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Physiological control of leaf methane emission from wetland plants

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Abstract

The transport of methane from the rhizosphere to the atmosphere takes place in the intercellular spaces and stomata of wetland plants, and foliar gas exchange is one of the critical steps of the transport process. The objectives of our research were to investigate: (i) variation in foliar gas exchange among four common wetland plant species (i.e., *Peltandra virginica* L., *Orontium aquaticum* L., *Juncus effusus* L., and *Taxodium distichum* L.), (ii) the role of key environmental factors (i.e., light, temperature, and carbon dioxide concentration) in controlling foliar methane emission, and (iii) physiological mechanisms underlying the variation in methane emission due to species and the environment. Experiments were conducted in an instantaneous, flow-through gas-exchange system that operated on a mass balance approach and concurrently measured foliar fluxes of methane, water vapor, and carbon dioxide. The chamber system allowed for the control of light, temperature, humidity, and carbon dioxide concentration. Diel patterns of methane emission varied among species, with daylight emissions from *P. virginica* and *O. aquaticum* 2–4 times those of *J. effusus* and *T. distichum* in saturating light. Foliar methane emission from *P. virginica* (1.80 μmol m^{-2} s^{-1}) under ambient daylight conditions was an order of magnitude higher than that of the other three species ($\sim$0.20 μmol m^{-2} s^{-1}). As leaf temperature was increased by 10 °C, methane emission increased by a factor of 1.5–2.2, and the temperature effect was independent of stomatal conductance. When data

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were pooled among the four species, varying the light and carbon dioxide concentrations in a stepwise manner produced changes in foliar methane emission that were associated with stomatal conductance ($r^2 = 0.52$). To scale our observations to other wetland plant species, a stepwise multiple regression model is offered that incorporates stomatal conductance and net carbon dioxide assimilation to estimate instantaneous methane emission from foliar surfaces. The model indicates that changes in stomatal conductance affect methane emission three times more than equivalent changes in net carbon dioxide assimilation.

Keywords: Methane; Wetlands; *Peltandra virginica*; *Orontium aquaticum*; *Juncus effuses*; *Taxodium distichum*

1. Introduction

Methane (CH_4) is produced in anaerobic soils by methanogenic bacteria, and released to the atmosphere through ebullition and the foliage or stems of emergent aquatic macrophytes (Schütz et al., 1991). Transport through plants often accounts for up to 90% of the CH_4 emissions from wetlands (Dacey and Klug, 1979; Singh and Singh, 1995; Whiting and Chanton, 1996), with much of this released from the plant foliage. Yet, the physiological mechanisms that control CH_4 release to the atmosphere from foliar surfaces are not fully understood. For example, some studies have reported that CH_4 emission was correlated with stomatal conductance (Frye et al., 1994; Thomas et al., 1996), but in other studies stomatal conductance did not significantly affect CH_4 emission (Nouchi et al., 1990; Whiting and Chanton, 1996).

Stomatal conductance and net carbon dioxide (CO_2) assimilation are physiologically coupled as plants respond to light, CO_2 concentration, and water vapor pressure (Lambers et al., 1998). Once stomata are opened, water vapor and other gases (e.g., CH_4) vent to the atmosphere through stomata. Because the factors that control stomatal conductance exhibit diel variation, it is not surprising that diel variation in CH_4 emissions have been observed. In plants where ventilation is driven by pressurized gas flow (Dacey and Klug, 1979; Brix et al., 1992), CH_4 emissions are strongly diel. Such responses have been documented in wetland angiosperms with cylindrical culms, and linear and floating leaves, including *Typha latifolia* L. (Brix et al., 1992; Knapp and Yavitt, 1995; Kaki et al., 2001), *Phragmites australis* (Cav.) (Arkebauer et al., 2001; Chanton et al., 2002), and *Nuphar luteum* L. (Dacey and Klug, 1979). Plant ventilation systems that operate primarily by diffusion may also exhibit diel CH_4 emission patterns (Whiting and Chanton, 1996), although these are less pronounced.

Temporal variation in CH_4 emissions can also be caused by changes in rhizosphere processes that influence methanogens and methanotrophs. Aquatic plant roots influence the activity of microorganisms through the production of organic matter and the transport of oxygen (O_2) to the rhizosphere. Radiolabelling studies have demonstrated that photosynthate is converted to CH_4 in 2–10 h (Megonigal et al., 1999; King and Reeburgh, 2002), and diel variations in CH_4 oxidation have been observed (King, 1996). These processes require a longer period of time (i.e., hours) to influence foliar CH_4 emissions than changes in stomatal conductance which can affect emissions within minutes.

Our objectives were to: (i) quantify differences in foliar gas exchange among four freshwater aquatic plant species of differing ecophysiological attributes, (ii) investigate the role of key environmental factors in controlling foliar CH_4 emission for individual species and among species, and (iii) investigate the physiological mechanisms underlying the variation in foliar CH_4 emission for individual species and among species.

2. Materials and methods

2.1. Plant descriptions and growth conditions

Three herbaceous emergent aquatic macrophytes, *Orontium aquaticum* L., *Peltandra virginica* L., *Juncus effusus* L., and one coniferous tree species, *Taxodium distichum* L., were purchased commercially. These species were chosen because they are common in the southeastern United States (Tiner, 1988), and they differ in morphological and ecophysiological traits (i.e., stomatal conductance, growth rates, and habitat). All species are native to eastern North America and are most abundant in freshwater wetlands of the southeastern coastal plain (Cronk and Fennessy, 2001). *O. aquaticum* and *P. virginica* are perennials in the Araceae with well-developed aerenchyma and photosynthetic stems. *J. effusus* is a soft rush that grows in clumps from short and has cylindrical photosynthetic stems that are filled with spongy pith. *T. distichum* is a woody coniferous tree.

Seedlings were potted in polyvinyl chloride (PVC) containers that were 42.5 cm-deep × 10 cm-diameter with a PVC end-cap on the bottom. The pots were filled with 38 cm of a 5:1 mixture of mineral soil and organic matter (commercial peat moss). The soil mixture contained a slow-release, all purpose fertilizer (12–12–12 NPK) that was added at a concentration of 40 mL per 0.03 m^3 of soil. The soil mixture became strongly methanogenic after eight weeks of submersion, producing >700 mg CH_4 m^{-2} soil d^{-1} (unpublished data).

All plants were maintained in an environmentally-controlled growth room at a temperature of 21 °C, relative humidity of 35–40%, and a 16-h photoperiod; each of the growth conditions was uniform in the room. High intensity multivapor lamps were located above the plant canopy, providing a photosynthetic photon flux density (PPFD) of 1050 μmol m^{-2} s^{-1}. Irradiance distribution was made uniform for a given species by adjusting the location and height of the light source. The four species were placed in separate tubs (44 cm × 76 cm) and filled with water submerging the soil surface to a uniform depth of 4 cm above the soil surface. To minimize microsite environmental differences, each plant was rotated weekly within a tub. Plant species were not randomized among tubs in order to assure uniform irradiance. We recognized the possibility that exposing each species to separate lamps and tubs could cause developmental differences that would be confounded with genetic differences among the species. However, we decided this design would provide the best control over both light and flooding conditions. A mixed distribution of plant species among tubs would have resulted in species-specific distances between the plant canopy and the lamps and thus variation among species in PPFD. There were six replicates of each species leading to a total of 24 experimental plants. Plant height at the time of gas exchange measurement ranged from 31 to 41 cm.

2.2. Gas-exchange system

An open gas exchange system was used to measure gas fluxes from the aboveground portion of each plant individually (Fig. 1). The system allowed 15-s interval measurements of CO_2 and H_2O vapor concentrations simultaneously at the inlet and outlet ports (Tarnay et al., 2002). The shoots of an individual plant were placed in the chamber under a closed positive-pressure glass cuvette (20 L). A circular glass plate, which could separate in half across a center opening to accommodate plant shoots, was placed at the base of the cuvette to isolate shoots from roots. Spaces between the plant stems and the glass plate were sealed with a CH_4-free dental impression material (GC America, Examix type 2, Chicago, IL, USA). A fan inside the cuvette continuously stirred the air inside the chamber to ensure uniform mixing and a negligible leaf boundary layer (Taylor et al., 1983).

A Metalarc lamp (Energy Technics Model MH1000, P.L. Light Systems, Grimsby, CA, USA) was suspended above the cuvette to supply the light. Irradiance inside the cuvette was measured with a radiation sensor (Li-COR Model LI-190SA) at 846 μmol m^{-2} s^{-1} for all herbaceous species. Due to height differences, PPFD was 1179 μmol m^{-2} s^{-1} for *T. distichum*. For all species, light saturation of net CO_2 assimilation was achieved at PPFD $\geq$ 756 μmol m^{-2} s^{-1} (unpublished data). Soil temperature was maintained at 21.0 °C ± 2.3 °C. A cooling system within the chamber provided a constant atmospheric temperature of 20.5 °C via a cooling coil attached to a recirculating isotemp chiller (PolyScience Model 625, Niles, IL, USA) and a temperature probe connected to a thermistor (Cole Parmer Model 02186-20, Niles, IL, USA). Leaf surface temperature was monitored using a T-type exposed-junction thermocoupler (OMEGA Engineering, Stamford, CT, USA) connected to a multipoint digital thermometer (OMEGA Model M642/1191).

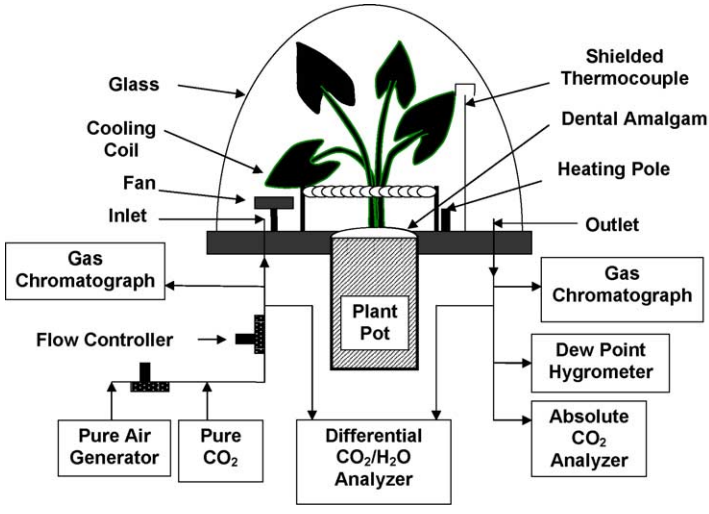

Fig. 1. A schematic of the chamber system for conducting gas exchange measurements from the aboveground portion of the wetland plant species. The aboveground portion of a plant was placed into the chamber under positive pressure so the soil column did not contribute to the CH_4 concentrations in the chamber's atmosphere.

Air from a pure air generator (Aadco Model 737-R, Clearwater, FL, USA) flowed into the chamber continuously at a rate of 4.5 L min^{-1}, resulting in a turnover time of 4.4 min. The generator was equipped with a reactor to remove CH_4 from the ambient air. To test for inadvertent CH_4 contamination, CH_4 measurements at the inlet port were taken daily and prior to environmental manipulations. Methane concentration in the inlet and outlet ports of the chamber was analyzed using a gas chromatograph (Varian Model 3700, Palo Alto, CA, USA) equipped with a flame ionization detector (Porapak Q 80/100 mesh column; Alltech Assoc., Deerfield, IL, USA). Injection/detection and column oven temperatures were maintained at 50 °C and 200 °C and helium carrier gas flow was 30 mL min^{-1}. Samples were injected on a 1 mL sample loop. Daily three-point calibrations were performed using reference CH_4 standards (Airgas East, Manassas, VA) and a 10 mL Glaspak syringe.

The CO_2 concentration at the outlet was maintained at 365 ± 15 μmol m^{-3} by passing pure CO_2 through a flow controller (Tylan Model FC-280, Torrance, CA, USA). The CO_2 concentration at the outlet was measured continuously by a CO_2 analyzer (Li-COR Model LI-800). A separate analyzer (Li-COR Model LI-6251) measured the difference between the inlet and outlet concentration of CO_2 and H_2O vapor. The CO_2 analyzer was routinely calibrated using reference CO_2 bottles (Airgas East, Manassas, VA). The outlet dew point was monitored with a calibrated dew-point hygrometer (EG&G Model 2000, Burlington, MA, USA).

2.3. Calculations

To quantify the amount of any gas (e.g., CH_4, CO_2, H_2O) reacting with or emitted from plant foliage (flux or J), J was calculated according to Taylor et al. (1983) and Tarnay et al. (2002): $J_x = \Delta_x[F/A]$, where J_x is the flux of gas (x) to or from the leaf, Δ_x is the change in gas (x) concentration between the chamber's inlet and outlet, F is the flow of air into the chamber, and A is the projected leaf area. The unit of J_x is moles m^{-2} s^{-1}, where area is the projected leaf area.

Calculations were made of net CO_2 assimilation (μmol CO_2 m^{-2} s^{-1}), stomatal conductance to H_2O vapor (G_s; mmol m^{-2} s^{-1}), transpiration (T_r; mmol m^{-2} s^{-1}), and foliar CH_4 emission (J_{CH_4}; μmol m^{-2} s^{-1}). The measured environmental factors included the outlet concentrations of CO_2, CH_4, and H_2O vapor, inlet/outlet concentration differential for the same gases, air and leaf temperature, and PPFD. Stomatal conductance to H_2O vapor was calculated as follows (Taylor et al., 1983; Tarnay et al., 2002):

$$G_s\,(\text{mmol}\,m^{-2}\,s^{-1}) = \left(\frac{T_r}{[H_2O]_{\text{leaf interior}} - [H_2O]_{\text{atmosphere}}}\right) \quad (1)$$

where $[H_2O]$ is the concentration of water in the leaf interior or the atmosphere. The leaf interior was assumed to be saturated at any given leaf temperature.

2.4. Experimental design

Measurements of J_{CH_4}, G_s and net CO_2 assimilation were made at daylight ambient conditions in order to assess differences among the four plant species at ambient conditions (i.e., temperature = 21 °C, CO_2 concentration = 365 μmol m^{-3}, and PPFD ≥ 846

μmol m^{-2} s^{-1}). Diel patterns of J_{CH_4} for all species were determined over a 24-h period (objective i). Methane samples were taken hourly for each species in the gas-exchange system. Both temperature and CO_2 concentration were held constant during the simulated 24-h period, while PPFD increased from 0 to ≥ 846 μmol m^{-2} s^{-1} at 6:00 a.m. and decreased to 0 μmol m^{-2} s^{-1} at 10:00 p.m. for all species. Methane emission was normalized to projected leaf area, which was determined using a leaf area meter (Li-COR model LI-3000A).

To address objectives (ii) and (iii), J_{CH_4} of each species ($n = 6$) was determined under simulated ambient daylight conditions by varying irradiance, temperature, or CO_2 concentration. Methane concentrations were determined by sampling gases ($n \geq 6$) from the inlet and outlet of the chamber system after the plant was acclimated (≥0.5 h). Irradiance, temperature, and CO_2 concentration were varied independently, and J_{CH_4} was calculated at pre-determined time increments for each environmental factor. Irradiance ranged from 0 to 846 μmol m^{-2} s^{-1} for the herbaceous species and 0 to 1179 μmol m^{-2} s^{-1} for *T. distichum* and was varied using neutral density filters. Carbon dioxide concentration varied from 20 to 748 μmol m^{-3} and was increased in 50 μmol m^{-3} increments. Temperature varied from 16 to 25 °C, with stepwise increases of 2 °C. The temperature coefficient (Q_{10}) was calculated using the Van't Hoff equation (Leonard et al., 1998):

$$\log Q_{10} = \frac{10}{t_2 - t_1} \log \frac{k_2}{k_1} \tag{2}$$

where k_2 was J_{CH_4} at the higher temperature (t_2), and k_1 was J_{CH_4} at the lower temperature (t_1).

2.5. Statistical analysis

All statistics were performed in Microsoft Excel (Version 2000) using the add-in statistical package and SAS (SAS Institute, 1987). Variation among species in J_{CH_4}, G_s, or net CO_2 assimilation (objective i) was tested with a one-way analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) ($p \leq 0.05$) was used for subsequent comparisons among means. No evidence of variance homogeneity of the raw data was identified using Bartlett's Test (Sokal and Rohlf, 1995).

Carbon dioxide concentration and PPFD were manipulated to induce changes of G_s and net CO_2 assimilation. This is a common approach for exploring the relationship between the flux of a gas at the leaf–atmosphere interface and plant physiological processes such as G_s and net CO_2 assimilation (Winner and Greitner, 1989). The relationship between J_{CH_4} and individual environmental factors for each species individually and for all species combined (objective ii) was evaluated using linear and nonlinear regression techniques (Sokal and Rohlf, 1995). Methane emission was normalized as percent of initial (under saturating light) for the analysis relating J_{CH_4} and PPFD. The nonlinear equation used to analyze the light-dependent data was adapted from Taylor and Gunderson (1988):

$$CH_4 = CH_{4(max)} = \left\{ 1 - \left[\frac{1 - CH_{4(min)}}{CH_{4(max)}} \right]^{\left(\frac{1 - PPFD}{l_0} \right)} \right\} \tag{3}$$

where l_0 is the non-zero x-intercept.

The relationship between J_{CH_4}, G_s and net CO_2 assimilation (objective iii) was evaluated using regression analysis (Sokal and Rohlf, 1995). The goodness of fit among the regression options was based on minimizing the residual sum of squares (Sokal and Rohlf, 1995). Because J_{CH_4} (dependent variable) and G_s or net CO_2 assimilation (independent variables) were experimentally controlled, a Model II regression was used.

To determine the relative contributions of G_s and net CO_2 assimilation to J_{CH_4} among the four species, we used a stepwise multiple regression model. Each variable was added or removed if it reduced the residual sum of squares by $\geq$5% or 10%, respectively (Sokal and Rohlf, 1995).

3. Results

3.1. Foliar gas exchange comparisons among species

Marked diel variation of J_{CH_4} was observed among species (Fig. 2A and B). As PPFD was changed in a step-wise function, J_{CH_4} from *P. virginica* doubled from 0.20 to 0.41 μmol m^{-2} s^{-1} and reached a plateau within 1.5 h from the onset of light at 6:00 a.m. Methane emission from *P. virginica* and *O. aquaticum* rose rapidly at 6:00 a.m. and 7:00 a.m., respectively and was 2–4 times that of *J. effusus* and *T. distichum* throughout the sampling period. *P. virginica* had the most pronounced decline in J_{CH_4} upon darkness, dropping from 0.40 to 0.18 μmol m^{-2} s^{-1} in $\leq$60 min. Among all species, nighttime J_{CH_4} ranged from 0.07 to 0.18 μmol m^{-2} s^{-1}.

During ambient daylight conditions (i.e., temperature = 20.5 °C, CO_2 concentration = 365 μmol m^{-3}, and PPFD $\geq$846 μmol m^{-2} s^{-1}), statistically significant variation was found among the four species in J_{CH_4}, G_s, and net CO_2 assimilation ($p \leq 0.0001$). *P. virginica* had the highest mean J_{CH_4}, G_s, and net CO_2 assimilation (Fig. 3A–C). *J. effusus* and *P. virginica* had significantly higher G_s than *O. aquaticum* or *T. distichum*, yet *J. effusus* net CO_2 assimilation was significantly lower for than for the other three species (Fig. 3B and C). Although the differences were not statistically significant, mean J_{CH_4} and G_s in *J. effusus* was three to four times that of *O. aquaticum* and *T. distichum* (Fig. 3A and B). *O. aquaticum* and *T. distichum* maintained similar mean levels of J_{CH_4} G_s, and net CO_2 assimilation.

3.2. Foliar J_{CH_4} emission as a function of environmental factors

The positive relationship between leaf temperature and J_{CH_4} (Fig. 4A) was observed for each species ($0.81 > r^2 < 0.99$). For *J. effusus*, J_{CH_4} doubled as leaf temperature increased from 16 to 27 °C. For each of the remaining species, the positive slope was less steep. The mean Q_{10} value was 1.82 (1.82 ± 0.32) and ranged between 1.50 and 2.22. The fact that all Q_{10} values were >1 indicates that the effect of temperature on J_{CH_4} was a function of diffusion. The prominent role of temperature in controlling J_{CH_4} was independent of changes in G_s. In two of the species (*J. effusus* and *T. distichium*) G_s increased with temperature, but in the other two species the opposite pattern was observed.

Response curves relating J_{CH_4} from individual species to PPFD were curvilinear (Fig. 4B). Data fit to Eq. (3) explained half of the variation in J_{CH_4} (r^2 = 0.49–0.50,

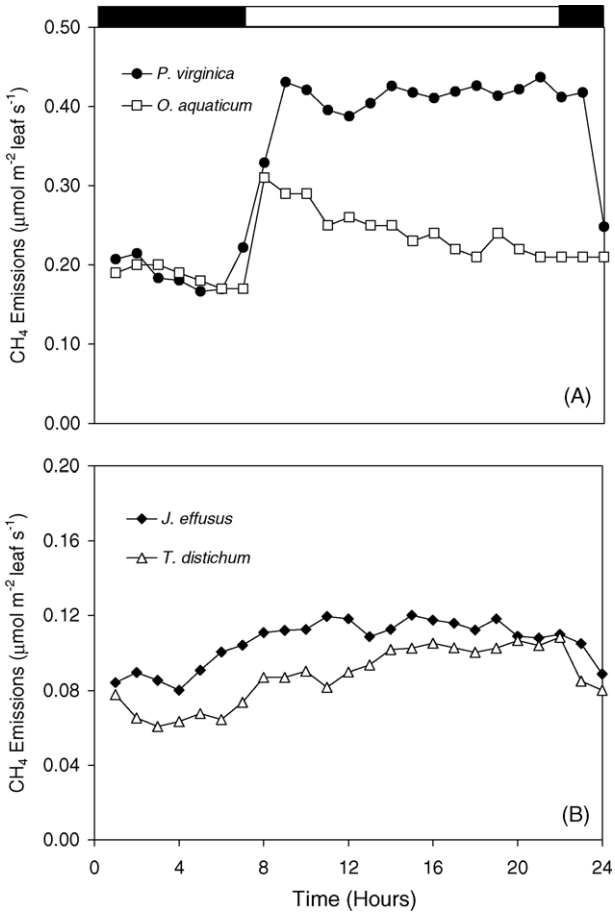

Fig. 2. Diel pattern of J_{CH_4} (μmol m^{-2} s^{-1}) in *P. virginica* (●) and *O. aquaticum* (□) (Panel A), and *J. effusus* (◆), and *T. distichum* (△) (Panel B). The black and open portions of the bar indicate dark (0 μmol m^{-2} s^{-1}) and light (≥846 μmol m^{-2} s^{-1}) PPFD conditions, respectively. All emissions are normalized to projected leaf area.

$p < 0.005$). When pooled among the four plant species, there was a significant positive relationship between J_{CH_4} and PPFD ($p \leq 0.0001$; Fig. 4B). However, J_{CH_4} among species was not related to CO_2 concentration ($r^2 = 0.14$, data not shown).

3.3. Physiological mechanisms underlying J_{CH_4}

Foliar J_{CH_4} from *T. distichum* was positively related to G_s ($r^2 = 0.65$, $p = 0.05$) and negatively associated with atmospheric CO_2 concentration ($r^2 = 0.70$, $p = 0.04$). This pattern of a positive relationship between J_{CH_4} and G_s and a negative relationship between J_{CH_4} and CO_2 held for the other three species, but none of the relationships were statistically significant.

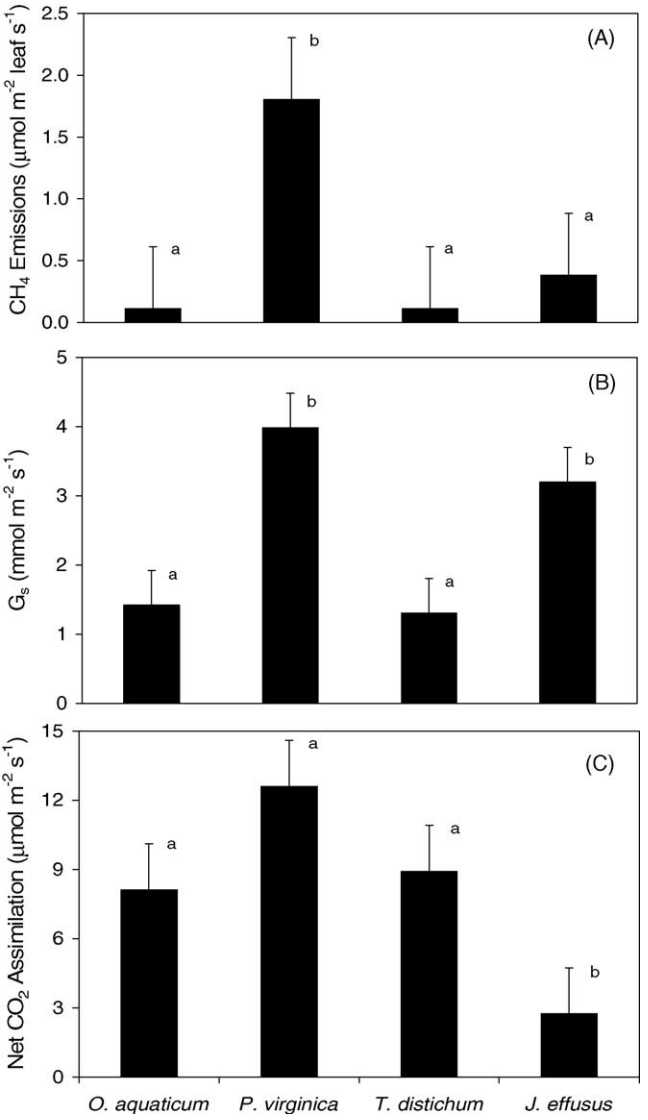

Fig. 3. Bar graphs of J_{CH_4} (Panel A; $F = 13.26$, $p \leq 0.0001$), G_s (Panel B; $F = 11.29$, $p \leq 0.0001$), and net CO_2 assimilation (Panel C; $F = 13.27$, $p \leq 0.0001$) in *O. aquaticum*, *P. virginica*, *T. distichum*, and *J. effusus* under simulated daylight conditions. Each bar is the mean ± L.S.D. of six measurements from different plants at saturating light (≥846 μmol m^{-2} s^{-1}), ambient CO_2 (365 μmol m^{-3}) and ambient air temperature (20.5 °C). Tukey–Kramer lettering indicates statistically significant differences among species. All emissions are normalized to projected leaf area.

When pooled among species, the relationship between J_{CH_4} and G_s (Fig. 4C) was positive ($r^2 = 0.52$), and the slope was statistically different from zero ($p < 0.01$). This relationship indicates that for every 1 mmol m^{-2} s^{-1} rise in G_s, J_{CH_4} increased by 0.43 μmol m^{-2} s^{-1}. The positive *x*-intercept suggests that CH_4 was not emitted from the

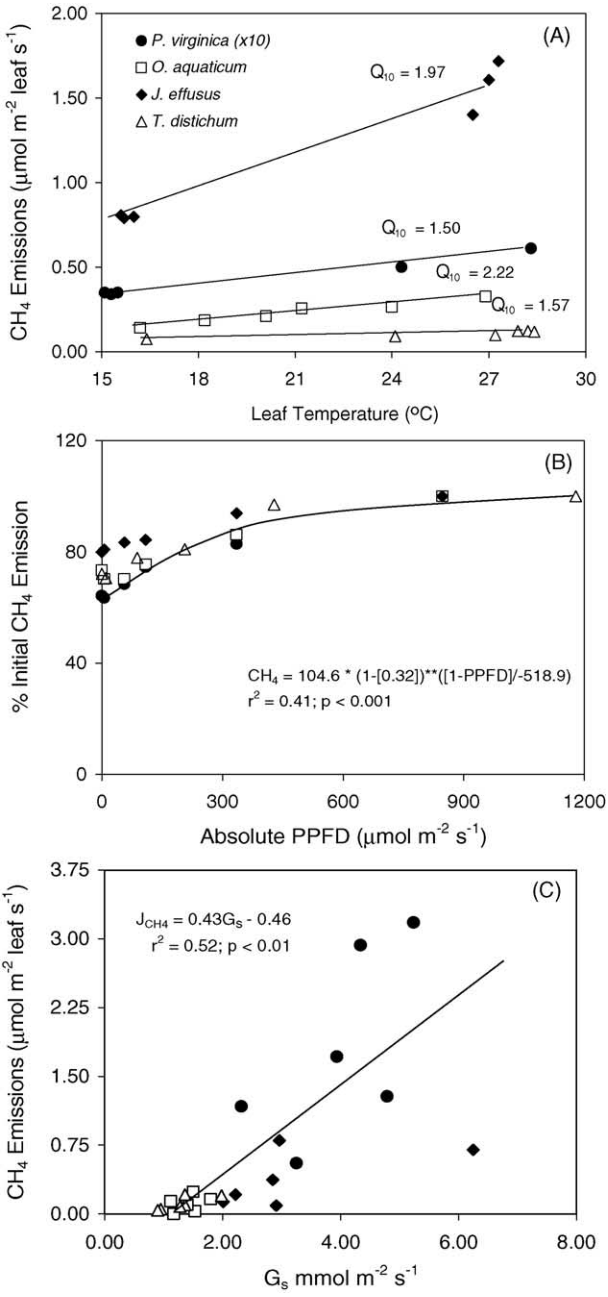

Fig. 4. Methane emission as a function of temperature (Panel A). Values for *P. virginica* are scaled by dividing by 10. Methane emission rates as a function of PPFD for all species (Panel B). PPFD was changed in a stepwise manner, whereas CO_2 concentration and temperature were held constant at ambient conditions. Methane emission was expressed as percent initial (full light of $\geq$846 µmol m^{-2} s^{-1}) to normalize the data. The relationship between J_{CH_4} and G_s among all four species (Panel C). Environmental conditions were the same as those in Fig. 3A–C. Each value is the mean of six plants per species.

foliage of these wetland plants (within our detection limit for CH_4) until G_s exceeded 1.1 mmol m^{-2} s^{-1}. This is consistent with a pathway for CH_4 emission through stomata and not the cuticle.

Using forward stepwise multiple regression on data pooled among species, both G_s and net CO_2 assimilation significantly contributed to the variation in J_{CH_4}, accounting jointly for 49% ($p = 0.01$) of the variation. Among these species, G_s accounted for 24% ($p = 0.01$) and net CO_2 assimilation accounted for 25% ($p = 0.004$) of the variation. The resulting equation is as follows: $J_{CH_4} = -0.97 + 0.31\ [G_s] + 0.10$ [net CO_2 assimilation]. Based on this model, changes in G_s affect J_{CH_4} three times more than equivalent changes in net CO_2 assimilation.

4. Discussion

Foliar CH_4 emission varied among four freshwater aquatic plant species of contrasting morphology when initial soil and environmental conditions were held constant. Diel changes in CH_4 emission were more dramatic for *P. virginica* and *O. aquaticum* than for *J. effusus* and *T. distichum* (Fig. 2A and B). There was no evidence to suggest the difference in diel patterns was due to differences in plant gas transport mechanisms. Patterns of increased CH_4 emission during daylight hours for *P. virginica* (Frye et al., 1994; Whiting and Chanton, 1996) and *O. aquaticum* (Megonigal et al., 1999) have been reported previously. All four species maintained patterns of CH_4 emission expected of diffusive gas transport and stomatal conductance control as influenced by PPFD. Because PPFD was the only environmental factor that was changed, the differences in diel patterns of CH_4 emission reflect underlying changes in physiology such as stomatal conductance and/or photosynthate supply to methanogens (i.e., CO_2 assimilation), or differences in morphology. If CH_4 emission had been driven by pressurized gas flow, there would have been a mid-morning CH_4 emission peak as CH_4 was rapidly flushed from stem and rhizome air spaces where it accumulated overnight (Sebacher et al., 1985; Chanton et al., 1993). Other characteristics of pressurized gas flow include a hysteretic CH_4 emission response curve, and changes in CH_4 emission with H_2O vapor or atmospheric temperature (Nouchi et al., 1990; Brix et al., 1992; Wang et al., 1999). Although diel CH_4 emission in *O. aquaticum* could be interpreted as showing a mid-morning peak and a hysteretic response, the pattern was less pronounced compared to other species that have been investigated (Chanton et al., 1993). Nonetheless, our interpretations should be tested with direct measurements of stem pressure, which have been reported previously to confirm diffusion-driven flow in *P. virginica* (Sebacher et al., 1985).

The relatively rapid increase in daylight CH_4 emission for *P. virginica* and *O. aquaticum* may reflect the fact that their rhizomes and stems are composed of spongy pith capable of rapidly conducting gases (Sebacher et al., 1985). Although we did not measure other morphological characteristics, the elevated CH_4 emission could also reflect larger aerenchyma cells or larger stomata than the other species. These characteristics enhance gas diffusion (Schütz et al., 1991). *J. effusus* and *T. distichum* have hard cuticle layers on foliar surfaces that restrict CH_4 transport to the atmosphere (Sebacher et al., 1985).

Foliar methane emission for *P. virginica* was 4–17 times higher than for the other species on a leaf area basis, including *O. aquaticum* (Fig. 3A). *P. virginica* also had the

highest mean stomatal conductance and net CO_2 assimilation rates of the four species, and morphology favorable to rapid transport. *J. effusus* and *P. virginica* had comparable rates of stomatal conductance, but not net CO_2 assimilation. Net CO_2 assimilation was comparable for *O. aquaticum*, *T. distichum,* and *P. virginica* but not stomatal conductance. Thus, high rates of methane emission could not be linked to a single variable, but only to a combination of interacting physiological and morphological characteristics.

Because temperature was held constant (21.0 °C) in studies addressing objective (i), diel patterns of CH_4 emission within a species can only be explained by variation in PPFD; in turn, PPFD affects stomatal conductance and net CO_2 assimilation (Figs. 2A and B, 4A–C). Changes in CH_4 emission were positively related to stomatal conductance when compared among species (Fig. 4C, $r^2 = 0.52$). Our results support the formulation of an empirical model for CH_4 emission from *P. virginica* in which stomatal conductance controlled CH_4 emission (Frye et al., 1994). Evidence of stomatal control of CH_4 emission has also been reported for *T. latifolia* (Knapp and Yavitt, 1995) and *Carex* (Morrissey et al., 1993). In comparison to these studies, our gas exchange system provided exceptional control of environmental factors and isolated whole-plant CH_4 emission from soil CH_4 emissions. We suggest that stomatal conductance plays an important role in CH_4 emission by regulating the diffusivity of the gas-phase pathway from the leaf interior to the atmosphere in many wetland plant species.

Even though stomatal conductance strongly affected CH_4 emission, environmental factors either indirectly (i.e., PPFD) or directly (i.e., temperature) affected CH_4 emission as well. When PPFD was varied in a step-wise manner, CH_4 emission increased with stomatal conductance (Fig. 4B). However, as leaf temperature increased, CH_4 emission also increased independently of stomatal conductance, and the effect showed a Q_{10} of 1.5–2.2. The temperature dependence of both methanogenesis and foliar gas exchange was normalized with the use of temperature coefficient (Q_{10}) values (Fig. 4A). The relationship between methanogenesis and temperature (i.e., seasonal, daily) has been documented with Q_{10} values from 2.1 to 4 (Whiting and Chanton, 1993; Thomas et al., 1996; Singh et al., 2000). However, Q_{10} values based on CH_4 emission at the leaf–atmosphere interface have only been determined in our study.

Given that the flux of any gas across an interface (e.g., liquid to gas) is governed by the product of the pressure potential and conductivity of the pathway, we propose that the CH_4 pressure potential is affected directly by temperature and that the physical site of action is the leaf interior at the interface between the mesophyll cell surface and the intercellular space. With a leaf temperature range from 15 to 29 °C, the Q_{10} values for CH_4 emission in *P. virginica* and *T. distichum* were 1.50 and 1.57, respectively, whereas the Q_{10} values for *O. aquaticum* and *J. effusus* were 2.22 and 1.97. Because kinetic energy (i.e., diffusion) increases only 3–4% for every 10 °C rise in temperature, the much larger 50–120% increase in CH_4 emission suggests that other processes underpin the temperature dependency, most likely thermochemical processes (Giese, 1973).

One mechanism for changes in atmospheric CO_2 to influence CH_4 emission is the alteration of the rate of organic carbon export from plants to fermentative and methanogenic microbial communities (Megonigal et al., 2004). If this was the case, atmospheric CO_2 would have a positive correlation with CH_4 emission (Vann and Megonigal, 2003). However, relationships between these two variables were absent or negative (significant solely for *T. distichum*) in the present study.

At the level of the individual leaf, the forward stepwise regression indicates that the variation in CH_4 emission among species under optimum environmental conditions is explained by a combination of stomatal conductance and net CO_2 assimilation, jointly accounting for 49% of the variation. Because stomatal conductance and net CO_2 assimilation were calculated independently, this result places a premium on quantifying stomatal conductance and net CO_2 assimilation as an a priori means of estimating CH_4 emission under daylight conditions. Of these two variables, it is noteworthy that the effect of stomatal conductance on CH_4 emission is three times that of equivalent changes in net CO_2 assimilation. Besides these two variables, one must also account for temperature effects on CH_4 emission in order to predict CH_4 emission under daylight conditions.

A portion of the variation in CH_4 emission could not be explained by the physiological and morphological variables we measured. The unexplained variation may be attributed to processes that operate sufficiently quickly to be relevant on a time-step of 1 h. For example, changes in rates of radial O_2 loss could also have influenced rhizosphere CH_4 oxidation (King, 1990; Calhoun and King, 1997). Other mechanisms such as changes in root exudation of organic carbon or root surface area for the uptake of dissolved CH_4 operate too slowly to explain CH_4 emission responses to our manipulations. These factors undoubtedly influenced differences among plant species in the absolute rates of CH_4 emission because they were all grown in the same soil mixture. However, such static differences have been investigated previously and were not the primary focus of the present study.

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