Biogeochemistry **51:** 259–281, 2000.

Evaluation of methane oxidation in the rhizosphere of a *Carex* dominated fen in north central Alberta, Canada

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Key words: carex, methane oxidation, methyl fluoride, peatlands, rhizosphere

Abstract. Rhizospheric methane oxidation was evaluated at a *Carex* (spp.) dominated fen in Alberta, Canada over three growing seasons. Aerobic incubations of bulk peat and live roots in the laboratory show a clear association between active methane oxidizing bacteria and the rhizosphere. Aerobic incubations also show an oxidation potential that far exceeds methane production potential measured in the laboratory. Quantitative estimates of how this oxidation potential is expressed *in situ* depend strongly on which of two common approaches are used. (1) Subtracting *in situ* methane emission rates from methane production rates measured in the laboratory with anaerobic incubations suggest that methane oxidation may attenuate emissions by 58 to 92%. (2) Applying the inhibitor methyl fluoride (CH_3F) to whole plants *in situ* suggest methane oxidation attenuates emissions by less than 20% seasonally. The production minus emission technique likely overestimates methane oxidation because methane production measured via anaerobic incubations in the laboratory are probably overestimates. Oxidation percentages measured by CH_3F were greatest early in the growing season when emission rates were low and fell to almost nondetectable levels as emission rates peaked in late summer. Estimates provided by the CH_3F technique were generally in better agreement with estimates of oxidation based on a stable isotope mass balance (0–34%) determined in a companion study (Popp et al. 1999).

Introduction

A top priority in understanding the current rise in atmospheric methane concentrations (Rassmussen & Khalil 1984) and its effect on climate is defining methane source and sink strengths. Wetland soils (excluding rice paddies)

are generally considered the greatest natural source of methane to the atmosphere, accounting for roughly 20% of the total emissions (IPCC 1994). The presence of emergent vascular plants in wetland systems is an important variable for methane dynamics and can influence methane flux by providing labile organic compounds available as methane precursors, facilitating methane transport and efflux, and shuttling oxygen below ground increasing the potential for methane oxidation (Chanton & Dacey 1991).

Microbial mediated aerobic methane oxidation occurs at oxic-anoxic interfaces as methane diffuses along concentration gradients from anaerobic methane production zones. In vegetated wetlands this can occur either at the sediment-floodwater interface when floodwaters are oxygenated or in the rhizosphere at points where oxygen leaks from roots of emergent plants (Armstrong & Armstrong 1991; Frenzel et al. 1992).

An association of methane oxidation with roots and rhizomes has been established for a number of aquatic vascular plant species (e.g. van der Nat et al. 1997; Calhoun & King 1997; Lombardi et al. 1997; King 1994, 1996; Gerard & Chanton 1993; King et al. 1990). The extent of oxidation in freshwater wetlands may be controlled by such factors as temperature (Crill et al. 1994; Dunfield et al. 1993), pH (Dunfield et al. 1993), and oxygen availability (Calhoun & King 1997; King 1992, 1994, 1996). Oxygen availability, in turn, may be limited by fluctuations in the water table level (Roslev & King 1996), the efficiency of the plants in oxygenating the rhizosphere (Calhoun & King 1997), and competition with other chemical and biological aerobic processes.

Quantitative estimates of rhizospheric methane oxidation vary widely from relatively small amounts or none (e.g. Lombardi et al. 1997; King et al. 1990; De Bont et al. 1978; Happell et al. 1993) to greater than 90% removal of potentially emitted methane (e.g. Schutz 1989; Holzapfel-Pschorn et al. 1985, 1986; Sass et al. 1990; Gerard & Chanton 1993). In addition to climate factors, the range of estimates may be related to differences in the systems studied such as plant species present or soil types. For example, King et al. (1990) found significant methane oxidation potential to exist in Everglades peat soils, but found no oxidation in Everglades marl sediments.

Much of the variability, though, appears to come from the various techniques used to estimate methane oxidation. Aerobic incubations of peat or sediment cores in the laboratory are useful in describing the spatial distribution of methane oxidizing bacteria, but likely grossly overestimate methane oxidation occurring *in situ* (Van der Nat et al. 1997; Gerard & Chanton 1993; King 1994). Oxidation rates calculated by subtracting *in situ* methane emission rates from methane production rates measured with anaerobic incubations in the laboratory tend to yield high estimates of methane oxid-

ation (Schutz 1989; Sass et al. 1990; Holzapfel-Pschorn et al. 1985, 1986; King 1994; Gerard & Chanton 1993). Methane oxidation estimates using the production minus emission technique could be considered upper limits (Gerard & Chanton 1993) as the production rate term is likely an overestimate of *in situ* methane production (Kelley et al. 1995; Denier van der Gon & Neue 1996). Biological inhibitors applied *in situ* provide a more direct measure of oxidation. An increasingly common inhibitor used to detect methane oxidation in soils is methyl fluoride (CH_3F, Van der Nat & Middelburg 1998, Lombardi et al. 1997; Schipper & Reddy 1996; Denier van der Gon & Neue 1996; Epp & Chanton 1993). The inhibitory effect of CH_3F on methanotrophic bacteria has been found to be nearly complete and reversible (Matheson et al. 1997, Oremland & Culbertson 1992). The effectiveness of CH_3F has been evaluated positively in greenhouse studies of whole plants (Epp & Chanton 1993; Schipper & Reddy 1996). When extending the application to field studies, CH_3F use generally has given lower estimates of oxidation than rate derived from laboratory incubation techniques (Epp & Chanton 1993; Lombardi et al. 1997; Van der Nat & Middelburg 1998; Denier van der Gon & Neue 1996). Recently, CH_3F has also been shown to partially inhibit methane production (Frenzel & Bosse 1996), potentially confounding oxidation estimates. Finally, stable isotope tracing has been used in field studies to detect methane oxidation (Popp et al. 1999; Alperin et al. 1988; King et al. 1989; Happell et al. 1993, 1994; Tyler et al. 1994, 1997; Chanton et al. 1997).

In this paper we evaluate methane oxidation at a fen site using techniques commonly found in the literature. (1) Aerobic peat incubations are used to qualitatively describe the distribution of methane oxidizers and their association with the rhizosphere, (2) CH_3F inhibition experiments in the field provide a direct quantitative measure of *in situ* oxidation. (3) The difference between *in situ* methane emissions and methane production potentials measured by anaerobic peat incubations in the laboratory provide an indirect quantitative estimate of methane oxidation. We consider the merits of each technique for quantifying rhizospheric methane oxidation. Comparisons of these techniques within the same system are useful for examining their validity and make each worth investigating. Indeed, we find estimates via the production minus emission technique are high relative to the more direct CH_3F inhibition method.

Methods

Site description

Work was conducted at a nutrient rich, pH neutral fen located in a mixed wood boreal forest approximately 110 km north of Edmonton, Alberta, Canada (54.6°N, 113.4°W). The fen was chosen for its homogeneity and its accessibility to the University of Alberta's Meanook Biological Research Station. The sedges *Carex aquatilis* and *Carex rostrata* were the dominant vegetation type. They reached maximum heights of 80 to 100 cm. Peat accumulation exceeded 60 cm in depth and in one instance a clay layer was found beginning at a depth of 70 cm. The rhizosphere in this system included approximately the top 20 cm of the peat column, based upon root profiles and visual inspection of peat cores.

At most times, the water surface was 10 to 20 cm above the peat surface. However, the water level fell below the peat surface during an extended dry period, from September 1994 into May 1995.

The fen site was manipulated to contain three distinct areas, namely control-vegetated (CV), fertilized-vegetated (FV), and nonvegetated (NV) sites. Sites were arranged so that control areas were always upstream of treated areas. NV sites were maintained free of vegetation by regularly clipping the plants below the water level over the three-year sampling period. Over the course of the study, progressively less clipping was necessary as plants failed to reestablish. FV sites were treated with a blend of urea and phosphate on roughly a bi-weekly basis. By the third year of treatment (1996) above ground biomass at FV sites was consistently greater than that at CV sites in number of stems, average stem height, and average mass per stem (Popp 1998). The sampling season was defined by the freeze-thaw cycle, generally spanning from the beginning of May to the end of October of each year (1994–1996).

Laboratory peat core experiments

Sample collection. Peat cores were obtained in 1995 with a thin walled aluminum pipe (inside diameter = 14 cm; length = 50 cm), and in 1996 with a stainless steel corer (i.d. = 16.5 cm; l = 75 cm). Cores were transported intact to the laboratory, usually within an hour of collection, where they were removed from the corer and sectioned into 5 or 10 cm depth intervals. A portion (one-quarter to one-half) of each subsection was immediately dried at 60 °C to a constant mass, and used to determine dry bulk density. The remaining portion of each core subsection was used for aerobic and anaerobic incubations.

Root profiles. Live roots were separated by hand from the bulk peat of three cores (FV-7/95, FV-9/96, and CV-9/96) to determine depth profiles of root density. The distinction between live and dead roots was made based on color and turgidity.

Aerobic incubations. Methane oxidation potential and the spatial distribution of methane oxidizers were determined using aerobic incubations of bulk peat. Wet bulk peat (approximately 1g dry mass) from each depth subsection was placed into 125-ml Erlenmeyer flasks in triplicate. Flasks were then sealed with a stopper fitted with a needle and three-way stopcock and briefly evacuated. A mixture methane and air was added to the headspace of each flask such that methane concentrations were 1–2% by volume and the internal pressure was 1.6 atm. Samples were incubated for three to five days, during which four to seven 5-ml aliquots of the headspace were taken at regular intervals to monitor the depletion of CH_4 concentrations over time. Samples were analyzed on a Shimadzu 8A gas chromatograph with a flame ionization detector, 1 ml sample loop and a 2 m Poropak Q column. Flasks were shaken vigorously before each sample was taken to ensure that the headspace was well mixed. Following the incubation period, a dry weight was determined for each peat sample. Only incubations performed at ambient soil temperature (as measured at 10 cm depth) are presented.

Methane oxidation rates were calculated using a linear regression of methane concentration versus time ($R^2 > 0.90$). Oxidation showed zero order kinetics above 2000 ppm CH_4 with no lag phase. Nonlinear portions of regressions below this threshold were omitted from calculations as our interest was in maximum potentials. Rates were converted to area units ($g(CH_4)m^{-2}d^{-1}$) using dry bulk density and integrating over the 40 or 50-cm depth of the core with a step function, permitting direct comparison to emission rates and oxidation estimates via the CH_3F approach (described below).

Live roots in the upper 10 cm of the core were separated from bulk peat, gently rinsed with distilled water to remove clinging wet peat and placed in incubation flasks and sampled as above.

Anaerobic incubations. Anaerobic incubations were used to measure CH_4 production potential. Each flask was filled with about 30-ml of O_2 free distilled water to cover the peat and the head space was evacuated and purged with 100% $N_2(g)$ mixture three times. With the third nitrogen addition, flasks were pressurized to 1.6 atm. To limit exposure to oxygen during sample preparation, the protocol was altered in 1996 to include the use of a nitrogen filled glove bag when sectioning the core. The effect of using the glove

bag was found to be significant in an experiment in which separate subsamples of homogenized peat were incubated after preparation with and without the glove bag, resulting in methane production rates of 268 ± 21 and 165 ± 15 mg(CH_4)$g^{-1}d^{-1}$ respectively (n = 5 for each average). Lower rates obtained without the use of the glove bag could be due to oxygen toxicity on methanogens. Since rates were linear over the incubation period there was probably very little re-growth of the methanogen population. Flasks were pre-incubated undisturbed for 1 to 2 days then sampled once or twice a day over a 3 to 5 day incubation period. Production rates were calculated with a linear regression of the CH_4 concentration in the headspace versus time ($R^2 > 0.90$). In very few cases an initial lag phase in methane production was observed, the nonlinear regions of the results were excluded from the regressions. Production rates were integrated to 40 or 50 cm as for oxidation rates.

Methyl fluoride experiments

In situ methane emissions and oxidation. To estimate rhizospheric methane oxidation directly, methyl fluoride was applied to whole plants at both FV and CV sites. Estimates of methane oxidation were calculated from the difference between emission rates before and after a CH_3F incubation period with the post-CH_3F treatment flux representing potential methane emissions in the absence of oxidation.

To measure uninhibited (control) methane emission rates, cylindrical Plexiglas chambers (diameter = 19.4 cm; volume = 19–25 L) open at both ends, were gently placed over 4 to 15 *Carex* stems and rested on the peat surface, forming an air tight seal at the base with the water table. Chambers were secured with bungee cords wrapped around stakes placed to fit just outside of the chamber. To minimize disturbance to the system and stress on the plants when sampling, open chambers were secured one hour to one day prior to control flux measurements. Each chamber was fitted with a sampling line and a small fan to ensure the headspace was well mixed. Because our interest was methane oxidation occurring in the rhizosphere, measurable by fluxes through plant stems, the floodwater was gently stirred with air bubbles for up to 30 minutes before sampling to reduce the diffusive flux across the floodwater/air boundary. Any remaining diffusive flux was assumed to be negligible compared to flux through the plant stems, which accounts for greater than 90% of the flux to the atmosphere in this wetland (Kelker & Chanton 1997). After the floodwater was stripped of CH_4 chambers were sealed with clear plastic wrap and rubber bands. Aliquots ($\sim$30 ml) of the headspace were sampled every 4 to 5 minutes over a 24 to 30 minute period beginning immediately after closing the chamber. One or more replicate flux measurements were

collected at each site, with an 8–10 minute venting period between each time series of samples. Within six hours after the final control flux measurement, CH_3F (1.5–3% by volume head space) was added to closed chambers and left to incubate overnight (roughly 16 hours). Chambers were shaded during the incubation period to reduce stress on the plants by keeping internal temperature near ambient and conserving CO_2 during daylight hours. The following day methane emissions were measured as before to determine the post CH_3F treatment flux rate. An effort was made to sample pre- and post-CH_3F treatment fluxes at the same time of day to limit any potential diurnal effects.

Unpaired one-tailed Student's *t*-tests were used to determine whether post-CH_3F treatment fluxes were higher than control fluxes at each individual plot, where $p < 0.05$ was considered significantly different. When flux differences were negative or nonsignificant methane oxidation levels were considered not detectable as an artifact of the technique.

Methane concentrations of all headspace samples were determined by GC analysis on the day of collection. Emission rates were calculated by best-fit linear regressions of CH_4 concentration versus time. Slopes were always positive and R^2 values were almost always greater than 0.99.

Methane production experiments with methyl fluoride. A potential limitation of the methyl fluoride technique is its tendency to partially inhibit methane production (Frenzel & Bosse 1996; Lombardi et al. 1997). The effect of CH_3F on methane production was examined using anaerobic incubations of peat samples. Roughly 10 g of bulk peat from the top 15 cm of a core was placed into each of fourteen 50-ml flasks and prepared as above. Control production rates were determined for each flask before CH_3F was added by removing 0.2 ml aliquots from the headspace periodically over a 3-day incubation. Aliquots were analyzed for CH_4 concentration by direct injection onto the GC column rather than through a sample loop as in previous incubation experiments. Flasks were then purged with N_2, and CH_3F was added to the headspace to bring concentrations to 1.5% by volume in five flasks and to 3% by volume in another five flasks. The remaining four flasks were not altered for the second incubation and served as controls to measure the effect of consecutive incubations. Incubations were restarted, and again the rate of CH_4 accumulation in the headspace was monitored over a 3-day incubation period.

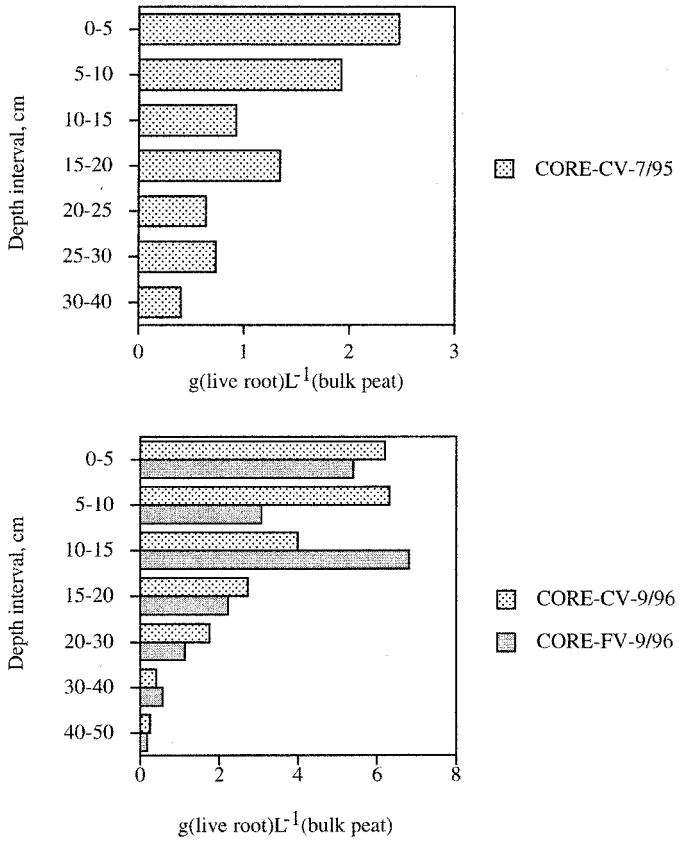

Figure 1. Live root density from three cores. Note the variation in depth intervals from the upper and lower panels. The upper 20 cm of the peat column was designated the rhizosphere.

Results

Laboratory peat core experiments

Root density. Live roots were found at all depths sampled up to 50 cm. Root densities were greatest near the surface and generally decreased with depth (Figure 1). Only a limited number or root profiles was measured (n = 3) and more should be done to properly study root distributions. Nevertheless the trend in Figure 1 is clear and visual inspections of dozens of peat cores confirm these profiles.

Methane oxidation in aerobic incubations. Methane oxidation potential as measured by aerobic incubations of peat in flasks exhibited a decreasing trend with depth in the peat column (Figures 2 and 3), matching the shape

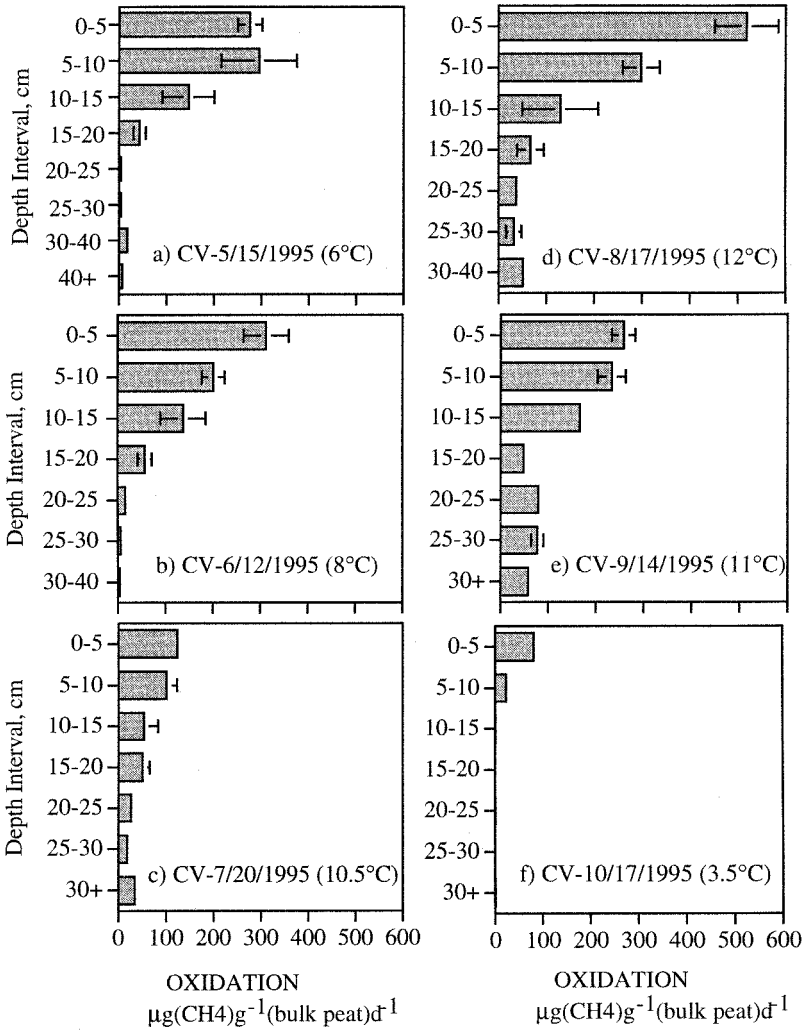

Figure 2. Depth profiles of methane oxidation measured in aerobic incubations of bulk peat from the 1995 CV-site series of cores. Incubations performed at ambient soil temperature as indicated.

of the live root profile (Figure 1). Methane oxidation measured for bulk peat of the non-vegetated site core (NV-7/96) was not significantly different than the oxidation rate of the CV (control vegetated) site cores of the same time period (CV1-CV3-7/96; ANOVA, $p > 0.05$; Figure 3).

Methane oxidation rates from incubations of rinsed fresh roots were highly variable, ranging from nondetectable levels to 610-mg(CH4)g^{-1}(roots)d^{-1} (Table 1).

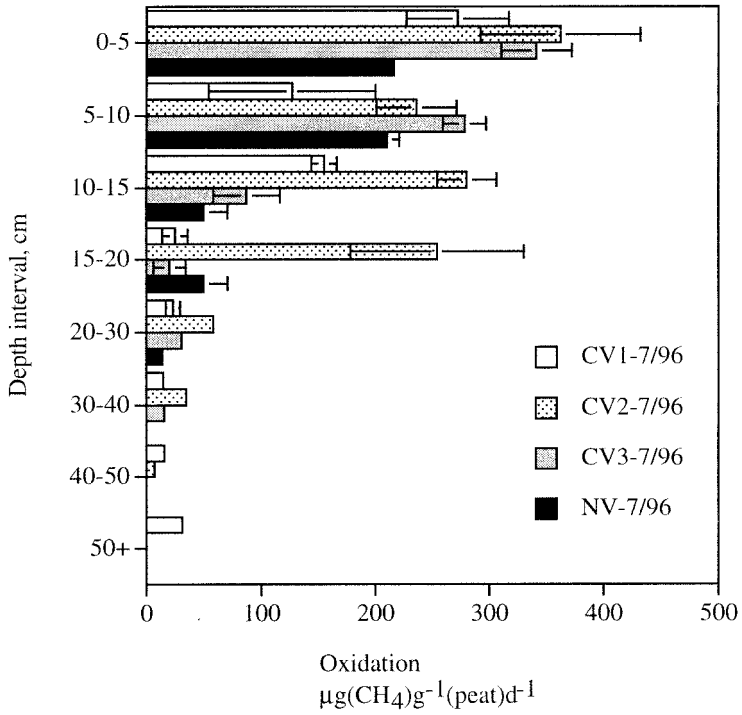

Figure 3. Depth profiles of methane oxidation measured in aerobic incubations of bulk peat from three cores from CV sites and one core from an NV site pulled in 7/96. Graph shows the spatial variability among cores. Incubations performed at ambient soil temperature (11 °C).

Cores taken from CV sites in 1995 yielded integrated methane oxidation rates of 400 to 5210 mg(CH_4)$m^{-2}d^{-1}$ (Table 2). Three CV cores pulled simultaneously in July 1996 (CV1-CV3-7/96) had integrated oxidation rates of 3210, 5220, and 4650 mg(CH_4) $m^{-2}d^{-1}$ (Table 2).

Methane production in anaerobic incubations. Like methane oxidation, depth profiles of methane production showed a decreasing trend with depth (Figures 4 and 5). In a few cases little or no methane production was detected in the 0–5 cm depth interval, suggesting that this interval may at times be suitably oxygenated *in situ* to almost completely inhibit methane production through elevated redox or direct oxygen toxicity to methanogens.

Cores taken from CV sites in 1995 yielded integrated methane production rates of 150 to 810 mg(CH_4)$m^{-2}d^{-1}$ (Table 2). Integrated production rates were higher for the three cores pulled in July 1996 (CV1-CV3-7/96), ranging from 830 to 2050 mg(CH_4)$m^{-2}d^{-1}$ (Table 2), and may reflect the change in protocol in 1996 to include the use of a N_2 filled glove bag during sample

Table 1. Incubations of rinsed live roots. Methane oxidation rate is per gram root. By rinsing roots only methanotrophs tightly attached to the root surface, or within the root, would be included.

Core	Temperture °C	Oxidation $\mu g(CH4)g^{-1}d^{-1}$
CV-5/15/95	6°	178 ± 20
	20°	515 ± 63
CV-6/12/95	8°	610 ± 254
	20°	200 ± 161
CV-7/4/95	20°	44 ± 23
FV1-7/4/95	20°	64 ± 21
FV2-7/4/95	20°	47 ± 54
CV-7/20/95	10°	119 ± 9
CV-8/17/95	12°	not detected
	20°	not detected

Table 2. Methane oxidation and production potentials integrated over the depth of each core incubated at ambient soil temperature. Methane emission rate from control flux of methyl fluoride experiments of the appropriate time. Estimate of oxidation determined by substracting emissions from production rate and dividing the difference by the production rate.

Core	T °C	Laboratory Oxidation (MO) $mg(CH4)m^{-2}d^{-1}$	Laboratory Production (MP) $mg(CH4)m^{-2}d^{-1}$	Methane Emissions (ME) $mg(CH4)m^{-2}d^{-1}$	Fraction Oxidized (MP-ME)/MP
CV-5/95	6	3980 ± 370	370 ± 60	29.5	0.92
CV-6/95	8	2690 ± 260	220 ± 50	43	0.80
CV-7/95	10.5	2360 ± 250	810 ± 100	118	0.85
CV-8/95	12	5210 ± 480	500 ± 50	174	0.65
CV-9/95	11	4090 ± 160	720 ± 40	243	0.66
CV-10/95	3.5	400 ± 70	150 ± 40	63	0.58
CV1-7/96	11	3210 ± 410	2050 ± 190	166	0.92
CV2-7/96	11	5220 ± 460	830 ± 120	166	0.80
CV3-7/96	11	4650 ± 300	1120 ± 100	166	0.85

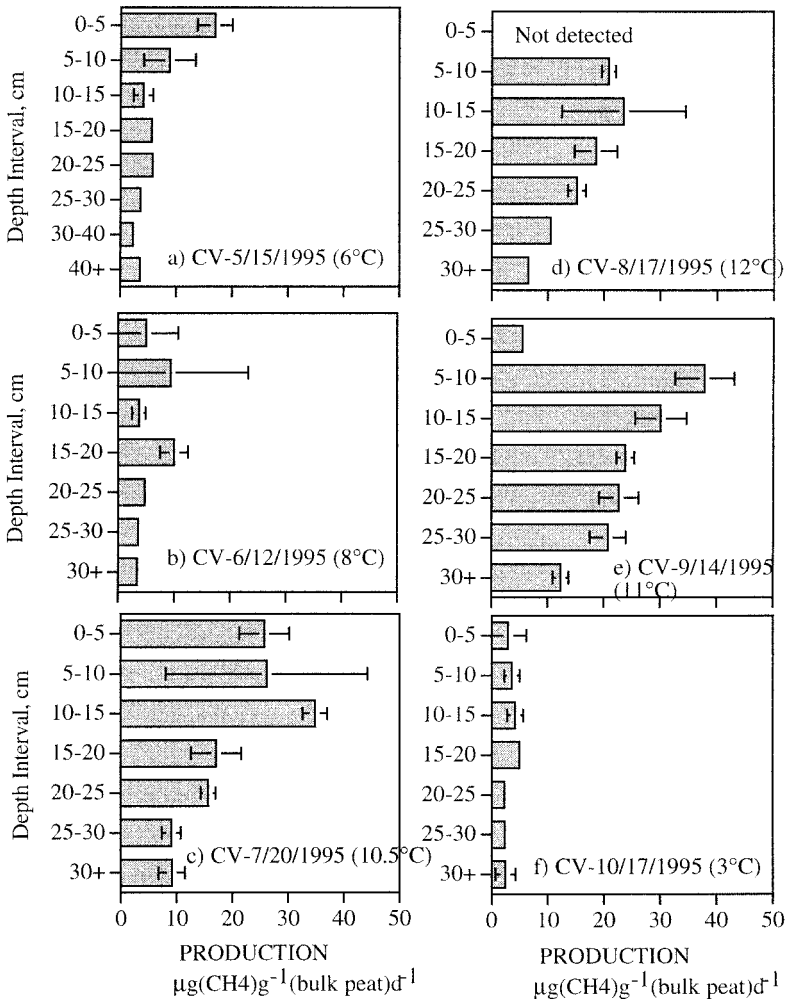

Figure 4. Depth profiles of methane production measured in anaerobic incubations of bulk peat from the 1995 CV-site series of cores. Incubations performed at ambient soil temperature as indicated.

preparation. Methane production rates were less than laboratory oxidation potentials by about two to ten fold (Table 2).

Oxidation estimates from production rates. In all cases methane production potentials measured in the laboratory were high relative to *in situ* emission rates. The percentage of methane oxidized in the rhizosphere implied by the difference of production and emission rates ranged from 58 to 92% (Table 2).

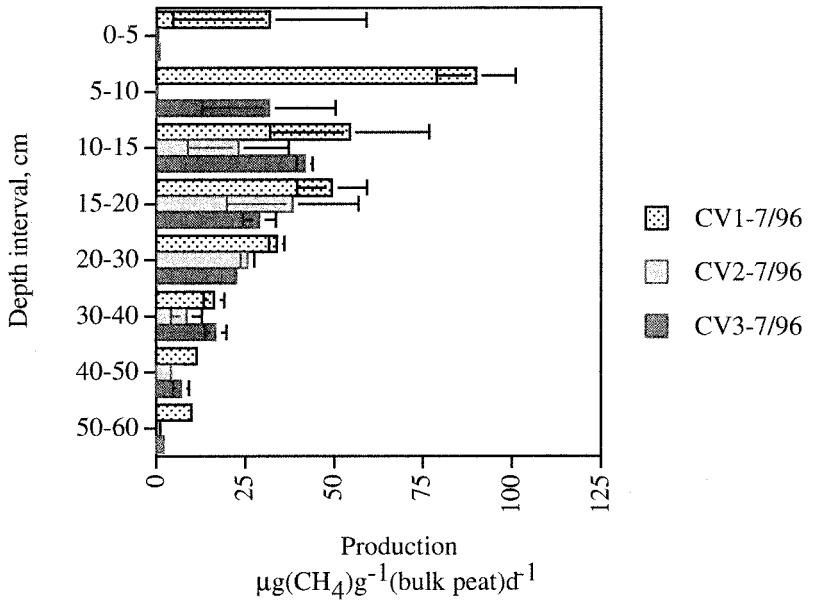

Figure 5. Depth profiles of methane production measured in anaerobic incubations of bulk peat from three cores pulled simultaneously on 7.8.96, showing the spatial variability among cores. Incubations performed at ambient soil temperature (11 °C).

Experiments with methyl fluoride

In situ emissions and oxidation. Post CH_3F incubation fluxes differed from control fluxes by –81 to +189 mg$(CH_4)m^{-2}d^{-1}$. On occasions when negative values were obtained for oxidation (25 of 105 cases), values were considered non-detectable by the CH_3F technique. The corresponding reduction in methane emissions due to oxidation in the rhizosphere was between nondetectable (0%) and 63% of the potential flux, with a median of 10% and a mean of 15 ± 15% (n = 105, average includes positive flux differences not significant at 95% confidence interval (n = 12) and values of 0% assigned to all negative "oxidation" cases (n = 25)).

Methane emission rates peaked in August of each year, generally coinciding with the lowest oxidation percentages and the highest frequency of non-detectable oxidation (Figures 6, 7, and 8). As emission rates increased to the late summer maxima, there was a decreasing trend in oxidation percentages, scattered roughly ±20% around the 0% baseline (Figures 6, 7, and 8). The CH_3F technique was less sensitive in detecting relatively small oxidation percentages associated with high emission rates, as the occurrence of nondetectable and nonsignificant values increased under these circumstances.

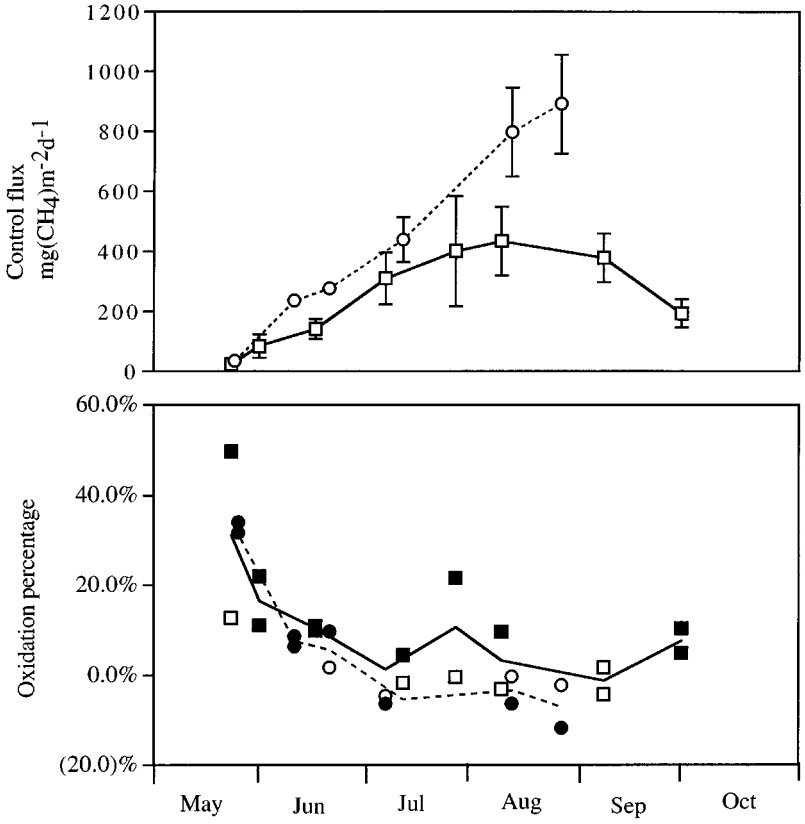

Figure 6. 1994. *Upper panel*: Control methane emission rates averaged for plots within CV and FV sites. *Lower panel*: Reduction in flux due to rhizospheric methane oxidation as determined by methyl fluoride inhibitor experiments. Squares = CV sites, circles = FV sites. Filled symbols in lower panel are values significant at 95% confidence. Solid line is average for CV site and dashed line is average for FV sites. Valus below 0% are considered nondetecable cases of oxidation.

Oxidation percentages at FV sites appeared higher than those at CV sites for methyl fluoride experiments in May, June, and August of 1996, with average values of 44.3% vs 2.2, 18.8 vs 4.3 and 29.5 vs 10.3 respectively (Figure 8). ANOVA analysis revealed that this difference between sites was weakly significant ($p < 0.05$) and that there was significant interaction between site and date of sampling ($p < 0.01$). The fertilizer treatment had no effect on oxidation percentages at all other times (Figures 6 and 7; $p > 0.05$ two-way ANOVA based on sampling date and site).

Seasonal trends in oxidation percentage were significant in 1994 and 1995, but not in 1996 (two-way ANOVA based on sampling date and site), although

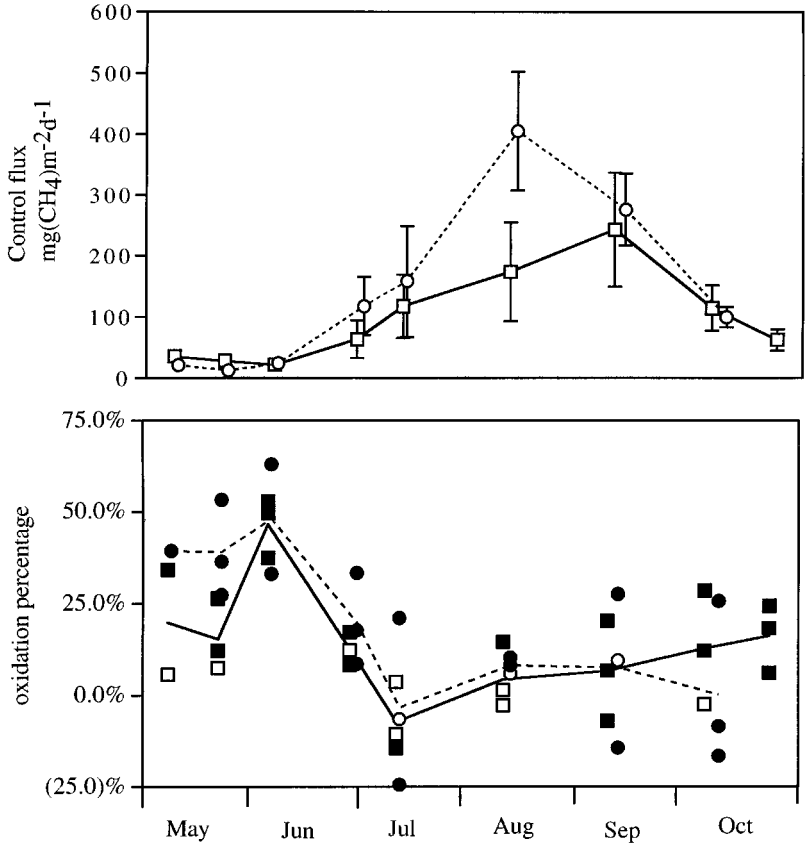

Figure 7. 1995. *Upper panel*: Control methane emission rates averaged for plots within CV and FV sites. *Lower panel*: Reduction in flux due to rhizospheric methane oxidation as determined by methyl fluoride inhibitor experiments. Squares = CV sites, circles = FV sites. Filled symbols in lower panel are values significant at 95% confidence. Solid line is average for CV site and dashed line is average for FV sites. Valus below 0% are considered nondetecable cases of oxidation.

the trend in 1996 at FV sites alone was significant. During May and June 1995, when the potential for oxidation was highest due to low surface water levels significant oxidation was detected in 12 of 14 cases (student's *t*-test, $p < 0.05$) with a mean of $34.1 \pm 17.4\%$, but these values may reflect changes in the O_2 gradient at the peat surface rather than plant associated oxidation.

Effect of CH_3F on methane production. On average, a 76% and 82% reduction in methane production occurred when the peat was exposed to 1.5% and 3% CH_3F respectively. A 15% reduction in production rate occurred in flasks when no CH_3F was added to the second incubation.

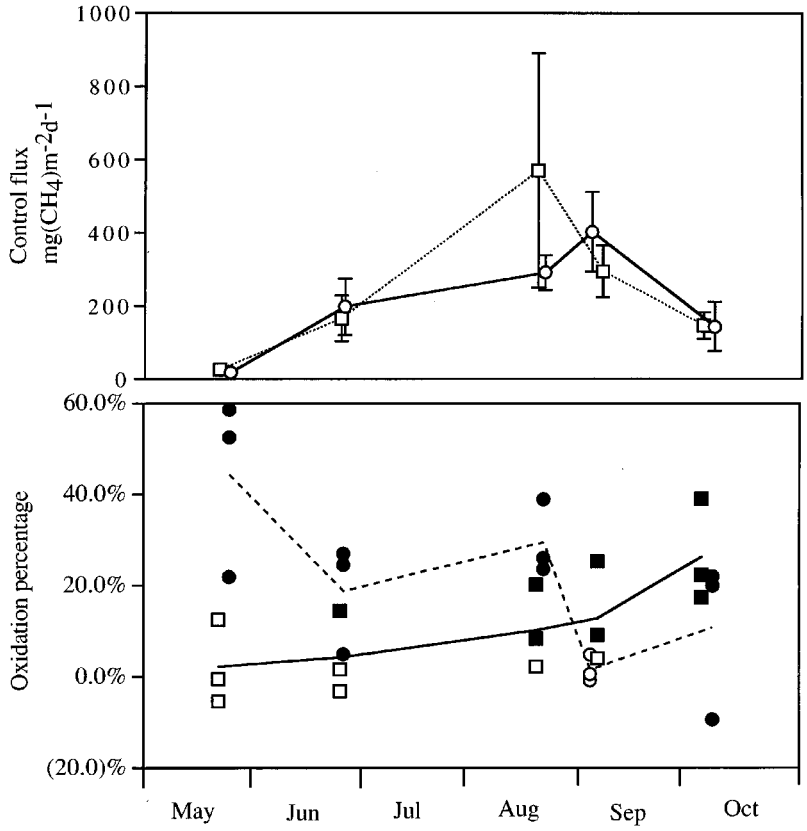

Figure 8. 1996. *Upper panel*: Control methane emission rates averaged for plots within CV and FV sites. *Lower panel*: Reduction in flux due to rhizospheric methane oxidation as determined by methyl fluoride inhibitor experiments. Squares = CV sites, circles = FV sites. Filled symbols in lower panel are values significant at 95% confidence. Solid line is average for CV site and dashed line is average for FV sites. Valus below 0% are considered nondetecable cases of oxidation.

Discussion

Aerobic incubations

In the aerobic incubations, initial conditions inside the flasks were such that both oxygen and methane were nonlimiting. Therefore calculated oxidation rates represent the maximum potential (Vmax) at a given point in the peat column and certainly overestimate *in situ* rates. Plant associated methane oxidation can be suppressed in natural waterlogged soils by oxygen limitation, which is linked to the ability of plant species to oxygenate their rhizosphere (Calhoun & King 1997; King 1994, 1996). Methanotrophs must

compete with other aerobic processes for free oxygen, if any, remaining after the oxygen demands of root respiration are met (Reddy et al. 1989). Methane concentrations may also limit methane oxidation (Gilbert & Frenzel 1995), but likely did not affect this system as porewater methane concentrations were generally high throughout the peat column (>2000 ppm when equilibrated with air, Whiting, unpublished data), although values were often lower in surface peat layers.

If not good quantitative measures of methane oxidation, aerobic incubations still reveal the importance of the plants in supporting methanotrophs. The spatial distribution of oxidation shows a clear association between methanotrophs and the rhizosphere (Figures 2 and 3), also demonstrated by similar methods in other vegetated wetland systems (e.g. Van der Nat et al. 1997; King 1994; King et al. 1990; Gerard & Chanton 1993). Furthermore, incubations of rinsed roots show significant oxidation potential directly associated with the root surfaces. These root incubations suggest a tight coupling between methanotrophs and the inside and/or outside of the root surface, but underestimate the potential indirect importance of the roots through oxygen delivery to areas nearby but away from the root surfaces.

Methanotrophic bacteria have been shown to survive extended periods of anoxia and carbon depravation, perhaps by utilizing a poorly defined anaerobic maintenance metabolism, while showing a relatively fast recovery time following starvation conditions (Roslev & King 1994, 1996). This ability is demonstrated by the existence of considerable oxidation potential at depths below the rhizosphere where conditions should be consistently and completely anaerobic (Figures 2 and 3) and at nonvegetated sites where the lack of emergent vegetation effectively shuts off delivery of oxygen to below the peat surface (Figure 3). The spike in oxidation percentages observed at the beginning of 1995 in the CH_3F experiments (Figure 7) is also consistent with this finding as fluctuations in the water table can cause changes in the oxygen gradients near the peat surface, thus increasing the potential for oxidation during drier times.

Estimates of methane oxidation

The two methods used to quantify methane oxidation contrast sharply. Estimates of oxidation calculated by subtracting emissions from production potentials measured in the laboratory are high relative to those measured directly *in situ* with the methyl fluoride technique (Table 3). Much of this discrepancy may lie in the limitations of each technique.

Methane oxidation via laboratory production rates. Methane oxidation calculated by subtracting emissions from production rates measured in incubations

Table 3. Rhizospheric methane oxidation estimates using three techniques at the fen site

Date	Oxidation estimate via: Production – emissions	Methyl Fluoride	Stable Isotopes*
May-95	92%	27%	—
Jun-95	80%	30%	16%
Jul-95	85%	not detected	33%
Aug-95	65%	6%	4%
Sep-95	66%	7%	—
Oct-95	58%	10%	not detected

*From Popp et al. 1999

(Table 2) has often given the highest estimates (e.g. Gerard & Chanton 1993; Holzapfel-Pschorn et al. 1985; Schutz et al. 1989). *In vitro* methanogenesis may not reflect rates *in situ* for several reasons, and are almost certainly over estimates as measured here. Within the incubation flasks strict anaerobic conditions prevailed, a condition that may not exist in all areas of the rhizosphere, especially where oxygen leaks from roots to fuel oxidation. Denier van der Gon and Neue (1996) concluded that the stimulation of methane emission rates from rice while exposed to a 100% N_2 atmosphere was in large part due to increased methane production because the inhibition of methanogenesis in the oxidized parts of the rhizosphere was no longer present. Furthermore, as an artifact of sample preparation, a greater amount of labile organic matter may become available for methanogenesis through the mechanical disruption of intact live roots and other biomass. For these reasons, the flask incubation results may represent upper limits to *in situ* methane production at the ambient temperature and thus would lead to an unrealistic quantitative estimate of methane oxidation when compared to *in situ* emission rates.

Methane oxidation via methyl fluoride and its limitations. The CH_3F technique showed that the relative importance of rhizospheric methane oxidation was greatest at the beginning of each growing season with a decreasing trend as emission rates increased through the summer months (Figures 6, 7 and 8). A similar seasonal trend has been reported in other freshwater wetlands (Lombardi et al. 1997; Van der Nat & Middelburg 1998). Although higher peat temperatures yield higher oxidation rates when measured in flasks (Popp 1998; Crill et al. 1994; Dunfield et al. 1993), the importance of methane oxidation in the field decreases during the warm months, as oxidation fails to keep pace with increases of *in situ* production and emissions. The supply of O_2 available to methanotrophs may vary seasonally due to an increased

demand for O_2 by aerobic heterotrophs in response to greater root exudation or by the increased demand for O_2 to support higher root respiration rates during the most productive times. The net oxidation determined by the CH_3F technique (<20% seasonally) contrasts sharply with those determined by using in vitro production potentials and are in general agreement with oxidation percentages via stable isotope mass balance at this site (Table 3, Popp et al. 1999).

In measuring methane oxidation at fertilized sites, it was hypothesized that higher productivity through fertilizer treatment could increase the potential for rhizospheric oxidation by increasing oxygen delivery to the roots to support higher respiration rates. Alternatively, N addition has been observed to inhibit methane oxidation in various soils, including peat (e.g. Crill et al. 1994; Van der Nat et al. 1997). The effect of the fertilizer application on aboveground biomass was significant by the beginning 1996 (Popp 1998). Aboveground biomass measured at a fertilized site at the end of May 1996 was roughly twice that of a control site, and remained substantially greater than control sites throughout the year. Only early in 1996 did methane oxidation at the fertilized sites appear to be greater than that at control sites (Figure 8). Apparently, relatively higher respiration rates at fertilized sites (Whiting, unpublished data), and the coincident relative increase of oxygen turnover below ground, was more important early in the season than later in 1996 when oxidation estimates between the two sites equalized or the effect began to be canceled out N induced inhibition of methane oxidation.

The effectiveness of the methyl fluoride technique should be discussed. Previously, in greenhouse studies the CH_3F technique predicted oxidation percentages up to more than 90% for *Pontederia cordata* and *Sagittaria lancifolia* potted in buckets (Lombardi et al. 1997; Schipper & Reddy 1996, Epp & Chanton 1993), on par with the high estimates predicted by laboratory production potentials (e.g. Gerard & Chanton 1993; Shipper & Reddy 1996). Experiments performed *in situ* on the same species, however, yielded much lower estimates; on the order of 20% (Epp & Chanton 1993; Lombardi et al. 1997). It is hypothesized that the difference in the greenhouse versus *in situ* measurements was due to higher root densities found in potted plants than in the field, and consequently more oxygen was available to methanotrophs (Lombardi et al. 1997).

The effectiveness of the CH_3F technique depends on the mechanism by which methane oxidation occurs. Lombardi et al. (1997) offer a conceptual model describing how oxidation must occur for the CH_3F technique to give an accurate measure of rhizospheric oxidation. Essentially, the methane signal must be attenuated as it diffuses across an oxic halo upon entering a root and it is at this point that CH_3F halts methane oxidation. The methane oxidation

observed to be associated with the root surface in rinsed root incubation is consistent with this model. Alternatively, if methane oxidation occurs in the bulk peat away from the root, CH_3F serves to increase the methane concentration of the bulk sediment and thus the diffusion gradient driving emissions. In this case, the measure of oxidation would depend on the reservoir size and turnover time, becoming less sensitive as each increased. Based on the spatial distribution of oxidation determined by aerobic incubations of peat, it seems likely that a combination of both mechanisms is at work, hence the potential that these oxidation estimates are underestimates.

While CH_3F completely inhibits methane oxidation, it also significantly inhibited methane production in laboratory (see also Frenzel & Bosse 1996; Janssen & Frenzel 1997). While here we found greater than a 50% reduction in production, other studies have shown less than 20% attenuation of production rates due to CH_3F in similar experiments (Lombardi et al. 1997; Schipper & Reddy 1996). If methanogenesis is equally inhibited by CH_3F *in situ* then oxidation estimates by this technique would again be underestimates, as an increase in flux due to the lack of oxidation may be offset by the decrease in flux due to lower production. Conditions *in situ*, however, may not allow for the full expression of this production effect. First, recently in culture studies methyl fluoride, at levels similar to those used in our incubations, was found to inhibit aceticlastic methanogens but not hydrogenotrophic methanogens (Janssen & Frenzel 1997). As stated previously, in flask peat incubations a greater amount of labile organic matter may become available for methanogenesis through the mechanical disruption of intact live roots and other biomass, potentially increasing the role of aceticlastic methanogens in the overall production of methane relative to its role *in situ*. Stable isotope tracing suggests that the relative role of aceticlastic methanogenesis is greater in more labile peat (Popp et al. 1999). Second, the turnover rate of the belowground methane reservoir must be fast enough for the lack of production during CH_3F exposure to effect emission rates over the incubation period. Essentially, with slow turnover the lack of methane production would be buffered by the size of the existing belowground methane reservoir. With typical CH_3F incubation times lasting roughly 16 hours, Lombardi et al. (1997) determined that with turnover rates greater than about 4 days, oxidation estimates would not be significantly effected by CH_3F induced inhibition of methanogenesis. Turnover rates calculated for the 1995 season at this site are generally between 2 and 4 days (Popp 1998) so if the production effect is expressed, oxidation estimates by the CH_3F method may again be underestimated. It follows that the negative values determined by some measurements may be a result of both the inhibition of production and experimental noise. When the partial inhibition of methane production could outweigh the full

inhibition of oxidation, resulting in negative flux differences after the CH_3F incubation, it could be argued that the actual oxidation percentages must have been relatively low (i.e. within the positive scatter of Figures 6, 7, and 8).

Conclusion

Aerobic incubations of bulk peat in flasks clearly show high oxidation potentials associated with the rhizosphere. To what extent this potential is expressed *in situ* is questionable. Methods used to quantify methane oxidation give conflicting results (Table 3). Estimates based on production potentials measured in the laboratory and emissions measured in the field suggest high levels of oxidation, ranging from 58 to 92% of the potentially emitted methane, but are likely unreliable due to overestimate of the production term. A more direct technique using CH_3F on whole plants *in situ* suggest methane oxidation may attenuate emissions by less than 20% seasonally with higher percentages occurring early in the growing season. With this technique methane oxidation appears to be less important during periods of high emissions and more so when emissions are low early in the growing season. The limitations of the CH_3F technique could most likely lead to underestimates of methane oxidation, however it seems to be a better measure than the production minus emission approach. One way to resolve the discrepancy is to model with system using methane stable isotopes (Popp et al. 1999; Tyler et al. 1997; Chanton et al. 1997). In a companion study, the stable isotope distribution at the fen site suggests lower estimates of oxidation (0–34%), in good general agreement with the CH_3F technique (Popp et al. 1999).

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