 1993). Further, an estimated 455 Pg of carbon (Pg = 10^{15} g), approximately one-third of the terrestrial soil carbon pool, is stored in peatlands. These ecosystems have the potential to release this stored carbon as additional carbon dioxide (CO_2) or CH_4, both of which are important greenhouse gases (Gorham, 1995; Bridgham et al., 1995; Moore et al., 1998; Wieder, 2001). As CH_4 has an estimated 26 times the global warming potential of CO_2 (Lelieveld et al., 1993), even small changes in CH_4 emissions could have important consequences for global climate change.

The net CH_4 flux from any ecosystem is the difference between CH_4 production and CH_4 consumption, minus any short-term change in storage. These processes are microbially mediated by methanogens and methanotrophs, respectively, and are controlled by a number of physical and environmental variables, including water-table level, substrate carbon quality, pH, redox state, nutrient availability, and plant community composition, biomass, and productivity. For example, the low pH of peatland ecosystems may limit methanogenesis (Williams and Crawford, 1984; Dunfield et al., 1993; Valentine et al., 1994; Hines and Duddleston, 2001), possibly due to

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the inhibition of hydrogen-producing and -consuming processes at low pH (Goodwin and Zeikus, 1987; Goodwin et al., 1988).

The role of nutrients in controlling CH_4 emissions in peatlands is important because many peatlands, like other natural systems, are currently experiencing increased nutrient (nitrogen and phosphorus) loading due to increased atmospheric deposition, agricultural inputs, and other anthropogenic activities (Vitousek et al., 1997; Richardson and Qian, 1999; Tilman, 1999; Noe et al., 2001; Galloway et al., 2003). Nutrient dynamics in these ecosystems are also likely to change in response to future climate change (Bridgham et al., 1995; Keller et al., 2004). The effects of nutrients on ecosystem CH_4 dynamics are complicated and may take place at biochemical, microbial, and ecosystem levels (Schimel, 2000). At the biochemical level, nitrogen (as NH_4) often inhibits CH_4 consumption (increasing net CH_4 flux) by competing for methane monooxygenase, the enzyme used to oxidize CH_4 (Hanson and Hanson, 1996). Nutrients can also act at a microbial level by directly stimulating or inhibiting methanogens or methanotrophs, with resulting increases or decreases in net CH_4 flux. Finally, nutrients can act at an ecosystem level over longer time scales by changing the dominant vegetation community. Such shifts in vegetation could affect CH_4 dynamics through changes in (1) litter inputs and resultant changes in soil quality (Chapin III et al., 1995; Moore and Dalva, 1997), (2) labile root exudates (Hutchin et al., 1995; Joabsson et al., 1999; Megonigal et al., 1999; Updegraff et al., 2001), or (3) CH_4 and oxygen transport through plant aerenchyma (Schütz et al., 1991; Whiting and Chanton, 1993).

Nitrogen fertilization has resulted in increased CH_4 emissions in peatlands (Aerts and Toet, 1997; Aerts and de Caluwe, 1999; Saarnio and Silvola, 1999), likely as a result of an inhibition of CH_4 consumption. A number of laboratory studies have supported nitrogen inhibition of CH_4 consumption in peatlands (Crill et al., 1994, Kravchenko, 1999a,b, 2002). However, recent work suggests that in some wetlands nitrogen may have the opposite effect and stimulate CH_4 consumption, and thus decrease CH_4 emissions. Bodelier et al. (2000a,b) found that the CH_4 emissions from rice paddy soil were reduced following nitrogen fertilization due to stimulation of methanotrophs. They suggested that high concentrations of CH_4 counterbalanced potential competitive inhibitory effects of nitrogen on methane monooxygenase. Updegraff et al. (2001) also observed a negative relationship between CH_4 flux and pore water NH_4 concentrations in bog and fen plots receiving several heating and water-table treatments. They hypothesized that one likely mechanism for this relationship was direct stimulation of a nitrogen-limited methanotrophic community.

While the role of nitrogen in controlling CH_4 dynamics has received considerable attention, the role of phosphorus has been less studied. Changes in phosphorus availability may affect CH_4 flux in peatlands because plant productivity in these ecosystems is often phosphorus limited (Bridgham et al., 1996; Bedford et al., 1999; Chapin et al., 2004). Phosphorus availability could also directly affect CH_4 flux by stimulating methanogens and/or methanotrophs.

This experiment was designed to examine the effects of six years of nitrogen and phosphorus fertilization and liming on CH_4 and CO_2 emissions in a fen in northern Minnesota. Specifically, we hypothesized that (1) nitrogen fertilization would stimulate CH_4 oxidation and thus decrease CH_4 emissions, (2) phosphorus fertilization would stimulate CH_4 production through increases in primary production and thus increase CH_4 flux, (3) CO_2 flux would increase in response to fertilization, and (4) liming would increase rates of CH_4 and CO_2 emissions by removing the pH limitation on microbial metabolism.

2. Materials and methods

2.1. Study site

Research was conducted at a fen in Alborn township in northeastern Minnesota (47° 00′ 42″ N, 92° 34′ 30″ W), which has been described in detail previously (Malterer et al., 1979; Santelmann, 1991; Bridgham et al., 1998; Chapin et al., 2003, 2004). This intermediate fen has a pH of ~4.9, with low areas (flarks) dominated by the graminoids *Carex exilis* Dewey, *C. livida* (Whal.) Wild., *C. limosa* L., *C. lasiocarpa* Ehrh., *Rhyncospora alba* (L.), and *R. fusca* (L.) Ait. F. The mean annual temperature in this region is 3.16 °C and the mean annual precipitation is 497 mm. Water levels in the fen during the growing season (May–October) range from about −10–+2 cm relative to the peat surface.

2.2. Fertilization treatments

The fertilization treatments utilized in this study are described in detail by Chapin et al. (2003, 2004), and a brief summary is provided below. Twelve, 3-m^2 circular plots were established in flarks (i.e. wet depressions), and 1-mm thick rubber roofing material was buried to of depth of 30–50 cm around the perimeter of the plots to prevent lateral loss of applied nutrients and calcium carbonate. These rings were installed in the winter to prevent unnecessary damage to surface vegetation of the fen. Wooden boardwalks were constructed to minimize further impacts to the plots.

Beginning in 1995, two nitrogen (N) levels (2.0 or 6.0 g N m^{-2} y^{-1} as NH_4Cl) and two phosphorus (P) levels (0.67 or 2.0 g P m^{-2} y^{-1} as a mixture of NaH_2PO_4 and Na_2HPO_4) were combined to produce four different fertilizer treatments: low N-low P ('LN-LP'), high N-low P ('HN-LP'), low N-high P ('LN-HP'), and high N-high P ('HN-HP'). Control (no fertilization) and liming (no fertilization with pH adjusted to ~6.4 with calcium

carbonate) treatment plots were also included in this study. All treatments have been maintained continuously since 1995. During this time, the pH of the liming treatment plots was measured each year and adjusted in late spring to 6.4 as necessary with additional calcium carbonate. Half of each fertilizer treatment was applied in early spring and half was applied in early summer of each year. There were two replicates of each treatment.

2.3. Sample processing and gas measurements

In the summer of 2001 (six years after the initiation of fertilization treatments), duplicate cores were taken from each plot to measure net flux of CO_2 and CH_4. Intact cores were removed to a depth of ~30 cm using 10-cm diameter PVC pipe. A sharp, serrated knife was used to guide the PVC pipe into the peat and avoid compression and damage to vegetation. Upon extraction, the PVC core (containing the intact peat section) was capped on the bottom.

In the laboratory, the water level in the PVC cores was adjusted to −7 cm below the peat surface (the average measured in situ water-table level) using pore water collected from the fen, and allowed to equilibrate in the dark at 15 °C for 2 h. Subsequently, the vegetation was gently folded inside of the headspace volume, and the PVC cores were capped and allowed to equilibrate for 1 additional hour. The cores were incubated in the dark at 15 °C (a typical summer soil temperature in this system), for 7 h. At 4 time points over this incubation, headspace volumes from the intact cores were sampled and analyzed for CO_2 and CH_4 using gas chromatographs equipped with a thermal conductivity detector and a flame ionization detector for CO_2 and CH_4, respectively. CO_2 accumulation was linear ($r^2 > 0.90$) in all cores. CH_4 accumulation was linear in all but 3 cores (13% of the total number of cores), which were excluded from subsequent analysis.

After net flux was determined, the PVC cores were transferred to a glove box with an anaerobic N_2 headspace. Cores were drained by gravity for approximately 5 min, the surface vegetation was removed, and the peat was sectioned into 5-cm increments: 0–5 ('2.5 cm'), 7.5–12.5 ('10 cm'), and 17.5–22.5-cm ('20 cm') depth increments were used for subsequent analysis of CH_4 dynamics.

Subsamples of each depth section (45.6 ± 2.1 g wet peat mass; mean ±1 SE) used to measure CH_4 production were placed in Mason jars in the glove box, and the headspace was vacuumed and filled with N_2 once more. The samples were capped and allowed to equilibrate for approximately 1 h at 15 °C, and then CH_4 production was measured several times over 5 h using gas chromatography. We defined CH_4 production as zero in cases where the maximum change in CH_4 concentrations over the incubation was <1.7 ppm CH_4, and excluded samples which did not show linear rates of CH_4 production (<3% of the total number of samples).

Additional subsamples from each depth section were used to measure aerobic CH_4 consumption. Thin slices from each depth (15.2 ± 0.6 g wet peat mass; mean ±1 SE) were placed in Mason jars and allowed to equilibrate with the ambient atmosphere at 15 °C for 6–10 h. Subsequently, the Mason jars were capped and the headspace CH_4 concentration was adjusted to 10,000 ppm. CH_4 consumption from the headspace was monitored at several time points over 7 h using gas chromatography. For CH_4 consumption, we defined the rate of consumption as zero in cases when the maximum change in CH_4 concentration was <10 ppm (i.e. a change of <0.1% over 7 h). Samples that exhibited non-linear rates of CH_4 consumption were excluded from analyses. In the 2.5 and 10-cm depth intervals, only 2 samples exhibited non-linear CH_4 consumption. However, the rate of CH_4 consumption was non-linear in half of the samples from the 20-cm depth interval. Statistical analyses of CH_4 consumption were run without data from the 20-cm depth, and the overall pattern of results did not change. Thus, we include the rates of consumption from the 20-cm depth interval in our discussion, but acknowledge that values from this depth should be interpreted with caution.

Dissolved CH_4 was calculated using Henry's Law, adjusting for solubility and temperature (Stumm and Morgan, 1995), in all depth-specific measurements of CH_4 production and consumption. The net flux measurements are expressed as the accumulation of gas in the headspace per volume of peat.

We utilized short incubation times (h) in this experiment to approximate rates of in situ microbial processes (i.e. we did not allow time for turnover of the microbial communities). A potentially important trade off of this approach was an increased sensitivity to variability in our measurements, especially considering the low number of replicates (discussed below). Thus, smaller effects of nutrient and liming treatments may have been missed by the variation encountered in our analysis. Additionally, potential sampling artifacts (e.g. disturbance from coring) may have been amplified by short incubation times. However, care was taken to maintain anaerobic conditions during sampling and processing to limit sampling effects on anaerobic microbial communities. Further, the generally linear rates of CH_4 and CO_2 flux from intact cores as well as CH_4 production and oxidation suggest that sampling artifacts may not have been dramatic in this experiment.

Nutrient availability was determined at all depths using 2 M KCl and acid fluoride extractions for nitrogen (as NH_4^+ and NO_3^-) and phosphorus, respectively (Kuo, 1996; Mulvaney, 1996). Extracts were analyzed for PO_4^{3-}, NO_3^- and NH_4^+ by standard spectrophotometric methods with a Lachat Quickchem 8000 autoanalyzer (Hach Corporation, Loveland, CO). Available NO_3^- and NH_4^+ were summed to calculate available nitrogen. Available NH_4^+ accounted for at least 91% (on average 98%) of the available nitrogen. Total nitrogen and phosphorus concentrations were determined using root-free peat from all depth increments. Peat samples were dried at 60 °C for 48 h and then ground to

a fine powder. Phosphorus content was determined by digestion with concentrated H_2SO_4 and 30% H_2O_2 (Allen, 1989) followed by spectrophotometric analysis on a Lachat Quickchem 8000 autoanalyzer. Nitrogen content was determined using a Costech 4010 Elemental Combustion System.

Living vegetation was clipped at the peat surface from each of the intact PVC cores following net flux measurements (prior to sectioning cores by depth). Belowground root biomass was removed by hand from an additional subsection of each of the depth increments. Aboveground and belowground biomass samples were oven dried at 60 °C to constant mass and expressed per surface area of the PVC cores (assuming a homogeneous distribution of belowground biomass over each 5-cm depth increment).

2.4. Statistical analyses

The net flux of CO_2 and CH_4 were analyzed for fixed effects of treatment in a one-way ANOVA with the duplicate cores nested within plots as a random effect (i.e. the plot was the true replicate unit and there were two replicates of each treatment) with the GLM procedure (SAS Institute, 1999). In cases where there were significant main effects in the ANOVA ($P<0.05$), we used Fisher's LSD to examine differences among individual treatments.

Treatment effects on depth-specific nutrient availability, CH_4 production and CH_4 consumption were analyzed using a repeated measure ANOVA (with depth as the repeated variable) using the MIXED procedure (SAS Institute, 1999). Individual cores were again nested within plots. Differences among depths were tested with the LSMEANS procedure. In cases where there was a significant depth×treatment interaction, soil chemistry variables at each depth were analyzed for fixed effects of treatment in a one-way, nested ANOVA using the GLM procedure (SAS Institute, 1999) and treatment differences were analyzed using Fisher's LSD. Prior to statistical analysis, all data were tested for normality and log-transformed where appropriate.

The treatments presented in this paper were initiated as part of a larger experiment examining the interactive roles of nutrient availability and pH/alkalinity in structuring peatland communities and nutrient mineralization dynamics (Chapin et al., 2003, 2004); thus a greater number of replicates were not logistically possible. While the number of individual replicates was only two, the nested sampling design utilized in this experiment gave reasonable power to detect overall treatment effects. For example, the one-way ANOVAs had 5 degrees of freedom (df) for treatment, 6 df for the effect of plot nested with treatment, and 12 error df. Although the statistical power was more limited in making pairwise comparisons among individual treatments, there was a lack of overall treatment effects on carbon mineralization in this experiment (see Results and Discussion). Thus, while we present pairwise comparisons where appropriate, we emphasize overall ANOVA results.

Regression between aboveground and belowground biomass and net flux of CO_2 and CH_4 were performed using the REG procedure (SAS Institute, 1999). Multiple, stepwise regressions were also performed of CH_4 production and CH_4 consumption rates from all depths against soil chemistry variables and aboveground and belowground biomass.

3. Results

Six years of fertilization with nitrogen and phosphorus generally increased nutrient availability, but only at the 2.5-cm depth interval (treatment×depth interaction $P<0.0002$, Table 1). Within the 2.5-cm depth increment, fertilization at the high N level always increased available nitrogen compared to other treatments (Table 1). Fertilization with phosphorus increased phosphorus availability by at least 10-fold compared to the control treatment within the 2.5-cm depth, but this difference was only significant in the high N-high P and high N-low P treatments (Table 1). Nitrogen and phosphorus availability were highest at the 2.5-cm depth, and there were no differences in nutrient availability between treatments at the 10-cm and 20-cm depth intervals (Table 1).

Total nitrogen did not differ among fertilization treatments at any depth, but fertilization tended to increase the total phosphorus content of peat at the 2.5 and 10-cm depth increments, although this increase was not always statistically significant (Table 1). Liming decreased total nitrogen and phosphorus concentrations at the 2.5 and 10-cm depths (Table 1).

Six years of liming significantly increased the pH at all depth levels to near the desired pH of 6.4 (Table 1). Across all fertilization treatments, the pH was lowest at the 2.5-cm depth interval. Fertilization with the high N-high P treatment lowered the pH compared to the control treatment in the 2.5 and 10-cm depth intervals (Table 1).

Despite increases in nutrient availability and total nutrient content following fertilization and increases in pH in response to liming, there were no treatment effects on CH_4 flux from intact cores ($P=0.52$, Fig. 1). Liming decreased the flux of CO_2 from intact peat cores approximately 3-fold compared to the unlimed control treatment ($P=0.01$), but the net flux of CO_2 did not differ among other treatments (Fig. 1). The net flux of CO_2 was significantly related to standing aboveground biomass ($P=0.02$, $r^2=0.23$), but CH_4 emissions were independent of aboveground biomass ($P=0.95$).

CH_4 production was highest at the 20-cm depth interval ($P<0.0001$), while CH_4 oxidation was highest at the 10-cm

Table 1
Mean (±1 SE) pH, available nitrogen (mg N g dry peat^{-1}), total nitrogen (mg N g dry peat^{-1}), available phosphorus (mg P g dry peat^{-1}), and total phosphorus (mg P g dry peat^{-1}) in fen peat following 6 years of fertilization with low N-low P (LN-LP), high N-low P (HN-LP), low N-high P (LN-HP), and high N-high P (HN-HP) treatments

	Control	LN-LP	HN-LP	LN-HP	HN-HP	LIME
pH						
2.5 cm	4.35±0.07^{a}	4.17±0.24^{ab}	3.95±0.27^{ab}	4.22±0.08^{ab}	3.77±0.08^{b}	6.82±0.19^{c}
10 cm	4.76±0.06^{a}	4.56±0.09^{a}	4.36±0.13^{ab}	4.67±0.06^{a}	4.12±0.22^{b}	6.53±0.15^{c}
20 cm	4.70±0.04^{a}	4.76±0.06^{a}	4.71±0.11^{a}	4.75±0.05^{a}	4.56±0.09^{a}	6.14±0.13^{b}
Available N						
2.5 cm	0.15±0.05^{a}	0.10±0.02^{a}	0.41±0.13^{b}	0.11±0.04^{a}	0.36±0.09^{b}	0.08±0.01^{a}
10 cm	0.07±0.02	0.04±0.01	0.12±0.05	0.03±0.00	0.09±0.04	0.06±0.02
20 cm	0.03±0.00	0.08±0.04	0.04±0.01	0.04±0.01	0.03±0.00	0.06±0.02
Total N						
2.5 cm	22.3±0.7^{a}	21.9±0.6^{a}	20.0±0.8^{a}	21.9±1.0^{a}	21.8±0.9^{a}	10.7±1.5^{b}
10 cm	23.7±1.1^{a}	24.1±0.5^{a}	24.1±1.7^{a}	24.0±0.8^{a}	22.2±1.0^{a}	15.0±0.8^{b}
20 cm	31.2±1.0	31.1±0.7	28.3±3.1	31.8±0.6	28.9±2.3	29.3±1.4
Available P						
2.5 cm	0.007±0.001^{a}	0.078±0.026^{ab}	0.130±0.040 ^{b}	0.089±0.036^{ab}	0.241±0.037^{c}	0.000±0.000^{a}
10 cm	0.005±0.001	0.030±0.012	0.024±0.008	0.020±0.018	0.101±0.047	0.009±0.004
20 cm	0.024±0.010	0.014±0.005	0.018±0.004	0.022±0.015	0.017±0.010	0.016±0.007
Total P						
2.5 cm	0.89±0.18^{a}	1.29±0.09^{a}	1.17±0.21^{a}	1.20±0.29^{a}	1.78±0.15^{b}	0.31±0.03^{c}
10 cm	0.69±0.05^{a}	0.76±0.04^{ab}	0.91±0.11^{b}	0.75±0.07^{ab}	0.80±0.06^{ab}	0.45±0.01^{c}
20 cm	0.75±0.06	0.72±0.03	0.61±0.04	0.73±0.02	0.76±0.07	0.64±0.02

Control (no fertilization) and liming (LIME) treatments are also included. Within each depth increment, means with the same letter are not significantly different (Fisher's LSD, $P>0.05$). Variables within a depth which are not followed by letters did not differ among treatments (GLM procedure, $P>0.05$).

depth interval ($P=0.01$, Fig. 2). However, there were no treatment effects on either CH_4 production or consumption at any depth ($P=0.71$ and $P=0.60$, respectively). Across all depths, total nitrogen concentration, pH, and aboveground biomass explained 62% in the variation of CH_4 production rates (Table 2). The rates of CH_4 consumption were less related to vegetation and soil chemistry variables, with available phosphorus concentration explaining only 5% of the total variation in rate measurements (and this relationship was not significant, $P=0.11$; Table 2). Regressions at individual depths did not improve their predictive power.

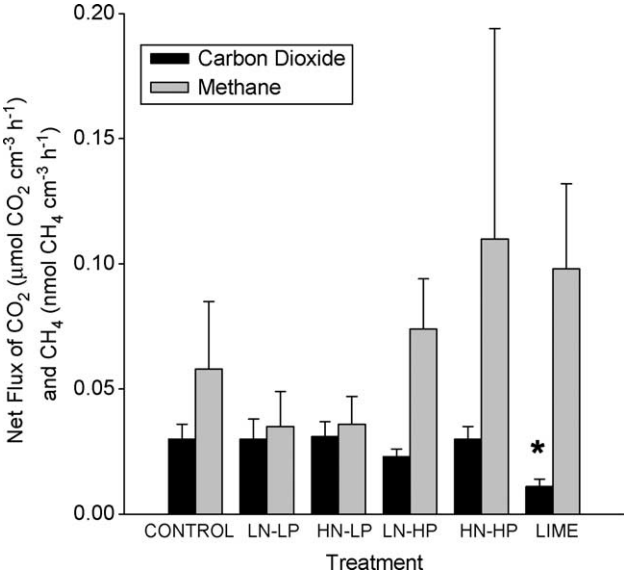

Fig. 1. Mean (±1 SE) net flux of CO_2 and CH_4 from intact fen cores. The asterisk indicates a significant decrease in net flux of CO_2 in the liming treatment ($P=0.01$). Note the different units for CO_2 and CH_4 flux. Treatments are as in Table 1.

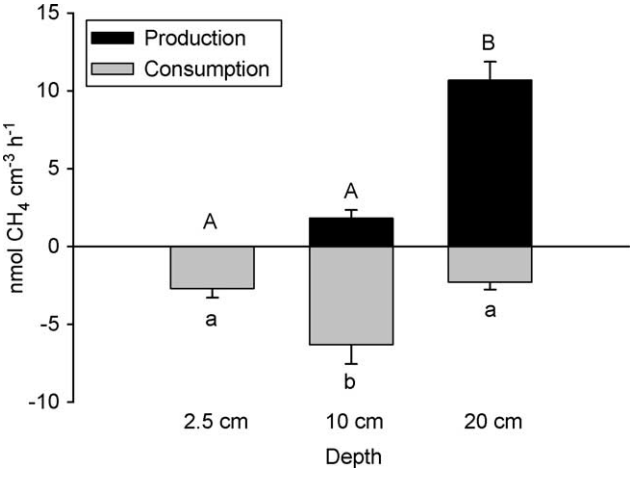

Fig. 2. Mean (±1 SE) production (as positive values) and consumption (as negative values) of CH_4 by fen peat at different depth intervals. Values represent the mean of all treatments at each depth increment. For each rate, means with the same letter are not significantly different (LSMEANS, $P>0.05$).

Table 2
Summary of significant ($\alpha < 0.10$) stepwise multiple regressions of CH_4 production (nmol CH_4 cm^{-3} h^{-1}) and CH_4 consumption (nmol CH_4 cm^{-3} h^{-1}) at all depth intervals against soil chemistry variables and aboveground ('Above') and belowground biomass

Step	Variable Entered	Partial r^2	Model r^2	Model df	Significance
CH_4 production					
1	Total N	0.46	0.46	64	<0.0001
2	pH	0.15	0.61		<0.0001
3	Above	0.01	0.62		0.16
CH_4 consumption					
1	Available P	0.05	0.05	53	0.11

Soil chemistry variables are as in Table 1.

4. Discussion

The microbial communities responsible for carbon cycling in this intermediate fen were allowed to develop in response to six years of continuous nitrogen and phosphorus fertilization and liming treatments. We hypothesized a priori that potential shifts in microbial community structure would have been dramatic in response to these multiple-year treatments. Thus, we utilized short incubation times in the laboratory to approximate in situ rates of carbon cycling, without allowing for microbial community turnover. Rates of CH_4 oxidation are often calculated using comparable incubation times (over the course of hours, e.g. Whalen and Reeburgh, 1996; Whalen, 2000). Similarly, rates of CH_4 production and efflux are often calculated over short sampling times (e.g. Valentine et al., 1994; Aerts and Toet, 1997; Aerts and de Caluwe, 1999; Saarnio and Silvola, 1999), although extended preincubation times (weeks) are common in such experiments to allow microbial communities to develop in response to laboratory treatments (i.e. nutrient amendments).

The overall paucity of effects of six years of fertilization and liming on CO_2 and CH_4 emissions, CH_4 production, and CH_4 consumption does not support our initial hypotheses and contrasts the findings of other studies in peatland ecosystems (Dunfield et al., 1993; Valentine et al., 1994; Aerts and Toet, 1997; Aerts and de Caluwe, 1999; Saarnio and Silvola, 1999). However, others have found that the effects of fertilization on annual CH_4 flux may be minor (Saarnio et al., 2000) and that CH_4 production and consumption are unaffected by nitrogen fertilization (Bridgham and Richardson, 1992; Saarnio and Silvola, 1999). Nykänen et al. (2002) also found no difference in CH_4 production or consumption potentials in a *Sphagnum fuscum* dominated peatland following nitrogen fertilization, although they did see an increase in CH_4 emissions which was driven by an increase in sedge cover. As carbon and nutrient dynamics differ across the ombrotrophic-minerotrophic peatland gradient (Bridgham et al., 1996, 1998; Chapin et al., 2003), it is possible that different peatland types may respond differently to similar fertilization and liming regimes, although these potential patterns have not yet been well studied.

CH_4 production was highest at the 20-cm depth interval ($P < 0.0001$, Fig. 2) where continuous anaerobic conditions are likely to favor populations of anaerobic methanogens. In contrast, CH_4 consumption was highest at the 10-cm depth interval, near the in situ water-table level ($P = 0.01$, Fig. 2). Conditions for the growth of methanotrophs are likely to be ideal near the water-table level where both CH_4 and oxygen are available. These patterns of vertical distribution of CH_4 production and consumption are consistent with other peatland studies (Krumholz et al., 1995; Edwards et al., 1998; Bellisario et al., 1999; Kettunen et al., 1999). Our treatments did not have significant effects on either CH_4 production or consumption at any depth increment, although minor treatment effects may have been missed by our short incubation times.

In this intermediate fen, fertilization and liming treatments have had dramatic effects on the plant community. Following three years of fertilization treatments, aboveground net primary production was stimulated by phosphorus addition, with this effect driven by the response of graminoid species (Chapin et al., 2004). Additionally, fertilization appears to lead to a shift in the dominant plant community structure on longer time scales. After six years of nutrient treatments, there was an increase in the overall percent cover of shrubs in all fertilization treatments, although this increase was not significant in the HN-HP fertilization treatment. Additionally, there was an increase in the cover of bryophytes in the LN fertilization treatments (Iversen and Bridgham, unpublished data).

These changes in plant community productivity and composition have not translated to differences in CH_4 dynamics between fertilization and liming treatments. CH_4 emissions were independent of aboveground biomass, and the rates of CH_4 production and consumption of CH_4 were at best poorly correlated to aboveground and belowground biomass (Table 2).

However, the net flux of CO_2 was at least 60-times greater than the net flux of CH_4 from intact peat cores (Fig. 1), suggesting that CO_2 was the dominant end product of carbon mineralization. This high CO_2–C to CH_4–C ratio is typical of other peatlands where fermentation is thought to dominate anaerobic carbon cycling (Bridgham et al., 1998; Vile et al., 2003; Keller et al., 2004). Further, processes in the aerobic portion of the intact peat cores (e.g. aerobic CH_4 oxidation) likely also contribute to this high CO_2–C to CH_4–C ratio. Although shifts in the vegetation community in response to fertilization treatments did not lead directly to concomitant treatment effects on CO_2 emissions from intact peat cores, the net flux of CO_2 was significantly related to standing aboveground biomass ($P = 0.02$, $r^2 = 0.23$). This relationship further demonstrates the potential importance of long-term, ecosystem-level effects of fertilization on carbon mineralization dynamics in peatlands.

The decrease in net flux of CO_2 following six years of liming (Fig. 1) suggests that the overall microbial

community was well adapted to the low pH of the site. This result, in addition to the lack of pH effects on CH_4 dynamics, is in contrast to previous work that suggests microbial metabolism is limited by the low pH of peatland ecosystems (Williams and Crawford, 1984; Goodwin and Zeikus, 1987; Goodwin et al., 1988; Dunfield et al., 1993; Valentine et al., 1994). However, Bridgham and Richardson (1992) found that increases in pH reduced CO_2 production under aerobic conditions in a North Carolina peatland. Liming also reduced available and total nitrogen and phosphorus compared to the control treatment in surface peat (although these differences were not always significantly different, Table 1). Further, the increased pH of the liming treatment may have altered the availability of a number of other trace nutrients (Lucas and Davis, 1961), which could have influenced the overall microbial community.

To conclude, six years of fertilization (with nitrogen and phosphorus) and liming increased the nutrient availability and pH, respectively, in this intermediate fen. However, despite these increases and subsequent shifts in the dominant plant community, there were no major effects on the soil carbon dynamics of this ecosystem in response to these fertilization treatments. Our results suggest that the microbial communities responsible for CH_4 cycling in this fen ecosystem are tolerant to wide ranges of nutrient concentrations and pH levels. If this is the case, future anthropogenic nutrient stress is not likely to directly alter the importance of these ecosystems in the context of the global carbon cycle in at least the intermediate term. Over longer periods of time, it is possible that changes in vegetation community structure and productivity will translate into changes in the quality and/or quantity of available soil carbon, which may subsequently affect respiratory CH_4 and CO_2 fluxes from fens.

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References

Aerts, R., de Caluwe, H., 1999. Nitrogen deposition effects on carbon dioxide and methane emissions from temperate peatland soils. Oikos 84, 44–54.

Aerts, R., Toet, S., 1997. Nutritional controls on carbon dioxide and methane emission from *Carex*-dominated peat soils. Soil Biology & Biochemistry 29, 1683–1690.

Allen, S.E., 1989. Chemical analyses of ecological materials. Blackwell Scientific Publications, Oxford pp. 565.

Bartlett, K.B., Harriss, R.C., 1993. Review and assessment of methane emissions from wetlands. Chemosphere 26, 261–320.

Bedford, B.L., Walbridge, M.R., Aldous, A., 1999. Patterns in nutrient availability and plant diversity of temperate North American wetlands. Ecology 80, 2151–2169.

Bellisario, L.M., Bubier, J.L., Moore, T.R., Chanton, J.P., 1999. Controls on CH_4 emissions from a northern peatland. Global Biogeochemical Cycles 13, 81–91.

Bodelier, P.L.E., Hahn, A.P., Arth, I.R., Frenzel, P., 2000a. Effects of ammonium-based fertilisation on microbial processes involved in methane emission from soils planted with rice. Biogeochemistry 51, 225–257.

Bodelier, P.L.E., Roslev, P., Henekel, T., Frenzel, P., 2000b. Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. Nature 403, 421–424.

Bridgham, S.D., Richardson, C.J., 1992. Mechanisms controlling soil respiration (CO_2 and CH_4) in southern peatlands. Soil Biology & Biochemistry 24, 1089–1099.

Bridgham, S.D., Johnston, C.A., Pastor, J., Updegraff, K., 1995. Potential feedbacks of northern wetlands on climate change. BioScience 45, 262–274.

Bridgham, S.D., Pastor, J., Janssens, J.A., Chapin, C., Malterer, T.J., 1996. Multiple limiting gradients in peatlands: a call for a new paradigm. Wetlands 16, 45–65.

Bridgham, S.D., Updegraff, K., Pastor, J., 1998. Carbon, nitrogen, and phosphorus mineralization in northern wetlands. Ecology 79, 1545–1561.

Bridgham, S.D., Ping, C.-L., Richardson, J.L., Updegraff, K., 2001. Soils of northern peatlands: histosols and gelisols, in: Richardson, J.L., Vepraskas, M.J. (Eds.), Wetland soils. Lewis Publishers, Boca Raton, FL, pp. 343–370.

Chapin III., F.S., Shaver, G.R., Giblin, A.E., Nadelhoffer, K.J., Laundre, J.A., 1995. Responses of arctic tundra to experimental and observed changes in climate. Ecology 76, 694–711.

Chapin, C.T., Bridgham, S.D., Pastor, J., Updegraff, K., 2003. Nitrogen, phosphorus, and carbon mineralization in response to nutrient and lime additions in peatlands. Soil Science 168, 409–420.

Chapin, C.T., Bridgham, S.D., Pastor, J., 2004. pH and nutrient effects on above-ground net primary production in a Minnesota, USA bog and fen. Wetlands 24, 186–201.

Crill, P.M., Martikainen, P.J., Nykänen, H., Silvola, J., 1994. Temperature and N-fertilization effects on methane oxidation in a drained peatland soil. Soil Biology & Biochemistry 26, 1331–1339.

Dunfield, P., Knowles, R., Dumont, R., Moore, T.R., 1993. Methane production and consumption in temperate and sub-arctic peat soils—response to temperature and pH. Soil Biology & Biochemistry 25, 321–326.

Edwards, C., Hales, B.A., Hall, G.H., McDonald, I.R., Murrell, J.C., Pickup, R., Ritchie, D.A., Saunders, J.R., Simon, B.M., Upton, M., 1998. Microbiological processes in the terrestrial carbon cycling: methane cycling in peat. Atmospheric Environment 32, 3247–3255.

Galloway, J.N., Aber, J.D., Erisman, J.W., Seitzinger, S.P., Howarth, R.W., Cowling, E.B., Cosby, B.J., 2003. The nitrogen cascade. BioScience 53, 341–356.

Goodwin, S., Zeikus, J.G., 1987. Ecophysical adaptations of anaerobic-bacteria to low pH - analysis of anaerobic digestion in acidic bog sediments. Applied and Environmental Microbiology 53, 57–64.

Goodwin, S., Conrad, R., Zeikus, J.G., 1988. Influence of pH on microbial hydrogen metabolism in diverse sedimentary ecosystems. Applied and Environmental Microbiology 54, 590–593.

Gorham, E., 1995. The biogeochemistry of northern peatlands and its possible responses to global warming, in: Woodwell, G.M., Mackenzie, F.T. (Eds.), Biotic feedbacks in the global climatic system. Oxford University Press, Oxford, UK, pp. 169–187.

Hanson, R.S., Hanson, T.E., 1996. Methanotrophic bacteria. Microbiological Reviews 60, 439–471.

Hines, M.E., Duddleston, K.N., Kiene, R.P., 2001. Carbon flow to acetate and C_1 compounds in northern wetlands. Geophysical Research Letters 28, 4251–4254.

Hutchin, P.R., Press, M.C., Lee, J.A., Ashenden, T.W., 1995. Elevated concentrations of CO_2 may double methane emissions from mires. Global Change Biology 1, 125–128.

Joabsson, A., Christensen, T.R., Wallén, B., 1999. Vascular plant controls on methane emissions from northern peatforming wetlands. Trends in Ecology and Evolution 14, 385–388.

Keller, J.K., White, J.R., Bridgham, S.D., Pastor, J., 2004. Climate change effects on carbon and nitrogen mineralization in peatlands through changes in soil quality. Global Change Biology 10, 1053–1064.

Kettunen, A., Kaitala, V., Lehtinen, A., Lohila, A., Alm, J., Silvola, J., Martikainen, P.J., 1999. Methane production and oxidation potentials in relation to water table fluctuations in two boreal mires. Soil Biology & Biochemistry 31, 1741–1749.

Kravchenko, I.K., 1999a. The inhibiting effect of ammonium on the activity of the methanotrophic microbial community of a raised Sphagnum peatland in West Siberia. Microbiology 68, 203–208.

Kravchenko, I.K., 1999b. Influence of nitrogen compounds on methane oxidation in a raised Sphagnum bog in West Siberia. Microbiology 68, 209–213.

Kravchenko, I.K., 2002. Methane oxidation in boreal peat soils treated with various nitrogen compounds. Plant and Soil 242, 157–162.

Krumholz, L.R., Hollenback, J.L., Roskes, S.J., Ringelberg, D.B., 1995. Methanogenesis and methanotrophy within a Sphagnum peatland. FEMS Microbial Ecology 18, 215–224.

Kuo, S., 1996. Phosphorus, in: Sparks, D.L. (Ed.), Methods of Soil Analysis: Part 3. Chemical Methods, 3. Soil Science Society of America, Inc, Madison, WI, pp. 869–920.

Lelieveld, J., Crutzen, P.J., Brühl, C., 1993. Climate effects of atmospheric methane. Chemosphere 26, 739–768.

Lucas, R.E., Davis, J.F., 1961. Relationships between pH values of organic soils and availabilities of 12 plant nutrients. Soil Science 92, 177–182.

Malterer, T.J., Olson, D.J., Mellem, D.R., Leuelling, B., Torne, E.J., 1979. Sphagnum moss peat deposits in Minnesota. Minnesota Department of Natural Resources 1979;.

Megonigal, J.P., Whalen, S.C., Tissue, D.T., Bovard, B.D., Albert, D.B., Allen, A.S., 1999. A plant-soil-atmosphere microcosm for tracing radiocarbon from photosynthesis through methanogenesis. Soil Science Society of America Journal 63, 665–671.

Moore, T.R., Dalva, M., 1997. Methane and carbon dioxide exchange potentials of peat soils in aerobic and anaerobic laboratory incubations. Soil Biology & Biochemistry 29, 1157–1164.

Moore, T.R., Roulet, N.T., Waddington, J.M., 1998. Uncertainty in predicting the effect of climatic change on the carbon cycling of Canadian peatlands. Climatic Change 40, 229–245.

Mulvaney, R.L., 1996. Nitrogen–inorganic forms, in: Sparks, D.L. (Ed.), Methods of Soil Analysis: Part 3. Chemical Methods. Soil Science Society of America, Inc, Madison, WI, pp. 1123–1184.

Noe, G.B., Childers, D.L., Jones, R.D., 2001. Phosphorus biogeochemistry and the impact of phosphorus enrichment: Why is the everglades so unique?. Ecosystems 4, 603–624.

Nykänen, H., Vasander, H., Huttunen, J.T., Martikainen, P.J., 2002. Effect of experimental nitrogen load on methane and nitrous oxide fluxes on ombrotrophic boreal peatland. Plant and Soil 242, 147–155.

Richardson, C.J., Qian, S.S., 1999. Long-term phosphorus assimilation capacity in freshwater wetlands: a new paradigm for sustaining ecosystem structure and function. Environmental Science and Technology 33, 1545–1551.

Saarnio, S., Silvola, J., 1999. Effects of increased CO_2 and N on CH_4 efflux from a boreal mire: a growth chamber experiment. Oecologia 119, 349–356.

Saarnio, S., Saarinen, T., Vasander, H., Silvola, J., 2000. A moderate increase in the annual CH_4 efflux by raised CO_2 or NH_4NO_3 supply in a boreal oligotrophic mire. Global Change Biology 6, 137–144.

Santelmann, M.V., 1991. Influences on the distribution of *Carex exilis*: an experimental approach. Ecology 72, 2025–2037.

SAS Institute Inc., 1999. SAS OnlineDoc®, Version 8, Cary, NC.

Schimel, J., 2000. Rice, microbes and methane. Nature 403, 376–377.

Schütz, H., Schröder, P., Rennenberg, H., 1991. Role of plants in regulating the methane flux to the atmosphere, in: Sharkey, T.D., Holland, E.A., Mooney, H.A. (Eds.), Trace gas emissions by plants. Academic Press, Inc, San Diego, CA, pp. 29–64.

Stumm, W., Morgan, J.J., 1995. Aquatic chemistry: chemical equilibria and rates in natural waters, Third Ed. Wiley, New York, NY pp. 1022.

Tilman, D., 1999. Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proceeding of the National Academy of Sciences 96, 5995–6000.

Updegraff, K., Bridgham, S.D., Pastor, J., Weishampel, P., Harth, C., 2001. Response of CO_2 and CH_4 emissions from peatlands to warming and water table manipulation. Ecological Applications 11, 311–326.

Valentine, D.W., Holland, E.A., Schimel, D.S., 1994. Ecosystem and physiological controls over methane production in northern wetlands. Journal of Geophysical Research 99, 1563–1571.

Vile, M.A., Bridgham, S.D., Wieder, R.K., Novák, M., 2003. Atmospheric sulfur deposition alters pathways of gaseous carbon production in peatlands. Global Biogeochemical Cycles 17, 1058–1064.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alterations of the global nitrogen cycle: sources and consequences. Ecological Applications 7, 737–750.

Whalen, S.C., 2000. Influence of N and non-N salts on atmospheric methane oxidation by upland boreal forest and tundra soils. Biological Fertility of Soils 31, 279–287.

Whalen, S.C., Reeburgh, W.S., 1996. Moisture and temperature sensitivity of CH_4 oxidation in boreal soils. Soil Biology & Biochemistry 28, 1271–1281.

Whiting, G.J., Chanton, J.P., 1993. Primary production control of methane emissions from wetlands. Nature 364, 794–795.

Wieder, R.K., 2001. Past, present, and future peatland carbon balance: an empirical model based on ^{210}Pb-dated cores. Ecological Applications 11, 327–342.

Williams, R.T., Crawford, R.L., 1984. Methane production in Minnesota peatlands. Applied and Environmental Microbiology 47, 1266–1271.