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Evaluation of a closed-chamber method for estimating methane emissions from aquatic plants

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ABSTRACT

Closed-flow chambers are commonly used to measure CH_4 emissions from aquatic plants. We monitored environmental and plant physiological responses to closed chambers during measurements of CH_4 emission from *Typha latifolia* (cattails) to evaluate potential errors in emission estimates. In 1990, two wetlands were studied: one in NE Kansas and one in central New York. Leaves and stems of *Typha* enclosed in gas-impermeable, collapsible bags were exposed to rapid increases in chamber air temperature and, thus, tissue temperatures (up to 9°C above ambient) as well as rapid reductions in both CO_2 partial pressure due to photosynthesis and the leaf-to-air water vapor pressure deficit due to transpiration. These rapid (<5 min) environmental changes within chambers are known to affect stomatal aperture through which CH_4 exits *Typha* leaves. Indeed, stomatal conductance (g) increased up to 42% compared to control leaves after 19 min of enclosure in chambers, and chamber effects on interactions between irradiance and g were detected. The duration of enclosure also critically affected the CH_4 gradient within chambers. Estimated CH_4 emission from leaves in chambers sampled after 19 min (3 nmol m^{-2} s^{-1}) was 10-fold lower than estimates based on 30-s sampling periods (30 nmol m^{-2} s^{-1}) due to reduction in the leaf-to-air concentration gradient for CH_4. Since closed chambers (1) induce alterations in g, (2) may result in rapid environmental changes, and (3) lead to almost instantaneous reductions in the leaf-to-air concentration gradient for CH_4 diffusion, we suggest that only very short enclosure times should be used when measuring CH_4 emission from plants.

1. Introduction

Methane is an important greenhouse gas that is microbially produced in significant quantities in anoxic sediments of wetland ecosystems (Svensson and Rosswall, 1984; Yavitt et al., 1987; Westermann and Ahring, 1987). Transport of CH_4 from sediments to the atmosphere can occur through vascular plants (Dacey and Klug, 1979; Sebacher et al., 1985), and this pathway allows CH_4 to bypass the oxic water-sediment interface where oxidative processes can consume much of the CH_4 produced (Yavitt et al., 1988; King et al., 1990). A substantial proportion (50–95%) of the total CH_4 emitted from wetlands may occur through aquatic plants (Bartlett et al., 1988; Schütz et al., 1989) driven by mass flow and/or diffusion processes through stomata (Dacey, 1981). Hence, accurate estimates of emissions from plants, and an understanding of the plant processes controlling CH_4 flux, are crucial for the evaluation of this vegetation-atmosphere exchange.

A standard method of estimating CH_4 emissions from plants involves sealing entire plants, or portions of them, into closed chambers (or gas-impermeable bags) and measuring the rate of increase in CH_4 concentration in the chamber over time (Dacey and Klug, 1979; Sebacher et al., 1985). While detailed procedures are seldom provided, enclosure times in many studies can be as long as several hours (Burke et al., 1988). Enclosure time is critical for accurate measurements of gas fluxes out of plants because: (1) changes in the leaf microenvironment within closed chambers can have both direct and indirect effects on plant physiological processes (Nobel,

1983); and (2) diffusion rates of gases out of the plant are determined, in part, by the concentration gradient from inside the plant to the chamber atmosphere. A reduction in this gradient in chambers that are sealed around plants for extended periods of time may affect estimates of CH_4 emission.

In green plant parts, as opposed to dead or senescent biomass, stomatal conductance (i.e., opening) largely determines gas exchange (Nobel, 1983). Reduction in LAVPD due to increased humidity, increased leaf temperature, and a lowering of ambient CO_2 partial pressure are all known to increase stomatal conductance in well-watered plants (Fig. 1; Schulze and Hall, 1982). Of particular relevance for CH_4 emission is the nonlinear response of stomatal conductance to alterations in CO_2, temperature, and LAVPD (Schulze and Hall, 1982). Stomatal responses to environmental variations may be nearly instantaneous or may require several minutes depending on the species and its physiological state (Losch and Tenhunen, 1981; Knapp and Smith, 1990). Thus, the potential for rapid environmental changes and physiological responses in plants enclosed in chambers must be evaluated before accurate estimates of gas exchange can be made. In species with stomata that respond rapidly to environmental changes, overestimates of CH_4 emissions could result from rapid increases in stomatal conductance due to reduced LAVPD and/or CO_2 partial pressure (Fig. 1).

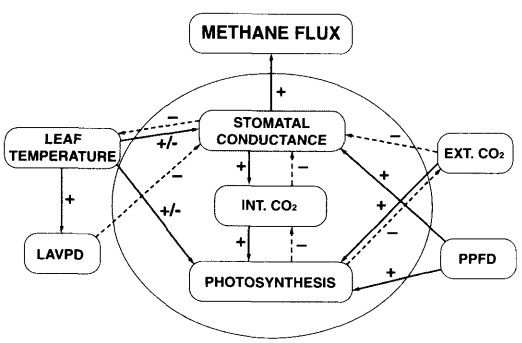

Fig. 1. Relationships between methane flux from plants and environmental and plant physiological variables that may be altered by closed chambers. PPF = Photosynthetic Photon Flux (0.3–0.7 μm wavelengths), INT CO_2 and EXT CO_2 = carbon dioxide partial pressure within intercellular air spaces in plant tissue and external to the plant, respectively, LAVPD = Leaf-to-air water vapor pressure deficit, a function of temperature and humidity. Changes in these variables may influence stomatal opening (conductance) ultimately altering emission of methane from plants. + 's and − 's next to arrows indicate the effect that an increase in one variable has on another variable.

The results of studies reported here focus on the common practice of enclosing plants in chambers to estimate CH_4 emissions and provide an analysis of potential errors that may result from chamber induced alterations in both the plant microenvironment and physiological processes.

2. Experimental procedures

Two *Typha latifolia* dominated wetlands were studied: one in NE Kansas at the Konza Prairie Research Natural Area (KPRNA) near Manhattan and one in central New York near Ithaca. Measurements were carried out on fully expanded, mature leaves and on stems. Stems are defined as the portion of the plant above the water line and composed of the basal culms of all of the leaves on an individual shoot.

Methane emissions were measured using 0.0675 mm thick (or 2.7 mil) plastic bags (referred to hereafter as "chambers") that enclosed portions of leaves or stems. Laboratory tests indicated that the chambers stored CH_4 and CO_2 satisfactorily. For example, chambers filled with either 1.09 ppmv CH_4 or 280 ppmv CO_2 remained unchanged for at least 24 h. Chambers filled with above-ambient concentrations of either CH_4 or CO_2 also did not show concentration changes during a 1 day period.

In the field, the distal 45 cm of an individual leaf varying in size from 40 to 120 cm^2 was placed in a chamber, and the chamber was sealed using plastic, gas-impermeable tape (Scotch Brand Inc., St. Paul MN, cat. no. 191). For plant stems, about 200 cm^2 was enclosed by a piece of plastic and the edges were heat sealed and taped to make an airtight enclosure. Exact volumes of chambers were determined at the end of any given enclosure by either: (1) withdrawing and quantifying the entrained air using a large-volume syringe, or (2) by injecting 1 mL of an inert gas (e.g., ethane) into the chamber and evaluating the resulting increase in gas concentration. Because the chambers were easily collapsible and of relatively large volume (at

least 250 mL), withdrawal of gas samples for CH_4 analysis during an enclosure did not alter chamber pressure or CH_4 emission.

2.1. *Environmental changes in closed chambers*

Within chambers with enclosed leaves, we monitored air temperature, leaf temperature, humidity and photosynthetic photon flux (PPF, 0.3 to 0.7 μm) to determine the magnitude and time course of changes in these factors that can affect stomatal conductance (Fig. 1). Air (inside and outside the chamber) and leaf temperatures were measured with fine-wire thermocouples, humidity with a capacitance sensor mounted in a temperature/humidity probe (Cole-Parmer, model 3309-50) and PPF with a quantum sensor (LI-COR Inc., model 190SB). All instruments were sealed in chambers simultaneously with leaves. These measurements were carried out daily between 11 and 14 June 1990 and again on 25 August 1990 using leaves on 5 different plants for each daily measurement.

To determine the time course of changes in CH_4 concentration within chambers, 5-ml air samples were withdrawn using plastic syringes at the beginning of each enclosure and at 30 s and 1, 2, 4, and 8 min thereafter. For each collection, the syringe was flushed twice with chamber air to mix the chamber atmosphere before drawing the sample. Syringes also served as storage vessels until analyses could be carried out within 24 h of collection. Leakage from the syringe was inhibited by capping the needle with a silicone stopper and by applying distilled water to the barrel to prevent diffusion of gases. Less than 2% of CH_4 in the syringe was lost during a 24-h storage period, as determined by tests using a 1.08 ppm CH_4 standard.

2.2. *Physiological responses of enclosed leaves*

For the June sampling dates, enclosure time varied in length from 8 to 68 min and physiological responses of enclosed leaves was measured after the enclosure. Air samples from the chambers were collected for CH_4 concentrations at the beginning of the enclosure and at 8-min intervals thereafter. Subsets of chambers were removed after enclosures lasting 8, 19, 39 and 68 min ($n = 5$ for each enclosure time), and within 40 s we measured net photosynthesis (A; μmol CO_2 $m^{-2} s^{-1}$) and stomatal conductance to water vapor diffusion (g; mmol $m^{-2} s^{-1}$) in leaves. Similar measurements were made on non-enclosed (control) plants. A and g were measured with an infrared gas analyzer coupled to a portable gas exchange system (LI-COR Inc., model 6200). Water vapor condensation on leaves and chamber walls due to saturation of the chamber atmosphere from transpiration occurred in < 1 min, but all measured portions of leaves were blotted dry (requiring about 20 s) prior to gas exchange measurements. Leaves enclosed within the chambers were collected to determine leaf area using a video leaf area meter (Decagon Devices). All gas exchange measurements were expressed on a projected ($\frac{1}{2}$ total) leaf area basis.

We also evaluated the effect of the closed-chamber technique on leaf physiological processes and on estimates of CH_4 emissions under high vs low PPF conditions. We shaded *Typha* leaves (PPF ca. 200 μmol $m^{-2} s^{-1}$) for 8–10 min and measured A, g, and CH_4 emissions under these low PPF conditions. We then compared these data to gas exchange measurements made on leaves enclosed in chambers and exposed to identical levels of PPF.

2.3. *Methane concentrations in Typha plants*

We determined concentrations of CH_4 within the aerenchyma (i.e., the internal air space) of *Typha*. Gas samples were withdrawn along entire plants staring at the water-atmosphere interface and continuing at 45 cm intervals to the top of the plant. A 1-ml syringe with an attached 25 gauge needle (about 16 mm long) was inserted into the leaf blade and held in place while the air sample was collected. We used different shoots for each sample in order to avoid any influence of wounding on gas concentrations within the plant.

Samples were collected between 27 August and 4 September 1990 and were taken at either predawm (630 h), mid day (1400 h) or following sun set (2200 h).

2.4. *Methane measurements*

Methane in each air sample was measured using a gas chromatograph (Shimadzu Inc., model Mini-2) equipped with a flame ionization detector. Gases were separated on a Poropak-Q column (80/100 mesh, 2m × 3 mm) maintained at 35°C using He carrier gas (30 ml min^{-1}). Methane concentrations were quantified by comparing peak

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areas for samples and standards. Certified CH_4 standards (1.08 to 1054 ppm CH_4 in N_2; Scott Specialty Gases, Plumsteadville, PA) bracketed every 5–10 samples.

3. Results and discussion

3.1. *Environmental changes in closed chambers*

When a physiologically active leaf or stem is sealed in a chamber under field conditions, several important environmental changes occur rapidly in the chamber (Fig. 2). Transpirational water loss from enclosed *Typha* leaves resulted in a rapid increase in relative humidity and atmospheric vapor pressure with concomitant reductions in the leaf-to-air vapor pressure deficit (LAVPD; the driving force for transpiration). Condensation of water vapor on chamber walls was noted in <1 min after enclosure. Because the bags were transparent to visible light, PPF incident on leaves in the chambers was not significantly reduced prior to, or after condensation occurred, but light scattering may have been affected by water droplet formation.

The energy input to this closed system resulted in increases in chamber air temperature and leaf temperature of up to 9°C during the initial 10 min of enclosure (Fig. 2). Leaf temperatures greater than chamber air temperature were observed throughout the enclosure period due to the high absorption of *Typha* leaves. Short-term variability in leaf and air temperature were caused by fluctuations in wind speed and hence convective heat exchange. We monitored temperatures over a longer 60-min period (data not shown) and found that air temperature differences between ambient and chamber values were quite variable depending on fluctuations in wind speed, but in general both air and leave/stem temperatures within the chambers were on average 4 to 5°C above ambient.

Upon enclosure, concentrations of CH_4 in the chamber air increased rapidly from the ambient value (Fig. 3) and reached a maximum value within about 1 min. Overall, the concentration increased by >1.5 ppm, which is much greater than the precision of the gas chromatograph (<0.02 ppm). We used these changes in CH_4 concentration in the chambers to calculate CH_4 emission rates for the enclosed *Typha* leaves (Fig. 3). The estimated rate, based only on the first minute of the enclosure and assuming a linear rate of emission during that time period, was 31 nmol m^{-2} s^{-1} on a leaf area basis. The rates for the 2-, 4- and 8-min enclosure periods were calculated only from initial ($t=0$ min) and final

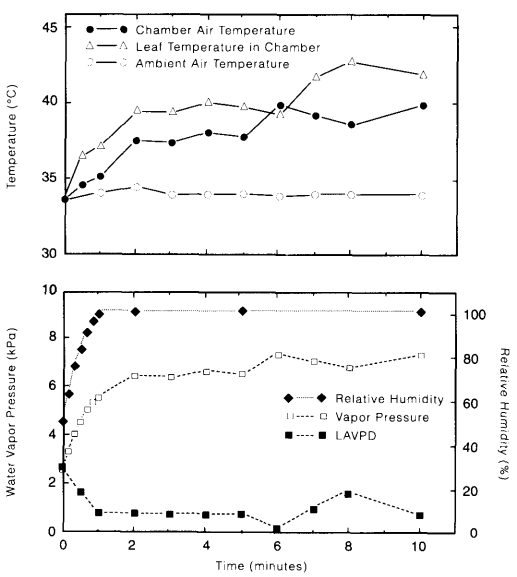

Fig. 2. Top: Time course of changes in air and leaf temperature after leaves of *Typha latifolia* were sealed in closed, transparent chambers (gas-impermeable bags) to estimate CH_4 flux. Ambient air temperatures are those measured outside the chambers, but adjacent to the enclosed plants. Bottom: Changes in relative humidity (RH), the atmospheric water vapor pressure and the leaf-to-air vapor pressure deficit (LAVPD) for leaves sealed in closed chambers.

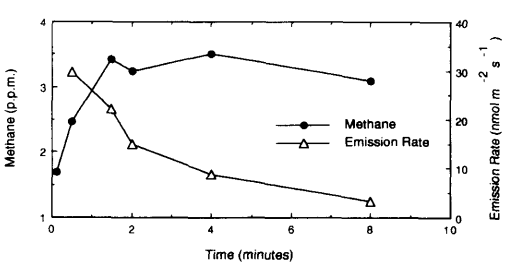

Fig. 3. Time course of changes in CH_4 concentrations within closed chambers with enclosed *Typha* leaves. Rates of CH_4 emission are calculated from CH_4 concentration after enclosure time minus initial CH_4 concentration.

values. These rates are low, in part, because we assumed linear rates of emission during the 2-, 4- or 8-min enclosure periods when, in fact, emission was linear only during the first minute of enclosure. If we assume instantaneous CH_4 emission from *Typha* leaves and extrapolate the relationship between calculated CH_4 flux and enclosure time to $t = 0$ min (an estimate of instantaneous flux), CH_4 emission rates are 50 nmol m^{-2} s^{-1}. Our value for *Typha* leaves (50 nmol m^{-2} s^{-1}) is in reasonable agreement with published values for rice plants (100 nmol m^{-2} s^{-1} on a leaf area basis; Nouchi et al., 1990), which are one of the largest global sources of atmospheric CH_4 (Matthews et al., 1991).

3.2. *Physiological responses of enclosed leaves*

Compared to responses for ambient leaves, stomatal conductance (g) was not affected until after 8 min of enclosure (Fig. 4). At >19-min enclosure time, g was significantly increased by as much as 42% relative to values measured prior to enclosure, and this difference persisted thereafter. Sealing a chamber or bag around a leaf or stem will increase the effective boundary air layer that surrounds enclosed objects (Gates, 1980) and may represent a significant additional resistance to the diffusion of water vapor (and CH_4) not present under well-mixed (windy) ambient conditions. By adjusting the chamber volume relative to the leaf/stem area enclosed, concentration gradients for water vapor within the chamber can be minimized. The rapidity with which transpired water vapor saturated the atmosphere within the chambers used in this study (Fig. 2) suggests that significant gradients were short-lived.

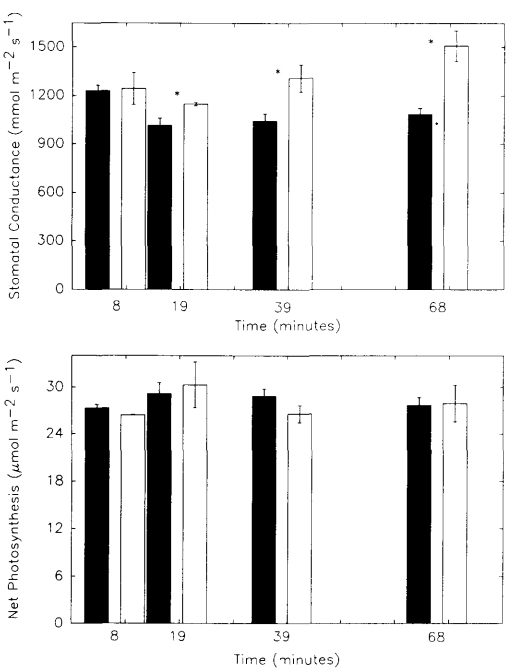

Fig. 4. Effect of enclosure time in closed chambers on stomatal conductance to water vapor diffusion (top) and net photosynthesis (bottom) in leaves of *Typha latifolia*. In both panels, solid bars refer to measurements made on leaves prior to enclosure and open bars refer to measurements made on the same leaves after they had been removed from the chamber. Vertical bars indicate one S.E. ($n = 3$) and * indicates that "initial" and "post" values were significantly different ($p < 0.05$).

Net photosynthesis (A) measured after various enclosure periods was not significantly different when compared to control leaves (Fig. 4). However, these results require a caveat. CO_2 concentrations were rapidly reduced within the chamber and were too low to support significant net CO_2 uptake after a few minutes of enclosure. For example, given the measured photosynthetic rates, the amount of leaf area enclosed, and the known volume of the chamber, we estimated that CO_2 partial pressure in the chamber was depleted to less than 100 ppmv within the first minute of the

Table 1. *Summary data (±S.E., $n = 3$) for CH_4 concentrations in closed chambers and CH_4 emissions from enclosed leaves as a function of enclosure time; compare with relevant data in Fig. 4*

	Time of enclosure (min)				
Parameter	0	8	19	39	68
CH_4 concentration	1.77 ±0.02	5.20 ±0.04	5.38 ±0.08	5.10 ±0.15	5.23 ±0.38
Amount of CH_4 released (μmol m^{-2} of leaf area) during entire enclosure time	0	4.0 ±0.20	4.4 ±0.20	4.5 ±0.22	4.2 ±0.22
Rate of CH_4 emission (nmol m^{-2} s^{-1})[a)]	0	8.3	3.8	1.9	1.0

[a)] Calculated from CH_4 concentration after enclosure time minus initial CH_4 concentration.

enclosure. This value is within the range of compensation points reported for most aquatic plants (cf., Nobel, 1983). When chamber air was analyzed for CO_2 via gas chromatography, CO_2 partial pressures were <40 μmol l^{-1} (limit of detection) after 2 min of enclosure. Therefore, A values given in Fig. 4, which measured using the LI-COR system that uses atmospheric CO_2 concentrations (about 350 ppmv), suggest that long-term enclosure did not affect the ability of *Typha* leaves to rapidly assimilate CO_2.

Methane concentrations in chamber air peaked during the first 8 min of the enclosure and did not change during the longer 68-min enclosure (Table 1). Therefore, CH_4 emission from these enclosed *Typha* leaves was more rapid than changes in g for the enclosed leaves. The lack of response in CH_4 emission, despite an increase in g during long-term enclosure, suggests that a decrease in the concentration gradient for diffusion from the leaf to the atmosphere limited CH_4 emission after the first 8 min of the enclosure period. Of course, the rapidity with which concentration gradients decrease will be a function of chamber volume, plant surface area enclosed and emission rates.

The results shown in Table 1 serve to emphasize the importance of time-series measurements when estimating CH_4 emission rates for enclosed leaves. When these concentration changes are expressed as emission rates, rates sampled after 19 min (3 nmol m^{-2} s^{-1}) are 10-fold lower than estimates based on the 30-s sampling periods (31 nmol m^{-2} s^{-1}) despite the increase in g that occurred during enclosure periods (Fig. 4).

It is important to stipulate that our results do not mean that CH_4 emissions are independent of changes in stomatal conductance (g). Rapid changes in g occur in response to rapid changes in PPF due to clouds or within canopy shading (Knapp and Smith, 1990). For example, both A and g were substantially reduced by about 75% for leaves (not enclosed in chambers) subjected to low PPF for 8–10 min (Fig. 5). With leaf enclosure, A measured at low PPF was similarly reduced but g was not significantly ($P < 0.05$) different from control leaves in full sunlight (Fig. 4). This suggests that decreased LAVPD or CO_2 partial pressure whithin chambers led to increases in g that offset the expected shade-induced reductions in g (Fig. 1).

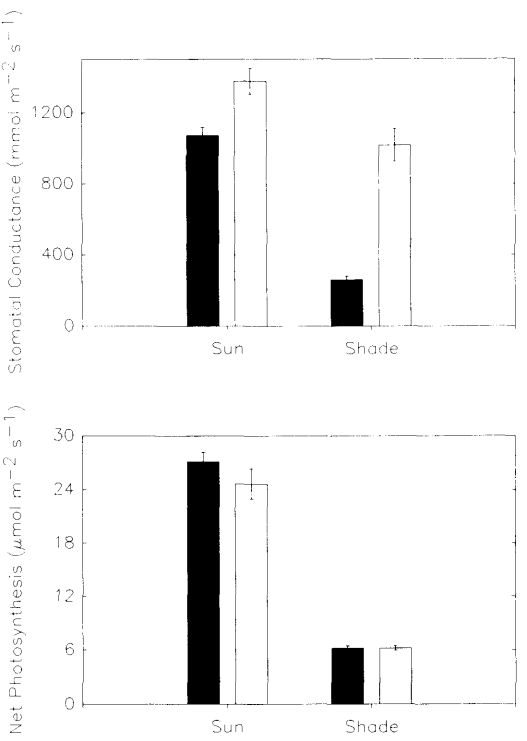

Fig. 5. Comparison of the response of stomatal conductance and net photosynthesis in *Typha* leaves to changes in sunlight from full sun levels (PPF > 1500 μmol m^{-2} s^{-1}) to shade (PPF ca. 200 μmol m^{-2} s^{-1}). In both panels, solid bars refer to measurements made on ambient (non-enclosed) leaves and open bars refer to measurements made on enclosed leaves. Note that the expected reduction in stomatal conductance due to shade did not occur if leaves were enclosed in chambers. Vertical bars indicate one S.E. ($n = 4$).

Enclosing *Typha* for 8–10 min with full sun did not affect CH_4 emission (Table 2), as indicated by similar emissions for enclosed leaves and ambient leaves (i.e., enclosed only briefly to obtain an emission value). However, enclosing *Typha* leaves for 8–10 min with shade led to a 3-fold greater emission than that for shaded, ambient leaves. These results for CH_4 emissions are consistent with the closed-chamber effect on g for shaded leaves (Fig. 5). Thus, the closed-chamber technique may not be suitable for estimating the effect that periods of reduced PPF may have on CH_4 emissions since levels of g in *Typha* did not reflect responses in control leaves exposed to ambient conditions.

Table 2. *Summary data (±S.E., n=4) for CH_4 emissions from enclosed and ambient (non-enclosed) leaves in sun or shade treatment; compare with relevant data in Fig. 5*

CH_4 released (μmol m^{-2})	Treatment	
	Sun	Shade
Enclosed[a)]	3.5 ± 0.12	3.8 ± 0.41
Ambient[b)]	3.8 ± 0.20	1.0 ± 0.04

[a)] CH_4 released during 8–10 min enclosure time.
[b)] CH_4 released during 1 min enclosure time following 8–10 min of treatment.

3.3. *Methane concentrations in Typha plants*

Knowing CH_4 concentrations within *Typha* plants is important since the concentration gradient for diffusion from the plant to the atmosphere appears to drive CH_4 emission. Not all portions of *Typha* plants have equivalent internal CH_4 concentrations, however (Sebacher et al., 1985). Measurements of CH_4 concentrations within *Typha* leaves and stems indicated that concentrations were greatest in the lowest portions of stems and leaves (e.g., the culms) and decreased with increasing height (Fig. 6). Concentrations within all portions of the plant were always greater than ambient atmospheric levels for CH_4 such that a diffusion gradient was always present from the plant to the atmosphere.

As an alternative to estimates of CH_4 emission rates based on closed chambers, we calculated a CH_4 emission rate using Fick's Law (Nobel, 1983). We used (1) the concentration gradient of CH_4 from the plant to the atmosphere for the mid-day values in Fig. 6, (2) measured stomatal conductance to water vapor diffusion, and (3) the ratio of diffusion coefficients of H_2O/CH_4 (1.19; Marrero and Mason, 1972; Nobel, 1983). Estimates of CH_4 emission from Fick's law varied between 60 and 100 nmol m^{-2} s^{-1} depending on variation in internal CH_4 concentration in leaves and stomatal conductance. The similarity between calculated and measured values indicates that known diffusional laws may be sufficient to describe CH_4 emission from *Typha* plants.

We also observed a strong diurnal pattern in CH_4 concentrations within *Typha* plants (Fig. 6). The lowest concentrations occurred at mid day on sunny days, and concentrations were significantly higher during the night. The diurnal changes appeared to occur quite rapidly, as indicated by concentrations that increased several fold between midday and soon after sunset. At the peak concentration, however, emission rates were <10 nmol m^{-2} s^{-1}. Since stomata close at night, we hypothesized that *Typha* regulate internal concentrations and emissions of CH_4 through changes in stomatal conductance. We tested this hypothesis by forcing stomatal closure on *Typha* leaves at mid-day. Opaque bags were used to shade leaves for >40 minutes, thereby diminishing ambient light on the leaf surface by more than 90% and forcing stomatal closure. The results of the experiment showed that CH_4 concentrations in aerenchyma increased from <3 to >620 ppmv for shaded leaves and from <45 to >550 ppm for stems of shaded plants. With relaxation of the

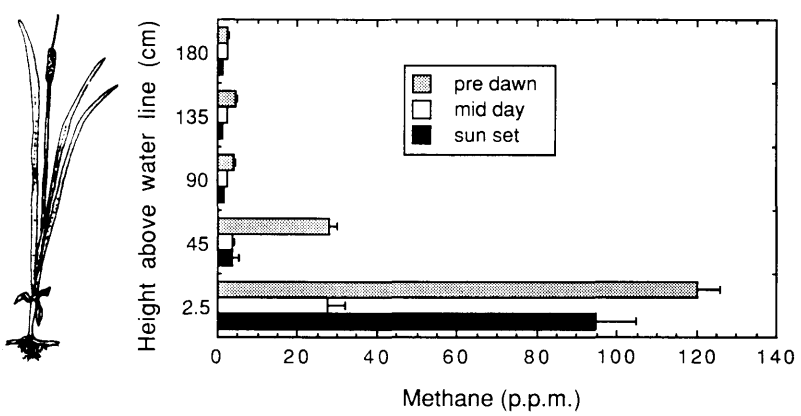

Fig. 6. Methane concentrations in the aerenchyma of *Typha* plants. Errors bars indicate one S.E. ($n=3$).

treatment (stomatal opening), a large pulse of CH_4 was emitted rapidly (within 10 min) at a rate of 1200 nmol $m^{-2} s^{-1}$!

Our results are consistent with the following diurnal pattern and control of CH_4 emissions from *Typha* plants. When stomata are open, CH_4 is rapidly released from *Typha* plants and internal concentrations reach relatively low values even though still high enough to support emission. When stomata are closed, emissions are severely depressed and continued CH_4 transport upward from CH_4-saturated sediments causes the large increase in CH_4 concentration within the internal air spaces. If true, CH_4 emission from *Typha* plants should be most rapid during the brief time in the morning when stomata open and release CH_4 that had accumulated during the night.

4. Conclusions

Chambers are commonly used to estimate gas exchange rates for CO_2 and water vapor between leaves and the atmosphere (Pearcy et al., 1989). Chambers have also been modified to simultaneously measure pollutant exposure and gas flux (Ennis et al., 1990 and references cited therein), but designs used to measure fluxes of trace gasses from leaves or entire plants are less well documented. Using chambers to estimate CH_4 emission from plants requires special considerations. Large, bulky static chambers may be inappropriate, especially if the concentration gradient of CH_4 from the leaf to the air reaches abnormal levels during extended enclosure times or as a result of chamber placement when investigator disturbance causes release of CH_4 from surrounding sediments. Open-flow chambers with continuous input of ambient air for the sweep gas may be more appropriate, but maintaining ambient conditions within such chambers is difficult and expensive (Pearcy et al., 1989). For these reasons, collapsible bags may be best suited for CH_4 emission estimates, provided short-term enclosures are used. Advantages of this technique are that bags can be fixed on leaves or around entire plants relatively rapidly, they are lightweight and do not damage plant tissues, and they are inexpensive allowing for several replicate samples to be collected.

If a closed-chamber system is used to estimate CH_4 emission from plants, chamber volume and plant surface area enclosed should be adjusted to avoid rapid reductions in concentration gradients for CH_4 diffusion, and the time of enclosure should be minimized to reduce changes in plant physiological processes. Simultaneous or post-enclosure measurements of CO_2 exchange and stomatal conductance can serve as indicators of physiological responses during enclosure.

Overall, our results for *Typha* plants show CH_4 emission rates of 30 nmol $m^{-2} s^{-1}$ for leaves and 130 nmol $m^{-2} s^{-1}$ for stems (expressed on leaf/stem area basis). Assuming that a typical *Typha* stand has a leaf area/ground area ratio of 5 (Longstreth, 1989) and about 24 stems m^{-2}, these rates extrapolate to about 280 nmol $m^{-2} s^{-1}$ (or 390 mg $m^{-2} d^{-1}$) on a ground-area basis. This value is in reasonable agreement with published values for wetlands with emergent vegetation (Aselmann and Crtuzen 1989), suggesting that closed chambers on leaves and stems can be used to accurately estimate CH_4 emission rates from aquatic vegetation.

Finally, we believe that ignoring the role of stomatal conductance in CH_4 emission estimates from aquatic plants may lead to erroneous values. For example, a typical approach for estimating CH_4 emissions through aquatic plants involves enclosing the whole ecosystem and measuring CH_4 emission before and again after removal of the plants by clipping. Certainly closed chambers, used during short-term enclosure, is less destructive and probably provides a more accurate emission estimate. An even more straightforward technique would involve measuring stomatal conductance to water vapor diffusion (with standard porometry techniques; Pearcy et al., 1989) and the concentration gradient for CH_4 from inside the plant to the atmosphere. Fick's Law may then be used to calculate CH_4 flux under ambient conditions.

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