*Biogeochemistry* **23**: 79—97, 1993


# Quantification of methane oxidation in the rhizosphere of emergent aquatic macrophytes: defining upper limits

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Received 5 November 1992; accepted 9 September 1993

**Key words:** aquatic macrophytes, methane, methane oxidation, plant/microbial interactions, rhizosphere, stable isotopes

**Abstract.** Rates of rhizospheric methane oxidation were evaluated by aerobic incubations of subcores collected in flooded anoxic soils populated by emergent macrophytes, by greenhouse whole plant incubations, and by $CH_4$ stable isotopic analysis. Subcore incubations defined upper limits for rhizospheric methane oxidation on an areal basis which were equal to or greater than emission rates. These rates are considered upper limits because $O_2$ did not limit $CH_4$ uptake as is likely to occur *in situ*. The ratio of maximum potential methane oxidation (MO) to methane emission (ME) ranged from 0.7 to 1.9 in Louisiana rice (*Oryza sativa*), from 1.0 to 4.0 in a N. Florida *Sagittaria lancifolia* marsh, and from 5.6 to 51 in Everglades *Typha domingensis* and *Cladium jamaicense* areas. Methane oxidation/methane emission ratios determined in whole plant incubations of *Sagittaria lancifolia* under oxic and anoxic conditions ranged from 0.5 to 1.6. Methane oxidation activity associated with emergent aquatic macrophytes was found primarily in fine root material. A weak correlation was observed between live root biomass and $CH_4$ uptake in *Typha*. Rhizomes showed small or zero rates of methane uptake and no uptake was associated with plant stems. Methane stable isotope data from a *S. lancifolia* marsh were as follows: $CH_4$ emitted from plants: $-61.6 \pm 0.3‰$; $CH_4$ within stems: $-42.0 \pm 0.2‰$; $CH_4$ within sedimentary bubbles: $-51.7 \pm 0.3‰$). The $^{13}C$ enrichment observed relative to emitted $CH_4$ could be due to preferential mobilization of $CH_4$ containing the lighter isotope and/or the action of methanotrophic bacteria.

## Introduction

Emergent aquatic macrophytes growing in flooded anoxic soils function in manners which can both enhance and attenuate wetland $CH_4$ emissions (Chanton & Dacey 1991). Enhancement occurs through production of leaf litter, root sloughing and exudate production, and by transportation of substantial quantities of $CH_4$ via molecular diffusion or pressurized ventilation through internal conduits (Sebacher et al. 1985; Dacey

1981a,b; Dacey & Klug 1979). Aquatic plants may attenuate $CH_4$ emission by providing $O_2$ to $CH_4$ oxidizing bacterial communities associated with the rhizosphere (De Bont et al. 1978; Holzapfel-Pschorn et al. 1985). It is difficult to directly quantify the activity of $CH_4$ oxidizing bacteria in the rhizosphere without disturbing factors which affect oxidation rates, so this term is one of the greater unknowns in the wider assessment of the importance of methylatrophy (Reeburgh et al. 1993). Methods applied in previous investigations of rhizospheric $CH_4$ oxidation have differed widely. For example, one method evaluates $CH_4$ oxidation as the difference between the amounts of $CH_4$ emitted *in situ* and that produced in anaerobic incubations of belowground material (Holzapfel-Pschorn et al. 1985; Schutz et al. 1989; Sass et al. 1990). A second method measures $CH_4$ oxidation by comparing fluxes measured under normal conditions (light-oxic) with fluxes measured under conditions to inhibit $CH_4$ oxidation (De Bont et al. 1978: Holzapfel-Pschorn et al. 1985). Methane stable isotopic composition may also be indicative of the importance of methane oxidation in the rhizosphere (Chanton et al. 1992a; Happell et al. 1993).

Both $O_2$ and $CH_4$ are present within aquatic macrophytes (Dacey 1981a, b; Chanton et al. 1992a, b), therefore $CH_4$ oxidizing bacteria could function within plant stems, rhizomes and roots. Although $O_2$ is consumed by root respiration (Bedford et al. 1991), some leaks from roots (Smits et al. 1990; Armstrong & Armstrong 1988), possibly fueling methane oxidizing bacteria in the sediments of the rhizosphere. Competition for $O_2$ must limit rhizospheric methane oxidation due to the demands of root respiration, aerobic oxidation of complex organic matter, oxidation of ferrous iron (Green & Etherington 1977; Macfie & Crowder 1987) and nitrification of ammonia (Reddy et al. 1989). Stable isotopic data has indicated $^{13}C$ enrichment of aquatic plant stem $CH_4$ consistent with the presence of microbial oxidation, but more likely due to mass dependent fractionation effects associated with gas diffusion (Chanton et al. 1992a, b).

Our first hypothesis was that $CH_4$ oxidation takes place in stems, rhizomes, roots and sediments of the rhizosphere. Our second hypothesis was that $CH_4$ oxidation associated with aquatic macrophytes is small relative to rates of $CH_4$ emissions. The justification for our second hypothesis is that stable isotope studies have shown no evidence for (but cannot rule out) $CH_4$ oxidation in the rhizosphere of *Peltandra virginica* and *Cladium jamaicense* (Chanton et al. 1992a; Happell et al. 1993). Additionally, $CH_4$ fluxes from vegetated areas have shown a positive correlation with plant biomass (Whiting et al. 1991; Whiting & Chanton 1992, 1993; Happell et al. 1993). The fact that $CH_4$ flux increases with plant biomass suggests that the processes of transport and production

which enhance $CH_4$ emissions outweigh $CH_4$ oxidation which attenuates emissions.

Our first hypothesis was tested by measuring $CH_4$ uptake rates in incubations of different parts of plants and roots with and without sediment under a $CH_4$/air mixture. The second hypothesis was tested using three different approaches: uptake rates, stable isotopes and whole plant inhibition studies. The first approach involved measurements of $CH_4$ uptake rates under an air/$CH_4$ mixture from portions of cores collected from the rhizosphere of different plants. We then converted these rates to an areal basis, and compared them to *in situ* $CH_4$ emissions. This aerobic incubation technique has the disadvantage of incubating material under conditions in which $O_2$ is present in greater quantities than *in situ*, however it serves to constrain the upper limit of $CH_4$ oxidation rates. Since our hypothesis was that $CH_4$ oxidation rates were small relative to emissions, we hoped to provide a convincing demonstration utilizing the upper limit value of these rates. As will be shown below, we failed to achieve this goal. To evaluate the role of roots in $CH_4$ oxidation, an attempt at correlating root density with $CH_4$ uptake rates was also made.

The second approach was to examine stable isotopic ratios of sedimentary and emitted $CH_4$ for evidence of $CH_4$ oxidation in the rhizosphere of *Sagittaria lancifolia* (bull tongue) following the procedures of Chanton et al. (1992a) and Happell et al. (1993). Methane oxidizing bacteria preferentially consume $CH_4$ containing the lighter isotope of carbon, (Coleman et al. 1981; Barker & Fritz 1981), resulting in $^{13}C$ enrichment in residual $CH_4$.

The third approach consisted of inhibiting rhizospheric $CH_4$ oxidation by anoxic treatment of whole growing plants following procedures described in Holzapfel-Pschorn et al. (1985). Methane emissions from *S. lancifolia* were compared under light-oxic and dark-anoxic conditions. The anoxic conditions inhibited $CH_4$ oxidation by cutting off the supply of $O_2$ to the methanotrophs. Darkness prevented photosynthesis and $O_2$ production. By quantifying the emission rate when $CH_4$ was not consumed in the rhizosphere, and comparing it to the flux obtained under light-oxic conditions, it was possible to evaluate the quantity of $CH_4$ oxidized.

## Procedure

Four sites were sampled in this study. Lake Hall, a fresh water lake near the campus of Florida State University, had fringes of *Pontederia cordata* and in deeper waters, patches of *Nymphaea odorata* and *Nelumbo*

*pentapetala.* The St Marks wildlife refuge, 40 km south of Tallahassee had a fresh water marsh containing *Sagittaria lancifolia.* Measurements on *Typha domingensis* and *Cladium jamaicense* were performed north of Everglades National Park in water conservation area #3. In both the northern Florida and Everglades sites, the plants were rooted in a layer of peat. Rice plants *(Oryza sativa),* rooted in clay, were sampled from the Louisiana State University Experimental Rice Station in Crowley, Louisiana.

Incubation experiments were generally performed in 125 ml-Erlenmeyer flasks sealed with rubber stoppers, however those from the Everglades utilized 60-ml syringes. Three to five replicates were run for each plant material or core subsection. Syringes and flasks were both tested to determine their effectiveness at retaining or absorbing gases. All $CH_4$ samples were analyzed on a gas chromatograph equipped with a flame ionization detector.

To determine if $CH_4$ uptake was of bacterial origin, five incubation flasks were initiated with approximately the same amount of root material, and their $CH_4$ concentration monitored. Twenty-five hours after the start of the incubation, one milliliter of a saturated $HgCl_2$ solution was added to three of the flasks. Methane concentrations were monitored for another 50 hours, and compared with the flasks to which no $HgCl_2$ addition was made.

Plant material was collected from the different sites described above, and experiments started on the day of collection. In each flask, one of the following amounts of plant material was incubated: two to three 10 cm-portions of stems, three to six 3 mm-slices of rhizome, four to five roots disconnected from the rhizome, and a small amount of sediment taken from the rhizosphere including roots. In order to expose the microbial flora to $O_2$ during the incubations and inhibit $CH_4$ production within the flasks, stems were cut in half, rhizomes were cut in thin slices and incubated portions of the sediments were broken apart. In some cases roots were cleaned of sediment with deionized water. Initial $CH_4$ mixing ratios varied from 500 to 3000 ppm and were monitored once or twice each day over 3 to 4 days until the flasks ran out of $CH_4$. Linear fits of mixing ratios vs. time were determined and rates of $CH_4$ uptake (slopes in ppm $h^{-1}$) were converted into $\mu mol\ g^{-1}_{(dry\ material)}\ h^{-1}$ by measuring the mass of material incubated and the headspace volume. Cores 20 to 40 cm in length, and 10 cm in diameter were collected near the base of several plants. Sections 2 to 5 cm thick were used to determine porosity, dry bulk density and $CH_4$ uptake rates. In these experiments replicates of core subsections of known volume were placed within a flask, broken apart to insure exposure to $O_2$, incubated under air and $CH_4$, and methane uptake

rates quantified. A step function was used to integrate these measured rates vs. depth, and porosity and bulk density used to convert uptake rates to g $CH_4$ $m^{-2}$ $d^{-1}$. Rates were generally integrated over the upper 20 cm of each core. This depth was chosen because most live roots were located in the upper 20 cm of the sediments (Gerard 1992).

In the Everglades experiments, core subsections were incubated in 60 ml syringes with initial headspace concentrations of about 1% $CH_4$. Ten ml gas samples were taken twice a day until the syringes ran out of headspace. Methane uptake rate was determined as loss of $CH_4$ vs. time. These rates were averaged for each replicate syringe and for each depth of a given core. Everglades material used for incubation was also used to determine live root density. Live roots from the subsections were separated from the sediment and dead plant material, dried and weighed. The mass of live roots per volume of wet sediment was computed for each depth interval.

Methane emissions were measured with temperature and $CO_2$ controlled chambers made of clear Plexiglas (Whiting et al. 1992). Methane mixing ratios were monitored by taking gas samples from the chambers every 4 to 10 minutes for periods varying from 30 minutes to 2 hours. For *Sagittaria lancifolia*, a sample for stable carbon $CH_4$ isotope analysis was collected in an aluminum cylinder at the end of the sampling period.

Pore water equilibration samplers or peepers (Hesslein 1976) were placed at the St Marks *Sagittaria lancifolia* site and at the Everglades sites and used to obtain profiles of pore water $CH_4$ concentration. These profiles were used to calculate the $CH_4$ flux across the sediment-water interface using the measured porosity values and diffusion coefficients from Chanton et al. (1989), adjusted for porosity.

Bubbles were collected by compressing the sediments and capturing the bubbles in an inverted water filled funnel. Stem gas was collected with a needle and syringe inserted into the stem under the waterline. This method has been shown to yield results identical to squeezing gas from stems (Chanton et al. 1992a). Methane from bubble and stem samples were transferred under $CH_4$-free water into vials for determination of $CH_4$ isotopic composition. Bubble and chamber headspace $CH_4$ were combusted and purified by vacuum distillation at FSU as described in Chanton et al. (1992a). Carbon isotope ratios were determined on a Finnigan Mat Delta D Isotope Ratio Mass Spectrometer in the laboratory of Dr. Neal Blair of North Carolina State University.

*Sagittaria lancifolia* with its surrounding sediments were transplanted from the St Marks Refuge to a greenhouse at Florida State University. Cylindrical chambers made of Plexiglas were inserted in the sediments around the plants. The systems were left idle for several weeks to allow

$CH_4$ production to resume and saturate the sediments as occurs *in situ.* For each plant-chamber, a series of flux measurements under light-oxic conditions, then dark-anoxic, and then light-oxic conditions were made. While held under the dark-anoxic conditions, the chambers were flushed continually with $N_2$. Flood waters were stripped of $CH_4$ prior to all measurements so that only plant mediated flux was measured.

## Results

Empty Erlenmeyer flasks did not leak or absorb $CH_4$; data were submitted to an f-test which showed that the slopes ($CH_4$ concentration or mixing ratio versus time) of all 66 tests were zero. Similar tests showed that little $CH_4$ was leaking out of syringes ($-0.0303$ $\mu$mol $h^{-1}$; $n = 8$). Methane consumption observed in the flasks was of bacterial origin. High rates of $CH_4$ uptake inside flasks ($n = 3$) containing roots was halted by the injection of mercuric chloride. Methane continued to be consumed in two other flasks to which no addition was made.

When $CH_4$ was consumed within incubation flasks its concentration decreased linearly. The uptake rates were independent of $CH_4$ concentration over the concentration ranges investigated. When the incubated material showed any activity, the coefficient of determination, $r^2$, derived from the linear equations that were fit to the data, was always greater than 0.9. No lag phase was observed at the beginning of the incubation experiments.

Average uptake rates from plant part incubations for the different sites are compiled in Table 1. Data from the incubations of stems collected from both Lake Hall and St Marks sites and rhizomes of *Nelumbo pentapetala* and *Nymphaea odorata* from Lake Hall were submitted to an f-test which revealed that none of these incubations showed any uptake, therefore showing that no methanotrophs were active in these stems and rhizomes. However, an f-test performed on the uptake data from rhizomes of *Pontederia cordata* from Lake Hall showed that the uptake rates were significantly different from zero ($-0.03$ $\mu$mol $g^{-1}_{(\text{dry material})}$ $h^{-1}$; Table 1). Average uptake rates from washed roots were $-1.119$ $\mu$mol $g^{-1}_{(\text{dry material})}$ $h^{-1}$ (SD = 1.80; $n = 19$) and from roots with sediments $-0.406$ $\mu$mol $g^{-1}_{(\text{dry material})}$ $h^{-1}$ (SD = 0.643; $n = 13$). Slopes of lines fit to the mixing ratios vs time plots had $r^2$ values greater than 0.95. Average rates were computed for individual experiments and showed a wide range of values for the different plants and for incubations of the same plant collected at different times (*P. cordata*: LH: 6/90B and 10/90; Table 1). The rates of $CH_4$ consumption of washed roots of *P. cordata* from Lake Hall were

*Table 1.* Average methane uptake rates from aerobic incubations of parts of plants collected from the different sites. Rates are in $\mu$mol $CH_4$ $g^{-1}_{(\text{dry incubated material})}$ $h^{-1}$ ($n$: number of replicates; SD: standard deviation).

| | | Stems | | Rhizomes | | | Washed roots | | | Roots + sediments | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | average | $n$ | average | $n$ | SD | average | $n$ | SD | average | $n$ | SD |
| St Marks | | | | | | | | | | | | |
| *Sagittaria lancifolia* | 5/90 | 0 | 4 | | | | | | | −0.98 | 3 | 1.02 |
| *Pontederia cordata* | 5/90 | 0 | 4 | | | | | | | −0.11 | 2 | 0.02 |
| Lake Hall | | | | | | | | | | | | |
| *Nelumbo pentapetala* | 9/90A | | | 0 | 2 | | −0.39 | 2 | 0.01 | | | |
| *Nymphaea odorata* | 6/90A | | | | | | −1.19 | 2 | 0.33 | | | |
| | 9/90A | | | 0 | 6 | | −0.30 | 3 | 0.28 | | | |
| | 9/90B | 0 | 3 | 0 | 3 | | −0.23 | 3 | 0.07 | | | |
| *Pontederia cordata* | 6/90A | 0 | 4 | | | | −1.92 | 2 | 0.02 | | | |
| | 6/90B | | | | | | −5.94 | 2 | 0.12 | −0.67 | 3 | 0.61 |
| | 10/90 | 0 | 5 | −0.03 | 5 | 0.03 | −0.16 | 5 | 0.05 | −0.02 | 5 | 0.01 |

significantly larger for each date than the rates associated with stems or rhizomes (Table 1). Rates associated with rhizomes and roots with sediments of *P. cordata* from Lake Hall (10/90) were not significantly different. Rates from washed roots of *Nymphaea odorata* and *Nelumbo pentapetala* were significantly larger than rates associated with rhizomes (9/90A and 9/90B). These data indicate that methanotrophs are present in the rhizosphere and are more likely to be associated with the roots. The apparently higher uptake rates per unit weight associated with washed roots relative to roots plus sediments are somewhat misleading due to the greater weight of dry sediments relative to dry root biomass.

From the incubations of core subsections under $CH_4$ and air, consumption profiles vs. depth were obtained for each site (Fig. 1). The potential (maximum, see below) uptake rates were integrated for each core, and were plotted beside measured $CH_4$ emission rates and $CH_4$ flux across the sediment water interface as calculated from profiles of dissolved $CH_4$ concentration (Fig. 2). In St Marks, $CH_4$ uptake on an areal basis integrated over 20 cm depth ranged from 0.31 to 1.20 g $CH_4$ $m^{-2}$ $d^{-1}$ with an average of 0.64 ($n$ = 6; SD = 0.30). Potential uptake rates of cores from Louisiana rice fields were only integrated over the top 10 cm because very few roots were observed to be in the dense clay sediments below this depth. Total potential uptake rates from cores from Louisiana ranged from 0.17 to 0.46 g $m^{-2}$ $d^{-1}$. Methane uptake rates for cores from the Everglades ranged from 0.79 to 7.19 g $m^{-2}$ $d^{-1}$.

It should be emphasized that these $CH_4$ consumption rates define the upper limit of *in situ* rates of rhizospheric methane oxidation because they

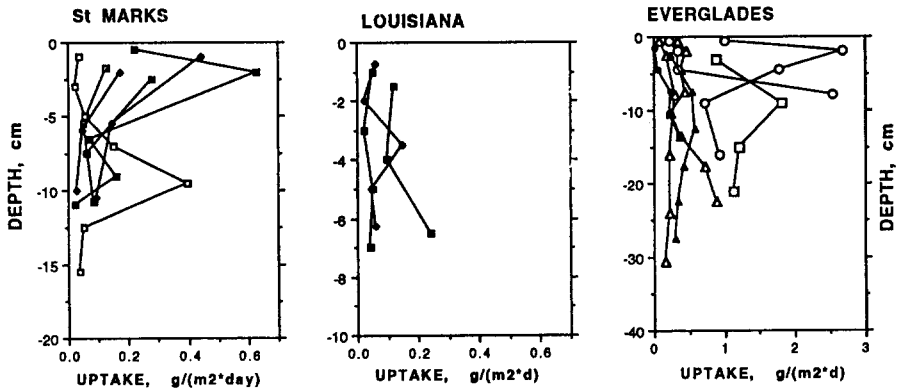


*Fig. 1.* Methane uptake profiles from aerobic incubations of core sections collected within St Marks *Sagittaria lancifolia* (left), Louisiana Rice (center) and Everglades (right) *(Typha domingensis* site, open symbols and *Cladium jamaicense* site, closed symbols).

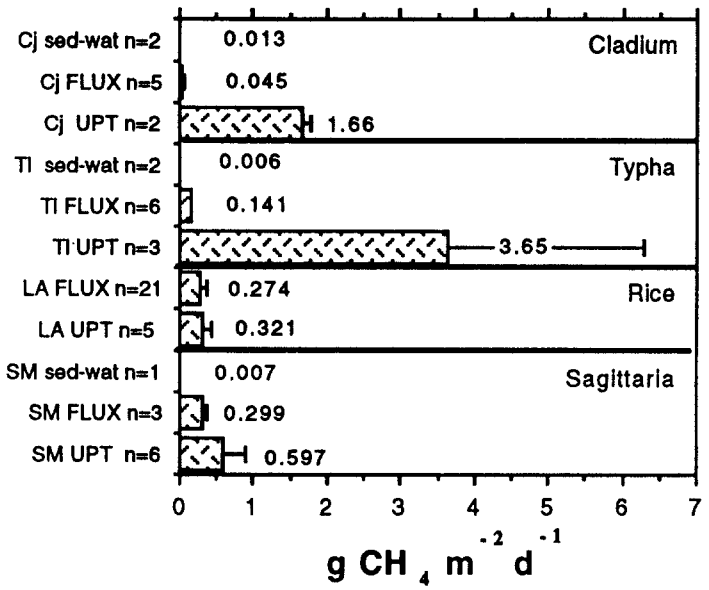


*Fig. 2.* Comparison between methane uptake (UPT) determined from aerobic incubations of core sections (Fig. 1) and extrapolated to an areal basis, methane flux across the sediment-water interface calculated from pore water profiles (SED-WAT) and total methane emissions (FLUX) measured in chambers for the different sites. St Marks *Sagittaria lancifolia* (SM), Louisiana Rice (LA), and Everglades *Typha domingensis*, (Tl), and *Cladium jamaicense* (Cj).

were performed under air. In situ, oxygen is likely to be scarce, while dissolved $CH_4$ concentrations range from 300—600 $\mu$M, and sedimentary bubbles contain 20 to 40% $CH_4$. In the rhizosphere, $O_2$ availability probably limits $CH_4$ oxidation. Whalen et al. (1992) measured high rates of $CH_4$ uptake (0.86 g $m^{-2}$ $d^{-1}$) in similar incubations of aerobic boreal forest soils under conditions of non-limiting $CH_4$ and $O_2$. Under *in situ* conditions in these forest soils, $CH_4$ oxidation was limited by $CH_4$ availability, opposite to the sediments in this study.

Methane uptake profiles (Fig. 1) exhibited peaks similar to peaks found in depth profiles of live root density (Gerard 1992). Therefore $CH_4$ uptake rates from subcore incubations were plotted vs. their respective live root densities to examine the relationship between the two variables (Fig. 3). The data indicate a weak positive relationship between live root density and $CH_4$ uptake rates for the *T. domingensis* site but no correlation was observed at the *C. jamaicense* site (Gerard 1992).

Methane fluxes were measured at all three sites where $CH_4$ uptake data from subcore incubations were obtained (Fig. 2). At the St Marks *S.*

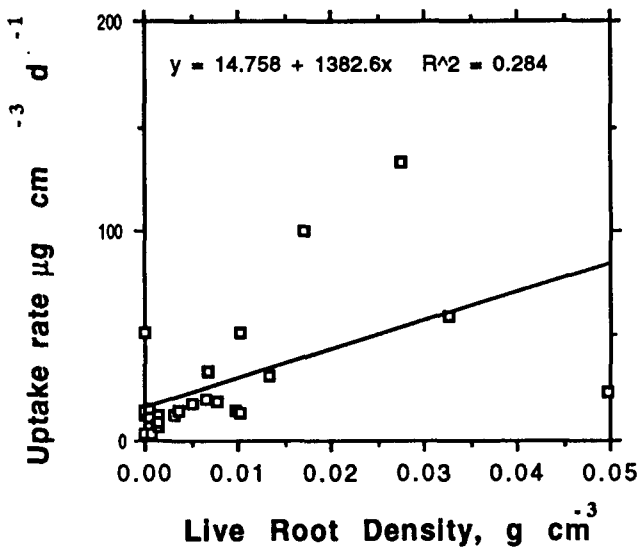


*Fig. 3.* Live root density and $CH_4$ uptake in Everglades *Typha domingensis* were positively correlated, $r = 0.54$, $n = 27$, $p < 0.001$. The regression would improve to $r = 0.77$ with the exclusion of the point in the lower right side of the graph.

*lancifolia* site, emissions ranged from 0.22 to 0.36 g $m^{-2}$ $d^{-1}$ with an average of 0.30 ($n = 3$; SD = 0.07). Methane emissions from the Louisiana rice were similar (0.27; $n = 21$; SD = 0.11). Emissions from the Everglades were smaller than emissions from St Marks and Louisiana, ranging from 0.11 to 0.17 g $m^{-2}$ $d^{-1}$ for the *T. domingensis* site and from 0.03 to 0.07 g $m^{-2}$ $d^{-1}$ for the *C. jamaicense* site. Methane oxidation rates (upper limit), as determined from the aerobic incubations, were larger than the measured emission rates for St Marks *S. lancifolia* and Everglades *C. jamaicense* and *T. domingensis*. In contrast, average flux from rice at the Louisiana site was found to be within the range of potential $CH_4$ oxidation rates derived from the aerobic subcore incubations (Fig. 2). The calculated diffusive flux of $CH_4$ across the sediment-water interface was small relative to the total flux; therefore 98% of the emitted $CH_4$ was through the plants (Fig. 2). All of the sites investigated were flooded with 5 to 15 cm of water overlying the sediments.

The average mixing ratio of stem gas from *S. lancifolia* from St Marks was 1.5 ± 0.9% $CH_4$ ($n = 28$). Bubbles from vegetated sites contained less $CH_4$ (21.8 ± 3.3%) and more nitrogen (59.4 ± 5.2%) than bubbles from unvegetated sites in the same marsh (65.4 ± 1.4% $CH_4$; 12.8 ± 0.2% $N_2$ respectively). This difference was mainly due to increased ventilation of the sediments by the plants. The isotopic composition of $CH_4$ within bubbles stirred from the sediments at the St Marks *S.*

*lancifolia* site (−51.7 ± 0.3‰ $n = 3$) was enriched in $^{13}C$ with respect to $CH_4$ emitted from this site (−61.6 ± 0.3‰ $n = 4$) and with respect to the average isotopic composition sedimentary $CH_4$ from freshwater environments (−60.1 ± 6.5‰, Whiticar et al. 1986). *S. lancifolia* stems contained $CH_4$ more enriched in $^{13}C$ (−42.0 ± 0.2‰ $n = 3$) than either the sediments or the emitted $CH_4$.

Methane fluxes measured from greenhouse *S. lancifolia* increased markedly from light-oxic to dark-anoxic conditions (Fig. 4). Methane

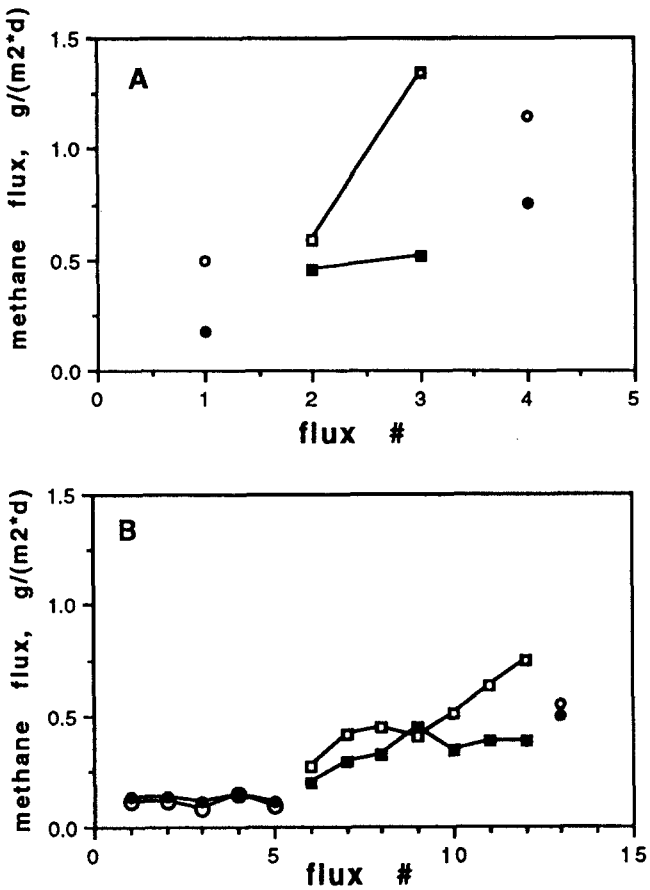


*Fig. 4.* Emissions from greenhouse *Sagittaria lancifolia*. Plants were collected on 6/24/91 (A) and 9/30/91 (B). Methane emission rates were plotted in the daily chronological order as they were measured. Open and filled symbols represent different plants. Circles represent light-oxic $CH_4$ emission measurements while squares represent emission under dark-anoxic conditions. Lines connect sequential light-oxic and dark-anoxic flux measurements. Only the first dark-anoxic flux was used to compute methane oxidation by difference from the light-oxic fluxes. Prolonged incubation under nitrogen and darkness enhanced methane emission. Light-oxic values failed to return to initial values after the dark-anoxic treatments.

emissions from greenhouse plants under light-oxic conditions were similar to the emissions measured *in situ* (Figs. 2, 4).

## Discussion

Methanotrophs were not active in the stems of any of the plants tested and in the rhizomes of *N. odorata* and *N. pentapetala*. Chanton et al. (1992a) reported small rates of $CH_4$ uptake in stems of *Peltandra virginica* but concluded it was not significant in terms of the quantities of $CH_4$ passing through the stems. Incubations of rhizomes of *P. cordata* showed an average uptake rate of $-0.03$ $\mu$mol $CH_4$ $g^{-1}$ $h^{-1}$. Incubations of washed roots showed large mean uptake rates but with high standard deviations. The high variability could be due to the association of $CH_4$ oxidizing bacteria with only specific parts of the roots. Smits et al. (1990) found that $O_2$ was released mostly at the tips of growing roots and methanotrophs would likely be concentrated there. Roots with sediments also showed large variability, possibly due to variations in the quantity of roots present in the incubated sediments, or because bacteria were present in very specific locations as described above. From these experiments, it appears that methanotrophs are not active in stems and their activity is closely associated with the roots. This is further supported by the relationship established between live root density and $CH_4$ uptake rate observed at the Everglades *T. domingensis* site (Fig. 3).

In a previous study, King et al. (1990) evaluated $CH_4$ oxidation at the root-soil interface of aquatic plants by stripping roots from their surrounding sediments and incubating them aerobically with $CH_4$. *C. jamaicense* and *S. lancifolia* growing in peat sediments showed rates of $CH_4$ consumption ($0.71 \pm 0.38$ $\mu$mol $CH_4$ $(g_{(\text{dry material})} \times h)^{-1}$ ($n = 3$) and $0.53 \pm 0.04$ $\mu$mol $CH_4$ $(g_{(\text{dry material})} \times h)^{-1}$ ($n = 3$; respectively, King et al. 1990) similar to the rates found in this work (all types of washed roots: 1.12 $\mu$mol $CH_4$ $(g_{(\text{dry material})} \times h)^{-1}$ ($n = 19$; SD = 1.80). Combining this data with estimates of belowground biomass, King et al. (1990) concluded that compared with the rates of $CH_4$ oxidation observed at the sediment-water interface, $CH_4$ oxidation due to below ground plant surfaces should not significantly increase the capacity of the system to consume $CH_4$ However, the King et al. (1990) study was done in an oligotrophic portion of the Everglades, which might have consequences for the amount of roots present in the sediments and therefore for the amount of rhizospheric $CH_4$ oxidation. The conclusion of King et al. (1990) was based on a calculation showing that 2.6 kg (dry weight) of roots would be required to equal the amount of oxidation observed at the sediment-water interface.

The observed live root biomass was only 0.5 kg $m^{-2}$ (M. Hardisky: personal communication to G. King). Root density from *C. jamaicense* growing in peat soils of the northern Everglades ranged from 0.6 to 6 kg $m^{-2}$ with most of the densities between 1.2 to 1.8 kg $m^{-2}$ (Gerard 1992). Methane oxidation in the rhizosphere of aquatic macrophytes may be dependent upon root density (Fig. 3).

To compare $CH_4$ oxidation (MO) to $CH_4$ emitted (ME) on an areal basis, a ratio MO/ME, defined as the quantity of $CH_4$ oxidized divided by the quantity of $CH_4$ emitted, was computed (Table 2). On an areal basis, maximum potential oxidation rates from the aerobic incubations were from 0.70 to 51 times the $CH_4$ emission rates. The potential oxidation rates were generally of similar magnitude at the St Marks and Louisiana sites (MO/ME = 1.03 to 4.01), but higher in the Everglades (MO/ME = 5.63 to 51.0).

The hypothesis that the maximum potential rates of $CH_4$ oxidation would be small relative to the emission rates was incorrect. Is this consistent with the two observations on which the hypothesis was based? Methane emissions generally increase with biomass (Whiting et al. 1991; Whiting & Chanton 1992, 1993; Happell et al. 1993), indicating that the positive consequences plants have on $CH_4$ emissions outweigh the attenuating effects of rhizospheric $CH_4$ oxidation. The presence of plants has been shown to enhance $CH_4$ production relative to unvegetated sediments probably because of increased substrate production (Whiting & Chanton 1992). We conclude that while the presence of macrophytes in a sediment may enhance methane production and flux relative to a similar unvege-

*Table 2.* Comparison of MO/ME ($CH_4$ oxidized/$CH_4$ emitted) values determined by a variety of techniques.

*Table 2A.* Methane emission and methane oxidation rates determined from aerobic incubations (this study). All Rates in g $m^{-2}$ $d^{-1}$.

| Sites | Oxidation range (MO) (g $m^{-2}$ $d^{-1}$) | Emission (ME) (g $m^{-2}$ $d^{-1}$) | MO/ME range |
|---|---|---|---|
| St Marks | | | |
| *Sagittaria lancifolia* | 0.31—1.20 | 0.30 | 1.0—4.0 |
| Everglades | | | |
| *Typha domingensis* | 0.79—7.19 | 0.14 | 5.6—51.0 |
| *Cladium jamaicense* | 1.66 | 0.04 | 36.7 |
| Louisiana | | | |
| rice | 0.17—0.46 | 0.27 | 0.7—1.9 |

*Table 2B.* Methane oxidation (MO) determined as the difference between methane production (MP) and methane emission (ME). Data are literature values for rice, units are as indicated.

| Rice<br>Studies | Production<br>MP | Emission<br>ME | MO/ME<br>{(MP-ME)/ME} |
|---|---|---|---|
| Holzapfel-Pschorn et al. 1985 | | | |
| lab ($\mu g\ h^{-1}$) | 301 | 52 | 4.8 |
| field ($mg\ m^{-2}\ h^{-1}$) | | | |
| highest (MO/ME) | 25 | 15 | 0.7 |
| lowest (MO/ME) | 28 | 25 | 0.1 |
| Schutz et al. 1989 ($ml\ m^{-2}\ h^{-1}$) | | | |
| late season | 297 | 8.3 | 35 |
| early season | 20 | 11 | 0.8 |
| Sass et al. 1990 ($mg\ m^{-2}\ d^{-1}$) | | | |
| | 850 | 350 | 1.4 |

*Table 2C.* Methane oxidation determined by inhibition experiments. Methane oxidation (MO) calculated as the difference of the average of the light-oxic fluxes (ME) and the first dark-anoxic flux (Fig 4).

| | Light-Oxic<br>($g\ m^{-2}\ d^{-1}$) | Dark-Anoxic<br>($g\ m^{-2}\ d^{-1}$) | MO/ME |
|---|---|---|---|
| 6/24/91 | | | |
| *S. lancifolia* #1 | 0.15 | 0.39 | 1.6 |
| *S. lancifolia* #2 | 0.51 | 0.60 | 0.2 |
| 9/30/91 | | | |
| *S. lancifolia* #3 | 0.10 | 0.23 | 1.3 |
| *S. lancifolia* #4 | 0.13 | 0.20 | 0.5 |
| Rice Studies | control | inhibitor | MO/ME |
| De Bont et al. 1978 (ml $CH_4$) | | | |
| $C_2H_2$ | 11.67 | 15.54 | 0.3 |
| Holzapfel-Pschorn et al. 1985 | | | |
| $N_2$ | 100% | 172% | 0.7 |

tated sediment, this enhancement does not exclude the possibility of a substantial quantity of $CH_4$ oxidation.

The second observation on which the hypothesis was based, was that previous stable isotope studies of macrophyte systems (Chanton et al. 1992a; Happell et al. 1993) have shown no evidence for methanotrophic

activity in the rhizosphere. If substantial methane oxidation was occurring, these authors suggested that it must occur quantitatively in discrete microzones and thus not impart a $^{13}C$ enriched signal to sedimentary or emitted $CH_4$. In contrast with these studies however, the stable isotope data from the St Marks *S. lancifolia* site ($CH_4$ emitted from plants: $-61.6 \pm 0.3‰$; $CH_4$ within stems: $-42.0 \pm 0.2‰$; $CH_4$ within sedimentary bubbles: $-51.7 \pm 0.3‰$) showed that the $CH_4$ from the sedimentary bubbles was more enriched in $^{13}C$ than the average sedimentary $CH_4$. This enrichment could be due to the action of methanotrophic bacteria or the apparent preferential mobilization of $CH_4$ containing the lighter isotope which could leave residual sedimentary $CH_4$ $^{13}C$ enriched as happens within plant stems (Chanton et al. 1992a, b). Emissions were $^{13}C$ depleted with respect to the sediment bubbles.

In addition to the technique of aerobic incubations used in this study, $CH_4$ oxidation in the rhizosphere has been estimated by other means. Estimates of $CH_4$ oxidation as the difference between $CH_4$ emitted and $CH_4$ produced in anoxic incubations of sedimentary material has produced a range of values summarized in Table 2 (Holzapfel-Pschorn et al. 1985; Schultz et al. 1989; Sass et al. 1990). From each of these studies, we computed a MO/ME ($CH_4$ oxidized/ $CH_4$ emitted) ratio and compared them with the ratios obtained from the aerobic incubation technique (Table 2B). St Marks *Sagittaria lancifolia* and Louisiana rice data showed oxidation to $CH_4$ emissions (MO/ME) ratios that were similar to the ratios observed in these studies. But as discussed above, our aerobic incubations should yield upper limits for the oxidation rates because $O_2$ is probably not as readily available to the methanotrophs *in situ* as it was in the incubations. Since aerobic incubations yield upper limits for the MO/ME ratio and since $CH_4$ production experiments yield results in the same range as the aerobic incubations, we suggest that $CH_4$ production experiments which determine methane oxidation (MO) as the difference between methane production (MP) and methane emission (ME) may yield upper limits for the ratio MO/ME.

This suggestion is reasonable because 1) these incubations may include live root material (now cut off from the rest of the plant) which can add substrate to the sediment, and 2) the anoxic incubations disrupt the natural interstitial interfaces present in the soil because they cut off the supply of $O_2$ to the sediments by the roots, thereby — 1) lowering sediment Eh, possibly stimulating $CH_4$ production and 2) stopping aerobic oxidation of complex organic substrates, possibly shunting the remineralization of these materials into the anaerobic food chain and stimulating $CH_4$ production.

Methane oxidation rates were also determined in this study by com-

paring fluxes made under light-oxic conditions and dark-anoxic conditions from plants collected from the field and grown in a greenhouse. Results indicated that the MO/ME ratios obtained were generally similar to or somewhat lower than the ratios obtained using aerobic or anaerobic incubations. These MO/ME ratios however, may also be upper limits because $CH_4$ production may have increased between the light-oxic and the dark-anoxic fluxes. The difference between the $CH_4$ emission rate during the dark-anoxic measurements and the rate under light-oxic conditions is interpreted as the quantity of $CH_4$ consumed by oxidation, assuming that $CH_4$ production remained the same during the two fluxes. However, the increase in emissions during subsequent dark-anoxic fluxes indicates that this assumption might not be valid (Fig. 4). The continued increase in $CH_4$ flux from plants held under prolonged dark anoxic conditions could be due to: (1) as $CH_4$ oxidation was inhibited, below-ground $CH_4$ concentration increased and therefore the flux increased, or (2) as $O_2$ supply to the rhizosphere was cut off, aerobic respiration of complex organic matter ceased, and these compounds were available in greater quantities to the anaerobic food chain resulting in increased rates of $CH_4$ production. It is also possible that under the stress of darkness and anoxic conditions the plant released more organic matter that might become available to methanogens. Fluxes measured under light-oxic conditions after the conclusion of the series of dark-anoxic flux measurements continued to be elevated relative to initial control fluxes (Fig. 4). Increase in production should have the least impact on the first dark-anoxic flux. For this reason, the first dark-anoxic flux was used to calculate $CH_4$ oxidation for these experiments (Table 2). These values must be regarded as upper limits because of the possibility of stimulation of $CH_4$ production rates.

Holzapfel-Pschorn et al. (1985) performed similar experiments on rice grown in the laboratory. Methane emissions through rice plants held under pure nitrogen were found to be 172% of the emissions when plants were held under air. If the increase in flux is only attributed to a decrease in $CH_4$ oxidation, this experiment gives a MO/ME of 0.72 similar to the ratios observed in our experiments. Holzapfel-Pschorn et al. (1985) concluded that the increase in emissions could be due to $CH_4$ production enhancement or $CH_4$ oxidation inhibition or both.

In similar experiments, De Bont et al. (1978) used an inhibitor of $CH_4$ oxidation (acetylene) on young rice that was transplanted from the field into pots. Pots were incubated in closed chambers under air or under air containing acetylene. Assuming that approximately the same amount of $CH_4$ was produced in each pot, their experiment showed that about 25% of the $CH_4$ produced was oxidized. This gave an uptake rate of $CH_4$ 0.3

times the $CH_4$ emission rate (Table 2). From their experiments, De Bont et al. (1978) concluded that little $CH_4$ oxidation was taking place in the root-soil interface compared with the amounts of $CH_4$ available. They hypothesized that the rhizosphere could be anoxic because other processes could quickly utilize the $O_2$ leaking out from the roots. However, the value of 0.3 obtained for MO/ME ratio has some uncertainties associated with it. This computation was based on the assumption that the two pots used in the comparison were producing the same amount of $CH_4$. Spatial variability in $CH_4$ oxidation rates or in the $CH_4$ production rates between the two pots could introduce uncertainty to this value. One way to overcome this problem would be to measure the two fluxes successively on each pot, first under air and then under acetylene, or some other inhibitor (for example methyl fluoride, Epp & Chanton 1993). Presumably short term temporal variability would be a smaller factor than spatial variability. Additional uncertainties are present because acetylene can disrupt microbial processes in addition to methane oxidation, including methanogenesis (Oremland & Capone 1988; Oremland & Culbertson 1992a). Recently in a series of greenhouse studies, Epp & Chanton (1993) have investigated the application of methyl fluoride, a more specific inhibitor of methane oxidation (Oremland & Culbertson 1992a, b), to the problem of rhizospheric methane oxidation.

*Speculations*

In this study, it was observed that the ratios MO/ME derived from the aerobic incubations of sedimentary material collected within *T. domingensis* and *C. jamaicense* Everglades sites were much larger than those derived from material collected within *S. lancifolia* from St Marks and rice collected in Louisiana. From these results, we speculate that mature plant communities as found in the sub-tropical Everglades oxygenate their rhizosphere in a more effective fashion than annually planted macrophytes like rice or *S. lancifolia* which recesses every winter in North Florida. This hypothesis is supported by the results of Schutz et al. 1989 (Table 2) which shows that the MO/ME ratio from the late season (35) was much larger than from the early season (0.8). This hypothesis could also explain why a large range of values have been observed for MO/ME. In order to evaluate the importance of $CH_4$ oxidation in the rhizosphere of plants, it will be necessary to make seasonal studies of $CH_4$ oxidation rates.

## Acknowledgments

We thank Susan Boehme for thorough review and criticism of this manuscript. Gary Whiting and Jim Happell assisted with fieldwork and experimental design. We thank Patrick Bollich, Chuck Lindeau and the Louisiana State University Rice Research Station in Crowley, La. for allowing us to sample and providing us with laboratory space. Our isotopic analyses were run in the laboratory of Neal Blair of North Carolina State University. This work was funded by the NASA Terrestrial Ecology/Biospherics Research Program and the NOAA Global Change Program.

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