Control of the Diurnal Pattern of Methane Emission from Emergent Aquatic Macrophytes by Gas Transport Mechanisms

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Abstract

Methane emissions from *Typha latifolia* (L.) showed a large mid-morning transient peak associated with rising light levels. This peak was also associated with a steep decline in lacunal CH$_4$ concentrations near the stem base. This pattern contrasted sharply with emissions from *Peltandra virginica* (L.) that gradually rose to a peak in the mid-afternoon corresponding to elevated air temperatures. Internal CH$_4$ concentrations within *P. virginica* stems did not change significantly over the diurnal period. Stomatal conductance appeared to correlate directly with light levels in both plant types and were not associated with peak CH$_4$ emission events in either plant. These patterns are consistent with a convective throughflow and diffusive gas ventilation systems for *Typha* and *Peltandra*, respectively. Further effects of the convective throughflow in *T. latifolia* were evident in the elevated CH$_4$ concentrations measured within brown leaves as contrasted to the near ambient levels measured within live green leaves. Experimental manipulation of elevated and reduced CO$_2$ levels in the atmosphere surrounding the plants and of light/dark periods suggested that stomatal aperture has little or no control of methane emissions from *T. latifolia*. 
Introduction

Emergent aquatic macrophytes are major conduits for gas exchange between the atmosphere and the reducing flooded soils where they are rooted. Relative to the water or soil surface, plants generally transport 80 to 90% of methane flux from a wetland through their aerenchymous tissue. Mechanisms of gas transport commonly employed by aquatic plants include molecular diffusion (Barber et al., 1962; Lee et al., 1981; Koncalova et al., 1988; Beckett et al., 1988), which is the passive "random walk" of gas molecules down a concentration gradient, and throughflow convection, in which gas flows from regions of high pressure to regions of lower pressure thus supplying oxygen to belowground organs. The pressurization of plant lacunal tissue (the mechanism driving throughflow convection) may be accomplished by either thermal transpiration (Dacey, 1981a,b; Schroder, 1989; Grosse et al., 1991) or induction from a humidity differential across the leaf boundary (Armstrong and Armstrong, 1991; Armstrong et al., 1992; Brix et al., 1992). When gas is transported belowground by either mechanism, methane, which is produced in organic rich sediments and flooded soils due to the anaerobic decomposition of organic matter (Zehnder, 1978), is vented to the atmosphere. Methane is a trace gas in the atmosphere, but none-the-less it plays an important role in atmospheric chemistry (Cicerone and Oremland, 1988).

Diurnal patterns of methane release from aquatic macrophytes have generally followed changes in soil and air temperature (e.g. Schultz et al., 1989a,b; Whiting & Chanton, 1992). However, Chanton et al. (1993) recently reported a pattern in *Typha domingensis* (Pers.) and *Typha latifolia* (L.) growing in Florida, USA that did not follow the temperature trend. Methane emission from these plants exhibited mid-morning maxima as great as a factor of four above emissions just prior to and following this period. Plant lacunal methane concentrations varied inversely with emission rates, showing a precipitous decline which was coincident with the emission peak. In an experiment in which light levels and temperature were varied, this mid morning maximum was shown to be associated with changing light levels rather than temperature. It was postulated that the maximum was associated with the conversion of *Typha* from a low efficiency diffusive system at night, to a more efficient convective throughflow driven circulation system during the day (Mevi-Schutz and Grosse, 1988; Sebacher et al., 1985; Brix et al., 1992). With solar input, pressurization and the advent
of internal convection flushed gas from the lacunae containing relatively high methane concentrations which had built up during the night. As an alternative hypothesis, the peak in methane emission and related drop in lacunal methane may be related to plant stomatal opening coincident with increasing daylight (Knapp and Yavitt, 1992). However, Armstrong and Armstrong (1990; 1991) in experiments using *Phagmites* noted an inverted peak (minimum) in the oxygen concentration of gases venting from the rhizome after the night period and during the initial renewal of convective flow. Such an observation would support the first explanation of the methane maxima early in the day.

Since natural wetlands and rice fields are two of the largest single sources of methane to the atmosphere (Cicerone and Oremland, 1988), many research teams are quantifying methane flux from vegetated wetlands. It is important to understand the causes underlying diurnal variations in methane emission from aquatic plants so that investigators can predict them and design proper field sampling strategies.

The goals of this study were to 1) replicate the findings of a strong mid-morning maximum in methane release from *Typha* in a temperate environment; and 2) demonstrate that the peak in emission was due to the switch-over from molecular diffusion at night to convective throughflow in daylight rather than simply due to the opening of plant stomata. We addressed the second objective in two ways. First, we compared diurnal variations in methane emission rate, plant lacunal concentration, and stomatal conductance in two plants growing adjacently with different transport mechanisms: *T. latifolia* is known to possess throughflow convection (Brix et al., 1992; Bendix et al., 1994; Tombjerg et al., 1994; Sebacher et al., 1985) and *Peltandra virginica* has a diffusive system (Frye, 1989; Frye et al., 1994). In a second set of experiments, we monitored methane emission rates while varying CO$_2$ concentrations in an attempt to affect stomatal opening without affecting the pressurized convective gas transport system. We also measured methane emission as a function of light level, which may affect both stomatal opening and pressurization.
Methods

Field Site

This study was conducted in the Newport News Swamp located within the Newport News Park and Reservoir, in the coastal plain of southeastern Virginia, United States. This site was the subject of a previous seasonal study of CH₄ emission (Wilson et al., 1989). This marsh and swamp was formed in 1862 during the War Between the States as an impediment to the movement of troops through this area and has been maintained as a reservoir since that time. A boardwalk which transects the marsh and swamp provides a stable platform for equipment and chamber deployment. *T. latifolia* and *P. virginica* are two of the more abundant species in this area. The water is typically 10 to 20 cm deep over a rich organic layer of plant debris (ca. 20 cm in depth) which grades into a silt and clay (Wilson et al., 1989). The overlying water pH is 7.8.

Measurement techniques

Two months prior to the initiation of these experiments, aluminum frames (collars, 0.43 m²) were placed in an area of *T. latifolia* located in the marsh. Collar sides extend 20 cm into the sediment and the flat lip of the collar was normally just below or at the water surface. This provided a solid base of support for the gas exchange chambers with minimal disturbance to the vegetation and sediments throughout the experimental period.

We utilized a portable, climate-controlled, instrumented phytochamber to control chamber air temperature and CO₂ and to capture gas for CH₄ emission measurements. It is a closed-system design made up of three major parts: a clear chamber with aluminum framing, a climate control system, and a sensor system. It has been used extensively over the past 5 years (see Bartlett et al., 1990; Whiting et al., 1992; Chanton and Whiting, 1995). The sensors measure chamber air temperature, relative humidity, incident light (PAR), and CO₂ concentrations utilizing a LI-COR Model 6200 Portable Photosynthesis System (LI-COR, Inc., Lincoln, Nebraska, USA). The chambers are adjustable in height, covering 0.43 m² of surface area, and range in volume from 700 to 1000 liters. Three sides and the top are covered with
transparent (~90% PAR transmission) Teflon film, with the remaining side composed of clear polycarbonate sheet. The chamber is placed on top of the collar during sampling and sealed with closed-cell foam gasket and clamps. The chamber used for the *P. virginica* site covered 1m² (800 liters in volume) and is not deployed on collars but is equipped to float over the plants. Chamber air temperature was regulated within 1°C of outside temperature by controlling the flow of cold water pumped from an ice-water reservoir through a heat exchanger attached to the inside wall of the polycarbonate side. Chamber air was blown through the heat exchange coils with brushless box fans with additional fans circulating chamber headspace at velocities normally encountered by plants at the top of the canopy (<1m/s). Care was taken to not directly blow air onto the water surface and cause gases to be stripped producing aberrant high emissions. CO₂ levels within the chamber were amended by controlling the flow of pure tank CO₂ attached to a rotometer and valve. To experimentally provide dark conditions, the chambers were covered by a blackout cloth.

Following deployment, chambers were vented to the atmosphere for a minimum of 20 minutes to allow for stabilization of the system in case of disturbance (i.e. bubbling) during set-up. CO₂ and temperature were monitored and adjustments made to keep levels within 10ppm and 1°C of ambient (or within desired experimental levels), respectively. Following chamber closure, the methane emission rates were determined by collecting 30ml gas samples (BD plastic disposable syringes with 3-way valves) of the chamber air every 5 minutes over 30 to 60 minutes and measuring the linear rate of CH₄ increase within the chamber. No tailing or flattening of the rate of methane increase was observed in these chambers (Chanton and Whiting, 1995). Following an emission rate measurement, the chamber was again flushed with ambient air for a minimum of 10 minutes assuring the return of chamber air methane concentrations to ambient levels prior to repeating the above procedure.

Methane concentrations of gas samples were determined utilizing a FID gas chromatograph with a 6-port gas sampling valve (1 ml loop) attached. Samples were analyzed within an hour of collection in a mobile field laboratory set up near the marsh. This provided a quick turnaround of results and a means to design or change parameters of the experiment while in the field. Scott standard gases (Scott Specialty Gases, Inc. Plumsteadville, PA, USA) were used to calibrate the GC for CH₄ and the LICOR for CO₂.
Stomatal conductances were measured with a LI-COR 1600 steady state porometer (LI-COR Inc., Lincoln, Nebraska, USA). For both plant types, a minimum of 10 leaves were measured for each sampling period. Conductance for T. latifolia is reported as a mean of both ad- and abaxial measurements. P. virginica conductance is reported only for the underside of leaf surfaces since minimal conductance values from leaf topside suggested the absence of stomata.

Lacunal methane was sampled by utilizing 10ml syringe fitted with a 3-way valve and an attached 16 ga needle (for stem/petiole sampling) or a 21 ga needle (for leaf sampling). Internal gas was obtained by inserting the needle into the stem/petiole at a point below the surface of the water. To prevent ambient air contamination in samples obtained from leaves above the water surface, the needle was inserted through a small amount of silicone grease placed on the leaf to ensure a tight seal.

To measure the response time of the stomata to dark or elevated CO$_2$ conditions, a LI-COR 6200 with a 1-liter leaf chamber (attached to a LI-COR 6200) was clamped near the midpoint of the leaf length. During the dark manipulative experiments, initial measurements were made under full sunlight conditions followed by immediate shrouding of the leaf with a blackout cloth which reduced PAR (light) levels to zero. The stomatal response to short term changes in elevated CO$_2$ levels was determined from initial measurements of stomatal conductance under ambient levels of CO$_2$ (mean=332 ppmv) followed by measurements made after the addition of pure CO$_2$ which raised the surrounding leaf and chamber air concentrations to near 1200 ppmv.

Results

$CH_4$ Emission Patterns

The methane emission from T. latifolia displayed a transient peak between 1000 and 1100 hrs (Fig. 1). This peak was over 50% greater than emission rates determined on either side of this peak period. This pattern of emission was similar to T. latifolia and T. domingensis Pers. emissions measured in the Everglades of Florida where emissions peaked about 400% above
adjacent base emissions (Chanton et al., 1993). Another minor peak of emission did occur near 1400h.

Air temperature rose rapidly during the morning hours reaching a maximum peak of 31°C in the early afternoon (ca.1400h; Fig. 1). This temperature peak did not coincide with the major peak of emission but was associated with the minor emission maximum event of the afternoon. The peak of emission near 1400h may be evidence of an underlying diffusive transport system affected by temperature.

In contrast, emissions associated with *P. virginica* did not display a mid-morning maximum event but rather exhibited a gradual rise throughout the daytime with a peak of emissions during the mid-afternoon (1500h; Fig.2). This pattern corresponds to rising air temperatures throughout the morning and midday with a maximum in the mid-afternoon (Fig. 2). This is a pattern found in other plants that employ primarily a diffusion-based ventilation system (Schutz et al., 1989a,b; Whiting and Chanton, 1992).

**Stomatal Conductance**

Leaf conductance for both *T. latifolia* and *P. virginica* peaked during the midday which paralleled changing light levels (Figs. 3 & 4). It is noteworthy that peak CH₄ emissions did not correlate with peaks in stomatal conductance. Maximum rates of methane emission in *T. latifolia* preceded the maximum in stomatal conductance while in *P. virginica*, maximum emission rates occurred following the peak in stomatal conductance. Both of these patterns accord with the convective throughflow and diffusive processes driving gas transport in *T. latifolia* and *P. virginica*, respectively.

**Diurnal Change of CH₄ Lacunal Concentrations**

The contrasting influence of the two types of transport mechanisms is further evidenced in the diurnal pattern of plant lacunal concentrations of CH₄. Concentrations within *T. latifolia* show a significant decrease (from 6175 ppmv to 255 ppmv) during the morning hours between 0500 and 1200 (Fig. 5). This negatively correlates with the transient peak of *T. latifolia* emission
rates during this time period. This decrease in *T. latifolia* stem concentration is contrasted with the insignificant change of CH$_4$ concentrations over the diurnal period within the *P. virginica* petioles (Fig 5).

*Profiles of CH$_4$ concentrations within the leaves of T. latifolia*

The process of convective throughflow also appears to affect the CH$_4$ concentration along the length of the leaves and between live and brown leaves within the same plant. Gas samples taken from live "green" leaves along their length indicate a flat profile of concentration (mean=2.2 ppmv; n=8) from near their tips (ca. 150 cm from basal insertion near the water line) to 50 cm above the waterline. At the waterline, CH$_4$ concentrations increased by a factor of 300 above those found in the distal parts of the leaf. Brown leaves had increasing CH$_4$ concentrations from 100 cm down to the stem base at the waterline. Except for the 150 cm sample, all other samples from brown leaves were 4 to 500 times greater than samples taken from the green leaves. A number of live and brown leaves were sampled at the 100 cm level and results indicated that brown leaf concentrations (mean=281 ppmv; std.=376; n=9) were over 2 orders of magnitude greater than green leaves (mean=2.34 ppmv; std.=0.20; n=10). Similar comparisons between CH$_4$ concentrations within lacunae in the petioles of green and senescent *P. virginica* failed to reveal any significant differences (Chanton et al., 1992).

*Response of Emissions to elevated CO$_2$ levels*

The response of plant-associated emission to elevated, ambient, and low levels of CO$_2$ was examined in *T. latifolia*. Elevation and reduction of chamber CO$_2$ will affect the stomatal aperture and thereby should help elucidate the possible role stomata play in controlling emission rates (Seiler et al., 1984). Two chambers (designated A & B) were used simultaneously to expose plants and to measure the associated response of CH$_4$ emission rates. Each treatment and sampling time extended over 1 hour. To lower the CO$_2$ levels within the chamber, tank CO$_2$ additions were halted and the photosynthesizing plants reduced chamber headspace concentrations (mean=214 ppmv) under sunlight. During elevated CO$_2$
manipulations, the headspace was amended with tank CO₂ to keep concentrations near 1140 ppmv. As also observed by Seiler et al. (1984), our results suggest that stomata appear to play a minor role in controlling CH₄ emission rates (Fig. 7). Rates were not significantly different (Kruskal-Wallis test: P=0.3) among the CO₂ treatments. Some caution is given in the definitiveness of these results since the number of trials was limited. However, similar conclusions were made from results obtained at a North Florida T. latifolia site (J. Chanton and E. Gaza, unpublished). At the N. Florida and the Virginia site, emission rates decreased when chambers were darkened which may be related to the cessation of sunlight- induced convective throughflow within these plants.

To determine that the stomata were responding within the time frame of the above manipulations (>1h), stomatal conductance was measured on individual leaves under similar conditions (Fig. 8). When leaves were darkened, stomatal conductance was reduced approximately 50% within the first 2 to 3 minutes. Within 20 to 25 minutes of darkness, conductance was 10% of its initial full sunlight values. The response of stomata to elevated CO₂ was near the same rate as manipulations under light and dark conditions (Fig. 8).

Discussion

The diurnal variation of CH₄ flux and its correlation to temperature has been observed in both natural and agricultural (rice) wetlands (Holzapfel-Pschorr et al., 1986; Sass et al., 1991; Whiting and Chanton, 1992; Chanton et al., 1993). Schutz et al. (1989b) observed daily fluctuations in methane flux of up to 5-fold from rice during early stages of growth, with the highest rates occurring during the afternoon. Later in the growing season, the magnitude of this day-to-night ratio was decreased to less than 1.5 to 2-fold. These investigators concluded that diel variation in emission resulted from changes in sediment temperature. A diurnal pattern was also observed for Peltandra virginica (Fig. 2) with peak emissions corresponding to elevated air temperatures. These results suggest a diffusion transport system; a conclusion also supported by evidence from stable isotope tracing of CH₄ associated with these plants (Chanton et al., 1992). In general, peak emissions are positively correlated to either soil or air temperature in plants considered to employ molecular diffusion for gas transport. The direct
response of methanogenesis to temperature changes has been shown in laboratory studies (Zeikus and Winfrey, 1976; King and Wiebe, 1978; Flanagan and Bunnell, 1980), but the extent to which the production of CH\textsubscript{4} (affecting the gradient from the soil to the root atmosphere) is directly regulated by temperature in the field is not known.

As contrasted to \textit{P. virginica}, peak emissions from \textit{Typha latifolia} preceded the elevated air temperatures of the afternoon (Fig. 1). This dissimilarity of emissions between a diffusion and a pressurized, ventilated plant was also observed for \textit{Cladium jamaicense} (sawgrass) and \textit{T. domingensis} in the Everglades (Florida, USA; Chanton et al., 1993). Pressurization in gas throughflow plants is driven by internal temperature changes induced by light absorption of the leaf or humidity gradients between the leaf and the air (Dacey, 1981 a,b.; Armstrong and Armstrong, 1991; Grosse et al., 1991; Schutz et al., 1991; Brix et al., 1992). The peak in mid-morning emissions (Fig. 1) appears to be a result of the pressurized system being activated by the rising light intensities (PAR ca. 600 to 1600 \(\mu\text{E m}^{-2}\text{s}^{-1}\); Fig. 3).

The lacunal gas concentrations reflect the differences in the type of transport system present. Lacunal concentrations within plants with diffusive transport do not significantly change over diurnal periods (Fig. 5) even though the emissions do positively correlate with temperature changes. As contrasted with convective throughflow of \textit{T. latifolia} which sweeps ambient gas through the plant reducing the internal CH\textsubscript{4} concentrations (Fig. 5), diffusion is accelerated during the mid-day as gas moves from the roots to the shoots. It is interesting to note that nighttime lacunal concentrations of \textit{T. latifolia} (mean= 6175 ppmv; std=2400) approach the night and early morning concentrations of \textit{P. virginica} (mean =8498 ppmv; std=1880; Fig. 5) when both plants operate in a diffusive transport mode.

There arises the question whether stomatal activity affects CH\textsubscript{4} emission. Cicerone et al. (1983) and Seiler et al. (1984) found no change in the rate of methane emission from day to night in rice. Similar results were found in a \textit{Spartina alterniflora} marsh near Lewes, Delaware (Bartlett et al., 1987), and in a \textit{Cladium} marsh in the Florida Everglades (Whiting et al., 1991). Utilizing CO\textsubscript{2} to stimulate the stomatal aperture, Seiler et al. (1984) observed that lowering (100 ppmv) or raising (40000 ppmv) carbon dioxide levels had little effect on emission rates.
from rice plants. They concluded that methane emission is independent of photosynthesis and stomatal aperture.

The conclusion that stomatal aperture is not a factor in methane emission is supported by comparison with the transport of oxygen through plants. The processes controlling oxygen flux from the atmosphere to the rhizosphere are similar to methane except for the reversal of transport direction. With a number of experiments and diffusion models, Armstrong (1979) concluded that leaves of aquatic plants were of minor consequence in regulating the diffusion of oxygen from the atmosphere to the rhizosphere. The largest resistance to oxygen transport was in the movement of gas from the root aerenchyma to the rhizosphere. It has been suggested (Chanton and Dacey, 1991; Chanton et al., 1993) that the effect is similar for methane emission.

The emission associated with the convective throughflow in *Typha* appears to be limited in the afternoon since afternoon rates are comparable to emission at night (data collected from other sites, not shown; also Chanton et al., 1993). Following the initiation of convective throughflow in the morning with an initial evacuation (sweep) of the lacunal spaces, CH$_4$ emission appears to be limited at the point of entry into the plant - the interface between the roots and the high concentrations of CH$_4$ dissolved in the interstitial sediment water (ca. 400µM CH$_4$, data not shown). Elevated lacunal concentrations during the night (mean= 6175 ppmv, std.=2400) and over an order of magnitude reduction of daytime concentrations (mean=255 ppmv; std.=143) support this postulate. The strongest gradient in methane concentration along the transport path is between the sediment and the root aerenchyma. Methane gas concentrations are typically measured in the range of 40 to 80% in unvegetated sediments, but are not likely to reach 10 to 50% within soils of rooted emergent vegetation (Chanton and Dacey, 1991). This steep gradient in concentration is indicative of the resistance to gas flow into the plant. Therefore, changes in leaf resistance in response to diurnal light changes will minimally influence methane emission.

A light and dark exposure experiment over rice plants for a number of days also did not show any differences in emissions (Holzapfel-Pschorr et al., 1986). Results from a similar field manipulation of light/dark and high/low CO$_2$ conditions over *T. latifolia* are also consistent with
a non-stomatal control of emissions (Fig. 7). Previous field manipulations of both light/dark and high/low CO₂ experiments on *P. virginica* also did not exhibit an effect on CH₄ emissions (Chanton et al., 1992). By contrast, Knapp and Yavitt (1992) presented evidence of stomatal activity affecting CH₄ emission from green leaves of *T. latifolia*. Others have also concluded that emission from *P. virginica* (Frye et al., 1994) and *Carex* (Morrissey et al., 1993) may be influenced by stomatal activity.

It is possible that one could interpret the present emission results of *Typha* as partially supporting the contention of stomatal control of methane emission. During the mid-morning (ca. 1000h), stomatal conductance rapidly increased to a noon maximum in response to light (Fig. 3). During the initial opening of the stomata, the gas collected during the night may vent to the atmosphere, thus creating the peak in emissions. However, since changes of stomatal conductance in response to light occurs for both *T. latifolia* (Fig. 3) and *P. virginica* (Fig. 4), the non-occurrence of an emission peak for *P. virginica* (Fig. 2) suggests a different process controlling the emission peak in *T. latifolia*, namely the most obvious difference between the plants, pressurized ventilation.

The difference in concentration profiles between green and brown leaves (Fig. 6) denotes a path of air-intake into the green leaves and release from the older brown leaves which is consistent with the flow pattern for plants with a pressurized throughflow ventilation (Dacey, 1981a). This pattern confirms the observations of Sebacher et al. (1985) who observed a similar pattern for green and brown leaves of *T. latifolia*. In examining convective flow within *Eleocharis spacelata*, Sorrell and Boon (1994) found a significant increase of CH₄ concentrations within the efflux culms over that in the influx culms. Petiole samples collected from the white water lily, *Nymphaea odorata*, at Lake Hall, Florida had a comparable pattern of elevated CH₄ concentrations in the older leaves (mean=4654 ppmv; std.=2548; n=6) as compared to the young green leaves (mean=254 ppmv; std.=193; n=5; Chanton unpublished data). This plant is considered to employ pressurized ventilation (Grosse et al., 1991). If brown leaves are considered "leaky" (Dacey, 1981a,b), then the higher concentrations within these leaves (as compared to the live, green leaves) would not be caused by stomatal closure. Within a diffusion transport system, these leaky leaves should be always lower in concentration than the live leaves since they are "more open" to the atmosphere. If stomata
affect emissions in convective flow-through plants, they would appear to have an effect on the
gas entrance side of the flow pattern (i.e. the green leaves).

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References


Figure Captions

Figure 1. Methane emission rates from *T. latifolia* (circles) and air temperature (triangles) plotted versus time of day. Methane emission shows a transient peak in emission rate in the mid-morning.

Figure 2. Methane emission rates from *P. virginica* (circles) and air temperature (triangles) plotted versus time of day. In contrast to the *T. latifolia* results, methane emission rates from *P. virginica* show a maximum in the afternoon associated with maximum temperatures.

Figure 3. Stomatal conductance in *T. latifolia* (upper panel) and light levels (PAR, lower panel) plotted versus the time of day. Each measurement of stomatal conductance represents 10 measurements on separate leaves. Error bars represent standard deviation. Maximum stomatal conductance was associated with maximum light levels.

Figure 4. Stomatal conductance in *P. virginica* (upper panel) and light levels (PAR, lower panel) plotted versus the time of day. Each measurement of stomatal conductance represents 10 measurements on separate leaves. Error bars represent standard deviation. Maximum stomatal conductance was associated with maximum light levels.

Figure 5. Lacunal-air methane concentration in syringe samples collected by inserting a needle into the plant stem just below the water line. Concentrations within *T. latifolia* (upper panel) varied by an order of magnitude with lowest concentrations during daylight and highest concentrations at night. This pattern was in stark contrast to that observed in *P. virginica* where concentrations did not vary over the diel cycle. Each symbol represents 5 to 6 measurements; error bars represent standard deviations.
Figure 6. Profile of methane concentration within leaves of *T. latifolia*. Samples collected from live green leaves did not vary with leaf height and had a mean value of 2.2 ppmv (n=8). Samples taken from green stems did have elevated methane concentrations (ca. 500 ppmv). Brown leaves, in contrast, had increasing methane concentrations approaching the water from the top of the plant. Except for the uppermost sample, methane concentrations within brown leaves were 4 to 500 times greater than samples collected from corresponding heights in green leaves. Three to five samples were taken at each height; error bars represent standard deviation.

Figure 7. Methane emission rates from *T. latifolia* were not significantly affected by variations in chamber air CO₂ concentrations. A and B represent parallel treatments in replicate chambers. Emission rates decreased when chambers were darkened, possibly due to the cessation of light-induced convective through-flow. Three determinations of emission rate were made at ambient CO₂ with duplicate determinations during other manipulations; error bars are standard deviation and ranges, respectively.

Figure 8. Response of stomatal conductance (*T. latifolia*) over time to changes in light to darkness (square and triangle) and from ambient (332 ppmv) to elevated (1180 ppmv) CO₂ (circle). Time represents the lapse time from the initiation of darkness (shrouded) and exposure to elevated concentrations of CO₂.
Fig. 2.

The diagram shows the diurnal variation of CH$_4$ flux (mg m$^{-2}$ h$^{-1}$) and air temperature (°C) for Peltandra. The CH$_4$ flux increases from early morning to midday and then decreases in the evening. The air temperature follows a similar pattern but with a peak around noon.
Typha

Conductance (cm s\(^{-1}\))

PAR (\(\mu E m^{-2} s^{-1}\))

Time (h)
Fig. 4
Figure 6: Graph showing the relationship between Height Above H$_2$O Surface (cm) and Internal CH$_4$ Concentration (ppmv) with Brown and Green lines.
CH₄ Emission (mg CH₄ m⁻² h⁻¹)

Ambient | High CO₂ | Low CO₂ | Dark

Chamber A | Chamber B
Methane Stable Isotopic Distributions as Indicators of Gas Transport Mechanisms in Emergent Aquatic Plants

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ABSTRACT

Methane stable isotope distribution patterns vary markedly between plants utilizing convective throughflow ventilation relative to those which primarily employ molecular diffusion. In diffusive plants, methane sampled from lacunal air was $^{13}$C-enriched by $10.6 \pm 3.7 \text{ o/oo}$ relative to sedimentary CH$_4$. In plants possessing the convective flow system, differences between plant lacunal methane collected in daylight and sediment methane were not apparent, and averaged $-0.9 \pm 2.1 \text{ o/oo}$. At night, as convection gave way to molecular diffusion, the isotopic distributions in Typha domingensis Pers. and Typha latifolia Crantz, convective-flow plants, became similar to distributions observed in diffusive plants, with plant lacunal methane becoming $^{13}$C-enriched. Diurnal variations in the isotopic signature of methane emitted from Typha were also observed; methane emitted in daylight was $^{13}$C-enriched by $4-7 \text{ o/oo}$ relative to night emissions. The results indicate that methane isotopic distributions are useful indicators of plant gas transport mechanisms. Diurnal variation in isotopic distribution patterns confirm observations that plants with convective throughflow ventilation switch to molecular diffusion in the absence of sunlight.
INTRODUCTION

Aquatic macrophytes are agents for gas exchange between the atmosphere and anaerobic environments. Wetland environments, both natural (tundra, bogs, fens, marshes and swamps) and agricultural (flooded rice fields or paddies) are the largest single source of methane to the troposphere, contributing over 40% of the annual atmospheric methane flux. A number of studies have demonstrated that plants mediate the bulk of the methane released from vegetated wetlands, generally accounting for 80-90% of the CH$_4$ emitted (Schutz et al., 1991; Muller et al., 1994). Strong correlations have been reported between CH$_4$ transport, live plant biomass and CO$_2$ exchange (Whiting and Chanton, 1992, 1993, Whiting et al., 1991; Happell et al., 1993). Plants have effects on the sediments in which they are rooted (Boon and Sorrell, 1991).

Wetland plants employ a number of differing strategies to transport air belowground to support root respiration (Dacey, 1981a). In the process of bringing O$_2$ to roots, CH$_4$ is vented to the atmosphere. The different gas transport modes have been shown to have a dramatic effect on diurnal patterns of methane emission (Chanton et al., 1993; Sebacher et al., 1985). Mechanisms of gas transport by emergent macrophytes include simple molecular diffusion (Armstrong, 1978, 1979; Lee et al., 1981), pressurized bulk flow-through convection (Dacey, 1981a,b; Grosse and Mevi-Schutz, 1987; Schröder, 1989; Grosse et al, 1991, Brix et al., 1992; Armstrong and Armstrong, 1990; Armstrong et al., 1991) and effusion (Dacey, 1987; Schröder, 1989). Diffusion is the result of a partial pressure differential between the sediment, plant and atmosphere. While initiated by diffusive type processes (Armstrong et al., 1991) internal pressurization and subsequent convective flow arise as a result of thermal transpiration (Dacey, 1981a,b) and humidity-induced pressurization (Armstrong and Armstrong, 1991). The total internal pressure is the sum of the two mechanisms. Gas flows from regions of high pressure (young leaves)
through the rhizome and returns to the atmosphere through more mature porous efflux leaves in the case of water lilies (Dacey, 1981a), or through broken or dead culms in *Pragmites australis, Eleocharis sphacelata* and possibly *Typha* (Armstrong et al., 1992; Sorrell and Boon, 1994; Bendix et al., 1994 and Tornbjerg et al., 1994). Effusion, also called free molecular or Knudsen flow, is a third gas transport mode which may be described as the passage of gas in the presence of a partial pressure differential (but not necessarily total pressure) through holes which are small relative to the mean free path of the gas molecules.

The first hypothesis we tested is that stable isotopic analysis of CH₄ can be used to discriminate between the different gas transport modes. While gas transport by convective throughflow (laminar flow) does not produce fractionations dependent upon molecular weight, effusion and molecular diffusion do (Schroder, 1989; Dacey, 1987; Mason and Marrero, 1970). When plant ventilation is occurring under pressurized convective throughflow, we hypothesize that the isotopic signature of methane within the lacunae of plant petioles and stems should be equivalent to sedimentary methane. During diffusion or effusion of methane from the plant, isotopic fractionation occurs via preferential loss of the lighter isotope (¹²C), leaving the methane remaining in the lacunal atmosphere ¹³C-enriched relative to sedimentary methane (Chanton et al., 1992a,b). The maximum theoretical isotope effects predicted for this fractionation are 19 and 31 o/oo for diffusion and effusion respectively (Harden and Chanton, 1994). Mass dependent fractionation during gas transport from diffusive plants has been confirmed in Southeastern wetlands by injecting the tracers ethane (C₂H₆) and propane (C₃H₈) into petioles (Harden and Chanton, 1994). Ethane left the petioles more rapidly leaving gas within petioles propane enriched.

It has been shown that internal pressurization and subsequent convective throughflow is light dependent (Dacey and Klug, 1982; Grosse and Mevi-Schutz, 1987; Armstrong and Armstrong, 1990). The degree of pressurization in *Nuphar* and
Phragmites has been shown to be dependent upon the quantity of photosynthetically active radiation incident to the leaf surface (Dacey, 1981b; Armstrong and Armstrong, 1990). Light dependence of pressurization has been observed whether the mechanism driving pressurization has been thought to be humidity or thermally induced. The convective ventilation system that dominates during daylight is thought to give way to molecular diffusion at night (Armstrong et al., 1991; Grosse and Mevi-Schutz, 1987). The second hypothesis we tested was that isotopic distribution patterns in plants with convective ventilation should exhibit strong diurnal variations: during daylight when gas transport is dominated by convection, internal and sedimentary methane should have similar isotopic composition, while at night the distributions should be disparate, similar to those observed in diffusive plants.

To test our first hypothesis we made measurements of the isotopic signature of CH$_4$ in daylight in both plants which utilize pressurized convective ventilation and those which do not. To test our second hypothesis, we examined the isotopic distributions under full sun and at night in a macrophyte which has been shown to use the pressurized throughflow ventilation system *Typha domingensis* Pers. and *Typha latifolia* L (Bendix et al., 1994; Tornbjerg et al., 1994; Brix et al., 1992; Sebacher et al., 1985). This investigation would also test the work of Chanton et al., (1993) who attributed strong diurnal patterns of CH$_4$ release from these species to diurnal variations in gas transport mechanisms.

METHODS

This study was conducted primarily at two field sites, both of which have been described previously. We examined *T. domingensis* at a site in the Florida Everglades water conservation area #3 which is north of US Highway 41 and near the L-67 water control structure (Chanton et al. 1993). Studies were conducted on *T. latifolia* in the Newport News Swamp located in coastal Virginia (Chanton et al., 1992a; Wilson et al.,
Results are also reported for plants sampled at other locations when we were able
to deduce from the literature that the plants were chiefly diffusive or showed convection.

Methane was collected and prepared for isotopic measurements as described in
Chanton et al. (1992a). Briefly, methane samples from within plant lacunae were
collected in a syringe by inserting a needle within 5 cm below the water line on the plant.
Gas samples were transferred to vials, sealed with butyl rubber stoppers and stored at 1-
2°C. Gas sampled by this technique has been shown to be representative of the actual
lacunal gas pool in comparisons of syringe samples with those collected by cutting the
stems and holding them underwater while pressing the gas from them (Chanton et al.
1992a). Methane emitted from plants was collected in light transparent, temperature and
CO2 controlled "phytochambers" as described by Whiting et al. (1992) and Chanton and
Whiting (1995). At both sites, over 85% of the methane was transported via the plants,
compared to the open water between plants (unpublished data). Evaluation and
comparison of gas collection techniques for isotopic samples were reported in Chanton et
al. (1992a). Sedimentary methane was collected by stirring gas from the sediments and
collecting it in vials sealed with butyl rubber stoppers. Bubble methane has been shown to
be representative of porewater methane and sedimentary methane in general (Chanton et
al., 1992b; Martens et al., 1986). To confirm the equilibrium between pore water and
sedimentary bubble methane, pore water was drawn from sediments with stainless steel
tubing perforated at the crimped lower end, extracted with N2 as described by Happell et
al. (1993) and analyzed for methane isotopic signature. In the Virginia swamp,
sedimentary methane was collected only in daylight as previous studies had shown that
there was no diurnal variation in sediment methane isotopic signature (Chanton et al.,
1992a).

Samples of methane were prepared for isotopic analysis by combusting methane to
CO2 in a He stream (50 mL/min) over 800°C copper oxide wire (Chanton et al., 1992a).
An in-line column of Schutze reagent (I2O5, part 761-747, Leco Corp., St. Joseph, MI,
USA) was used to remove any carbon monoxide. Purified CO₂ samples were run on a
Finnigan MAT Delta E isotope ratio mass spectrometer in the laboratory of Neal Blair at
North Carolina State University.

Stable isotope results are reported in the δ notation:

\[ \delta^{13}C (\text{o/oo}) = \left( \frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right) \times 1000 \]

where \( R_{\text{sample}} \) and \( R_{\text{std}} \) refer to the \( ^{13}C/^{12}C \) ratio of the sample and a known standard,
Peedee Belemnite. Methane is depleted in \( ^{13}C \); values for biogenic methane often range
from -60 to -70 o/oo. Organic carbon values are less depleted in \( ^{13}C \) and range from -30
to -12 o/oo. Marine carbonates are \( ^{13}C \) enriched relative to methane, typical values are
about 0 o/oo, close to the value of the standard (Hoefs, 1987).

RESULTS AND DISCUSSION

Clear differences were observed between the isotopic signature of plant lacunal
and sedimentary methane in diffusive plants: methane within diffusive plants was
enriched in \( ^{13}C \) 10.6 ± 3.7 o/oo relative to sedimentary CH₄ (Table 1). The isotopic
signature of methane emitted from diffusive plants has been shown to be \( ^{13}C \)-depleted
relative to plant lacunal methane. Methane emitted from diffusive plants is similar to or
\( ^{13}C \)-depleted relative to sedimentary methane (Chanton et al., 1992a,b; Happell et al.,
1993). Isotopic fractionation occurs as the lighter isotope diffuses from the plant at a more
rapid rate, causing the retention of the heavier isotope within the plant. As an analogy, we
have likened diffusive plants to pipes, with restrictors at their exit ports that discriminate
against \( ^{13}CH_4 \). The \( ^{13}CH_4 \) concentration builds up within the pipe until its overpressure
compensates for the discrimination against it. At steady state, what goes into the pipe is
what comes out of the pipe, and the buildup of \( ^{13}CH_4 \) within the pipe ultimately
compensates for the degree of discrimination against it at the exit.
In plants possessing the convective flow system, differences between plant internal methane and sediment methane samples collected in full daylight were not as apparent, and if they existed, lacunal methane was $^{13}$C depleted relative to sediment methane. The average of the difference between lacunal CH$_4$ and sediment CH$_4$ in convective plants was $-0.9 \pm 2.1 \, \text{o/oo}$ (Table 1). These results confirm our first hypothesis: stable isotopic distributions of CH$_4$ appear to be useful indicators of gas transport modes employed by emergent aquatic plants.

The question arises as to whether the diffusion path into and through the roots leads to some isotopic differences in the lacunal system. One would expect the lighter isotope to diffuse to the lacunae more rapidly. Perhaps this effect accounts for the occasional $^{13}$C depleted values found within plants with convection. The effect is apparently overwhelmed in the diffusive plants.

The results of diurnal measurements of methane isotopic signatures within Typha, emitted from Typha and within flooded soils where Typha grew are presented in Table 2 and Figure 1. First, at Everglades site E-1, there was no difference in the isotopic signature of CH$_4$ sampled from flooded soils at night versus that sampled during the day. These results were pooled for each site for statistical analysis. At both the Virginia and Everglades sites, the isotopic signature of CH$_4$ within plant stems at night was significantly $^{13}$C-enriched relative to the isotopic signature of CH$_4$ within plant stems in daylight, to CH$_4$ within flooded soils, and to emitted (day or night) CH$_4$. These differences were significant at the 99% confidence level. Alternatively, the isotopic signature of CH$_4$ within plant stems during daylight was not significantly different from emitted (in daylight) or sedimentary CH$_4$. The results support the second hypothesis. Changes in isotopic distributions are associated with the plants' transition from non-fractionating pressurized convection during daylight to passive diffusion, which fractionates isotopes, at night. Methane emitted from Typha in daylight was $^{13}$C-enriched by 4-7 o/oo relative to night emissions (Figure 1, Table 2). At night, as diffusion became
the dominant gas exchange process, the lighter isotope diffused out at a more rapid rate and $^{13}$CH$_4$ was retained within the plant lacunae (Figure 1, Table 2).

Pore water methane ($-58.3 \pm 2.6$, n=5) was not significantly different from bubble methane ($-61.6 \pm 2.3$, n=4) in comparisons in the Virginia swamp. Collections of sediment CH$_4$ were only made in daylight in Virginia as previous analyses in this swamp had not revealed diurnal variations (Chanton et al., 1992a). For consistency, bubble methane values were used to represent sediment CH$_4$ in calculations, figures and tables.

Chanton et al. (1992a) compared a number of differing static uncontrolled chamber designs against the temperature-CO$_2$ controlled phytochamber for the measurement of CH$_4$ emission rate and the collection of stable isotope samples of methane emitted by plants. *Peltandra virginica* (L), a diffusive plant was used for these studies. The results indicated that simple uncontrolled chambers would suffice for the collection of isotope samples from plants that employ molecular diffusion as a gas transfer mechanism. However, the authors cautioned that uncontrolled chambers might not serve for the collection of isotope samples from convective plants. Our results confirm this contention (Fig. 1). Use of light chambers without temperature regulation reduces humidity induced pressurization, as the humidity within the chamber quickly reaches 100% upon closing, reducing the water vapor gradient which drives pressurization (Armstrong and Armstrong, 1991). Furthermore, if the chamber is dark, both thermal and humidity induced pressurization will be halted or reduced (Whiting and Chanton, this issue). Cessation of convective throughflow will alter the $\delta^{13}$C of released CH$_4$ (Figure 1). Use of uncontrolled chambers may also cause an underestimate of methane emission rates from pressurized plants due to attenuation of convective-flow (Chanton and Whiting, 1995). The transparent chambers used in this study were temperature regulated, resulting in relative humidities within the chamber that were within 10% of ambient conditions.

The diurnal variations of CH$_4$ concentration within the plant stems are consistent with our hypothesis (Figure 2, Table 2). The methane concentration was significantly greater at
night, when the less efficient mechanism of gas transport, diffusion, was employed. During daylight, the more efficient convective flow system swept gas from the plant stems reducing CH$_4$ concentration. The mid-day peak in methane emission rate observed in *Typha* has been attributed to flushing of plant interior atmosphere accompanying the transition in gas transport mode and the greater mobilization of methane by the more efficient pressurized ventilation system (Chanton et al., 1993; Whiting and Chanton, this issue).

Diurnal variations in the CO$_2$ content of the lacunal atmosphere of *Phragmites australis*, a convective plant, have been observed with daytime concentrations (~0.2% by volume) being a factor of 5 times lower than night time concentrations (~1%, Brix, 1988). These variations were attributed to changes in gas transport mechanism, from convection in daylight to diffusion at night.

The results of this study indicate that methane isotopic distributions may be a useful indicator of the presence of convective gas transport in wetland macrophytes. Convective throughflow does not result in significant isotopic fractionations between sedimentary methane and plant lacunal methane in contrast to distributions observed in diffusive plants. The isotopic distributions confirm the diurnal variations of gas transport mechanism in convective plants, as they switch from convective-throughflow during daylight to molecular diffusion at night.

Acknowledgments

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Water Management District, and the City of Newport News for allowing us to conduct field work in wetlands under their management.
References


Table 1. Isotopic fractionation between plant stem and sediment methane as an indicator of gas transport mode. Plants in the table below exhibiting $^{13}$C enrichment in methane sampled from the hollow air-filled plant lacunal atmosphere or petiole aerenchyma relative to the methane in the sediment bubble reservoir are diffusive plants—those without convective flow transport systems. In plants using a convective throughflow ventilation system, little isotopic fractionation is observed between lacunal and sediment methane. Replicates range from 2 to 8 for each sample. All of the measurements reported in Table 1 were on samples conducted in daylight.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Site</th>
<th>lacunal CH$_4$ °/oo</th>
<th>Sediment Bubble CH$_4$ °/oo</th>
<th>$\Delta$ °/oo</th>
<th>through-flow convection?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pontederia cordata</td>
<td>L. N. Carolina</td>
<td>-47.0 (2.3)</td>
<td>-63.1 (0.5)</td>
<td>16</td>
<td>No$^1$</td>
</tr>
<tr>
<td>Pontederia cordata</td>
<td>N. Florida</td>
<td>-44.4 (1.7)</td>
<td>-52.9 (0.1)</td>
<td>9</td>
<td>No$^1$</td>
</tr>
<tr>
<td>Peltandra virginica</td>
<td>Virginia</td>
<td>-51.4 (2.9)</td>
<td>-57.1 (1.0)</td>
<td>8</td>
<td>No$^2$</td>
</tr>
<tr>
<td>Peltandra virginica</td>
<td>Everglades</td>
<td>-51.1 (0.1)</td>
<td>-63.1 (0.2)</td>
<td>12</td>
<td>No$^2$</td>
</tr>
<tr>
<td>Sagittaria lancifolia</td>
<td>L. Virginia</td>
<td>-51.7 (0.3)</td>
<td>-42.0 (0.2)</td>
<td>10</td>
<td>No$^1$</td>
</tr>
<tr>
<td>Sagittaria sp.</td>
<td>Everglades</td>
<td>-59.7 (0.6)</td>
<td>-63.8 (0.1)</td>
<td>4</td>
<td>No$^1$</td>
</tr>
<tr>
<td>Carex rostrata</td>
<td>Stokes Alaska</td>
<td>-48.3 (1.4)</td>
<td>-61.0 (1.9)</td>
<td>13</td>
<td>No$^3$</td>
</tr>
<tr>
<td>Cladium jamaicense</td>
<td>Everglades</td>
<td>-54.8 (1.2)</td>
<td>-68.0 (0.5)</td>
<td>13</td>
<td>No$^4$</td>
</tr>
<tr>
<td>Nymphaea odorata Ait</td>
<td>N. Florida</td>
<td>-64.0 (2.0)</td>
<td>-62.0 (0.5)</td>
<td>-2</td>
<td>Yes$^5$</td>
</tr>
<tr>
<td>Nymphaea odorata</td>
<td>N. Florida</td>
<td>-55.4 (3.2)</td>
<td>-55.0</td>
<td>0</td>
<td>Yes$^5$</td>
</tr>
<tr>
<td>Typha latifolia L.</td>
<td>Virginia</td>
<td>-55.2 (1.5)</td>
<td>-56.0 (1.0)</td>
<td>1</td>
<td>Yes$^{1,6}$</td>
</tr>
<tr>
<td>Typha latifolia</td>
<td>Virginia</td>
<td>-60.8 (1.5)</td>
<td>-56.8 (0.3)</td>
<td>-4</td>
<td>Yes$^{1,6}$</td>
</tr>
<tr>
<td>Typha latifolia</td>
<td>N. Carolina</td>
<td>-62.8 (2.6)</td>
<td>-63.0 (0.6)</td>
<td>0</td>
<td>Yes$^{1,6}$</td>
</tr>
<tr>
<td>Menyanthes trifoliata</td>
<td>L. Quebec</td>
<td>-58.5 (0.8)</td>
<td>-57.7 (0.2)</td>
<td>1</td>
<td>Yes$^7$</td>
</tr>
</tbody>
</table>

Table 2. The isotopic signature of methane ($\delta^{13}$C o/oo) within *Typha*, within the sediments where they grew, and emitted from *Typha*. The concentration of methane within *Typha* is also reported (% by volume). E1 and E2 are adjacent Everglades sites. V1 is a site in the Virginia coastal swamp. Standard deviation and the number of replicates are indicated in parentheses.

<table>
<thead>
<tr>
<th>site</th>
<th>time</th>
<th>lacunal % CH₄</th>
<th>lacunal $\delta^{13}$C</th>
<th>emitted $\delta^{13}$C</th>
<th>sediment $\delta^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>full sun</td>
<td>0.14 (0.08, 4)</td>
<td>-64.6 (2.2, 4)</td>
<td>-66.1 (1.2, 7)</td>
<td>-62.4 (0.3, 3)</td>
</tr>
<tr>
<td>E2</td>
<td>full sun</td>
<td>0.11 (0.05, 5)</td>
<td>-64.7 (1.9, 5)</td>
<td>-66.0 (0.7, 2)</td>
<td>-63.4 (1.8, 6)</td>
</tr>
<tr>
<td>E1</td>
<td>night</td>
<td>0.48 (0.12, 4)</td>
<td>-59.4 (2.1, 4)</td>
<td>-73.0 (0.2, 2)</td>
<td>-62.9 (2.0, 3)</td>
</tr>
<tr>
<td>V1</td>
<td>full sun</td>
<td>0.04 (0.01, 3)</td>
<td>-61.7 (1.6, 3)</td>
<td>-60.6 (0.2, 3)</td>
<td>-61.6 (2.3, 4)</td>
</tr>
<tr>
<td>V1</td>
<td>night</td>
<td>0.40 (0.08, 3)</td>
<td>-54.3 (2.4, 3)</td>
<td>-64.3 (0.0, 2)</td>
<td>-61.6 (2.3, 4)</td>
</tr>
</tbody>
</table>
Figure 1. Isotopic signature of methane (δ^{13}C oo/oo, y axis) sampled in sediments, lacunal air and emitted from *Typha* (x axis). The upper panel (A) is Everglades data (*T. domingensis*) while the lower panel (B) is from a coastal Virginia swamp (*T. latifolia*). Data represented by lightly filled bars were collected in full sunlight and data represented by dark bars were collected at night. Lacunal and emitted methane had isotopic signatures which were distinctly different from sediment methane in darkness but under full sun conditions lacunal and emitted methane were similar to sediment methane. The data reflect a transition from non-fractionating throughflow convection during daylight to molecular diffusion, which does fractionate isotopes, at night.

Figure 2. Lacunal atmosphere methane concentration (% methane, x axis) was distinctly higher at night (dark bars) than during daylight (light bars) at both field sites (E1 & E2, Everglades sites, V1, Virginia site).
**Everglades, T. domingensis**

![Graph A](image1)

**Virginia, T. latifolia**

![Graph B](image2)
Figure 2