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Investigation of the methyl fluoride technique for determining rhizospheric methane oxidation

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Abstract. Methane oxidation rates in the rhizosphere of *Pontederia cordata, Sagittaria lancifolia,* and *Typha latifolia* were quantified in field studies using the methyl fluoride inhibition technique. An average oxidation of 22.9 ± 17.7% (sd, n = 44) was found for all field experiments (oxidation is expressed as a % of total potential emission in the presumed absence of oxidation). Greenhouse experiments using the same technique gave an average rhizospheric oxidation of 64.9 ± 17.0% (sd, n = 44). Comparison of a subset of greenhouse plants with the methyl fluoride (MF) and a light oxic/dark anoxic (LO/DA) technique for suppressing CH_4 oxidation yielded similar percentages (57.7 ± 15.0% for MF and 58.5 ± 13.9% for LO/DA, n = 11). Rhizospheric oxidation displayed a seasonal trend in *Typha latifolia* with decreasing oxidation percentages during warmer months as the importance of rhizospheric CH_4 oxidation declined relative to CH_4 emission (46.5 ± 13.8% in December and 13.5 ± 1.7% in July). However, the absolute rate of methane oxidation was highest during the warmer months (44.2 ± 3.4 mg m^{-2} d^{-1} in December and 318.7 ± 151.4 mg m^{-2} d^{-1} in July). As methane emission rates increased, the sensitivity of the methyl fluoride technique decreased due to the larger error between replicate flux measurements.

Introduction

The annual flux of CH_4 to the atmosphere has been estimated to be roughly 540 × 10^{12} g CH_4 (Cicerone & Oremland, 1988). With their high organic productivity and the attenuation of O_2 diffusion due to flooded or waterlogged conditions in soils, natural wetlands and rice paddies represent the largest single source of CH_4, providing approximately 40% of the total CH_4 released to the atmosphere annually (Cicerone & Oremland 1988). The large values for natural wetlands and rice paddies are, in part, a consequence of the plants found there. Emergent aquatic plants facilitate gas transport to the atmosphere and can account for 80 to 90% of the total emitted CH_4 from both natural wetlands and rice fields (Schütz 1989a, b; Holzapfel-Pschorn & Seiler 1986; Holzapfel-Pschorn et al. 1985, 1986; Whiting et al. 1991; Bartlett

et al. 1992; Chanton et al. 1992; Happell et al. 1993; Morrissey & Livingston 1992). In addition to acting as conduits for CH_4 release, these plants also provide organic material to methanogenic microbes in the form of leaf litter, root litter and exudates.

Microbial methane oxidation serves as a sink for atmospheric CH_4 in dry soils (Tyler 1991; Crill 1991) and serves to attenuate CH_4 release from wetlands (King 1992; Reeburgh et al. 1993). In waterlogged soil, CH_4 oxidizers (methanotrophs) act as a sink for CH_4 which is produced in the anoxic layers of the soil, thus shielding the atmosphere from the release of additional CH_4 (King 1990b; King 1992). Oxidation in these environments (Figure 1) occurs at the oxic-anoxic interface which is generally (1) near the water table surface in unsaturated sediments and peats or at the sediment-water interface in flooded wetlands and (2) in the rhizosphere, the area of soil influenced by the roots through organic matter exudation (Curl & Truelove 1986), where O_2 supplied to fuel root respiration leaks outward (Armstrong & Armstrong 1988; Bedford et al. 1991; Smits et al. 1990). Through the use of microelectrodes, Frenzel et al. (1992) have shown that considerable O_2 may be present in the rhizosphere of rice microcosms. Consequently, methanotrophic bacteria could live within the rhizosphere or along the rhizoplane and oxidize the CH_4 that would otherwise travel unimpeded through the plant (King et al. 1990; Chanton & Dacey 1991).

Experiments quantifying rhizospheric CH_4 oxidation have yielded oxidation percentages ranging from little or no methane oxidation (De Bont et al. 1978; King et al. 1990; Gerard 1992; Happell et al. 1993) to 70 to 90% of the potential CH_4 emitted (e.g. that quantity which would be emitted in the absence of any oxidation; Schütz 1989a; Holzapfel-Pschorn et al. 1985, 1986; Sass et al. 1990). This disparity could be a result of variation in O_2 availability to the methanotrophic bacteria caused by competing demands of root respiration (Chanton et al. 1992; Conrad 1993), nitrification of ammonia (Reddy et al. 1989), and ferrous iron oxidation (Green & Etherington 1977; Macfie & Crowder 1987). Further variability could result from seasonal changes associated with temperature or plant development. Relationships between plant ripening and flooding and CH_4 oxidation have been demonstrated (Schutz et al. 1989a; Sass et al. 1992; Conrad 1993). The number of CH_4 oxidizing bacteria in the rhizosphere of rice has also been shown to increase over the growth period (Gilbert & Frenzel 1995).

Still, some of the variability in oxidation estimates could arise from the methods used to determine oxidation. Techniques such as (1) the calculation of rhizospheric methane oxidation by difference between methane production, as determined from anaerobic incubation of sedimentary material, and methane emission, or (2) the aerobic incubation of live root material may provide

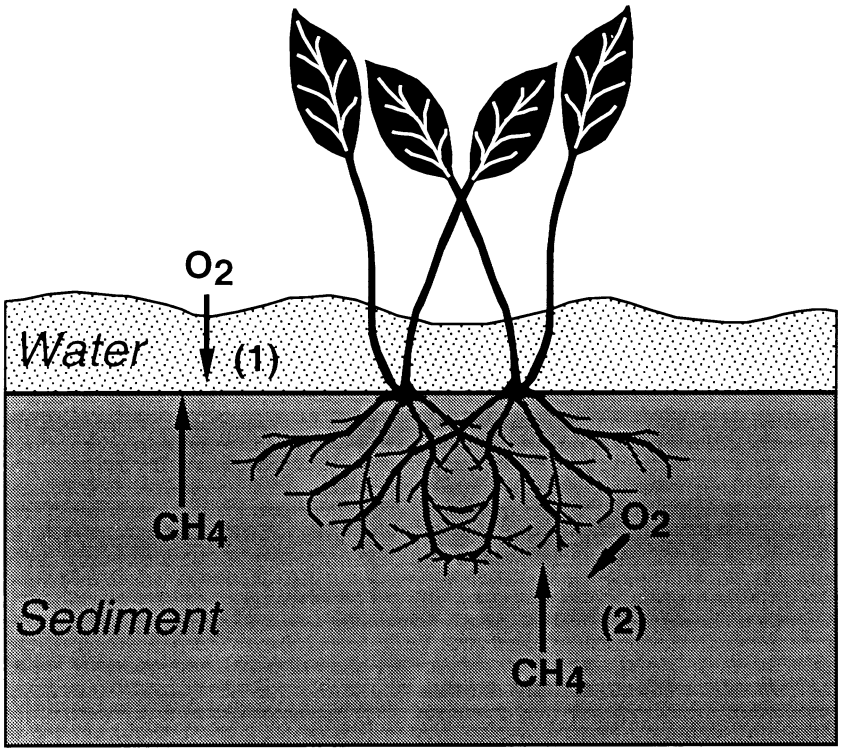

Figure 1. Methane oxidation in freshwater sediments occurs within two zones: (1) the sediment – water interface and (2) the oxygen – methane interface in the rhizosphere.

inaccurate measurements of oxidation because they may alter the balance of organic material or O_2 provided to microbial communities (Gerard & Chanton 1993).

The use of biological inhibitors to aid in the determination of rhizospheric methane oxidation has until recently been inadequate due to the lack of an inhibitor that specifically targets methanotrophs. Using a method employing methyl fluoride (MF, Oremland & Culbertson 1992a, b) as a rhizospheric oxidation inhibitor, Epp & Chanton (1993) found oxidation percentages of 23–90% for *S. lancifolia* and *P. cordata* growing in 20 liter soil mesocosms in a greenhouse. Also employing the MF technique on potted plants, Schipper & Reddy (1996) found an oxidation percentage of 65 $\pm$ 24% (sd, n = 14) for *S. lancifolia*. When using the mass balance approach, where rhizospheric methane oxidation was calculated as the difference between methane production (as measured in anoxic incubations) and methane emission, Schipper and Reddy found oxidation percentages of 79 $\pm$ 20% (sd, n = 14) in the same plants. Thus MF determined rates of rhizospheric methane oxidation in

greenhouse *P. cordata* and *S. lancifolia* are in general agreement with some of the highest estimates as determined by other techniques.

Initial results suggested a quantitative difference between field estimates of oxidation and those obtained in the greenhouse studies on potted plants (Epp 1993). Nevertheless, the greenhouse study proved an excellent location in which to develop the MF technique as oxidation was clearly occurring within the rhizosphere of the potted plants, and the studies also showed that plants could be used to deliver the methyl fluoride to the rhizosphere. Given the potential effectiveness of the technique (Epp & Chanton 1993), the goal of this study was to conduct additional tests to further confirm its reliability and to apply it to field conditions to provide a more realistic estimate of methanotrophic oxidation within the rhizosphere of aquatic macrophytes.

Methods

Oxidation experiments were undertaken on *P. cordata* at Lake Hall, Maclay Gardens, Tallahassee, Florida and on *S. lancifolia* at the St. Mark's Wildlife Refuge, St. Mark's, Florida. Experiments were performed in the same manner as the initial greenhouse study described elsewhere (Epp & Chanton 1993). Briefly, measurements of CH_4 emission were taken before and after incubation with MF, a CH_4 oxidation inhibitor, in the headspace of circular plexiglass chambers (20.3 cm in diameter and 0.7 to 1.8 m high which contained on average about 33.5 ± 7.4 L (sd, $n = 108$)). The open ended chambers, which were fitted with a sampling tube and a small fan for air circulation, were placed over plants a day prior to sampling to reduce the possibility of stress and bubble ebullition from the water saturated soil during sampling.

Because of the strict interest in plant CH_4 emission and to allow differention between rhizospheric methane oxidation and oxidation at the soil-water interface, the CH_4 diffusion through the water column was reduced by gently bubbling the water over the soil with air for 1/2 hour to strip dissolved CH_4. After aeration, diffusive CH_4 flux was calculated from the remaining water CH_4 concentration using a method by Sebacher et al. (1983; see Epp & Chanton 1993). This value was then subtracted from the methane flux value determined via headspace sampling to give an emission value more representative of flux through the plant rather than the soil. The water CH_4 concentration after bubbling was small and plant transport dominated CH_4 emission, generally representing 94% or more of the measured flux. Sampling was done in the morning to lessen the effects of heat and direct sunlight on the plants. Because biomass has been linked to CH_4 emission (Whiting et al. 1991; Whiting & Chanton 1992, 1993; Sass et al. 1990), plants were chosen on the basis of similar biomass as judged visually.

Prior to utilization of the inhibitor of CH_4 oxidizing bacteria, control incubations were undertaken to determine CH_4 emission through the plant. After aeration, chambers were closed using rubber bands and Saran wrap, and headspace samples were withdrawn using 30 ml syringes fitted with plastic stopcocks. Sampling occurred immediately after closure and every 4 to 5 minutes thereafter for 28–30 minutes. Chambers were vented 10 to 15 minutes between collection of two replicate measurements on the same plant. Since in prior experiments (Epp & Chanton 1993, Epp 1993) no statistical difference was observed between controls that had been left open or sealed for the 24 hours preceding flux measurement, an opened control was used in all experiments.

To determine CH_4 emission without the attenuating affects of CH_4 oxidation, plants were incubated with methyl fluoride. Twenty minutes after the last replicate control flux was sampled, MF was added to the closed chambers via the sampling tube along with CO_2 to bring the headspace concentration to 1.5% MF or 3.0% MF depending on the experiment. Past work has shown no significant difference in the effectiveness of the two concentrations (Epp 1993; Epp & Chanton 1993). CO_2 was added to ensure continued plant O_2 production. A tarp was used to shield the chambers from the sun during incubation, thereby reducing the stress to the plants. After an average 22 hour incubation, the chambers were vented and aerated. Sampling of CH_4 emission was performed as described above. The difference between the mean value of CH_4 emission determined under inhibition of CH_4 oxidizers (total potential flux) and emission without inhibition (control) was taken to indicate the amount oxidized by methanotrophs. By dividing this difference by the inhibited flux (total potential flux) the percent of rhizospheric CH_4 oxidation was calculated.

Concentrations of CH_4 and MF for all flux and water samples were quantified by either a Shimadzu Mini-2 or a Shimadzu 8A gas chromatograph with a flame ionization detector (FID), 1 ml sampling loop, and a 2 m Poropak Q column. Scott (Plumsteadville, Pennsylvania, USA) CH_4 standards were used for calibration.

Experiments at Lake Hall began at the end of March, 1993, when *P. cordata* was just emerging from the water, and continued periodically through the beginning of May, 1993 at which time the plants were 19 to 28 cm taller and in full bloom. Water level during sampling was on average 1.0 m.

S. lancifolia at St. Mark's were sampled for two experiments during March, 1994 and one experiment in October, 1994. The same site was utilized both months but 2 different stands of plants were used in March. The water depth during the two months ranged from approximately 0.8 to 1.0 m.

We tested the hypothesis that O_2 diffusing through the walls of the 20L buckets in which the greenhouse plants were growing was responsible for elevated rates of rhizospheric methane oxidation observed in greenhouse plants relative to field plants. Oxidation measurements with MF were conducted on two *P. cordata* growing in buckets and then the buckets containing the plants were buried in a trough containing a flooded soil compost mixture. This prevented O_2 from reaching the roots through diffusion through the plastic walls of the buckets. Four weeks following burial, methane oxidation was remeasured on the same plants.

In addition, after an overnight incubation of 3.0% MF, belowground MF concentrations were examined in five greenhouse pots to determine the extent of diffusion of MF into the sediments. After the MF incubation, pore water samples were obtained using a "swampsucker"; a 0.3 cm stainless steel tube, 0.3 m long, which had several small holes in one end designed to draw in water. The swampsucker was placed next to the chamber and 30 ml pore water samples were taken at a depth of 5 cm. Methane and MF were quantified using the headspace equilibration technique (McAuliffe 1971).

Methane oxidation was also measured for 11 greenhouse plants using the light-oxic/dark-anoxic technique (LO/DA). This technique has been utilized by King et al. (1990), Conrad & Rothfuss (1991), Frenzel et al. (1992), and Gerard & Chanton (1993). The control fluxes were measured as for the MF technique. Plants were then incubated under N_2 overnight and covered with a black-out cloth. The flow of N_2 over this period kept the chambers O_2 free. Darkness prevented photosynthesis and O_2 production thus halting methanotrophic activity. After 12 hours of incubation, the methane flux was measured and the methane oxidation percentage calculated as for the MF technique. The plants did not appear to be stressed by this treatment. After allowing time for O_2 to return to sediment, methane oxidation was determined using methyl fluoride and the two percentages compared.

For clarity, SM was added to a plant's notation to indicate experiments at St. Mark's. Similarly LH represented Lake Hall experiments, GR represented typical greenhouse experiments and GRB greenhouse pots buried in wet-organic soil to test for O_2 leakage through the pot's walls.

A seasonal study of CH_4 oxidation in the rhizosphere of cattails (*Typha latifolia*) growing in a local pond was also undertaken using the MF technique. Two chambers were monitored monthly from February, 1993 to January, 1994. Three replicate flux measurements were taken on each chamber on each day of sampling except for August and September where $n = 1$ and $n = 2$ respectively. Cattails, which utilize a pressurized bulk flow ventilation system (Dacey 1981, Brix et al. 1992), show a peak in CH_4 emission coincident with incoming solar radiation (Chanton et al. 1993). Therefore, oxidation values

for these experiments were determined from paired individual control and treatment fluxes taken at the same time each day to adjust for the light intensity effect. Pond water level was maintained at an average depth of 30 cm throughout sampling. The 3 flux measurements for each of the 2 days (control day and MF day) yielded 3 oxidation percentages which were then averaged. An average for the two chambers was then calculated and one-half the range between the two reported.

Pore water samples were taken at the 20 cm depth to determine a CH_4 inventory for the greenhouse pots. By dividing the methane inventory (moles) by the average open control flux (mol d^{-1}), the days needed for complete turnover of belowground methane were calculated. For St. Mark's and the *Typha* pond porewater equilibrators (Hesslein 1976) were used to obtain CH_4 concentration versus depth profiles, which were integrated over the top 20 cm of sediment to obtain the methane inventory (mol m^{-2}) and then divided by the average open control flux (mol $m^{-2}d^{-1}$) to calculate turnover. All inventories were adjusted for porosity. At St. Mark's and the *Typha* pond gas bubble volume was accounted for by multiplying by a correction factor of 1.2 (Chanton et al. 1989).

Statistical analysis for greenhouse and field experiments was performed using an unpaired one-tailed Student's t-test where $p \leq 0.05$ was considered statistically significant. A paired one-tailed t-test was used to examine the effect of MF in the cattails due to the dependence of the emission on the time of day. In statistical analyses, pre-MF CH_4 flux values were compared to those measured after MF exposure. For comparison, oxidation values were calculated even if t-tests indicated no significant difference at the 95% confidence level between pre-MF and MF treatments. Reduced confidence levels are always indicated. At high rates of methane emission, rhizospheric CH_4 oxidation became difficult to detect due to variability in replicate CH_4 flux measurements. Nonetheless, post-MF treatment rates of CH_4 emission were, except on one date, always greater than pre-MF treatment or control rates. F-tests were also performed to assure that the regressions plotted for each 5 minute sample were significantly different from zero within a 95% confidence interval.

Due to questions regarding the effect of MF on CH_4 production in addition to CH_4 oxidation (R. Conrad, personal communication, 1995), this possible impact was investigated. Rhizosphere material containing abundant fresh roots was placed in 125 ml Erlenmeyer flasks and covered with deoxygenated water. Oxygen was stripped from the flasks by evacuating them briefly and then flushing with N_2 three times. The headspace volume and the dry weight of incubation material was determined. To establish steady state conditions, the flasks were left undisturbed for the 48 hours preceding the experiments.

After 48 hours 1% MF was added to 1/2 the flasks and the other 1/2 were kept as controls. One percent MF was used in this experiment for comparison to Schipper & Reddy (1996) and because over the entire incubation period methanogenic bacteria are likely exposed to lower concentrations than are present in the headspace of the chamber (see below). The rate of production in μg CH_4 g^{-1} dry d^{-1} was monitored by sampling 5 times over the next 48 hours. The rate of methane production for control and MF flasks was compared to determine the impact of MF on CH_4 production rates. An additional experiment was also performed using sediment containing no roots obtained from a nearby lake.

Results

Lake Hall, P. cordata

March results from site 1 at Lake Hall showed that rhizospheric methane oxidation was occurring in the field (Table 1). Fluxes under control treatments ranged from 151.4 to 439.7 mg m^{-2} d^{-1}. In all four plots, fluxes after treatment with 3% MF were significantly higher than control values, resulting in oxidation percentages that ranged from 41.5 $\pm$ 6.8% to 53.0 $\pm$ 6.1%. After an interval of one week, oxidation percentages ranged from 14.9 $\pm$ 9.2% to 32.6 $\pm$ 8.4%, although control and post-MF methane fluxes were not significantly different at the 95% confidence level for one of the plots (Table 1). The average oxidation percentage for the four plots on March 23 was 45.4 $\pm$ 5.1%, while on March 31 the average for the three plots was 24.6 $\pm$ 8.9%.

In April, we utilized a second site at Lake Hall after a tree fell within the first field site. At this time, this site showed no evidence of CH_4 oxidation, unlike the data from site 1 (Table 1). The control fluxes varied from 223.0 to 789.2 mg m^{-2} d^{-1}. In May, at the second Lake Hall site, control and post MF fluxes for two plots were significantly different and yielded oxidation values of 22.0 $\pm$ 10% and 19.7 $\pm$ 7.5% respectively (Table 1). *P. cordata* #P7-LH fluxes were not significantly different at the 95% confidence level but an oxidation percentage of 20.8 $\pm$12.4% could be calculated. The average for all three plots in May was 20.8 $\pm$ 1.1%.

Water temperatures increased over the period. The average water temperature for the March 23 experiment was 18.8 $\pm$ 0.3 °C (sd, n = 4). For the March 31 experiment the average water temperature was 20.9 $\pm$ 0.3 °C (sd, n = 4). Temperatures for sampling at April 8 and May 5 were 20.0 $\pm$ 0 °C (sd, n = 4) and 24.3 $\pm$ 0.9 °C (sd, n = 4) respectively.

Table 1. Field and Greenhouse MF incubation results. A lack of significant difference at the 95% confidence level is indicated by an *. LK-Lake Hall; SM-St. Mark's; GR-unburied greenhouse; and GRB-buried greenhouse. Standard deviation is given by sd and 1/2 the range is given by s.

Date	Plant	Pre-MF Flux $mg\ m^{-2}d^{-1}$	sd $n = 2$	Post-MF Flux $mg\ m^{-2}d^{-1}$	sd $n = 2$	Oxidation %	s	Significance p
3/23/93	#P1-LH	208	11	369	11	44	4	0.00
	#P2-LH	162	15	287	14	44	7	0.01
	#P3-LH	366	25	626	32	42	7	0.01
	#P4-LH	422	26	897	43	53	6	0.00
3/31/93	#P1-LH	291	32	431	16	33	8	0.01
	#P2-LH	321	34	378	8	15	9	0.08*
	#P4-LH	988	126	1341	10	26	10	0.03
4/8/93	#P6-LH	789	16	756	60	–4	8	0.27*
	#P7-LH	370	86	284	35	–30	33	0.16*
	#P8-LH	223	33	224	17	0	17	0.48*
5/5/93	#P6-LH	1420	160	1819	83	22	10	0.05
	#P7-LH	421	50	532	42	21	12	0.07*
	#P8-LH	380	35	474	5	20	8	0.03
3/22/94	#S1-SM	221	15	249	24	12	12	0.15*
	#S2-SM	351	1	394	14	11	4	0.02
	#S3-SM	199	7	228	4	13	4	0.02
	#S4-SM	305	1	391	15	22	4	0.01
3/24/94	#S5-SM	583	11	841	42	31	5	0.01
	#S6-SM	278	8	404	53	31	14	0.04
	#S7-SM	454	21	576	38	21	8	0.03
	#S8-SM	212	10	278	8	24	5	0.01
10/19/94	#S9-SM	1028	20	1244	107	17	9	0.05
	#S11-SM	1167	37	1500	112	22	8	0.03
	#S12-SM	761	0	828	80	8	10	0.18*
8/8/94	#P3-GR	57	1	410	11	86	4	0.00
	#P4-GR	36	1	281	3	87	1	0.00
9/20/94	#P3-GRB	105	20	365	7	71	6	0.00
	#P4-GRB	33	15	185	2	82	8	0.00

St. Mark's, S. lancifolia

March sampling of *S. lancifolia* at St. Mark's also showed evidence of rhizospheric CH_4 oxidation. In the March 22 experiment three of the four plots

showed a significant difference between the control and MF incubation (Table 1). *S. Lancifolia* #S2-SM, #S3-SM, and #S4-SM indicated oxidation percentages of 10.8 ± 3.5%, 12.6 ± 3.5%, and 21.9 ± 3.9%. *S. lancifolia* #S1-SM results were not significant at the 95% confidence level but an oxidation percentage of 11.5 ± 11.5% was calculated. In the March 24 experiment, results from all four plots were significantly different (Table 1). Oxidation values of 30.7 ± 5.4%, 31.2 ± 13.8%, 21.1 ± 7.7%, and 23.8 ± 4.7% were found. The average oxidation percentage for experiment one was 14.2 ± 5.2% and for experiment two 26.7 ± 5.0% with the average for March being 20.5 ± 8.2%. The average water temperature for March was 19.6 ± 1.1 °C (sd, n = 8).

October St. Mark's data also indicated methane oxidation but only one of the plots showed a significant difference between the control and post-MF flux corresponding to an oxidation percentage of 22.2 ± 8% (Table 1). Results for the two other plots were not significantly different but oxidation percentages of 17.4 ± 8.9% and 8.2 ± 9.7% were calculated. The average oxidation percentage for all three plots was 15. ± 7.1% and the average water temperature was 20.3 ± 0.3 °C (sd, n = 4).

Greenhouse Measurements

The buried 20 L pots experiment was designed to test for diffusion of O_2 through the pot walls in the greenhouse experiments (Table 1). *P. cordata* #P3-GR and #P4-GR were sampled prior to burial and oxidation percentages of 86.1 ± 3.6% and 87.1 ± 1.3% were determined. After burial in water-saturated organic-rich soil for four weeks the oxidation percentages were 71.1 ± 6% for *P. cordata* #P3-GRB and 82.4 ± 7.9% for #P4-GRB. The oxidation percentages before and after burial were not significantly different (average before 86.6 ± 0.7% and after burial 76.8 ± 8.0%, Table 1). The average water temperature was 23.5 ± 0.5 °C for unburied and 22.2 ± 0.3 °C for buried (sd, n = 4).

Belowground MF samples indicated that MF was present in the sediment immediately adjacent to the chamber. After an overnight incubation with 3.0% MF, the MF concentration in 5 cm deep porewater samples ranged from 186.6 to 380.5 μM. The extraction efficiency of MF was determined to be lower than that of CH_4, 0.40 and 0.96 respectively. This is consistent with the reported solubility of MF (Budavari 1989). The concentration of MF in air that was at equilibrium with the measured porewater value ranged from 0.30 to 0.62% MF by volume. For all experiments (greenhouse and field) the concentration of MF remaining in the headspace at the end of the incubation period was 0.75 ± 0.4% (sd, n = 85) less than the amount of MF in the chamber headspace at the initiation of incubation.

Table 2. Comparison of MF and Light-oxic/Dark-anoxic oxidation percentages.

Plant	MF Oxidation % [sd] $n = 2$	LO/DA Oxidation % [sd] $n = 2$
Pont 7	76.7 [8.1]	72.6 [13.0]
Sag 8	42.5 [9.2]	55.8 [26.0]
Sag 4	52.5 [4.1]	48.0 [14.8]
Pont 6	68.9 [2.7]	71.1 [1.5]
Sag 6	43.9 [2.6]	62.4 [13.6]
Pont 9	38.9 [2.6]	57.1 [10.4]
Sag 5	47.2 [1.2]	24.0 [25.4]
Sag 1	68.1 [18.8]	64.8 [8.0]
Sag 2	56.0 [7.6]	61.6 [4.0]
Pont 1	84.8 [0.4]	71.6 [4.4]
Pont 2	55.3 [2.4]	54.0 [7.5]
Average	57.7 [15.0]	58.5 [13.9]

Oxidation percentages calculated by both the MF and LO/DA techniques are shown in Table 2. For the eleven greenhouse plants, the average oxidation percentage for the light-oxic dark-anoxic method was $58.5 \pm 13.9\%$ and for MF $57.7 \pm 15.0\%$. These results were not significantly different. The average water temperature for the light-oxic dark-anoxic and MF methods were $23.6 \pm 1.6\,^{\circ}C$ and $24.6 \pm 0.7\,^{\circ}C$ respectively. Frenzel et al. (1992) reported an oxidation rate of 80–90% in rice mesocosms determined with the LO/DA technique.

Seasonal Variations in Methane Oxidation

The seasonal study of oxidation associated with *T. latifolia* showed a decreasing trend for percent oxidation from cooler to warmer months (Figure 2). The CH_4 emission rate (pre-MF flux) increased from 39.9 mg m^{-2} d^{-1} in February to 3563.2 mg m^{-2} d^{-1} in June. Following June the CH_4 flux decreased (Figure 2a). In general the smallest fluxes corresponded to the largest relative amounts of oxidation (Figure 2a, b). The oxidation percentages decreased from an average of $46.9 \pm 2.73\%$ in February (1/2 range, $n = 2$ plots) to a baseline of $10.4 \pm 6.5\%$ in the warmer months (1/2 range, $n = 8$, June through September, Figure 2b). Results were not always significantly different at the 95% confidence level because as the CH_4 fluxes increased the differences between replicate measurements grew as large as the apparent oxidation rate. However, the post-MF fluxes were consistently greater than the pre-MF or control fluxes. Following summer the oxidation percentages then increased

from 22.7 ± 2.8% in October to 46.5 ± 13.8% in January (1/2 range, n = 2, Figure 2b).

The absolute rate of methane oxidation (calculated as the difference between the post-MF methane emission rate and the pre-MF methane emission rate) was greatest in the warmer months with the largest absolute rate occurring in July and the smallest absolute rates corresponding to the largest CH_4 oxidation percentages (Figure 2c). Water temperatures changed during the sampling period from 13.6 ± 0.5 °C in February to 26.0 ± 0.0 °C in July and back to 11.0 ± 1.1 °C in January (sd, n = 6).

Methane Turnover

The days necessary for turnover of CH_4 in the sediments are shown in Table 3. The average turnover for all 11 greenhouse pots was 4.1 ± 3.7 (sd, n = 11) days with the largest turnover being 12.9 days and the smallest 1.0 day. For St. Mark's the turnover time for the March 24 experiment was 2.6 days and for October 0.9 days. In the *Typha* pond a seasonal trend was shown for CH_4 turnover time. The fastest turnover was in June and the slowest in February (0.8 days and 22.7 days respectively, Table 3). The times of highest methane flux corresponded with the fastest turnovers.

Effect of MF on CH_4 production

For roots plus sediment, the average rate of CH_4 production for the control was 237.4 ± 58.4 μg g^{-1} dry d^{-1} (n = 6) and for the MF additions 193.2 ± 43.2 μg g^{-1} dry d^{-1} (n = 7). This MF rate was 81.4% of the control rate corresponding to a 18.6% attenuation in CH_4 production. For lake sediment free of roots the rate of production was 2.3 ± 0.7 μg g^{-1} dry d^{-1} (n = 3) for the control and 1.8 ± 0.8 μg g^{-1} dry d^{-1} (n = 4) for the MF addition treatment. This corresponded to a 21.7% attenuation in CH_4 production since the MF rate was 78.3% of the control rate. However, in both experiments the two rates were not significantly different (p = 0.20 and 0.42 for roots plus sediment and sediment only, respectively).

Discussion

Rhizospheric methane oxidation was found to occur in field experiments with *P. cordata*, *S. lancifolia*, and *T. latifolia*. However, all field determined oxidation percentages were significantly smaller than greenhouse values determined in this study and by Epp & Chanton (1993). The oxidation percentages for *S. lancifolia* were 56.2 ± 16.1% (sd, n = 19) and 19.2 ± 7.8% (sd, n

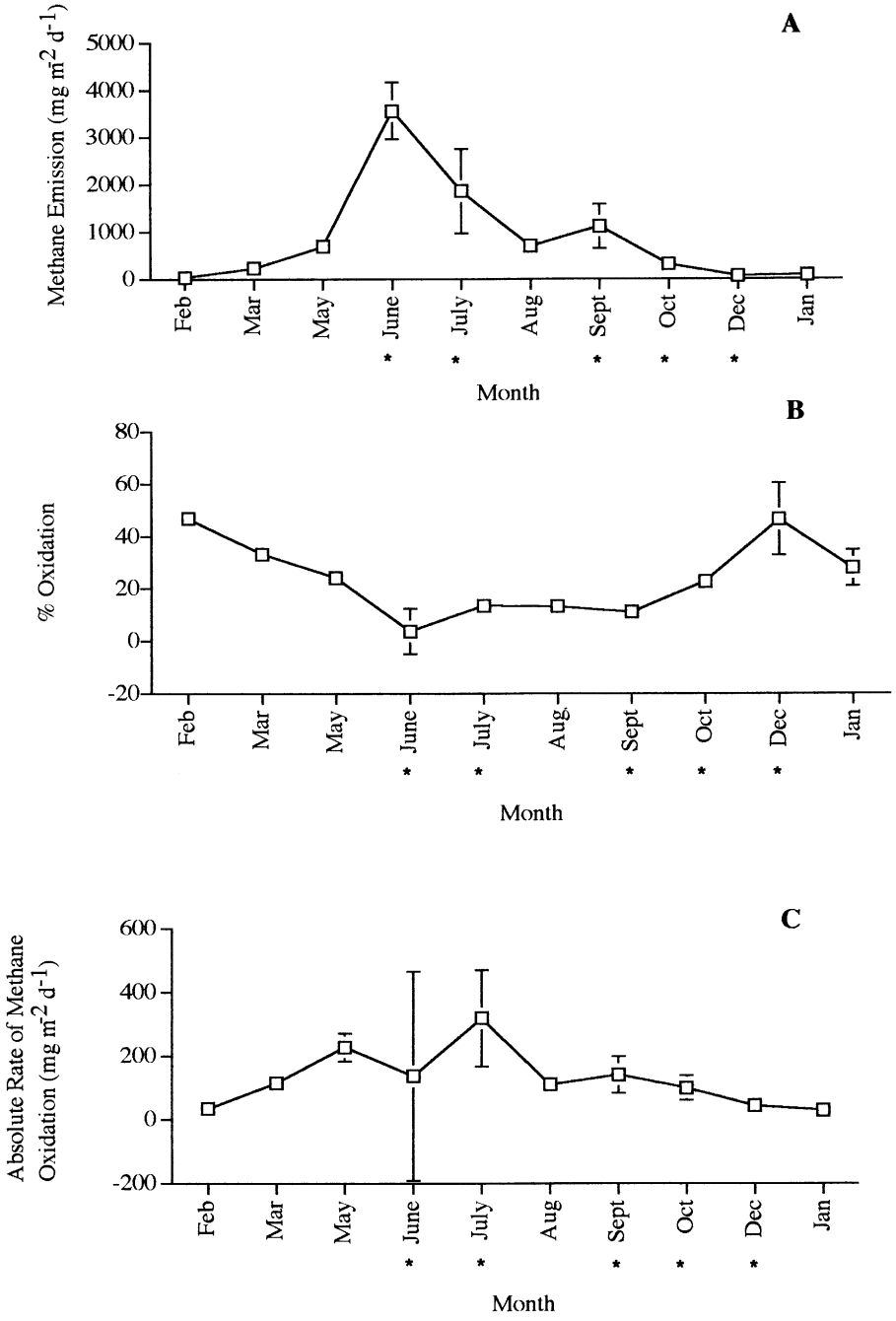

Figure 2. A seasonal study of CH_4 oxidation associated with the rhizosphere of *T. latifolia*, A, Methane Emission; B, Oxidation Percentage; C, Rate of Methane Oxidation. $n = 3$ replicates per chamber per day except for August and September where $n = 1$ and $n = 2$. An * indicates no significant difference for at least one of the two chamber for that month.

= 11) for greenhouse and field experiments respectively. For *P. cordata* the percentages were 71.5 ± 14.7% (sd, n = 25) and 21.9 ± 22.9% (sd, n = 13) for greenhouse and field experiments. In both cases, the percentages for the greenhouse and field were statistically different at a 99% confidence level. Based on the *T. latifolia* seasonal results, these field experiments were performed when the CH_4 oxidation percentages should have been relatively large (March, October).

We hypothesize that the difference between field and greenhouse measurements is a result of the increased root density in the potted plants relative to plants growing in the field. Visual examination of greenhouse plants growing in the buckets showed that the plants were root bound. Increased root density could result in a more oxygenated rhizosphere (Armstrong & Armstrong 1988). Correlation between root density and rate of methane oxidation in incubations of Everglades peat have been reported (Gerard & Chanton 1993). The possibility that the increased oxidation observed in the greenhouse experiment was due to O_2 diffusion through the pot sides was eliminated in the experiment in which two pots were buried in flooded organic-rich soil (Table 1).

Methane oxidation in the rhizosphere of *T. latifolia* showed a clear seasonal response in the percentage of CH_4 oxidation with a decreasing trend from the end of winter to mid summer (Figure 2b). This decrease in the relative importance of CH_4 oxidation corresponded with the timing of the seasonal increase in CH_4 emission rates found in this and in other wetland environments (Seiler et al. 1984; Wilson et al. 1989; Conrad 1993). In an Alberta fen, Popp et al. (in prep) found a similar trend in CH_4 oxidation.

The seasonal changes in percent oxidation may be related to the production of root exudates in addition to changes in soil temperature. Plant roots produce exudates which are released to the rhizosphere where they can support a community of aerobic and anaerobic microorganisms (Curl & Truelove 1986; Rovira 1969). Methane oxidation percentages declined and CH_4 emission rates increased as the plants developed toward their flowering stage (Figure 2a, b). These results are consistent with the suggestion of King (1990b) that rhizospheric CH_4 oxidation could decrease with increased CH_4 production because the release of root exudates may increase the demand for O_2 by aerobic heterotrophs, thereby reducing the amount of O_2 available to methanotrophs. Temperature increases should also elevate root respiration increasing the demand for O_2, further decreasing O_2 availability to methanotrophs.

An additional explanation could be seasonal changes in the solubility of methyl fluoride. Methanotrophic bacteria might have been exposed to differing MF concentrations as temperature changed over time, since gas solubility

is inversely related to temperature. This could have resulted in incomplete inhibition of methane oxidizing bacteria. Alternately, methyl fluoride could be degraded by the anaerobic sediment thus decreasing the amount available for inhibition of methanotrophs (Oremland et al. 1994a, b; Lovely & Woodward 1992). However, during the course of the experiment most of the MF was retained in the headspace and porewater samples showed MF present in the porewater next to the chamber. Epp & Chanton (1993) clearly demonstrated with dose response curves that the bacteria were exposed to MF concentrations within limits shown by Oremland & Culbertson (1992a) to inhibit bacterial activity.

Even though CH_4 oxidation percentages declined from winter to summer, absolute rates of rhizospheric methane oxidation increased seasonally (Figure 2c). King (1994) reported similar seasonal trends in the maximum potential uptake rate of CH_4 in aerobic incubations of rhizospheric material. The MF incubation technique apparently lost sensitivity as CH_4 emission rates increased and rhizospheric CH_4 oxidation became relatively less important. Rates of rhizospheric CH_4 oxidation failed to keep pace with methane emission rates. At high summer rates of CH_4 emission, rhizospheric methane oxidation rates are similar in magnitude to the error of replicate methane flux measurements. Rhizospheric CH_4 oxidation is apparently limited by the delivery of O_2 belowground by roots as suggested by Chanton et al. (1992) and King (1994).

In the incubation studies, MF was found to attenuate CH_4 production by 18 to 22%, although the differences were not significant given the variability of the results. These results concur with Schipper & Reddy (1996) who only found a 15% inhibition of methanogenesis when studying soil samples where *S. lancifolia* was growing and 1% MF had been added. This 1.0% incubation value is larger than the belowground MF concentrations (0.3 to 0.6% MF by volume).

If attenuation of CH_4 production by MF does occur, this could explain the difference between field and greenhouse oxidation percentages and the apparent seasonal effects. Reduced CH_4 production due to attenuation by MF could deplete the belowground reservoir of CH_4 depending upon the residence time of CH_4 belowground (Table 3) and the time scale of the experiment. During the 22 hours of MF incubation, on plants having a 2–4 day turnover time, this attenuation would be relatively small because of the larger belowground reservoir of CH_4. A 20% reduction in CH_4 production over the incubation period might only reduce the CH_4 concentration by 5 to 10%, or less as CH_4 producing bacteria would not be exposed to MF for the entire length of incubation. Under these circumstances the belowground CH_4 would buffer the system and the potential 20% attenuation of CH_4 production

Table 3. Belowground methane turnover time for greenhouse and field experiments.

Location	Turnover days [sd]
Greenhouse	4.1 [3.7]
St. Marks – March	2.6
St. Marks – October	0.9
Dean's Pond – February	22.7
Dean's Pond – March	9.1
Dean's Pond – May	3.5
Dean's Pond – June	0.8
Dean's Pond – July	2.0
Dean's Pond – August	5.5
Dean's Pond – September	3.6
Dean's Pond – October	14.6

by MF would be less important. For the greenhouse and March St. Mark's experiment, the turnover time for the belowground CH_4 was similar. The attenuation of CH_4 production by MF becomes more relevant as CH_4 emission increases and turnover times decrease. Both the June *Typha* and October St. Mark's CH_4 turnover times were less than one day, providing reduced buffering by the system. The belowground CH_4 would have completely turned over during the course of these experiment and 20% attenuation in production approaches and exceeds the values determined for attenuation by methane oxidation. The relationship between turnover time of belowground CH_4, the duration of the incubation period, and the response of CH_4 production must be considered when employing this technique (R. Conrad, personal communication, 1995).

For the MF technique to give an accurate measure of rhizospheric methane oxidation, we envision the following conceptual model. Methane must diffuse across an oxic zone where its signal is attenuated before it enters the plant on its way to the atmosphere. In the rhizosphere, this assumes that, although different spatially, the anoxic-oxic interface at the sediment-water interface and rhizoplane are somewhat similar in configuration (Figure 3). The use of MF halts the consumption of CH_4 within the oxygenated zone surrounding the root. Therefore, the flux of CH_4 into the plant in the presence of MF is equivalent to the flux in the absence of CH_4 oxidation. Possible attenuation of CH_4 production by MF occurs in the anoxic zone and may affect the CH_4 concentration gradient only by 5 to 10% over the time scale of these experiments. However, if CH_4 oxidation occurs by an alternate model, such as leakage of O_2 into the bulk sediment away from the root, and if the MF

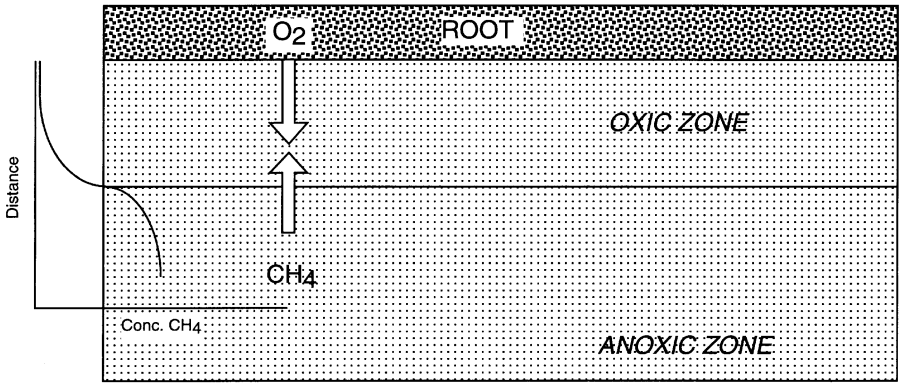

Figure 3. Conceptualization of the oxygen-methane interface in the rhizosphere. Oxygen is confined to zones or haloes around the root. Methane is produced outside of these zones and is oxidized as it diffuses towards the roots through the oxic zones.

application acts to increase CH_4 flux by increasing the concentration of CH_4 in the bulk sediment and thus the diffusion gradient for CH_4 into the plant, then the MF technique will not be a sensitive measure of CH_4 oxidation. In the latter case, the measure of CH_4 oxidation would also be dependent upon the turnover time of belowground CH_4 and would lose sensitivity as turnover time and belowground inventories increased. The possible attenuation of CH_4 production and the cessation of CH_4 oxidation by MF would be competing processes in terms of their effects on CH_4 concentration in the bulk sediment.

In applying the MF technique, we assume that the sites where O_2 leaks from the roots are the sites where CH_4 enters the roots (as conceptualized by Conrad 1993, Figure 2, pp. 325) and that rhizospheric CH_4 oxidation occurs at these locations. We further assume that oxygen in the rhizosphere occurs in zones or haloes around the roots (as described by Armstrong & Armstrong 1988 and Smits et al. 1990) and is not dispersed throughout the soil. Epp & Chanton (1993) showed that MF compared effectively to the use of picolinic acid in attenuating CH_4 oxidation at the unvegetated sediment-water interface. To the extent that the oxic-anoxic zone interface in the rhizosphere is similar to this interface, the MF technique for determining rhizospheric CH_4 oxidation should serve to yield estimates (within 10 to 20%) of a process that is extremely difficult to quantify otherwise without vastly perturbing the system. The dose-response curves for MF's effect on rhizospheric CH_4 oxidation suggest that inhibition of CH_4 production does not begin to affect the measurement until MF percentages greater than 3.0% are reached (Epp 1993; Epp & Chanton 1993).

Furthermore, the comparison of MF and LO/DA validates the use of the MF technique in the greenhouse studies. Oxidation percentages were

the same for both methods. The LO/DA method should not attenuate CH_4 production. Gerard & Chanton (1993) argued that the CH_4 flux might increase after incubation under dark/anoxic conditions since aerobic respiration of organic matter would cease and excess organic matter would then be available to be degraded anaerobically. Additionally, the stress of being under these conditions might cause plant to release surplus organic matter for use by methanogens (Gerard 1992; Gerard & Chanton 1993). The results of this study support continued investigation of the use of MF for quantification of rhizospheric methane oxidation in the greenhouse and the field.

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References

Armstrong J & Armstrong W (1988) *Phragmites australis* – A preliminary study of soil oxidizing sites and internal gas transport pathways. New Phytologist 108: 373–382

Bartlett KB, Crill PM, Sass RL, Harriss RC & Dise NB (1992) Methane emissions from tundra environments in the Yukon-Kuskokwim Delta Alaska. J Geophys Res 97: 16645–16660

Bedford BL, Bouldin DR & Beliveau BD (1991) Net oxygen and carbon dioxide balances in solutions bathing roots of wetland plants. J Ecology 77: 943–959

Brix H, Sorrell BK & Orr PT (1992) Internal pressurization and convective gas flow in some emergent freshwater macrophytes. Limnol. Oceanogr. 37: 1420–1433

Budavari S (Ed) (1989) The Merck Index. Merck & Co., Inc., Rathway, NJ

Chanton JP & Dacey JWH (1991) Effects of vegetation on methane flux reservoirs and carbon isotopic composition. In: Sharkey T, Holland E & Mooney H (eds) Trace Gas Emissions from Plants (pp 65–92). Academic Press, San Diego

Chanton JP, Martens CS & Kelley CA (1989) Gas transport from methane-saturated, tidal freshwater and wetland sediments. Limnol Oceanogr 34: 807–819

Chanton JP, Whiting GJ, Showers WJ & Crill PM (1992) Methane flux from *Peltandra virginica*: stable isotope tracing and chamber effects. Global Biogeochem Cycles 6: 15–31

Chanton JP, Whiting GJ, Happell JD & Gerard G (1993) Contrasting rates and diurnal patterns of methane emission from emergent aquatic macrophytes. Aquat Bot 46: 111–128

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

Coleman DD, Risatte JB & Schoel M (1981) Fractionation of carbon and hydrogen isotopes by methane-oxidizing bacteria. Geochim et Cosmochim Acta 45: 1033–1037
Conrad R (1993) Mechanisms controlling methane emission from wetland rice fields. In: Oremland RS (ed) Biogeochemistry of Global Change (pp 317–335). Chapman and Hall, New York
Conrad R & Rothfuss F (1991) Methane oxidation in the soil surface layer of a flooed rice field and the effect of ammonium. Biol. Fertil Soils 12: 28–32
Crill PM (1991) Seasonal patterns of CH_4 uptake and CO_2 release by a temperate woodland soil. Global Biogeochem. Cycles 5: 319–334
Curl EA & Truelove B (1986) The Rhizosphere. Springer-Verlag, New York
Dacey JWH (1981) How aquatic plants ventilate. Oceanus 24: 43–51
De Bont JAM, Lee KK & Bouldin DF (1978) Bacterial oxidation of methane in a rice paddy. Biol Bull (Stockholm) 26: 91–96
Epp MA (1993) Rhizospheric Methane Oxidation. MS Thesis. The Florida State University Tallahassee, Florida
Epp MA & Chanton JP (1993) Rhizospheric methane oxidation determined via the methyl fluoride inhibition technique. J Geophys Res 98: 18413–18422
Frenzel P, Rothfuss F & Conrad R (1992) Oxygen profiles and methane turnover in a flooded rice microcosm. Biol Fertil Soils 14: 84–89
Gerard G (1992) Role of aquatic macrophytes in methane dynamics. MS Thesis. The Florida State University Tallahassee, Florida
Gerard G & Chanton JP (1993) Quantification of methane oxidation in the rhizosphere of emergent aquatic macrophytes: defining upper limits. Biogeochem 23: 79–97
Gilbert B & Frenzel P (1995) Methanotrophic bacteria in the rhizosphere of rice microcosms and their effect on porewater methane concentration and methane emission. Biol Fertil Soils 20: 93–102
Green MS & Etherington JR (1977) Oxidation of ferrous iron by rice (*Oryza sativa* L) roots: a mechanism for waterlogging tolerance? J Exp Botany 28: 678–690
Happell JD, Chanton JP, Whiting GJ & Showers WS (1993) Stable isotopes as tracers of methane dynamics in Everglades marshes with and without active populations of methane oxidizing bacteria. J Geophys Res 98: 14771–14782
Hesslein RH (1976) An *in situ* sampler for close pore water studies. Limnol Oceanogr 21: 912–914
Holzapfel-Pschorn A, Conrad R & Seiler W (1985) Production oxidation and emission of methane in rice paddies. FEMS Microbiol Ecol 31: 343–351
Holzapfel-Pschorn A, Conrad R & Seiler W (1986) Effects of vegetation on the emission of methane from submerged paddy soil. Plant and Soil 92: 223–233
Holzapfel-Pschorn A & Seiler W (1986) Methane emission during a vegetation period from an Italian rice paddy. J Geophys Res 91: 11803–11841
King GM (1990a) Regulation by light of methane emission from a wetland. Nature (London) 345: 513–515
King GM (1990b) Dynamics and controls of methane oxidation in a Danish wetland sediment. FEMS Microbio Ecol 74: 309–324
King GM (1992) Ecological aspects of methane oxidation a key determinant of global methane dynamics. In: Marshall KC (Ed) Advances in Microbial Ecology, Vol. 12 (pp 431–468). Plenum Press, New York
King GM (1994) Associations of Methanotrophs with the Roots and Rhizomes of Aquatic Vegetation. Appl Env Microb 60: 3220–3227
King GM, Roslev MP & Skovgaard H (1990) Distribution and rate of methane oxidation in sediments of the Florida Everglades. Appl Env Microb 56: 2902–2911
Lovely DR & Woodward JC (1992) Consumption of freons CFC-11 and CFC-12 by anaerobic sediments and soils. Environ. Sci. Tech. 26: 925–929
Macfie SM & Crowder AA (1987) Soil factors influencing ferric hydroxide plaque formation on roots of *Typha latifolia* L. Plant and Soil 102: 177–184

McAuliffe C (1971) Gas chromatographic determination of solutes by multiple phase equilibrium. ChemTechnol 1: 46–51
Morrissey LA & Livingston GP (1992) Methane emissions from Alaska arctic tundra: and assessment of local spatial variability. J Geophys Res 97: 16661–16670
Oremland RS & Culbertson CW (1992a) Evaluation of methyl fluoride and dimethyl ether as inhibitors of aerobic methane oxidation. Appl Environ Microbiol 58(9): 2983–2992
Oremland RS & Culbertson CW (1992b) Importance of methane-oxidizing bacteria in the methane budget as revealed by use of a specific inhibitor. Nature (London) 356: 421–423
Oremland RS, Miller LG, Culbertson CA, Connell TL & Jahnke L (1994a) Degradation of Methyl Bromide by Methanotrophic Bacteria in Cell Suspensions and Soils. Appl Environ Microbiol 60(10): 3640–3646
Oremland RS, Miller LG & Strohmaler FE (1994b) Degradation of Methyl Bromide in Anaerobic Sediments. Environ Sci Tech 28: 514–520
Popp TJ, Chanton JP, Grant NJ & Whiting GJ (1996) Evaluation of Rhizospheric Methane Oxidation in a Boreal Fen (in prep)
Reddy KR, Patrick WH & Lindau CW (1989) Nitrification-denitrification at the plant root-sediment interface in wetlands. Limnol Oceanogr 34: 1004–1013
Reeburgh WS, Whalen SC & Alperin MJ (1993) The role of methylotrophy in the global methane budget. In: Microbial Growth on C1 Compounds (pp 1–14). Intercept Ltd, Andover Hampshire England
Rovira AD (1969) Plant root exudates. The Botanical Review: 35–57
Sass RL, Fisher FM & Harcombe PA (1990) Methane production and emission in a Texas rice field. Global Biogeochem Cycles 4: 47–68
Sass RL, Fisher FM, Wang YB, Turner FT & Jund MF (1992) Methane emission from rice fields: the effect of floodwater management. Glob Biogeochem Cycles 6: 249–262
Schipper LA & Reddy KR (1996) Methane oxidation in the rhizosphere of *Sagittaria lancifolia*. Soil Sci Soc Amer Journal 40: 611–616
Schütz H, Holzapfel-Pschorn A, Rennenberg H, Seiler W & Conrad R (1989b) A three year continuous record on the influence of daytime season and fertilizer treatment on methane emission rates from an Italian Rice Paddy. J Geophys Res 94: 16405–16416
Schütz H, Seiler W & Conrad R (1989a) Processes involved in formation and emission of methane in rice paddies. Biogeochem 7: 33–53
Sebacher DI, Harriss RC & Bartlett KB (1983) Methane flux across the air-water interface: air velocity effects. Tellus 35B: 103–109
Seiler W, Holzapfel-Pschorn A, Conrad R & Scharffe D (1984) Methane emission from rice paddies. J Atmos Chem 1: 241–268
Smits AJM, Laan P, Thier RH & Van der Velde G (1990) Root aerenchyma, oxygen leaking patterns and alcoholic fermentation ability of the roots of some nymphaeid and isoetid macrophytes in relation to the sediment type of their habitat. Aquatic botany 38: 3–17
Tyler SC (1991) The global methane budget. In: Rogers JE & Whitman WB (Eds) Methane Nitrogen Oxides and Halomethanes (pp 7–38). American Society for Microbiology, Washington DC
Whiting GJ & Chanton J (1992) Plant-dependent methane emission in a subarctic Canadian fen. Global Biogeochem Cycles 6: 225–231
Whiting GJ & Chanton J (1993) Primary production control of methane emission from wetlands. Nature 364: 794–795
Whiting GJ, Chanton J, Bartlett D & Happell J (1991) Methane flux, net primary productivity and biomass relationships in a sub-tropical grassland community. J Geophys Res 96: 13067–13071
Wilson JO, Crill PM, Bartlett KB, Sebacher DI, Harriss RC& Sass RL (1989) Seasonal variation of methane emissions from a temperate swamp. Biogeochem 8: 55–71