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Methane oxidation associated with submersed vascular macrophytes and its impact on plant diffusive methane flux

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Abstract. Methane oxidation associated with submersed vascular plants and its effects on diffusive CH_4 release from plants were examined through a series of laboratory and field incubation experiments. In laboratory analyses, measured rates of epiphytic oxidation (i.e., oxidation associated with aboveground tissues) ranged from 0.3 to 32.9 pmol mm^{-2} plant tissue h^{-1} with significant CH_4 consumption associated with basal (i.e., near sediment) leaves and stems for all six species tested. Basal stem tissue also showed greater oxidation activity than basal leaves. Oxidation activity for washed roots of three submersed species ranged from 0.18 to 7.01 μmol g^{-1} root ash-free dry mass h^{-1} with higher rates associated with two rhizomatous/stoloniferous species than with a non-rhizomatous one. In field incubations of a single species (*Myriophyllum exalbescens*), intact plants showed net CH_4 consumption during the day and net release at night. When a specific inhibitor of CH_4 oxidation was applied (methyl fluoride – MF), net daytime release from plants was observed and nighttime flux increased, indicating that diffusive CH_4 release from submersed plants is significantly curtailed by the activity of epiphytic methanotrophs.

Abbreviations: AFDM – ash-free dry mass; E_h – redox potential; gdw – grams dry weight; MF – methyl fluoride – CH_3F; PVC – polyvinyl chloride; SWI – sediment-water interface

Introduction

Identification and quantification of atmospheric sources and sinks of radiatively-active CH_4 are crucial to understanding future changes in Earth's climate. Emissions from wetlands and other natural freshwater environments represent approximately one quarter of total global CH_4 release to the atmosphere (IPCC 1995). Identified pathways for atmospheric CH_4 release from organic-rich freshwater sediments include direct diffusion or ebullitive

release (Chanton et al. 1989; Sebacher et al. 1983; Smith & Lewis 1992) and transport through gas lacunae (intercellular air spaces) of emergent vascular macrophytes (Dacey & Klug 1979; Chanton et al. 1993; Sorrell & Boon 1994). Much of the CH_4 produced in deeper sediments can be oxidized to CO_2 by CH_4-oxidizing bacteria at oxic-anoxic boundaries, such as interfaces between sediment and oxic overlying water (Kuivila et al. 1988; Frenzel et al. 1990; King 1990) or between sediment and the roots of vascular plants (Gerard & Chanton 1993; Epp & Chanton 1993; King 1996; Schipper & Reddy 1996; Lombardi et al. 1997).

Although much work has examined the role of emergent vascular plants in the production, oxidation, and transport of sediment CH_4, few studies have addressed the effects of submersed vascular macrophytes on these processes. Boon and Sorrell (1991) documented reduced *in vitro* methanogenesis rates in sediments from a bed of submersed *Vallisneria gigantea*. Along with findings of higher sediment redox potential (E_h) associated with roots of submersed plants (Wium-Anderson & Anderson 1972; Jaynes & Carpenter 1986; Flessa 1994; Wigand et al. 1997), direct oxygen release from the roots of some species has also been documented (Sand-Jensen et al. 1982; Kemp & Murray 1986; Sorrell & Dromgoole 1987; Caffrey & Kemp 1991; Christensen et al. 1994). These studies of root-zone O_2 status did not examine the possible impact of oxygenation on rhizospheric CH_4 cycling. Sebacher et al. (1985) and Schuette and Klug (1995) measured CH_4 release from emergent inflorescences of submersed macrophytes. We report here the results of measurements of diffusive CH_4 release from completely submersed vascular plants.

In emergent and submersed vascular plants, CH_4 diffuses into the roots and then the lacunar network of the plants. Atmospheric emission of lacunar CH_4 from emergent species is well documented (see Schutz et al. 1991). However, until now, the fate of lacunar CH_4 within submersed plants had not been determined. Söhngen (1906) first isolated CH_4-oxidizing bacteria from the leaves of the submersed vascular plant *Elodea* (King 1992). However, the commonality and functional significance of epiphytic methanotrophs (i.e., CH_4-oxidizers associated with leaves and stems of plants) were previously unexamined. Well-developed communities of epiphytic methanotrophs could function much like those found at oxic-anoxic boundaries (e.g., sediment-water interface or SWI), acting as biofilters that decrease diffusive CH_4 flux from plants to surrounding littoral water. In addition, as with many emergent species, lacunar O_2 transport to and out of roots may support root-associated methanotrophs that could decrease diffusion of sediment CH_4 into plants. In this study, laboratory incubations of various tissues from several plant species were conducted in conjunction with *in situ* incubations of intact plants of a

single species with the goal of determining the prevalence and functional role (i.e., impact upon plant diffusive CH_4 flux) of methanotrophs associated with submersed vascular plants.

Materials and methods

Plant tissue incubations

Plants for incubation experiments were grown in a laboratory mesocosm (1.5 m-long, 0.15 m-wide, 0.7 m-high aquarium) or taken directly from Pleasant Lake, a small ($\cong$ 4 ha), relatively shallow (max. depth = 9 m, mean depth $\cong$ 3 m), mesotrophic lake located in the town of Edwardsburg in southwest lower Michigan (USA). The mesocosm was filled to a depth of 25 cm with sediments taken from Pleasant Lake (depth $\cong$ 2 m). Overlying water was a 1:1 mixture of tap water and distilled water. With weekly changing of roughly half of the mesocosm water, plants grew extremely well without the addition of any added nutrients. Illumination of 70 μmol quanta m^{-2} s^{-1} of photosynthetically active radiation was supplied with a combination of fluorescent and Gro-Lux® lamps. Mesocosm transplants were grown over a 10- to 12-week period before initiating experiments.

Six different species of submersed vascular plant were examined for epiphytic methanotrophy: *Myriophyllum exalbescens*, *Potamogeton angustifolius*, *P. crispus*, *P. robbinsii*, *Megalodonta beckii*, and *Sagittaria cristata*. The first five species were tested for effects of tissue type (leaf or stem) and position relative to the sediments (basal or apical) upon oxidation activity. Due to its small size (max. leaf length $\cong$ 20 cm) and rosette growth form, effects of tissue type and position were not tested for *S. cristata*. Only mesocosm-grown *M. exalbescens* and *P. angustifolius*, and field-sampled *S. cristata* were used in root tissue studies. These three species were chosen due to the marked differences in their morphology: *M. exalbescens* and *P. angustifolius* are caulescent, non-rhizomatous and rhizomatous species, respectively, while *S. cristata* is a stoloniferous, rosette species.

In all laboratory incubation studies, plant tissues were incubated in 30 mL of lake or mesocosm water contained in 38-mL glass serum vials (8 mL of air for 1:3.75 headspace:water volume) sealed with butyl rubber stoppers (Supelco, Cat. No. 3-3201M) and aluminum crimp seals. Once vials were sealed, calculations of air:water partitioning were used to determine appropriate volumes of CH_4 to add to each vial such that initial water CH_4 concentration was the same for each vial in individual experiments. All vials were lightly mixed throughout incubations using a slow-moving shaker table. Changes in total vial CH_4 through time during 48- to 96-hour incub-

ations were monitored via calculations of air-water partitioning (McAuliffe 1971) following analysis of headspace concentrations using a Hewlett Packard 5890 series II Gas Chromatograph equipped with a GS-Q column (53 mm-diameter; 30 m-long) and a flame ionization detector.

For epiphytic studies, tissue incubations for various species were started at slightly different initial CH_4 concentrations based on ambient CH_4 levels in the system (i.e., mesocosm or Pleasant Lake) at the time of tissue harvesting. Initial vial concentration was set at roughly double that observed in each system to assure maximal oxidation rates. Tissues of *M. exalbescens* and *P. angustifolius* taken in late May from Pleasant Lake (lake water CH_4 = 2.2 μM) were incubated at an initial water concentration of 4.5 μM. Tissues of *S. cristata* were taken from Pleasant Lake in July (lake water CH_4 = 4.5 μM) and incubated at an initial concentration of 9.0 μM. Aboveground tissues of other species were taken from mesocosm-grown plants (mesocosm water CH_4 = 1.5 μM) and incubated an initial concentration of 3.0 μM.

In root studies, starting CH_4 concentration was 8–9 μM. This initial level was much lower than porewater CH_4 concentrations observed in the upper 25 cm of mesocosm sediment with *P. angustifolius* and*M. exalbescens* (50–200 μM) but roughly double the concentrations observed in sediments with *S. cristata* (3–5 μM). The initial level of 8–9 μM for all root incubations was selected to maintain similar methodology in root and epiphytic studies.

For all studies, distilled and unfiltered lake (or mesocosm) water controls without plant material were run along with treatment vials with plant tissue. For most work, an additional set of inhibited controls (with and without plant material) was also used. Inhibited controls were treated with acetylene (headspace C_2H_2 concentration = 50 mL L^{-1}; aqueous concentration = 2.2 mM).

In tests of epiphytic methanotrophy, incubation vials contained 3–5 leaves or pieces of leaf (e.g., area of pieces of *Potamogeton* leaf = 2–3 cm^2), or 2–4 short ($\cong$ 2 cm) pieces of stem. Leaf surface area was determined by examining enlarged (200%) photocopies of pressed leaves using a computer-based image analysis system (OPTIMAS 2.03 – BioScan, Edmonds, WA). Stem surface area was calculated from measured diameters and lengths of stem pieces. For species examined for effects of tissue position, basal samples of aboveground tissues were taken from the first 15–20 cm of plant growth above the SWI. For mesocosm plants, samples of apical tissues were taken from fresh growth approximately 0.5 m above the SWI. For field samples of *M. exalbescens* and *P. angustifolius*, apical tissue originated from fresh growth approximately 1.0 and 0.8 m above the SWI respectively. Results of stem and leaf incubations are expressed as CH_4 loss per mm^2 of incubated tissue.

In analyses of root-associated methanotrophy, incubation vials contained 3–5 pieces of root (length ≅ 4 cm) or rhizome (length ≅ 3 cm). Various belowground tissues of rhizomatous *P. angustifolius* were incubated: pieces of rhizome (3–5 pieces, each 3-cm long), tissue from roots near their connection with the rhizome (basal roots in Results and Discussion), and tissue from roots at their tips (apical roots). For *M. exalbescens*, which does not produce a rhizome, we examined tissue from adventitious roots near their connection with stems below the SWI (basal) and tissue from roots at their tips (apical). For *S. cristata*, root and stolon tissues of several individual clones were sampled and incubated together. The densely intertwined root growth of *S. cristata* prevented sampling of fully intact root systems, precluding analysis of positional effects on root oxidation activity. Before incubation, roots tissues of all plants were cleansed of sediment and other debris by gentle repeated submersion in distilled water. Surface area of incubated root tissue was not measured. Results of root experiments are expressed as CH_4 loss per gram of root ash-free dry mass (AFDM = loss on ignition @ 500 °C for 6 hours).

In analysis of results from each incubation experiment, treatments and controls (inhibited and/or without plant material) were first compared for total cumulative CH_4 loss without correction for tissue surface area or AFDM. Analysis of variance and Tukey multiple comparison tests (SigmaStat 2.03: SPSS, Chicago, IL) were employed to determine specific treatments (i.e., combinations of tissue position and type: basal stem, apical leaves, basal root, apical root, rhizome, etc.) showing detectable CH_4 oxidation activity. Rates of CH_4 loss per unit surface area (leaf or stem) or AFDM (root) were then calculated from linear regression of corrected cumulative loss versus time. In separate analyses for each species, differences in cumulative CH_4 loss per unit surface area or AFDM among treatments were determined using analysis of variance and Tukey tests.

In situ *incubations of intact plants*

Diffusive CH_4 release from intact plants of *M. exalbescens* was determined *in situ* in Pleasant Lake. After preliminary studies showed considerable variability in diffusive release among randomly-selected plants, *M. exalbescens* shoots were transplanted into plots located in a single area of the littoral zone of Pleasant Lake to reduce any variation due to differences in plant age and sediment characteristics. Attempts to transplant *P. angustifolius* to allow comparisons between species were unsuccessful. In mid-May 1997, fresh *M. exalbescens* (30-cm-long pieces of new spring growth) were transplanted into several cleared, 5-m^2 areas within a nearly homogenous bed of *P. robbinsii*. Some *M. exalbescens* grew in this area, indicating that the sediments were

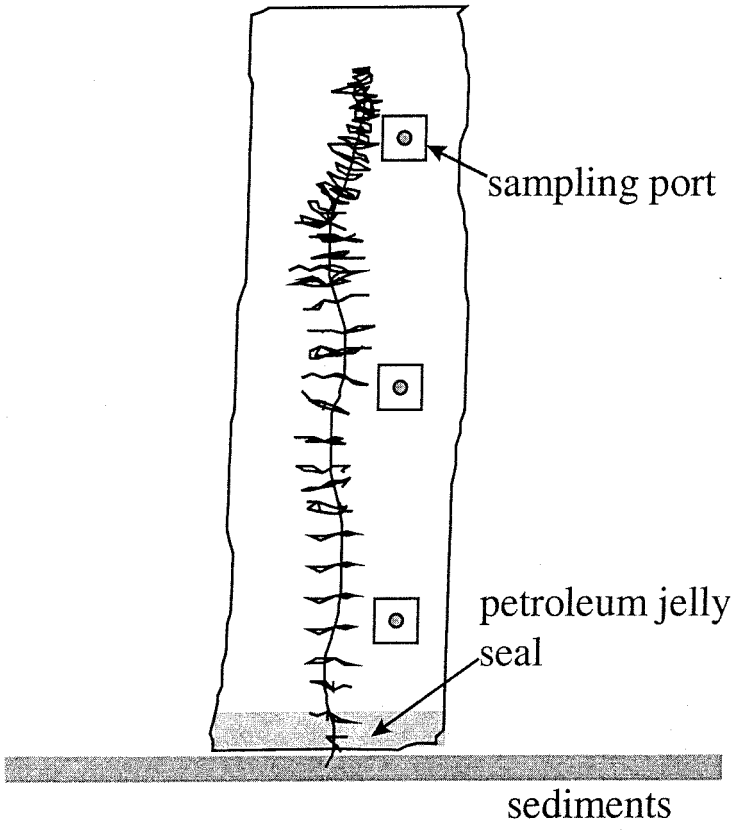

Figure 1. Design of bags used to sample *in situ* diffusive CH_4 release from intact plants of *Myriophyllum exalbescens*.

capable of supporting growth of the species. *M. exalbescens* transplants were allowed two months to become established before *in situ* diffusive release experiments were conducted.

Diffusive release from transplanted *M. exalbescens* was captured in bags (Figure 1) made of a multi-layer clear film of saran and PVC with low CH_4 permeability (Viskase Corp., Chicago, IL). Laboratory tests revealed that diffusive CH_4 loss was $< 3\%\ d^{-1}$ (in air and starting with 10 mL $CH_4\ L^{-1}$). The cylindrical bags were open at one end and 1.0 m in length. Total water volume enclosed during experiments was 3–5 liters. Each bag had three sampling ports consisting of butyl rubber septa (Alltech, Cat. No. 15418) attached to the bag using a combination of silicone glue and clear plastic tape. At the start of diffusive release experiments, bags were lowered over the top of plants with care taken to prevent plant damage. Just above the sediments, bags were

sealed around plant stems using petroleum jelly. The petroleum jelly seal was several mm thick and approximately 3-cm wide, thus limiting any diffusive loss through the CH_4-permeable jelly. Empty control bags showed negligible changes in water CH_4 concentration over intervals of up to 48 hours. During plant incubations, samples of enclosed water were taken from each of the three ports using 12-mL-draw, evacuated tubes (3 mL headspace; total volume = 15 mL). CH_4 concentration of aqueous samples was calculated from headspace CH_4 concentration as before. Total bag CH_4 was determined by averaging measured water CH_4 concentrations at the three sampling ports and multiplying the result by the total internal volume of the bag (determined as described below). The rate of CH_4 release per plant was calculated as the change in total bag CH_4 between sampling times divided by the time of the sampling interval.

During the mid-morning (9:00-11:00) of July 24, 1997, eight *M. exalbescens* plants were bagged and initially sampled. Bags were not sampled again until sunset (ca. 19:00). Final sampling was done around sunrise (6:30–7:30) on the following morning. Just before each bag was sampled at the end of the study, 60 mL of a 7 mM solution of methyl fluoride (MF) were added (20 mL at each sampling port) and mixed within the bag to determine bag internal volume via calculation of a dilution factor. MF was used because its extremely high solubility in water allowed for additions of manageably small volumes of aqueous solution to bags. Although MF is an inhibitor of CH_4 oxidation (Oremland & Culbertson 1992), it is unlikely that results were confounded since MF was present in bags for less than five minutes before final sampling. After final sampling, bags were carefully removed from study plants. On the morning of July 29, nine *M. exalbescens* plants (the original eight plus one) were bagged and 60 mL of 7 mM MF solution were added to each bag before initial sampling to inhibit epiphytic CH_4 oxidation during the experiment. The length of incubation and the timing of sampling during the inhibition study were the same as before. Weather conditions, water temperature, and wind/wave activity were nearly identical on the two proximal sampling days, supporting an assumption of similar plant function. Effects of time of incubation (day or night) and MF inhibition upon rate of CH_4 release were examined through analysis of variance and a Tukey test. A separate set of plants was also incubated to examine effects of incubation on O_2 levels of enclosed water. Water O_2 concentration was determined using headspace analysis as before and a Varian Star 3600 gas chromatograph equipped with a PoraPack-Q column and a thermal conductivity detector.

Lacunar CH_4 concentrations were also measured for *M. exalbescens* growing at a depth of 1.5 m. Sampled plants were similar in size and maturity to those used during *in situ* incubations of intact plants. Lacunar gas samples

Table 1. Calculated rates of CH_4 loss from treatment vials during experiments examining epiphytic oxidation associated with stem and leaf tissues of six species of submersed vascular plants. Loss rates are expressed as pmol mm^{-2} plant tissue h^{-1} ($\pm$ 1 SE; n = 4). Letters (superscript a–d) indicate results of Tukey multiple comparison of loss rates among treatments for each individual species tested

Species[1]	Basal stem	Basal leaves	Apical stem	Apical leaves
M. exalbescens (PL)	27.1 ± 2.4^{a}	17.3 ± 1.0^{b}	2.9 ± 0.3^{c}	0.6 ± 0.1^{dns}
P. angustifolius (PL)	32.9 ± 1.1^{a}	14.8 ± 2.1^{b}	2.7 ± 1.0^{c}	2.0 ± 0.5^{c}
S. cristata (PL)	ND	7.7 ± 1.4	ND	ND
P. robbinsii (M)	5.7 ± 0.5^{a}	2.3 ± 0.2^{b}	1.1 ± 0.6^{bc}	0.3 ± 0.2^{c}
P. crispus (M)	4.1 ± 0.3^{a}	0.6 ± 0.3^{bns}	0.3 ± 0.1^{cns}	0.6 ± 0.2^{bcns}
M. beckii (M)	29.3 ± 2.0^{a}	6.7 ± 0.3^{b}	0.2 ± 0.3^{cns}	0.8 ± 0.2^{cns}

ND = Not determined due to short rosette form of *S. cristata*.
[1]PL = taken directly from Pleasant Lake; M = grown in mesocosm.
[ns]cumulative CH_4 loss not significantly different from <1% loss observed in controls ($p > 0.05$).

were taken using 1-mL glass tuberculin syringes equipped with 25-gauge needles and were analyzed in the laboratory within six hours of sampling. Several tests revealed that syringe CH_4 loss was insignificant over that interval. Lacunar gas samples were taken from stem sections located 1 m above and 2 cm below the SWI. Plants were randomly sampled in mid-afternoon under clear, sunny conditions and then again at sunrise the next day. The effect of sampling time upon lacunar CH_4 concentration was tested through separate analyses of shoot and root data using Student t-tests.

Results

Plant tissue incubations

Laboratory incubation experiments identified epiphytic methanotrophy in association with all six species of submersed vascular macrophytes examined. In the absence of repeated sampling (samples taken only at start and end of incubation), all inhibited controls (with or without plant material) and uninhibited controls with only lake or mesocosm water exhibited negligible CH_4 loss during one-week trial incubations. Repeated sampling during actual incubation experiments (3–5 additional samples taken) produced slight decreases in control vial CH_4 (< 1% initial volume). When cumulative CH_4 loss (uncorrected for tissue surface area) from control and treatment vials was compared, significant CH_4 oxidation was detected for most tissues. Areal-

Table 2. Calculated rates of CH_4 loss from treatment vials during incubation experiments examining oxidation associated with root tissues of three species of submersed vascular plants. Loss rates are expressed as μmol g^{-1} root AFDM h^{-1} ($\pm$ 1 SE; n = 4). Tukey comparison tests of cumulative loss among treatments (basal, apical, or rhizome) did not detect positional differences in oxidation for either *M. exalbescens* or *P. angustifolius*. *S. cristata* root tissue included stolon and was not tested for positional differences (listed as basal)

Species[1]	Basal root	Apical root	Rhizome
M. exalbescens (M)	0.18 ± 0.13	0.06 ± 0.09^{ns}	ND
P. angustifolius (M)	3.95 ± 0.48	2.18 ± 0.49	2.79 ± 0.38
S. cristata (PL)	7.01 ± 0.82	ND	ND

ND = Not determined.
[1]PL = taken directly from Pleasant Lake; M = grown in mesocosm.
[ns]cumulative CH_4 loss not significantly different from <1% loss observed in controls ($p > 0.05$).

corrected rates ranged from 0.3 to 32.9 pmol mm^{-2} plant tissue h^{-1} (Table 1). Since significant oxidation could not be detected for any inhibited controls, surface area of tissues in inhibited vials was not determined. Therefore, calculations of loss per unit surface area for inhibited controls with plant tissue are not included in Table 1. Assessment of CH_4 loss among uninhibited treatments indicated that basal tissues growing near the sediments showed much greater epiphytic CH_4 oxidation than apical tissues, and that stems, particularly basal stems, generally had greater oxidation activity per mm^2 than leaves (Table 1). Due to the various sources (field or mesocosm) and sizes of study plants (see above), a direct statistical comparison of epiphytic methanotrophy among the various species was not conducted.

In studies of root-associated CH_4 oxidation, comparisons of control and treatment vials for total CH_4 loss indicated detectable oxidation for all root tissues except apical roots of *M. exalbescens*. Dry mass-corrected rates ranged from 0.18 to 7.01 μmol g^{-1} root AFDM h^{-1} (Table 2). The incubation water in root experiments was initially air-saturated, and therefore, calculated oxidation rates should be considered maximum potentials with respect to O_2 availability. Roots of *S. cristata* directly from Pleasant Lake showed the highest rates of root-associated CH_4 oxidation. Mesocosm-grown plants showed lower oxidation rates, perhaps as an artifact of incubation at CH_4 concentrations that were lower than ambient levels in porewater of mesocosm sediment (see Materials and methods). Due to differences in source of plant material, direct statistical comparison of field-grown *S. cristata* with the two mesocosm-grown species was not conducted. However, similar size and age of mesocosm-grown *P. angustifolius* and *M. exalbescens* allowed for direct

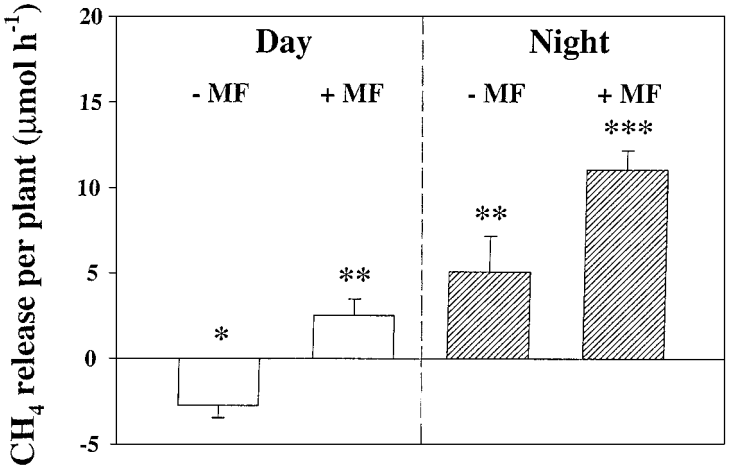

Figure 2. Diffusive CH_4 release during the day and at night from *Myriophyllum exalbescens* during an *in situ* study conducted in Pleasant Lake (Michigan, U.S.A.) in late July 1997. Release was monitored with and without an inhibitor of CH_4 oxidation (methyl fluoride – MF). Error bars equal + or – 1 SE (n = 8 for treatments without MF; n = 9 for treatments with MF). Asterisks represent equivalent release rates based on results of Tukey multiple comparison test ($p \leq 0.05$).

comparison that confirmed a significant effect of species (one-way ANOVA; $p < 0.001$) suggested by the markedly greater oxidation activity associated with roots of *P. angustifolius*. Separate Tukey comparison tests for each mesocosm species failed to detect significant differences between types of root tissue, but a more sensitive analysis of variance with repeated measures did indicate a significant effect of root type on vial CH_4 loss for *P. angustifolius* ($p = 0.017$).

In situ *incubations of intact plants*

Mean diffusive CH_4 release from intact plants of *M. exalbescens* ranged from –2.7 to 11.0 μmol h^{-1}, was markedly greater at night than during the day, and increased significantly in presence of MF (Figure 2). Negative mean values for release from plants during the day in the absence of MF indicated net consumption of CH_4 from enclosed water. Analysis of variance revealed significant effects of incubation period and MF application on release rate from intact plants of *M. exalbescens* without significant interaction between the two treatment effects ($p < 0.001$ for both treatments, interaction $p = 0.784$). During the separate test of plant bag O_2 status, O_2 concentration of enclosed water rose from an initial morning value of 11.5 ± 1.3 (1 SE; n = 3) mg L^{-1} to 26.5 ± 5.1 mg L^{-1} (>300% saturation) just before sunset before falling back

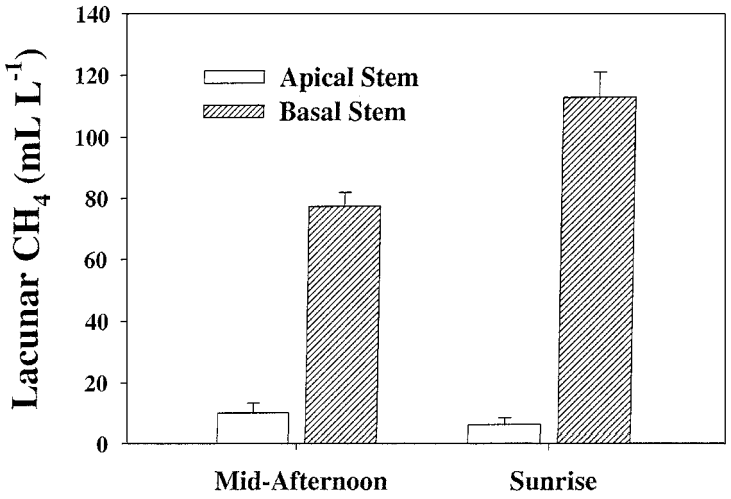

Figure 3. Stem lacunar CH_4 concentration in *Myriophyllum exalbescens* from Pleasant Lake (Michigan, USA) at mid-afternoon on August 24, and at sunrise on August 25, 1994. Apical stem samples were taken from stem lacunae ca. 1 m above the sediments, while basal stem samples were taken from stem lacunae ca. 2 cm below the sediment surface. Error bars represent + 1 SE (n = 18).

to 11.8 $\pm$ 2.8 mg L^{-1} at sunrise. Lacunar gas sampling of *M. exalbescens* (Figure 3) revealed diel changes in CH_4 concentration in belowground stem lacunae (basal in Figure 3). CH_4 concentration in these lacunae was significantly higher at sunrise than at mid-afternoon (Student t-test: $p < 0.001$). No significant difference in CH_4 concentration was observed between sunrise and mid-afternoon samples of apical-stem lacunar gas ($p = 0.29$).

Discussion

Plant tissue incubations

Results of tissue incubation studies that show epiphytic CH_4 oxidation for all tested species indicate that methanotrophs are commonly associated with leaves and stems of submersed vascular macrophytes. Older basal tissues closer to the CH_4-rich sediments showed strong oxidation activity, whereas younger apical leaves and stems well away from the sediments showed markedly less oxidation potential (Table 1). In addition, CH_4 oxidation appeared greater for stems than for leaves of the same position relative to the sediments. These results suggest that sediment CH_4, diffusing into the lower portions of plants via root and stem lacunae, is released from stem and leaf

tissues in quantities sufficient to support epiphytic communities of methanotrophic bacteria. Oxidation activity is greater for lower stems than for leaves since stem aerenchyma are the ultimate source of CH_4 diffusing up from the sediments. Consumption by CH_4oxidizers on older basal tissues combined with potential diffusive loss to surrounding water (Figure 2) limits the availability of lacunar CH_4 to biofilms developing on younger apical leaves and stems (see lacunar CH_4 data in Figure 3). This apparently precludes substantive growth of epiphytic CH_4-oxidizers on tissues well removed from the sediment (0.5 m or more above the SWI). Results showing highest oxidation activity in association with basal stem tissue agree with work by Harden and Chanton (1994) on two emergent species (*Pontedaria cordata* and *Sagittaria lancifolia*) that showed stem-like petioles closest to the sediments were the locus of plant CH_4 release.

Comparison of rates of epiphytic oxidation with literature values for rates of methanotrophic activity is difficult since results of previous studies are expressed in terms of consumption per unit dry weight of cells in pure culture, per unit volume of sediment or soil samples, or per unit weight of dry root material (King 1992; Gerard & Chanton 1993; King 1994). The latter expression is probably the most applicable to measurements described here. While not measured for all incubation studies, total dry mass (AFDM) of tissue with periphyton was measured for field samples of *M. exalbescens* and *P. angustifolius* (results per unit surface area in Table 1). Expressed in terms of dry mass, observed oxidation rates for basal tissues were 0.7–2.0 μmol g^{-1} AFDM h^{-1}. These rates are similar to oxidation rates measured for washed roots of emergent species (1.12 and 0.1–6.4 μmol gdw^{-1} root h^{-1}: Gerard and Chanton (1993); King (1994)). Along with results of incubations of intact *M. exalbescens* (discussed below), this similarity suggests findings that – like CH_4-oxidizing bacteria limiting uptake by roots of emergents – epiphytic methanotrophy has the capacity to limit diffusive CH_4 flux from submersed species.

Oxidation rates observed in laboratory incubations of submersed plant roots (0.18–7.01 μmol g^{-1} root AFDM h^{-1} – Table 2) also agree favorably with previous measurements using roots of emergent plants (see above), indicating that root-associated methanotrophy has the potential to limit CH_4 uptake by roots of submersed plants in a manner similar to that documented for emergent species. Since O_2 availability often determines rates of CH_4 oxidation in sediments (King & Blackburn 1996), similar oxidation activity for roots of submersed and emergent plants suggests similar flux of O_2 into the rhizosphere for both macrophyte groups. This seems unlikely given the direct connection between atmosphere and rhizosphere for emergent species. However, lacunar O_2 in submersed macrophytes has been shown to reach

levels as high as 340 mL L^{-1} in the late afternoon under sunny, calm conditions (*Myriophyllum heterophyllum* – Schuette 1996), while lacunar O_2 in emergents does not increase above the atmospheric concentration of 210 mL L^{-1} (Dacey 1981; Brix 1988). Elevated lacunar O_2 in submersed species should enhance diffusion O_2 to root systems during the day relative to emergent species that rely primarily on diffusion for gas transport (e.g. *Scirpus lacustris* – van der Nat et al. 1998). Plants can also use accumulated O_2 at night to meet respiratory needs (e.g. Sorrell & Dromgoole 1987) and maintain O_2 flux to the rhizosphere.

Higher oxidation rates observed here for rhizomatous *P. angustifolius* and stoloniferous *S. cristata* in comparison to the non-rhizomatous *M. exalbescens* (Table 2) may simply reflect species-specific differences. However, these results may indicate fundamental differences between rhizomatous/stoloniferous and non-rhizomatous submersed plants with respect to root oxygenation. Possible increased root oxygenation in rhizomatous/stoloniferous species could be the result of enhanced lacunar gas exchange via convective flow between connected clones on the same rhizome (Schuette & Klug 1995; Heilman 1998). More direct comparisons of non-rhizomatous and rhizomatous plants must be made to determine what, if any, relationship exists between root system architecture, root oxygenation, and root-associated CH_4 oxidation.

In situ *incubations of intact plants*

Results of incubations of intact plants growing in Pleasant Lake (Figure 2) corroborate the conclusion suggested by results of tissue incubations that epiphytic methanotrophs play an important role in the limitation of diffusive release of sediment CH_4 into littoral waters. Large increases in CH_4 release from *M. exalbescens* occurred when plants were exposed to MF. In the absence of inhibitor, average bag CH_4 concentration decreased during the day, signifying that consumption via epiphytic CH_4 oxidation was greater than loss from plant lacunae, but increased at night, indicating significant CH_4 release from plants. These findings have important consequences for CH_4 cycling in Pleasant Lake and other freshwater ecosystems. They suggest that the effects of submersed vascular plants on littoral zone water CH_4 concentration may alternate on a diel basis, with plants being sinks for CH_4 during the day and sources of CH_4 at night. Since bag O_2 concentrations remained high during nighttime incubations, greater diffusive CH_4 flux from plants at night did not result from decreased aboveground methanotrophy caused by reduced O_2 availability. Therefore, the increased nighttime flux was likely due to enhanced CH_4 flux from plant lacunae. Lacunar sampling of *M. exalbescens* indicated higher CH_4 concentrations within belowground

stem lacunae at sunrise than during mid-afternoon (Figure 3). This indicates that enhanced CH_4 diffusion into plant roots at night intensifies the concentration gradient driving CH_4 diffusion up through stem lacunae and out of plants.

Increased diffusion of sediment CH_4 into roots at night could be caused by two different mechanisms: inhibition of rhizospheric CH_4 oxidation or stimulation of rhizospheric methanogenesis. Photosynthesis during the day produces higher lacunar O_2 concentrations (Oremland & Taylor 1977; Sorrell & Dromgoole 1986; Schuette & Klug 1995, Schuette 1996) and concomitantly greater root O_2 release (Sand-Jensen et al. 1982; Sorrell & Dromgoole 1987; Christensen et al. 1994). O_2 released from roots may support CH_4 oxidation, or inhibit methanogenesis via direct toxicity to methanogens or enhancement of rhizospheric E_h through regeneration of electron acceptors (e.g., Fe^{2+} to Fe^{3+}). Either mechanism would decrease porewater CH_4 in the rhizosphere and reduce the gradient promoting diffusion of sediment CH_4 into the plant. At night, plant respiration reduces lacunar O_2. Schuette (1996) showed that O_2 within lacunae of *Myriophyllum heterophyllum* at sunrise is 35–125% lower than in the late afternoon (170 v. 230 or 150 v. 340 mL L^{-1} in low or high current flow respectively). At night, decreased lacunar O_2 will promote less root O_2 release and should enhance CH_4 diffusion into plant roots by reducing CH_4 oxidation and/or promoting CH_4 production in the rhizosphere.

Since MF concentrations as high as 0.5 mL L^{-1} were found in lacunar gas taken from stems at the SWI after MF incubation, enhanced nighttime CH_4 flux with MF (Figure 2) during *in situ* studies of intact plant may have resulted from inhibition of rhizospheric methanotrophy. However, this would not explain diel variation in the absence of MF, and results of tissue incubation studies suggest low levels of root-associated methanotrophy for *M. exalbescens*. Therefore, enhanced rates of rhizospheric methanogenesis are likely the driving force behind higher CH_4 levels in belowground lacunae and greater release from *M. exalbescens* at night. While perhaps insufficient to support high levels of CH_4 oxidation in organic-rich sediments, increased daytime O_2 release from roots of *M. exalbescens* may be sufficient to inhibit methanogenesis through direct toxicity and elevation of rhizospheric E_h. Flessa (1994) observed light-mediated increases in sediment E_h around roots of *Ranunculus circinatus* growing in strongly reduced sediment. With respect to CH_4 production, Boon and Sorrell (1991) showed reduced rates of *in vitro* methanogenesis for sediments colonized by *Vallisneria gigantea*, but failed to detect associated E_h increases. However, using finer scale sampling (1 mm versus 5 mm), Wigand et al. (1997) demonstrated increased sediment E_h in the root zone of *Vallisneria americana* in productive estuarine sediments,

indicating that the effect observed by Boon and Sorrell (1991) may have been derived from root O_2 release and associated E_h increases.

It should be noted that two potential artifacts might confound the results of experiments on intact plants. First, long incubations were necessary to accurately measure plant CH_4 flux due to low flux rates. The length of incubations resulted in elevated O_2 around plants in bags throughout the experiment. While under normal conditions, plant and biofilm photosynthesis regularly exposes epiphytic communities to supersaturated O_2 concentrations (Carlton & Wetzel 1987), elevated water O_2 concentrations in bags may have reduced the overall gradient driving O_2 diffusion from plants and epiphyton into the surrounding water. As a result, daytime and nighttime lacunar O_2 concentrations (not measured in this study) may have been higher than normal, promoting increased diffusive flux of O_2 to roots with associated effects on rhizospheric CH_4 processes: more oxidation and/or less CH_4 production translating into less CH_4 release from plants. However, while these effects may lower confidence in the magnitude of release, they would not nullify findings of significant differences in plant CH_4 release on a diel basis and in the presence of MF since all plants were subject to the same bagging effects. Similarly, the second potential artifact – altered flow regime around bagged plants – may again reduce confidence in the magnitude of responses, but conclusions of significant treatment effects should remain valid.

Impact of plant-associated methanotrophy upon CH_4 release from submersed, vascular plants

In summary, results indicate that well-developed communities of CH_4-oxidizing bacteria reside within the periphyton layer on leaves and stems of submersed vascular macrophytes and play an important role in the regulation of CH_4 emissions from these plants. Communities of epiphytic methanotrophs on basal tissues oxidize CH_4at rates comparable to the roots of emergent species. While root-associated methanotrophs may also play a role, particularly with rhizomatous or stoloniferous species, our findings suggest that epiphytic CH_4 oxidation is the dominant mechanism limiting CH_4 emission from submersed vascular plants. Support for this conclusion comes from incubations of intact plants of *M. exalbescens* – a species shown here to have limited potential for root-associated CH_4 oxidation – in which diffusive CH_4 flux from plants increased by 100% when epiphytic oxidation was inhibited. *In situ* incubations of intact *M. exalbescens* also indicated that submersed vascular plants might alternately act as sources of CH_4 to the littoral at night and as sinks during the day. Lacunar sampling indicated that reductions in potential daytime flux were due to decreased transport of CH_4 into root systems. Additional investigations of root-zone processes including rhizospheric

O_2 status and the roles of root:shoot ratio and root system architecture (e.g., rhizomatous or non-rhizomatous) should answer many questions that still remain about submersed vascular plants and their role in littoral CH_4 cycling and release.

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