Biogeochemistry **52:** 133–153, 2001.

Influence of Ni, Co, Fe, and Na additions on methane production in *Sphagnum*-dominated Northern American peatlands

NATHAN BASILIKO & JOSEPH B. YAVITT*
*Department of Natural Resources, Fernow Hall, Cornell University, Ithaca, NY 14853, U.S.A. (*author for correspondence, e-mail: jby1@cornell.edu)*

Key words: anaerobic respiration, bog, chelation, methanogenesis, peat soil, wetland

Abstract. Although *Sphagnum* (moss)-dominated, northern peatland ecosystems harbor methane (CH_4)-producing microorganisms (methanogens) and are a significant source of atmospheric CH_4, rates of CH_4 production vary widely among different systems. Very little work has been done to examine whether concentrations of cations and metal elements may account for the variability. We examined rates of CH_4 production in peat from five geographically and functionally disparate *Sphagnum*-dominated peatlands by incubating peat samples *in vitro* with and without additions of trace metals (Fe, Ni, Co) and base cations (Ca, Li, Na). In peat from the most mineral poor sites, the addition of metals and Na enhanced CH_4 production beyond that observed in controls. The same treatments in mineral rich sites yielded no effect or an inhibition of CH_4 production. None of the treatments affected anaerobic respiration, measured as CO_2 production, in the *in vitro* incubations of peat, except added citrate, suggesting that methanogens, and not the entire anaerobic community, can be limited by the availability of metal elements and cations.

Introduction

Although *Sphagnum* (moss)-dominated, northern peatlands harbor CH_4-producing microorganisms (methanogens) that emit CH_4 into the atmosphere (Cicerone & Oremland 1988), results of research have shown very high variation in rates of CH_4 production and CH_4 emission among different systems (Bartlett & Harriss 1993; Segers 1998; and references cited therein). Differences in climate (temperature, precipitation, etc.) account for some, but not all of the variability, leading researchers to consider other factors. These controlling factors include pH, energy (carbon quality), competition with other anaerobic processes (e.g., sulfate reduction), degree of anoxia, and chemical composition of the peat (Svensson & Sundh 1992; Segers 1998). Control of CH_4 production by the availability of a chemical element is particularly inter-

esting because methanogens have somewhat unique requirement for certain metal elements and major cations (Jarrell & Kalmokoff 1988). In particular, all methanogens require nickel, cobalt, and iron for growth, and some have a high requirement for sodium.

The availability of a chemical element could be either too high causing toxicity or too low causing limitation of a microbial process. Limitation by a chemical element is shown if the rate of a process increases by addition of that element, based on the logic of Chapin et al. (1986) for nutrient limitation of plant production. The addition of an element could increase the conversion rate of substrates into CH_4 by the population of methanogens *in situ*, or it could allow growth in the population of methanogens that occur in the peat but at too small of a population size *in situ* to produce much CH_4. Since the substrates for CH_4 production are actually products of other microbial processes, such as fermentation (see Cicerone & Oremland (1988) for details of CH_4 production), alleviating chemical element limitation of a fermentation process could also stimulate CH_4 production, even though the added element did not affect methanogens directly.

We manipulated the concentrations of Ni, Co and Fe, as well as Na, Ca and Li, in samples of peat from five different *Sphagnum*-dominated peatlands to examine effects on CH_4 production. We used peat from bog peatlands in which microorganisms in the surface peat rely largely on chemical elements deposited from the atmosphere, and peat from more mineral-rich fen peatlands that were more connected to the local groundwater than bog peatlands (Moore & Bellamy 1974). Study sites occurred in North America and ranged from a site in west central Canada to ones in northeastern U.S.A, where polluted air should be a large source of trace metal elements. Specific predictions were that addition of chemical elements would stimulate methanogens directly and that peat from bogs would display greater limitation than fens given lower chemical concentrations in bog waters than fen waters (Gorham et al. 1985).

Methods

The five study sites occurred in North America (Table 1). Each had been studied in the past, providing additional information about vegetation and hydrology. Bleak Lake is described in Vitt et al. (1995). Labrador Hollow is described in Paratley and Fahey (1986). McLean is described in Oswald (1970). Buckles is described in Maxwell and Davis (1972), and Big Run is described in Wieder (1985). Bleak Lake had the deepest water table, and the peat deposits at Bleak Lake and McLean were clearly separated from local surface water and ground water. Although Big Run sits topographically in

Table 1. Characteristics of the study sites

Site	Location	Peatland type	Plant species	Mean depth to water table (m)	pH
Bleak Lake	610-m elevation near Athabasca, Alberta (54°41′N, 113°28′W) MAT = 1.4 °C MAP = 490 mm	Open low-shrub bog	*Sphagnum* *S. fuscum, S. magellanicum* *S. angustifolium* *Vascular* *Ledum groenlandicum* *Vaccinium oxycoccos* *Smilacina trifolia, Rubus chamaemorus*	0.30	3.9
Labrador Hollow	300-m elevation near Tully, New York (42°45′N, 76°01′W) MAT = 8.1 °C MAP = 989 mm	Forested fen	*Sphagnum* *S. girgensohnii, S. henryensis* *S. russoweii* *Vascular* *Acer rubrum, Pinus strobus* *Osmunda cinnamomea*	0.15	6.3

Table 1. Continued

Site	Location	Peatland type	Plant species	Mean depth to water table (m)	pH
McLean	115-m elevation near Cortland, New York (42°45′N, 76°01′W) MAT = 13.1 °C MAP = 899 mm	Open low-shrub bog	*Sphagnum* *S. angustifolium, S. magellanicum* *Vascular* *Andomeda glaucophylla* *Chamaedaphne calyculata* *Eriophorum virginicum*	0.03	4.1
Big Run	980-m elevation near Parsons, West Virginia (39°07′N, 79°35′W) MAT = 7.5 °C MAP = 1330 mm	open graminoid bog	*Sphagnum* *S. fallax* *Vascular* *Eriophorum virginicum*	0.05	3.9
Buckles	880-m elevation in Garret County, Maryland (39°34′N, 79°16′W) MAT = 7.9 °C MAP = 1330 mm	open low-shrub bog	*Sphagnum* *S. fallax* *Vascular* *Andromeda glaucophylla* *Kalmia latifolia* *Gaultheria hispidula*	0.10	4.1

MAT = Mean Annual Temperature; MAP = Mean Annual Precipitation.

the lowest part of a relatively large forested watershed, and receives drainage from the surrounding forest, the predominant bedrock in the region is acid sandstone (Losche & Beverage 1967) and regional surface water and soil have low concentrations of major cations. Labrador Hollow also sits at the bottom of a large watershed, but unlike Big Run Bog, surface water passes through shale, slate and glacial till before reaching the wetland and accumulates high concentrations of major cations.

Peat was collected during May to December 1998. In each site, we collected samples of bulk peat from three places chosen randomly by cutting through the ground vegetation and collecting approximately 500-cm^3 of peat located at the surface of the water table. The three samples of peat per site were combined in a large plastic bag, which was placed in another plastic bag, and taken immediately to the laboratory at Cornell University. Peat samples were stored at 4 °C for one day. We homogenized the bulk peat sample by hand, wearing sterile gloves, and removed large roots, then approximately 40-g subsamples (wet weight) were placed in pre-weighed 350-ml Mason jars.

We carried out three experiments. In the first experiment, we examined the effect of added trace metals on CH_4 production. Sets of three jars were assigned to one of four treatments. The first was 150-ml distilled, de-ionized water, which served as the control. The second was 150 ml of a trace metal solution containing 12 μM $NiCl_2$, 5.5 μM $CoCl_2$, and 11.6 μM $FeCl_3$. The $FeCl_3$ was prepared according to the recipe of Ghiorse (1994). We did not test the effect of each metal individually is this study, in part, because the idea of trace metal limitation in *Sphagnum*-derived peat is new (cf., Segers 1998). However, our findings were intriguing enough that future studies should focus on the individual chemical elements added at different concentrations. We further examined trace metals effects on CH_4 production in the third treatment, which was 150 ml of 10 mM EDTA buffered to 6.0 (Nozoe et al. 1994), and the fourth treatment of 150 ml of 10 mM sodium citrate buffered to 6.0. Citrate and EDTA are strong complexing agents for metal elements (synonymous with chelators). Chelated metal elements remain soluble, which can increase their availability to organisms, or toxicity. In some cases, chelation prevents toxicity by making a complex too large to be taken up by an organism (Taylor 1988). The buffering is necessary to prevent low pH and organic acid toxicity to methanogenic microorganisms (Russell & Diez-Gonzalez 1998).

In the second experiment, we examined the effect of added cations on CH_4 production. Sets of three jars were assigned to one of four treatments. The first was 150-ml distilled de-ionized water. The others were 150 ml of either 2 mM NaCl, 2 mM LiCl, or 2 mM $CaCl_2$. These concentrations are a

bit higher than concentrations found in peat porewater in fens (Gorham et al. 1985), except for Li, which was included as an analogue of Na.

In the third experiment, we tested different combinations of peat and pore water. Sets of three jars were randomly assigned to one of the following treatments. Peat from Bleak Lake and 150 ml of either (1) de-ionized water or (2) pore water from McLean. Peat from McLean and either 150-ml of either (3) de-ionized water or (3) pore water from Bleak Lake Bog. The final treatment was peat from McLean and Bleak Lake and 150-ml de-ionized water. The purpose of this experiment was to examine whether pore water from a peatland near the southern extent of northern peatlands (i.e., McLean) would affect methane production in a northern counterpart (i.e., Bleak Lake). We did not use pore water from the other three sites with peat from Bleak Lake, because in those cases the pore water chemistry depends much more on the mineral composition of the bedrock or till in the region than on regional chemistry of precipitation. We assumed that the chemical composition of pore water in McLean and Bleak Lake depends mostly on atmospheric conditions in their regions.

The solution to peat ratio in each experiment was large enough to maintain a liquid layer of about 1-cm above the peat in the jar. We sealed each jar with a lid that had a thick rubber O-ring to ensure a tight seal and a septum to facilitate sampling of gases in the headspace to the jar. The headspace gas was removed for 4 min, while shaking the jar continuously, using a vacuum pump and replaced immediately with O_2-free N_2. This procedure was repeated two more times to remove residual CH_4 and to establish anoxic conditions within each jar. This procedure removes about 50% of the CO_2 in the peat slurry (Yavitt et al. 1990), with the remainder remaining dissolved in the solution.

We incubated each flask without agitation at 22 °C for up to 30 days and maintained a layer of water over the lid in the septum during the entire incubation to reduce gas exchange between ambient air and the jar's headspace. At 1 to 5-d intervals during the period, we took a 5-ml gas sample from the headspace of each flask for analyses of CH_4 and CO_2 and immediately added 5-ml of N_2 to maintain gas pressure. Samples were analyzed within 4-h of collection by gas chromatography using a flame ionization detector for CH_4 and a thermal conductivity detector for CO_2. The gas chromatograph had a 2.75 m $\times$ 3.18 mm column of Poropak Q (80/100 mesh, Waters Chromatography, Milford, MA, U.S.A.) maintained at 50 °C to separate the gases. The flow rate of the He carrier gas was 30 ml/min. Injector temperature was 110 °C. We used certified CH_4 standards (1.0, 9.8, 102, and 10400 μmol/mol CH_4 in N_2) and CO_2 standards (1024, 10000 μmol/mol CO_2 in N_2) for calibration and calculated concentrations of methane and CO_2 by comparing peak areas for samples and standards.

After the last gas sample, the volume of the jar headspace was determined by filling the jars with water and weighing the volume added. The water was then poured off and the peat was dried and weighed.

We also collected three samples of peat pore water at each site either from surface water, if available, or from water that filled the hole after we removed a peat sample. For each sample, we measured pH using a glass electrode and concentrations of metals and cations. We also measured concentrations of metals and cations in peat extracted with 150 ml of 10 mM EDTA over night (18 h). A 5-ml aliquot was removed, filtered through acid-rinsed glass fiber filters, and analyzed for metal and ion concentration analysis using a Jarrell Ash ICP-ES.

We used repeated-measures analysis of variance (ANOVA) to test effects of adding metals, EDTA, citrate, and cations on CH_4 production and CO_2 production. The repeated measurement of gas concentrations in the jars was the within-subjects factor. This was necessary because some peat samples showed a lag before the onset of CH_4 production. In some cases, the treatment reduced the lag time although the treatment and control samples produced the same amount of CH_4 after a long 30-d incubation period, resulting in a significant treatment X incubation time interaction. Relationships between CH_4 production and CO_2 production and concentrations of chemical elements were examined using correlation analysis. Significant differences are reported for $P < 0.05$.

Results

Methane production differed significantly among sites for peat incubated *in vitro* at 22 °C without added metal or cation elements (Figure 1, Table 2). The production of CO_2 measured concomitantly with CH_4 production did not differ significantly among sites (Figure 1, Table 2). In both cases, the significant site X incubation time interaction indicates different temporal patterns in gas production during the incubation period. For example, peat from Bleak Lake had a distinctive 12-d lag before the onset of CH_4 production. We were particularly interesting in testing whether this lag in CH_4 production was related to trace metal and (or) cation limitation.

Methane production in peat from Bleak Lake was much greater with either added trace metals or added citrate (Figure 2). In both cases, the increase in CH_4 production occurred quickly after a 7-d lag, resulting in a significant treatment X incubation time interaction ($P(s) < 0.001$). The trace metal mixture also increased CH_4 production in peat from McLean (Figure 3). Otherwise, CH_4 production was unaffected or inhibited by the addition of either the trace metal mixture or the chelators (Figure 3). Neither added

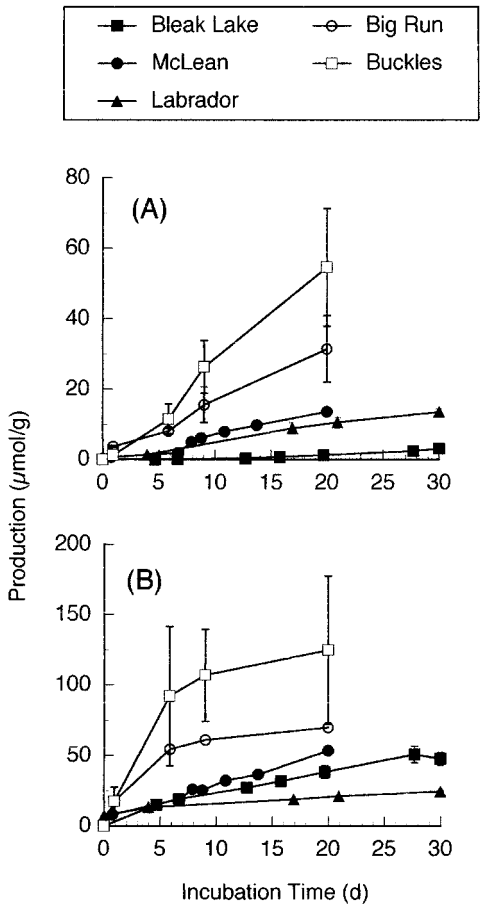

Figure 1. Production of CH_4 (A) and CO_2 (B) in control peat from each study site incubated *in vitro* at 22 °C. Values are mean of three replicates. Error bars are one standard error.

Table 2. Results (F values and significance) of repeated measures ANOVA testing effects of sites and incubation time on CH_4 production and CO_2 production. Sampling of gas accumulated in each flask was treated as the within-subjects factor, time

	df	CH_4 production	CO_2 production
Site	4	7.05**	3.12
Time	6	40.29***	15.96***
Site X Time	24	6.95***	3.32***
Error	60		

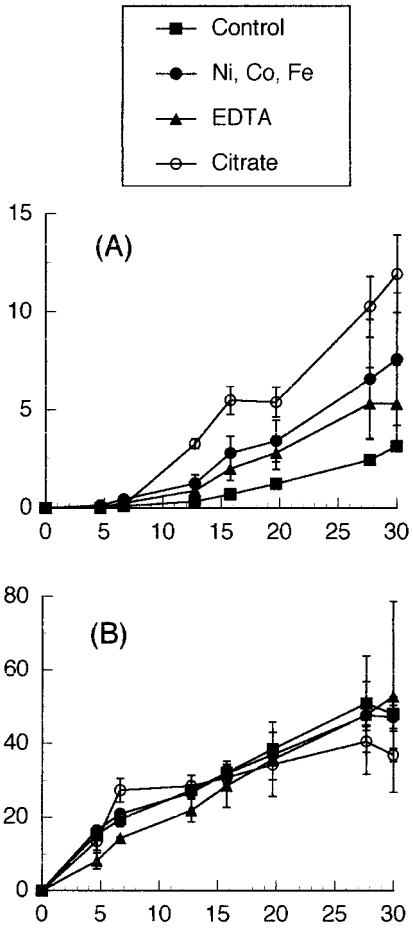

Figure 2. Production of CH_4 (A) and CO_2 (B) in peat from Bleak Lake incubated *in vitro* at 22 °C with de-ionized water (control), trace metals, EDTA, or citrate. Values are mean of three replicates. Error bars are one standard error.

metals nor EDTA affected CO_2 production, although CO_2 production was 2-fold greater in each site with added citrate.

Additions of Ca, Na or Li also increased CH_4 production in Bleak Lake peat (Figure 4), again, after an initial 7-d lag. The largest increase in CH_4 production occurred with the addition of Na plus the mixture of trace metals (Figure 5). This combination also increased CH_4 production in peat from McLean. The production of CO_2 was not affected by any of the added cations.

Methane production, but not CO_2 production, increased significantly in peat from Bleak Lake when incubated *in vitro* with pore water from McLean versus distilled, de-ionized water (Table 3). The reciprocal experiment, i.e.,

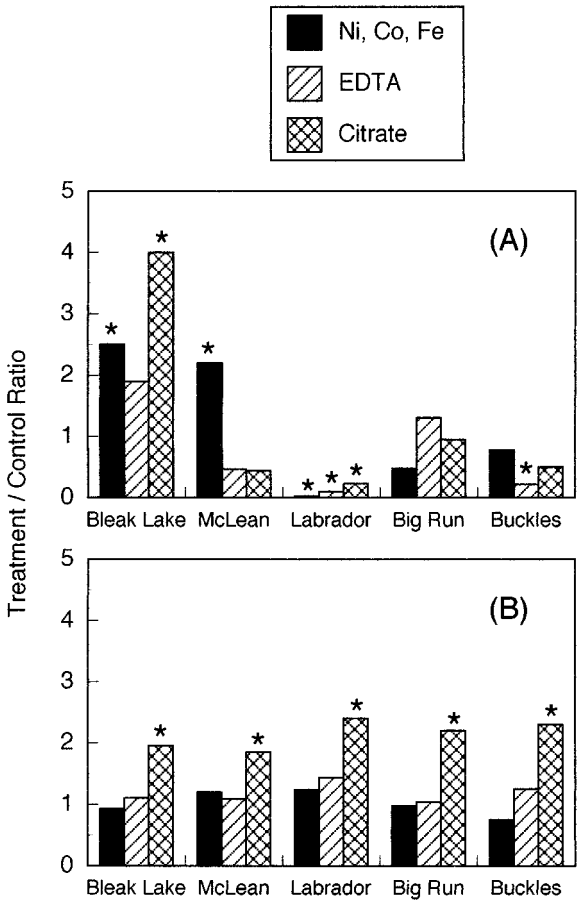

Figure 3. Treatment / control ratio of CH_4 production (A) and CO_2 production (B) in peat incubated *in vitro* at 22 °C. Treatments were trace metals, EDTA, or citrate. Control was de-ionized water. Ratios were determined from slopes of gas production versus time. Bars are mean of three replicates of treatment peat and three replicates of control peat. Stars above bars indicate that the ratio is significantly different than 1.0.

peat from McLean incubated *in vitro* with pore water from Bleak Lake, did not show a significant change in either CH_4 production or CO_2 production versus the control. A mixture of peat from Bleak Lake and from McLean had an intermediate value of CH_4 production, as expected.

Concentrations of cations and metals in peat pore water differed significantly among sites (Table 4). Porewater concentrations of Ca^{2+} and Mg^{2+}were significantly greater in Labrador Hollow, intermediate in McLean, Buckles and Big Run, and significantly less in Bleak Lake (site difference $p < 0.0001$ for Ca^{2+} and Mg^{2+}). Concentrations of K^{+} in pore water were more uniform

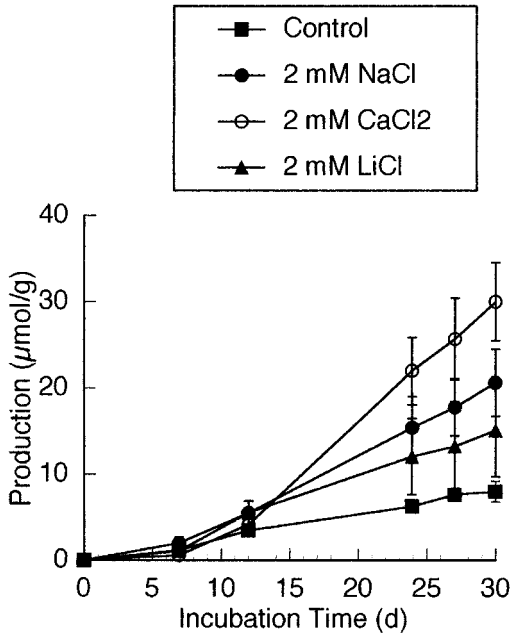

Figure 4. Production of CH_4 in peat from Bleak Lake incubated *in vitro* at 22 °C with de-ionized water (control) or 2 mM additions of NaCl, LiCl or $CaCl_2$. Values are mean of three replicates. Error bars are one standard error.

among the sites, but significant site differences still existed ($F_{4,10}$ = 15.96, p = 0.0002). Pore water concentrations of Na^+ were much lower in Bleak Lake than in the other sites ($F_{4,10}$ = 2.5, p = 0.11). Pore water concentrations of Ni^{2+}, Co^{2+}, and Fe^{3+} showed a much different pattern with significantly higher values in Big Run and in Buckles, especially for Co^{2+} and Fe^{3+} (site difference $p < 0.0001$). However, pore water in Labrador Hollow had an elevated Ni^{2+} concentration (site difference $F_{4,10}$ = 8.79, p = 0.0026). These site differences were much more evident in the EDTA extracts of peat samples, with the exceptionally high extractable concentrations of cations in Labrador Hollow peat and high extractable concentrations of metals for peat from Big Run and Buckles. The site differences in CH_4 production were correlated to concentrations of several chemical elements in peat pore water (Table 5).

Discussion

There are several environmental factors that can constrain CH_4 production in *Sphagnum*-dominated peatlands (Svensson & Sundh 1992; Segers 1998), but

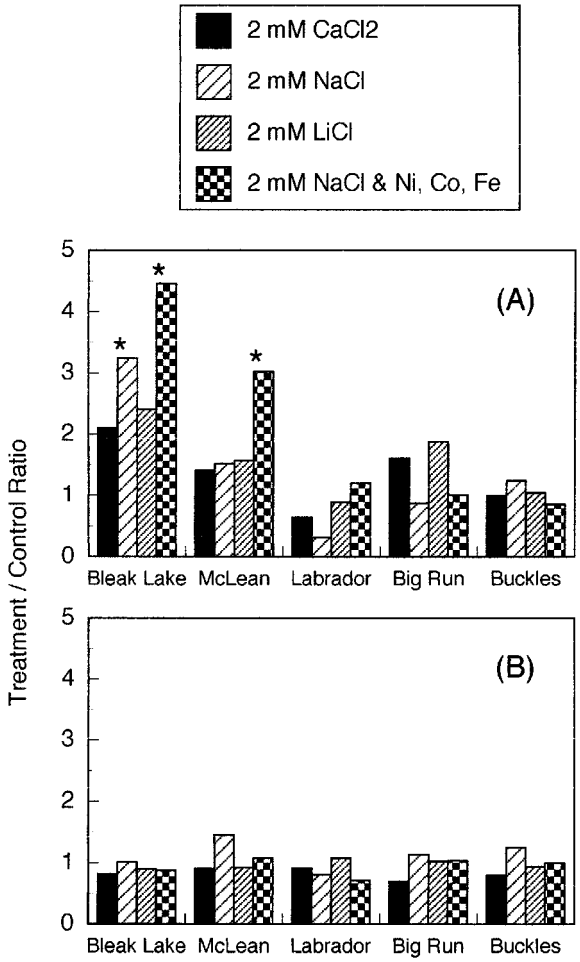

Figure 5. Treatment / control ratio of CH_4 production (A) and CO_2 production (B) in peat incubated *in vitro* at 22 °C. Treatments were 2 mM additions of NaCl, LiCl or $CaCl_2$. Control was de-ionized water. Ratios were determined from slopes of gas production versus time. Bars are mean of three replicates of treatment peat and three replicates of control peat. Stars above bars indicate that the ratio is significantly different than 1.0.

the availability of chemical elements for nutritional requirements of methanogens has not been implicated widely. Yet studies with pure cultures of methanogens have demonstrated a physiological need for relatively moderate levels of Ni, Co and Fe, and Na in some cases (Jarrell & Kalmokoff 1988). Studies of peat chemistry rarely report levels Ni and Co in either peat or porewater, and levels of Fe and Na are generally very low, except in peatlands impacted by marine air (Gorham et al. 1985). Our primary objective was to

Table 3. Rates of CH_4 production and CO_2 production in peat samples incubated for 30 days in vitro at 22 °C with different porewater. Values are mean of three replicates, with one standard error given in parentheses below each value

Peat	Water	CH_4 production μmol/g	CO_2 production μmol/g
Bleak Lake	control	5.0 ± 0.6	153 ± 30
Bleak Lake	McLean porewater	14.5 ± 2.0	147 ± 15
McLean	control	21.0 ± 4.2	99 ± 6
McLean	Bleak Lake porewater	28.7 ± 4.5	78 ± 9
McLean/Bleak Lake	control	11.2 ± 0.9	66 ± 9

examine trace metal and cation limitation of CH_4 production in peat samples from *Sphagnum*-dominated peatlands. Our results indicated that additions of trace metals and Na could stimulate CH_4 production in bog peatlands, especially, in a site located in continental central Canada.

All of the sites, except Labrador Hollow, classified as mineral-poor bogs or very poor fens which have low pH and low concentrations of Ca^{2+} and Mg^{2+} in surface pore water (Glaser et al. 1990; Vitt & Chee 1990). Although all bog peatlands have low pH and low Ca^{2+}concentrations, the availability of trace metals is less obvious. *Sphagnum* mosses have very high surface to mass ratios, making them unusually efficient at trapping metal-containing atmospheric gases and particles (Santelmann & Gorham 1988). Indeed, peatlands in regions with atmospheric pollution have higher pore water concentrations of Ni^{2+} and other trace metals than counterparts in regions with cleaner atmospheric conditions (Blancher & McNicol 1987). Presumably, the extreme atmospheric pollution in West Virginia (Spengler et al. 1996) accounts for the high porewater concentrations of Ni^{2+} and Co^{2+} in Big Run and in Buckles. New York State also has moderate air pollution, although McLean is a very small peatland surrounded by trees, and it is likely that the trees intercept much of the atmospheric pollutants and prevent deposition on the surface of the peatland. Although Ni is ubiquitous in the air, soil, and water of Canada, levels near Bleak Lake are very low and not influenced by the Ni-mining region of eastern Canada (Zoltai 1988; Chau & Kulikovosky-Cordiero 1995). None of the sites are influenced by marine air, which accounts for the low concentrations of Na^{+} in pore water.

Table 4. Concentrations of chemical elements in peat pore water (top part) and in EDTA extractions of peat (bottom part) from the five study sites. Values are mean of three replicates, with one standard error. Note different units

	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	Ni	Co	Fe
Porewater	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(μg/L)	(μg/L)	(mg/L)
Bleak	0.45 ± 0.19	0.07 ± 0.02	0.42 ± 0.05	0.56 ± 0.36	0.1 ± 0.1	1.6 ± 0.8	0.02 ± 0.01
Mclean	1.49 ± 0.01	0.27 ± 0.01	0.47 ± 0.01	3.46 ± 0.20	1.1 ± 0.4	2.0 ± 0.5	0.12 ± 0.01
Labrador	21.09 ± 0.34	2.83 ± 0.01	1.22 ± 0.05	6.29 ± 1.68	2.5 ± 1.0	105.5 ± 104.8	0.04 ± 0.01
Big Run	1.72 ± 0.16	0.29 ± 0.02	1.13 ± 0.14	3.49 ± 1.89	3.5 ± 1.0	285.0 ± 17.3	1.08 ± 0.04
Buckles	4.06 ± 0.12	0.90 ± 0.03	1.96 ± 0.32	3.33 ± 2.64	3.0 ± 0.9	160.5 ± 83.6	1.52 ± 0.13
EDTA extractable	(mg/g)	(mg/g)	(mg/g)	(mg/g)	(mg/g)	(mg/g)	(mg/g)
Bleak	1.03 ± 0.03	0.08 ± 0.01	0.18 ± 0.02	0.01 ± 0.01	0.18 ± 0.09	0.18 ± 0.05	0.25 ± 0.01
Mclean	0.97 ± 0.12	0.05 ± 0.01	0.12 ± 0.03	0.08 ± 0.02	5.43 ± 4.10	0.33 ± 0.28	0.48 ± 0.04
Labrador	6.18 ± 0.18	0.20 ± 0.01	0.11 ± 0.01	0.40 ± 0.04	0.11 ± 0.01	0.50 ± 0.41	0.58 ± 0.05
Big Run	1.07 ± 0.04	0.04 ± 0.01	0.21 ± 0.02	0.05 ± 0.01	43.38 ± 21.42	49.50 ± 19.64	2.44 ± 0.01
Buckles	1.04 ± 0.04	0.08 ± 0.01	0.79 ± 0.21	0.20 ± 0.02	5.22 ± 0.32	50.10 ± 4.42	2.37 ± 0.08

Table 5. Correlation coefficients for relationships between concentrations of chemical elements in peat pore water and CH_4 production and CO_2 production

	CH_4 production	CO_2 production
B	0.59	0.70
Ca	0.34	0.26
Co	0.92	0.92
Cr	–0.33	–0.55
Cu	–0.02	–0.21
Fe	0.99	0.92
K	0.93	0.93
Mg	0.71	0.63
Mn	0.98	0.97
Mo	0.31	0.12
Na	0.88	0.63
Ni	0.99	0.94
P	–0.42	–0.28
Pb	0.83	0.74
Se	–0.80	–0.60
Zn	0.94	0.87

Control of methane production

Although site differences in CH_4 production in *Sphagnum*-dominated peatlands are well documented, many causes have been proposed. Bog peatlands dominated by *S. fuscum*, like Bleak Lake, typically have very low rates of CH_4 production (Bubier et al. 1993; Bergman et al. 1998) and, accordingly, low rates of CH_4 emission to the atmosphere (Bubier et al. 1995). The reason is thought to be the relatively dry conditions in the surface peat since *S. fuscum* sits several cm above the water table level. Methanogens that reside deeper in the peat deposit near the water table have to deal with much older peat that has lost most of its easily decomposable material. Consequently wetting of *S. fuscum* peat, alone, does not induce large rates of CH_4 production, as in our control treatment, due to carbon quality limitation (Yavitt et al. 1997). Despite this hypothesis, we found that CH_4 production in *S. fuscum* peat was increased 3-fold by the addition of Ni, Co, and Fe, suggesting a nutritional limitation of CH_4 production. Furthermore, assuming that metals enter bog peatlands primarily from atmospheric deposition, they probably become bound to the surface peat (Vile et al. 1999), and they do not leach down in

high enough concentrations to affect the active methanogens in the subsurface peat.

Peat from McLean produced a moderate amount of CH_4, which agrees with intermediate levels of CH_4 emission from *S. angustifolium* peat (Bubier et al. 1995). Nevertheless, we found that CH_4 production in McLean peat responded to the addition of Ni, Co, and Fe, indicating a trace metal limitation. Although the water table in McLean stays very near the surface, and thus the active methanogens are close to the atmospheric source of trace metals, unlike in Bleak Lake, the input of trace metals in McLean is still low enough to limit availability to the methanogens. On the other hand, the slightly greater concentrations of trace metals in McLean pore water than in Bleak Lake pore water (Table 4) were able to simulate CH_4production in Bleak Lake peat (Table 5), upon addition. This indicates greater trace metal limitation of CH_4 production in bogs in continental Canada than in counterparts located southward. We can only speculate, but it seems likely that the greater air pollution and, accordingly, input of trace metals to peatlands in the United States has alleviated some of the trace metal limitation.

The relatively low rate of CH_4 production in peat from Labrador Hollow has a much different explanation. Methane production is very slow in forested peatlands (Yavitt et al. 1997), like Labrador Hollow, because of the very poor quality of the peat derived from woody roots and tree leaf litter in addition to *Sphagnum*. It is not clear why the addition of the trace metals inhibited CH_4 production in Labrador Hollow peat. However, since the inhibition did not cover CO_2 production, the effect was specific to CH_4 production and not anaerobic respiration in general. Furthermore, the amount of added metals did not bring concentrations up to the levels found in Big Run and in Buckles, and thus trace metal toxicity does not seem likely. It is possible that the added trace metals alleviated a limitation of some other anaerobic process that, in turn, robbed substrates used for CH_4 production, but without increasing CO_2 production, i.e., by stimulating cell growth.

Methane production in peat from the two sites in West Virginia was typical of peat in bog peatlands dominated by *S. fallax* (Nedwell & Watson 1995). *Sphagnum fallax* occurs close to the water table (Bubier et al. 1995), and the saturated conditions are thought to be the reason for high rates of CH_4 emission from these sites (Bubier et al. 1995). However, our sites also had high concentrations of the nutritional trace elements, suggesting no chemical element limitation of CH_4 production. Furthermore, no lag in CH_4 production upon incubation of peat from Big Run and Buckles indicates a relatively large *in situ* population of methanogens and no other anaerobic processes competing for energy and nutritional substrates (cf., Mayer & Conrad 1990; Peters & Conrad 1996).

We did not investigate control of CH_4 production by Ni, Co, and Fe added individually, although our findings suggest this should be addressed in future research. One reason why we used the mixture of trace metals is the likelihood of co-limitation: no single chemical element limits CH_4 production, but rather a suite of trace metal elements is required for active metabolism and growth. Furthermore, the concentration of each chemical element required for metabolism and growth may vary among different species of methanogen (Jarrell & Kalmokoff 1988), whereas methanogen species in *Sphagnum*-dominated peatlands are virtually unknown (cf., Lloyd et al. 1998). Notwithstanding, Gonzalez-Gil et al. (1999) found that Ni was critical for growth of methanogens, while Ni and Co are essential components in the enzymes producing CH_4. We included Fe in the metal mixture despite high Fe content of the peat because methanogens have very high Fe requirement for growth (Takashima & Speece 1990). Furthermore, most of the Fe in peat is likely bound to the peat and not available for uptake. Jarvis et al. (1997) argued that the availability of Co seems to be more important than Fe for the growth of methanogens in some anaerobic digestion systems. However, it is not clear whether this is true in *Sphagnum*-derived peat.

The strong correlation between CH_4 production and concentrations of some other trace elements in peat pore water, such as dissolved Mn and Zn, suggests a nutritional role for these elements, although the correlation could stem from the co-variance of their concentrations in peat pore water. On the other hand, virtually all methanogens require moderate levels of Mo, which is an essential component in the first enzyme involved in CO_2 reduction in methanogenesis, yet we found a fairly poor correlation between site differences in CH_4 production and the pore water concentrations of dissolved Mo. Clearly, some trace metals are toxic to microbial activity, and accordingly, we found negative relationships between dissolved concentrations of Cr and Se and both CH_4 production and CO_2 production. Based on these relationships, further work with individual trace elements in *Sphagnum*-derived peat is certainly warranted.

The metal chelators EDTA and citrate had much less effect on methane production, except in Bleak Lake peat. While chelators remove ions bound to peat and make them more soluble, it is not clear whether a metal bound to a chelator is actually more available to a microorganism, or even in a level that is too high causing toxicity. For instance, Nozoe et al. (1994) showed that EDTA added to paddy soil incubated *in vitro* increased CH_4 production, but only after a 6-d lag. In this case, it is possible that metals bound to EDTA were too large to be available to methanogens, but that degradation of the complex after six days released the elements to pore water in an available form. In our case, it is possible that citrate increased the availability of Fe(III).

However, the degradation of this in complex would increase the solubility of Fe(III), favoring the energetically more profitable reduction of Fe(III) and inhibiting CH_4 production (Roden & Wetzel 1996). The large increase in CO_2 production with added citrate provides this evidence.

The much greater concentrations of chemical elements in the EDTA extract of peat than in peat pore water (Table 4) confirms the existence of a large pool of elements bound to peat. *Sphagnum*-derived peat has a strong capacity to bind ions and remove them from solution, making them essentially unavailable to plants and, presumably, microorganisms. Van Breemen (1995) suggests that this binding has a functional significance because *Sphagnum* can grow in peat with very low nutrient levels, whereas most vascular plants cannot. In this way, *Sphagnum* 'prevents' the growth of taller, vascular plants that would block sunlight and limit its productivity. Therefore, the trace metal binding inadvertently also limits CH_4 production, particularly, in peatlands without a continuous input of elements.

The addition of Na increased CH_4 production in peat from Bleak Lake. Lithium had much less effect, suggesting the effect is specific for Na and not simply the need for a monovalent cation. Many methanogens have a high Na requirement for CH_4 production and growth (Perski et al. 1982; Jarrell & Sprott 1985). Apparently, Na functions in ATP formation by a Na^+/K^+ transport system. When Na is not available, some methanogens can use methanol and H_2 or formaldehyde (Blaut et al. 1986), but the importance of this in peat has not been studied. Because, the level of the Na requirement varies considerably among different methanogenic species (Jarrell & Kalmokoff 1988), predicting effects of Na concentrations on CH_4 production depends on the species of methanogen present. Indeed, the increase in CH_4 production in Bleak Lake peat with added Ca might be related indirectly to Ca. Since divalent Ca^{2+} would replace monovalent Na^+ on peat exchange sites, the added Ca could actually increase Na levels in solution.

The effects of added trace metals on CH_4 production, but not CO_2 production, in Bleak Lake and McLean is evidence for a direct effect on methanogens rather than on fermentation processes that supply substrates for methane production. Indeed, we found no relationship between CH_4 production and CO_2 production measured concomitantly, with a correlation coefficient of –0.11. This poor relationship between CH_4 production and anaerobic carbon metabolism in *Sphagnum*-derived peat has been noted (Segers & Kengen 1998), although there is no simple explanation. We suggest that trace metal limitation of CH_4 production, but not CO_2 production may influence the relationship in some cases. In contrast, plant production and organic matter decomposition in bog peatlands is likely constrained by availability of nitrogen and (or) phosphorus (Bridgham et al. 1996). Aerts and de

Caluwe (1999) found that adding nitrogen to intact columns of vegetation and peat increased CH_4 production, implying a CH_4 limitation of methanogens. Although we did not measure N in this study, we found a weak negative correlation between dissolve PO_4^{3-} concentrations and CH_4 production. It is likely that the effect of added N or P on CH_4 production is indirect, by stimulating plant production, and methanogens simply benefiting from the increase in fresh energy.

In contrast to our findings for CH_4 production, studies of CH_4 emission from *Sphagnum*-dominated peatlands to the atmosphere have shown no significant relationship with pore water chemistry (Moore et al. 1994; Bubier 1995). However, CH_4 emission is a complex phenomenon that depends on the balances between CH_4 production, microbial consumption of CH_4, and gas transport through peat and emergent aquatic vegetation (Yavitt 1997).

Finally, we can provide only a very rough estimate of the global extent of trace metal limitation of CH_4 production in *Sphagnum*-dominated peatlands because so few studies that have measured CH_4 production as well as concentrations of trace metals in the peat. However, if our findings for Bleak Lake apply to other bog peatlands in continental Canada and in Siberia, away from areas with fossil fuel burning and trace metal pollution of the air, then trace metal limitation of CH_4 production could be widespread.

Acknowledgements

Financial support came from the National Science Foundation. We thank Mike Rutzke (Dept. Plant Biology, Cornell Univ.) for help with ICP analyses.

References

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

Bartlett KB & Harriss RC (1993) Review and assessment of methane emissions from wetlands. Chemoshpere 26: 261–320

Bergman I, Svensson BH & Nilsson M (1998) Regulation of methane production in a Swedish acid mire by pH, temperature and substrate. Soil Biol. Biochem. 30: 729–741

Blancher PJ & McNicol DK (1987) Peatland water chemistry in central Ontario in relation to acid deposition. Water Air Soil Pollut. 35: 217–232

Blaut M, Mueller V & Gottschalk G (1985) Mechanism of ATP synthesis and role of sodium ions in *Methanosarcina barkeri* growing on methanol. Syst. Appl. Microbiol. 7: 354–357

Bridgham DS, Pastor J, Janssens JA, Chapin C & Malterer TJ (1996) Multiple limiting gradients in peatlands: A call for a new paradigm. Wetlands 16: 45–65

Bubier JL (1995) The relationship of vegetation to methane emission and hydrochemical gradients in northern peatlands. J. Ecol. 83: 403–420

Bubier JL, Moore TR & Juggins S (1995) Predicting methane emission from bryophyte distribution in northern Canadian peatlands. Ecology 76: 677–693

Bubier J, Costello A, Moore TR, Roulet NT & Savage K (1993) Microtopography and methane flux in boreal peatlands, northern Ontario, Canada. Can. J. Bot. 71: 1056–1063

Chau YK & Kulikovsky-Cordiero OTR (1995) Occurrence of nickel in the Canadian environment. Environ. Rev. 3: 95–120

Chapin FS III, Vitousek PM & Van Cleve K (1986) The nature of nutrient limitation in plant communities. Am. Nat. 127: 48–58

Cicerone RJ & Oremland RS (1988) Biogeochemical aspects of atmospheric methane. Global Biogeochem. Cycles 2: 299–327

Ghiorse WC (1994) Iron and manganese oxidation and reduction. In: Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties (pp 1079–1096). Soil Science Society of America Publication, Madison, Wisconsin

Glaser PH, Janssens JA & Siegel DI (1990) The response of vegetation to chemical and hydrological gradients in the Lost River Peatland, northern Minnesota. J. Ecol. 78: 1021–1048

Gonzalez-Gil G, Kleerebezem R & Lettinga G (1999) Effects of nickel and cobalt on kinetics of methanol conversion by methanogenic sludge as assessed by on-line CH_4 monitoring. Appl. Environ. Microbiol. 65: 1789–1793

Gorham E, Eisenreich SJ, Ford J & Santelmann MV (1985) The chemistry of bog waters. In: Stumm W (Ed.) Chemical Processes in Lakes (pp 339–363). John Wiley & Sons, New York

Jarrell KF & Kalmokoff ML (1988) Nutritional requirements of the methanogenic archeabacteria. Can. J. Microbiol. 34: 557–576

Jarrell KF & Sprott GD (1985) Importance of sodium to the bioenergetic properties of *Methanococcus voltae*. Can. J. Microbiol. 31: 851–855

Jarvis A, Nordberg A, Jarlsvik T, Mathisen B & Svensson BH (1997) Improvement of a grass-clover silage fed biogas process by the addition of cobalt. Biomass Bioenergy 12: 453–460

Lloyd D, Thomas KL, Hayes A, Hill B, Hales BA, Edwards C, Saunders JR, Ritchie DA & Upton M (1998) Micro- ecology of peat: minimally invasive analysis using confocal laser scanning microscopy, membrane inlet mass spectrometry and PCR amplification of methanogen-specific gene sequences. FEMS Microbiol. Ecol. 25: 179–188

Losche CK & Beverage WW (1967) Soil survey of Tucker County and northern Randolph County, West Virginia. US Soil Conserv. Serv., Forest Serv., and West Virginia Agric. Exper. Sta. Washington DC

Maxwell JA & Davis MB (1972) Pollen evidence of Pleistocene and Holocene vegetation of the Allegheny Plateau, Maryland. Quat. Res. 2: 506–530

Mayer HP & Conrad R (1990) Factors influencing the population of methanogenic bacteria and the initiation of methane production upon flooding of paddy soil. FEMS Microbiol. Ecol. 73: 103–112

Moore PD & Bellamy DJ (1974) Peatlands. Springer-Verlag, New York

Moore TR, Heyes A & Roulet NT (1994) Methane emissions from wetlands, southern Hudson Bay lowland. J. Geophys. Res. 99: 1455–1467

Nedwell D & Watson A (1995) Methane production, oxidation and emission in a U.K. ombrotrophic peat bog: Influence of SO_4^{2-} from acid rain. Soil Biol. Biochem. 27: 893–903

Nozoe T, Yoshida K & Yasuda M (1994) Effect of EDTA on reduction process in submerged paddy soil with rice straw. Soil Sci. Plant. Nutr. 40: 155–164

Oswald H (1970) Vegetation and stratigraphy of peatlands in North America. Academiae Ubsaliensis, Uppsala

Paratley RD & Fahey TJ (1986) Vegetation-environment relations in a conifer swamp in Central New York, USA. Bull. Torrey Bot. Club 4: 357–371

Perski HJ, Schoenheit P & Thauer RK (1982) Sodium dependence of methane formation in methanogenic bacteria. FEBS Lett. 143: 323–326

Peters V & Conrad R (1996) Sequential reduction processes and initiation of CH_4 production upon flooding of oxic upland soils. Soil Biol. Biochem. 28: 371–382

Roden EE & Wetzel RG (1996) Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnol. Oceanogr. 41: 1733–1748

Russell JB & Diez-Gonzalez F (1998) The effects of fermentation acids on bacterial growth. Adv. Microbial Physiol. 39: 205–234

Santelmann MV & Gorham E (1988) The influence of airborne road dust on the chemistry of *Sphagnum* mosses. J. Ecol. 76: 1219–1231

Segers R (1998) Methane production and methane consumption: a review of processes underlying wetland methane fluxes. Biogeochemistry 41: 23–51

Segers R & Kengen SWM (1998) Methane production as a function of anaerobic carbon mineralization: a process model. Soil Biol. Biochem. 30: 1107–1117

Spengler JD, Koutrakis P, Dockery DW, Raizenne M & Speizer FE (1996) Health effects of acid aerosols on North American children: Air pollution exposures. Environ. Health. Persp. 104: 492–499

Svensson BH & Sundh I (1992) Factors affecting methane production in peat soils. Suo 43: 183–190

Takashima M & Speece RE (1990) Mineral requirements for methane fermentation, a critical review. Environ. Control 19: 465–479

Taylor G (1988) The physiology of aluminum tolerance in higher plants. Commun. Soil Sci. Plant Anal. 19: 1179–1194

van Breemen N (1995) How Sphagnum bogs down other plants. Trends Ecol. Evol. 10: 270–275

Vile MA, Wieder RK & Novak M (1999) Mobility of Pb in Sphagnum-derived peat. Biogeochemistry 45: 35–52

Vitt DH & Chee W-L (1990) The relationships of vegetation to surface water chemistry and peat chemistry in fens of Alberta, Canada. Vegetatio 89: 87–106

Vitt DH, Bayley SE, Jin T-L (1995) Seasonal variation in water chemistry over a bog-rich fen gradient in continental western Canada. Can. J. Fish Aquat. Sci. 52: 587–606

Wieder RK (1985) Peat and water chemistry of Big Run Bog, a peatland in the Appalachian Mountains of West Virginia. Biogeochemistry 1: 277–302

Yavitt JB (1997) Methane and carbon dioxide dynamics in *Typha latifolia* (L.) wetlands in central New York State. Wetlands 17: 394–406

Yavitt JB, Williams CJ & Wieder RK (1997) Production of methane and carbon dioxide in peatland ecosystems across North America: Effects of temperature, aeration, and organic chemistry of peat. Geomicrobiol. J. 14: 299–316

Yavitt JB, Downey DM, Lancaster E & Lang GE (1990) Methane consumption in decomposing *Sphagnum*-derived peat. Soil Biol. Biochem. 22: 441–447

Zoltai SC (1988) Distribution of base metals in peat near a smelter at Flin Flon Manitoba, Canada. Water Air Soil Pollut. 37: 217–228