Biogeochemistry **41:** 1–22, 1998.

Diel methane emission patterns from *Scirpus lacustris* and *Phragmites australis*

FRANS-JACO W.A. VAN DER NAT, JACK J. MIDDELBURG*, DANIËLLE VAN METEREN & ANNETTE WIELEMAKERS
Netherlands Institute of Ecology, Centre for Estuarine and Coastal Ecology, P.O. Box 140, 4400 AC Yerseke, The Netherlands (Corresponding author)*

Accepted 22 September 1997

Key words: methane emission, plant mediated methane transport, *Phragmites australis*, *Scirpus lacustris*

Abstract. In mature *Phragmites australis* and *Scirpus lacustris* vegetated sediment methane was emitted almost exclusively by plant-mediated transport, whereas in unvegetated, but otherwise identical sediment, methane was emitted almost exclusively by ebullition. Diel variations in methane emission, with highest emission rates at daytime and emission peaks following sunrise, were demonstrated for *Phragmites* and *Scirpus*. The diel difference and magnitude of the emission peaks were much smaller for *Scirpus* than for *Phragmites*. In contrast to *Phragmites*, methane concentrations within *Scirpus* stems did not change significantly over the diel period. These patterns are consistent with a two-way transport mechanism for *Phragmites* (convective at daytime and diffusive at night-time) and an all day diffusive mechanism for *Scirpus*. The patterns could not be accounted for by diel variation in air and sediment temperature, plant transpiration, or photosynthetically coupled methane production. Comparison of the experimentally derived ratio of methane emission in helium and nitrogen under light and dark conditions with the theoretical derived ratio (calculated according to the kinetic theory of gases) confirmed the exploitation of the different transport mechanism for *Phragmites* and *Scirpus*. Methane emission from *Phragmites* correlated significantly with incident light, which probably drove the pressure differential associated with thermally induced convection. Decrease of the radial resistance of *Scirpus* stems for methane transport under light compared to dark conditions, in combination with morphological characteristics of the plant species, suggested that stomatal aperture, regulated by light, controls methane emission from *Scirpus*. Diel variation in bubble emission from the non-vegetated sediment coincided with sediment temperature changes. The results have important implications for sampling and scaling strategies for estimating methane emission from wetlands.

Introduction

Emissions from wetlands contribute significantly to the atmospheric budget of methane, an important greenhouse gas (Aselman & Crutzen 1989). Methane produced in the sediment can be transported to the atmosphere through three mechanisms: ebullition, molecular diffusion and via the vegetation present (Chanton & Dacey 1991). Emergent wetland plants typically have large

internal gas spaces which allow gas transport between the methane-rich sediment in which they are rooted and the atmosphere (Sebacher et al. 1985).

Oryza sativa and wetland species like *Peltandra virginica*, *Carex gracilis* and *Cladium jamaicense* employ a methane transport system based on molecular diffusion (Seiler et al. 1984; Koncalová et al. 1988; Chanton et al. 1993; Denier van der Gon & van Breemen 1993; Frye et al. 1994). Other wetland species such as *Typha ssp* and *Eleocharis sphacelata* (Bendix et al. 1994; Sorrell & Boon 1994; Chanton & Whiting 1996; Whiting & Chanton 1996), employ an additional system based on convective throughflow, in which methane flows from regions of high pressure to regions of lower pressure. The necessary pressure differential may be accomplished by a variety of mechanisms including humidity (humidity induced convection), thermal (thermo-osmosis) and wind speed (venturi induced convection) differential across plant lacunal tissue (Grosse et al. 1991; Schütz et al. 1991; Armstrong et al. 1992; Brix et al. 1992). The total internal pressure is created by the sum of these mechanisms. In general, rates of methane transport are higher when plants employ the more efficient convective gas transport mechanism compared to when methane transport is based on molecular diffusion (Dacey & Klug 1979; Sebacher et al. 1985; Mevi-Schutz & Grosse 1988; Chanton et al. 1993; Sorrell & Boon 1994; Whiting & Chanton 1996).

For several wetland plant species, methane emission rates are higher under light than dark conditions. These diel patterns in methane emission can be ascribed to changes in sediment and air temperature (Schütz et al. 1989a, b; Mikkela et al. 1995), plant transpiration rates (Chanton et al. 1997) and light intensity levels (Chanton et al. 1993; Whiting & Chanton 1996). Besides its effect on sediment and plant temperature, light may enhance methane emission rates by (1) accomplishing a shift from diffusive-driven towards pressure-driven transport (Sebacher et al. 1985; Brix et al. 1992; Chanton et al. 1993; Chanton & Whiting 1996; Whiting & Chanton 1996), (2) by increasing stomatal conductance (Knapp & Yavitt 1992; Morrissey et al. 1993; Frye et al. 1994) and, (3) by increasing photosynthetically coupled methane production rates (Whiting & Chanton 1993; Minoda & Kimura 1994; Chanton et al. 1995). Methane oxidation may potentially induce reverse diel emission patterns, but methyl fluoride inhibition flux based oxidation rates do not change significantly as a result of changing light regimes (van der Nat & Middelburg 1998).

Towards the goal of refining global budgets, it is essential to quantify the methane efflux from vegetated wetlands and its temporal variation, as well as to understand the factors and causes controlling such variations. In this study we document that *Phragmites australis* and *Scirpus lacustris* both show typical diel emission patterns for methane. We present data proving

the capability of *Phragmites* to exploit a diffusive and a convective transport mechanism for methane emission and we describe the importance of the mechanism of gas transport exploited by the two plant species as a controlling factor of the extent of diel variation.

Material and methods

Experimental approach

Measurements have been made in large constructed wetlands, not only outdoors, but also in a light, temperature, humidity and carbon dioxide controlled room (mesocosm) so that the sole effect of light could be determined. We have done a number of experiments to quantify and understand the causes underlying diel variations in methane emissions. First, methane emission measurements have been made over a 24 hour period and as a function of light level to quantify diel variations and to separate illumination effects from other factors. Second, lacunal methane concentrations have been determined to complement the flux measurements. Third, in a 'gas kinetic' experiment, we have applied nitrogen and helium atmospheres to trace the diffusional component of methane transport. If the methane flux enhancement is similar to that predicted by gas kinetic theory, a diffusive transport mechanism is operating, otherwise there is convective transport as well. Fourth, *in vitro* stem fluxes have been measured in the dark and light in an attempt to affect stomatal opening in the absence of pressure driven convection.

Outdoor constructed wetlands

Three containers ($l \times w \times h = 1 \times 0.8 \times 0.7$ m) filled with compost derived soil (Barenburg garden products, The Netherlands) were placed outdoors, at the inner court of our research institute. While acclimatising, the sediment was wetted with tap-water to assure similar waterlogged conditions. Hereafter the sediment was submerged with a water layer of 3 to 4 cm. Two containers were planted with *Scirpus* and *Phragmites* seedlings respectively, whilst one container was left unvegetated. The sediment was allowed to settle for a period of 4 months. Polypropylene collars (Ø = 24 cm) were installed to ensure consistent placement of the gas collecting chambers and minimise disturbance during successive methane flux measurements. At the end of the first plant growth cycle dead plant material was removed from the sediment surface. Before a new plant growth cycle commercial fertiliser (Barenbrug garden products, The Netherlands) was added to the flood water. Successive plant growth cycles were separated by winter.

Mesocosm wetlands

Essentially the same procedure as followed for installation and maintenance of the outdoors containers was used for the indoor mesocosm wetlands, except that the sediment used was collected from an intertidal freshwater marsh in the Schelde estuary and mixed with drainage soil, and the containers were larger (l×w×h = 1.25×1.25×0.85 m). A 16-hour day period with a light intensity of 0.48 mE.m^{-2}.s^{-1} at 40 cm below the lamps was set. The distance between lamps and sediment was 180 cm. Temperature, humidity and CO_2 concentration were set at 18 °C, 70% and 380 ppmv, day and night. Variation in the levels was less than 3% for temperature and less than 10% for humidity and CO_2 gas concentrations. Light was supplied by high frequency TL-tubes (Philips 32W/84) which were selected for their property of emitting photosynthetic active light (PAR) at relatively low heat production. Air between the lamps and the sediment surface was constantly mixed by fans to minimise local deviations of the conditions set. Successive plant growth cycles were separated by a 3-week period of darkness. For the outdoor and mesocosm wetlands, results are presented for the mid-season of plant growth cycle 2.

Methane emission

Methane emission is based on the enclosed chamber technique (van der Nat & Middelburg 1998). Briefly, methane concentrations in the chamber were measured by circulating chamber air between the chamber and a multi-gas monitor (Brüel & Kjaer type 1302) through Teflon tubes. Methane concentrations were measured every 1.5 minutes. Emission rates were calculated by linear regression analysis from the change of methane concentration in the chamber with time over at least a 15 minute period (n = 9). Emissions were usually highly linear ($R^2 > 0.98$ ($n \geq 9$)). Otherwise the measurement was discarded to assure that methane emission rates reported are true initial rates. The amount of methane transported through ebullition was distinguishable from the total methane flux because bubble events caused a discrete shift in the monitor output. Methane emission due to diffusion was calculated by subtracting bubble emission from total emission in the non-vegetated sediments. The value obtained was used to partition the amount of methane transported by the plants and by diffusion in the vegetated sediments. Unless stated otherwise, carbon dioxide levels inside the chamber were maintained within the range of ambient values during periods of carbon dioxide uptake by injecting small amounts of bottle carbon dioxide into the chamber. During the diel emission measurements the chamber was vented on a regular basis (usually each 40 to 50 minutes) by flushing with compressed atmospheric air

in order to re-establish initial chamber air conditions. Flushing was done via the inlet and outlet adapters from the gas monitor, so that the chambers were left in place and potential disturbance was minimised. During the experiment with increasing light intensities the chamber was flushed with atmospheric air between replicate measurements. After every change in light in this experiment, there was an overnight adjustment period to ensure steady-state conditions during measurements.

Lacunal methane

Lacunal gas of outdoor grown plants was obtained by inserting a 20 μl syringe into the stem of *Scirpus* or *Phragmites*. Immediately after sampling, the contents of the syringe were injected into a gas chromatograph (Carlo-Erba high resolution MEGA 5340 equipped with a FID detector). Between successive samples the syringe was flushed with ambient air. Afterwards, diameter, length, fresh and dry weight and number of yellow and green leaves were determined. Stem volumes were estimated using the formula $v = 0.33.\pi.h.(R^2 + r^2 + R.r)$, with h = height, R = radius stem base, r = radius stem top. Daytime sampling was done between 1200 and 1600 h and night-time sampling between 0100 and 0500 h. This time schedule was chosen to allow enough time for light adjustment. Dry gas samples obtained from underwater portions of the plants showed that no leakage occurred around the syringe. The volume per cm stem averaged approximately 1 ml for *Scirpus* and 0.2 ml for *Phragmites*. Hence, the gas volume extracted from the stem (20 μl) is very small compared to total stem volume and corresponds to a stem length of 0.1 cm length for *Phragmites* and even less for *Scirpus*.

Gas kinetic experiment

The 'gas kinetic' experiment was performed in the mesocosm wetlands and involved flushing of chambers with nitrogen (99.9%) or helium gas (99.99%). During flushing, the oxygen content of the chamber was monitored by an oxygen electrode inside the chamber. Anoxic conditions were reached within one hour. Flushing was continued at a lower rate for another 5 hours before the first emission measurement was started. Due to 'thin air' and subsequent pump failure of the gas monitor, methane concentrations in the helium gas treatment were determined utilising the FID equipped gas chromatograph. Eight gas samples (2.5 ml; $<0.005\%$ of chamber volume) were collected using gastight syringes and analysed within 1.5 hour of collection. Prior to the determination of the emission rate in a nitrogen and helium atmosphere, the emission rate was measured in normal air. Depending on the specific experiment, the chamber was flushed with either atmospheric air, nitrogen or helium between replicate

measurements (n = 3). After a change in light, an overnight adjustment period ensured steady-state conditions during measurements.

When diffusion of methane through the plant is independent of the mole fraction of the gas, then the binary diffusion coefficient (D) for methane (i) in another gas (j) may be calculated according to Hirschfelder et al. (1964):

$$D_{ij} = 0.0026280 \frac{\sqrt{T^3(M_i + M_j)/(2M_iM_j)}}{p\sigma_{ij}^2\Omega_{ij}} \quad (1)$$

with, D_{ij} binaire diffusion coefficient of trace gas i in gas j ($cm^2.s^{-1}$), T temperature (°K), p atmospheric pressure (Pa), M_i mol mass of methane, M_j mol mass of air, nitrogen or helium, σ_{ij} collision diameters for combined gas ij (Å) and Ω_{ij} collision integral for combined gas ij. The values for the collision diameters and integrals are given by Hirschfelder et al. (1964) as well and are recalculated for the combined gas (ij) using the empirical laws given by Leffelaar (1987). This approach is valid when lacunal methane concentrations are relatively small. The lacunal methane concentrations of *Scirpus* and *Phragmites* indicate that this would result in a deviation of the binary diffusion coefficient of maximal 4%. No attempt was made to correct for this deviation.

Replacement of air with nitrogen and helium causes not only a change in methane diffusion, but also results in inhibition of methane oxidation. Methane oxidation has been estimated from the difference between methane fluxes in air and nitrogen. Diffusion enhancement of methane in helium is compared to that in nitrogen to eliminate the effect of methane oxidation. The theoretical He/N_2 ratio (3.13) is calculated from the binary diffusion coefficients obtained using equation 1 ($D_{CH_4-H_e}$ 0.621 $cm^2.s^{-1}$ and $D_{CH_4-N_2}$ 0.198 $cm^2.s^{-1}$). The contribution of diffusion to total methane transport has been calculated from the difference between methane fluxes in nitrogen and helium divided by the theoretical ratio (3.13) $-$ 1.

In vitro stem flux rates

Stem parts of *Scirpus* and *Phragmites* plants grown outdoors were placed between 1 litre source and sink flasks. The source flask was continuously flushed with 100% methane gas at a low rate. Increase of methane in the sink flask was measured for a period of time, depending on the rate of gas transport, but no longer than 1 hour, using the multi-gas monitor. The increase of methane in the sink flask was usually linear (otherwise the measurement was discarded), indicating that the increase of methane in the sink flask was not affected by a reduction in the methane concentration gradient between the

source and sink flask, and possible deviation from ambient pressure values within the flasks. The desired stem length was excised from plants grown in the outdoors containers, and left for three hours to allow equilibration of lacunal methane with ambient methane levels. The stem extremities were coated with silicon grease, surrounded by a sleeve of tygon tubing and connected to the flasks with the stembase always connected to the source flask. Each stem part was only used once to prevent autocorrelation between measurements. Dark conditions were provided by means of a black-out cloth.

Environmental parameters

Incident light (PAR) was measured with a Macam type SD 101 light sensor. Light intensity inside the chamber was more than 90% of the light intensity measured outside the chamber. Air and sediment temperature were measured by simple mercury glass thermometers. Sediment temperature was measured 3 cm below the sediment-water interface. Chamber air temperature followed outside air temperature closely and deviated usually no more than 2 °C. Humidity inside the chamber was also monitored by the multi-gas monitor.

Statistical analysis

Errors are based on a normal distribution and are combined errors using the square root of sum-of-squares technique for propagation of errors assuming independence of errors. Statistical analysis was done using one- or multi-way ANOVA tests, depending on the number of factors, so possible interactions between factors would be revealed. Multiple comparisons are based on post-hoc Tukey contrasts. In all analysis where $p < 0.05$, the factor tested was considered statistically significant.

Results

Methane emission (outdoors)

Methane emission from *Phragmites* exhibited two peaks over the course of the day, one directly following 'sunrise' and a smaller one directly following clearing of the sky after a severe summer storm (Figure 1a). Both peaks closely followed the amount of light reaching the plants. Air temperature maxima did not coincide with the emission peaks. Methane emission from outdoor *Scirpus* exhibited a small peak following sunrise, but a rather gradual rise and fall over the course of the day, corresponding with the rising and subsequently decreasing irradiation levels and air temperatures over the course

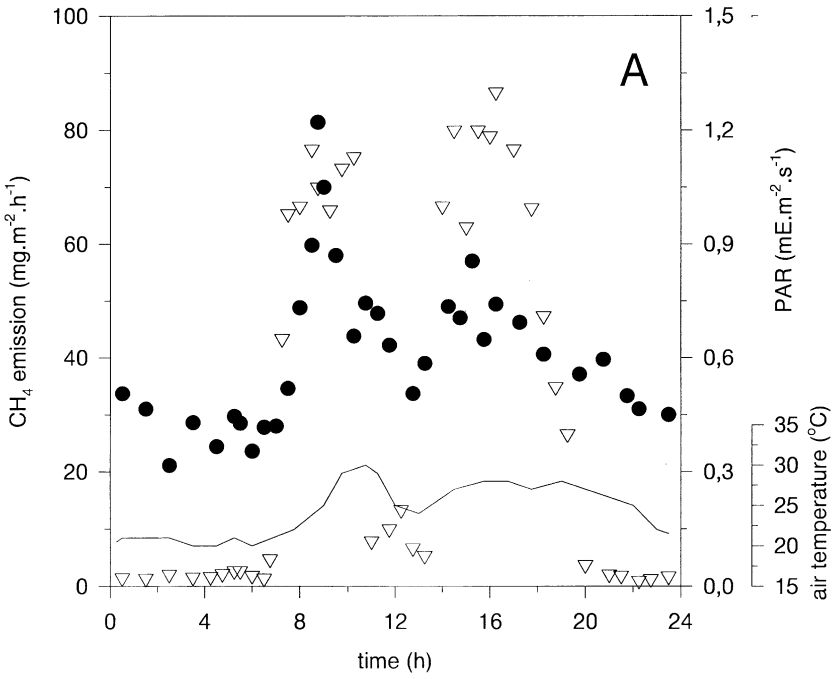

Figure 1. Diel variation of methane emission (solid circles) from *Phragmites* (panel A), *Scirpus* (panel B) and non-vegetated sediment (panel C) under naturally varying light (open inverted triangles), air temperature (solid line) and sediment temperature (dotted line) conditions. The decrease in PAR around midday (panel A) was caused by a severe summer storm. The *Scirpus* and *Phragmites* sediment temperatures during the day and night averaged 26 °C and 24 °C, respectively.

of the day (Figure 1b). Both plant species showed higher day- than night-time emissions. However, the diel difference was much smaller for *Scirpus* plants than for *Phragmites* plants. Methane emission from the non-vegetated sediment was largest between 16.00 h and 20.00 h, coinciding with the highest sediment temperatures (Figure 1c). Emission was almost completely mediated by ebullition, molecular diffusion contributed less than 0.2 $mg.m^{-2}.h^{-1}$. Methane influxes were only observed when methane concentrations within the chamber were higher than 7 ppmv.

Methane emission (mesocosm)

Differences between day and night time emission were also observed in the indoor mesocosm experiments, again less pronounced for *Scirpus* than for *Phragmites* (Figure 2). The dark to light transition resulted in emission peaks for *Scirpus* and *Phragmites* similar to those observed outdoors following

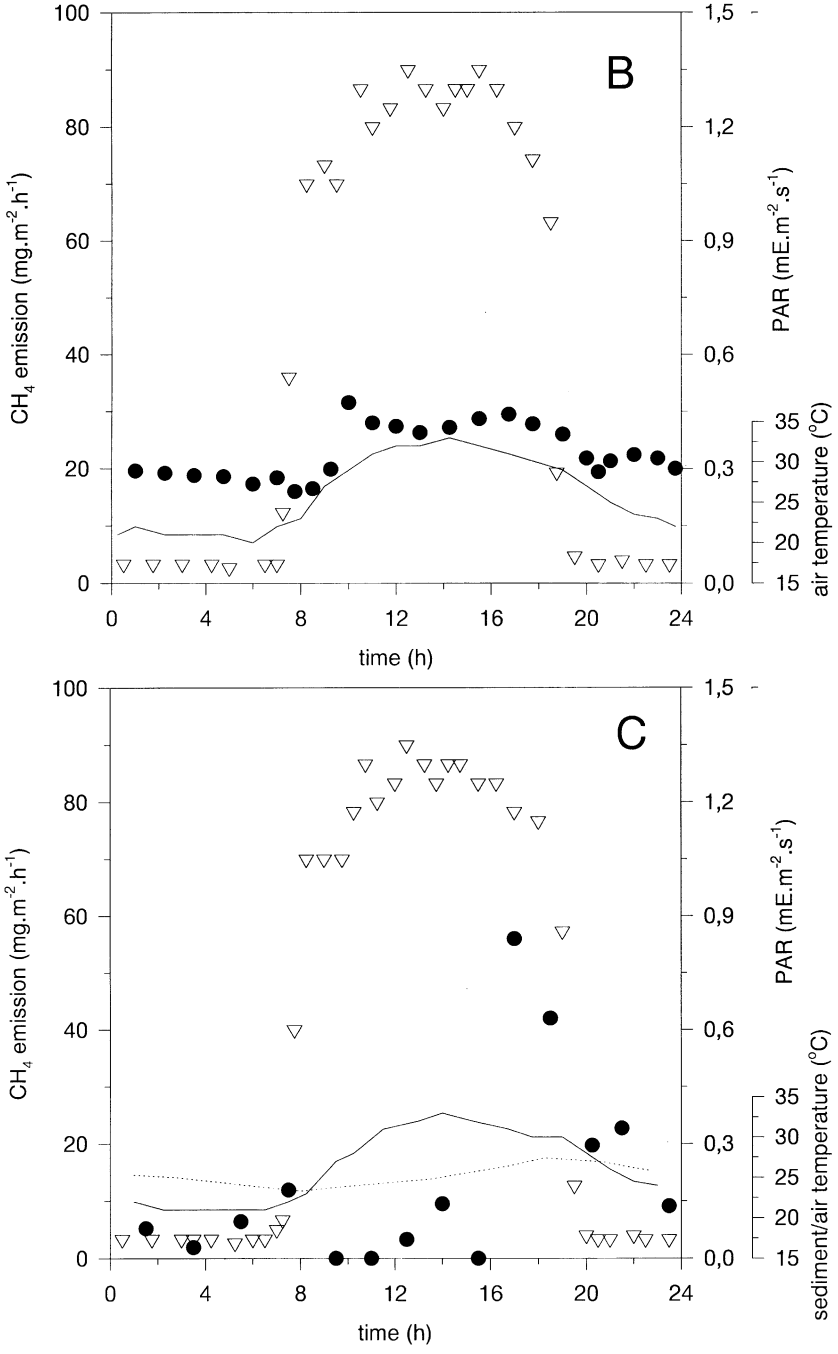

Figure 1. Continued.

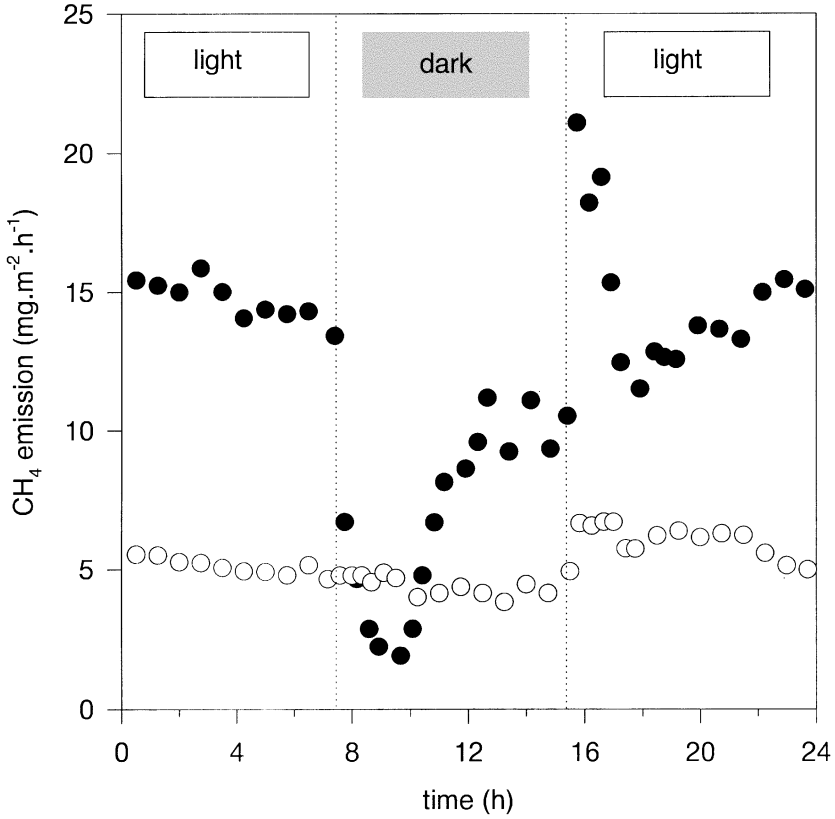

Figure 2. Effect of light and dark periods and light/dark transitions on methane emission from *Phragmites* (solid circles) and *Scirpus* (open circles) under controlled conditions. CO_2 uptake during light was not compensated for.

sunrise. The light to dark transition resulted in an initial sharp decrease of emission from *Phragmites*. Humidity variation inside the chamber under light and dark conditions was relatively small (Table 1). Unlike methane emission, changes in illumination were not immediately followed by changes in chamber humidity levels. The first changes were observed more than 1 hour after change of light (data not shown). The variation observed in the non-vegetated container corresponds with a 0.6 °C temperature increase under light conditions compared to dark conditions. Gradually increasing light intensities resulted in a gradual increment of methane emission from *Phragmites* (standardised coefficient of the increase was 0.603 (p = 0.017)), but not from *Scirpus* (p = 0.386, Figure 3).

Table 1. Chamber air humidities (RH; %).

	Illuminated (n = 48)	Dark (n = 51)
Bulrush	79.5 ± 1.42	72.7 ± 0.75
Reed	77.8 ± 2.65	68.4 ± 1.40
Non-vegetated	79.3 ± 1.84	77.0 ± 1.19
Climate room	70.0 ± 7.00	70.0 ± 7.00

± represents the standard error of the mean (n = 3).

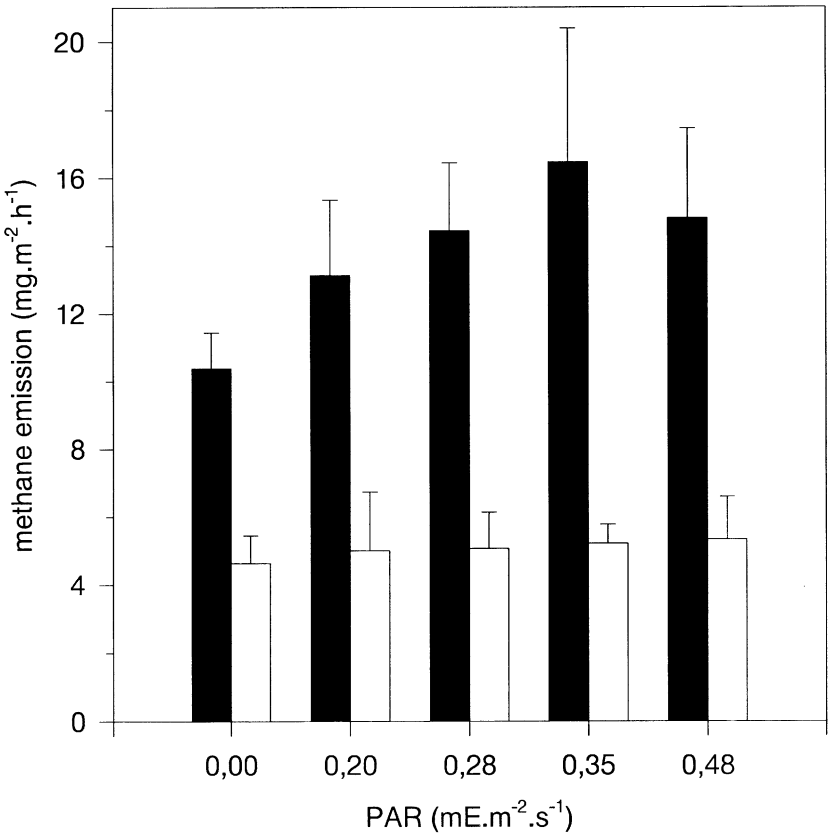

Figure 3. Effect of increasing light on methane emission from *Phragmites* (black bars) and *Scirpus* (open bars) under controlled conditions. The error bars represent standard error of the mean (n = 3).

Lacunal methane (outdoors)

Methane concentrations within *Phragmites* and *Scirpus* stems decreased rapidly relative to stem height, starting at the stem base (Figure 4). The stem

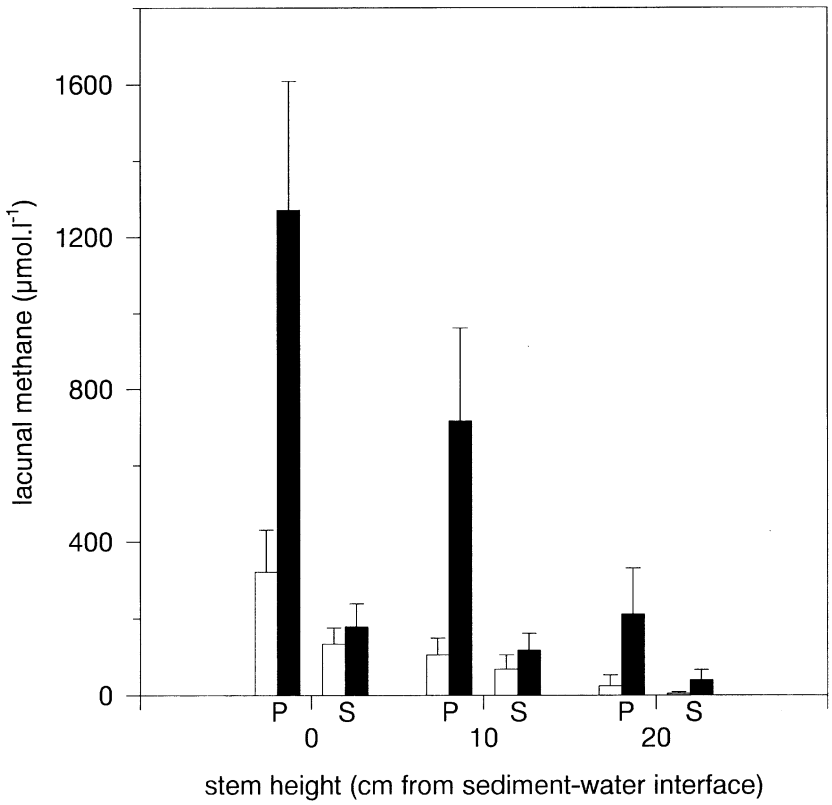

Figure 4. Methane concentrations within *Phragmites* (P) and *Scirpus* (S) stems at daytime (open bars) and night-time (black bars) under naturally varying conditions. At daytime, maximum PAR, air temperature and sediment temperature were 1 mE.m^{-2}.s^{-1}, 25 °C and 22 °C, respectively. At night-time: 0.04 mE.m^{-2}.s^{-1}, 18.3 °C and 20.9 °C, respectively. The error bars represent standard error of the mean (n = 15).

height at which methane was found in excess of ambient levels ($\pm$0.1 μM), depended on light levels but never exceeded 60 cm (both plant species). The length of the *Scirpus* and *Phragmites* stems averaged 111 and 161 cm, respectively. In contrast to *Phragmites*, daytime and night-time lacunal methane concentrations of *Scirpus* were not significantly different, at any of the three sampling heights (Figure 4). The volume of the first 30 cm of *Phragmites* and *Scirpus* stems averaged 1.92$\pm$0.65 and 9.32$\pm$4.53 ml, respectively. The absolute methane content within the lower 30 cm of stem were similar at night-time (*Phragmites* 3.64, *Scirpus* 3.49 μmol), but dissimilar at daytime (*Phragmites* 0.97, *Scirpus* 2.45 μmol). The number of *Phragmites* and *Scirpus* stems per m^2 in the outdoors containers, were 1060 and 640, respectively.

Gas kinetic experiment (mesocosm)

Light significantly increased methane emission in air for *Phragmites*, but not for *Scirpus*, and for both plant species, methane emission in nitrogen was higher than that in air, and methane emission in helium was higher than that in air and nitrogen (Table 2). For *Scirpus*, the experimental ratios of emission in helium relative to nitrogen were equal to or exceeded the theoretical value and diffusion could account for all methane transport. For *Phragmites*, the experimental ratios remained below the theoretical value and diffusion contributed between 13 and 46% to methane transport. The difference between the light and dark ratio was relatively small for *Scirpus* and relatively large for *Phragmites*. However, these differences proved not to be significant for both plant species. Emission in helium displayed much less light/dark variation compared to the emissions in air and nitrogen, especially for *Phragmites*. Methane oxidation rates were estimated from the difference between the emission in nitrogen and air, analogues to the anoxic/oxic flux chamber technique (Frenzel et al. 1992; Gerard & Chanton 1993; Denier van der Gon & Neue 1996). Oxidation rates were higher in the *Scirpus* container than in the *Phragmites* container (22% and 6%, respectively). Control experiments showed no consistent variation of the emission rate in air before and after treatment with nitrogen or helium.

In vitro stem flux rates

The amount of methane transported through the stems from the source to the sink flask decreased rapidly relative to ratio of stem part length and stem base area (Figure 5a,b). Hence, the longer the stem parts were, or the smaller the stem base area was, the lower the flux of methane through the stem part. Methane fluxes through stem parts longer than 40 cm were nil. The differences between the flux rates measured under light and dark conditions proved not to be significant for both plant species. However, when taking into account the lower 40 cm of the stem parts of *Scirpus*, fluxes were significantly higher ($p < 0.03$) under dark than light conditions. The absolute amount of methane transported through *Phragmites* stems was higher than through *Scirpus* stems.

Discussion

Almost all ($>98\%$) of methane emitted from the vegetated sediments was mediated by the plants present. Since temperature plays an important role in the offset of bubble emission (Fechner-Levy & Hemond 1996), the diel

Table 2. Methane emission in air, nitrogen and helium.

		Emission ($mg.m^{-2}.h^{-1}$)			Ratio He/N_2	Diffusion (%)	Oxidation (%)
		Air	N_2	He		$(He-N_2)/2.13$	$1-air/N_2$
Scirpus	Dark	3.96 ± 0.46	5.00 ± 0.32	17.2 ± 2.36	3.45 ± 0.52	115 ± 24	20.8 ± 2.74
	Light	4.48 ± 0.27	5.78 ± 0.45	18.1 ± 3.21	3.13 ± 0.61	100 ± 27	22.5 ± 1.79
Phragmites	Dark	9.84 ± 1.62	10.3 ± 0.40	20.4 ± 2.38	1.98 ± 0.24	46 ± 11	4.55 ± 0.77
	Light	14.8 ± 1.36	15.8 ± 1.19	20.3 ± 1.46	1.29 ± 0.13	13 ± 6	6.24 ± 0.74
Theoretical	Light/dark				3.13		

Measurements were conducted under controlled conditions. CO_2 uptake during light was not compensated for. $\pm$ represents the standard error of the mean ($n = 3$).

variation in sediment temperature (outdoors) was probably responsible for the methane emission pattern in the non-vegetated container.

The response to changing light conditions was similar for the outdoors (Figure 1a,b) and temperature controlled diel cycles (Figure 2), which suggested that variations in air and sediment temperature did not play an important role in controlling the *Phragmites* and *Scirpus* diel emission patterns. In addition, the diel change in air temperature outdoors was, according to the kinetic gas theory (Equation 1), responsible for no more than 35% of the observed variation. The relatively small variation in humidity under light and dark conditions (Table 1) and the weak correlation between humidity and methane emission patterns in the mesocosm wetlands do not imply an important role for plant transpiration or artificial alteration of humidity levels due to chamber deployment as well. The nearly instantaneous response to changing light conditions and the lack of difference between CO_2 compensated and non-compensated emissions indicate that possible variation in direct photosynthetically coupled methane production can be excluded. The absolute differences in methane emission between the outdoors and mesocosm containers are probably related to differences in labile organic matter content of the sediments and temperature conditions, both factors being important for methane production (Conrad 1989).

The emission pattern observed for *Phragmites* is similar to patterns observed for emergent plants exploiting diffusive transport during periods of low illumination or darkness and additional (pressurised) convective transport during periods of sunshine or illumination (Sebacher et al. 1985; Chanton et al. 1993; Chanton & Whiting 1996; Whiting & Chanton 1996). For such two-way transport species, shifts in illumination are typically accompanied by a significant emission peak (the ‘sunrise’ emission peak and ‘after storm’ peak, Figure 1a). Diel differences in emission (Figures 1a, 2) and lacunal methane concentrations (Figure 4) were relatively large compared to plants using solely diffusive transport, probably as a result of the shift from diffusive towards more convective transport. The emission peaks following increases in illumination are likely caused by initial ventilation of the enhanced lacunal methane concentrations that have accumulated during darker periods (Figure 4) when diffusion dominated gas transport. Similarly, the very low fluxes following darkening (Figure 2) can be attributed to the gradual accumulation of methane within the stems until a new steady-state level with respect to diffusive transport was established.

It has been shown that the degree of pressurisation in *Phragmites* depends upon the quantity of light incident to the leaf surface (Armstrong & Armstrong 1990). This would explain the significant correlation between emission and irradiance for *Phragmites* (Figure 3) and indicates that convective transport

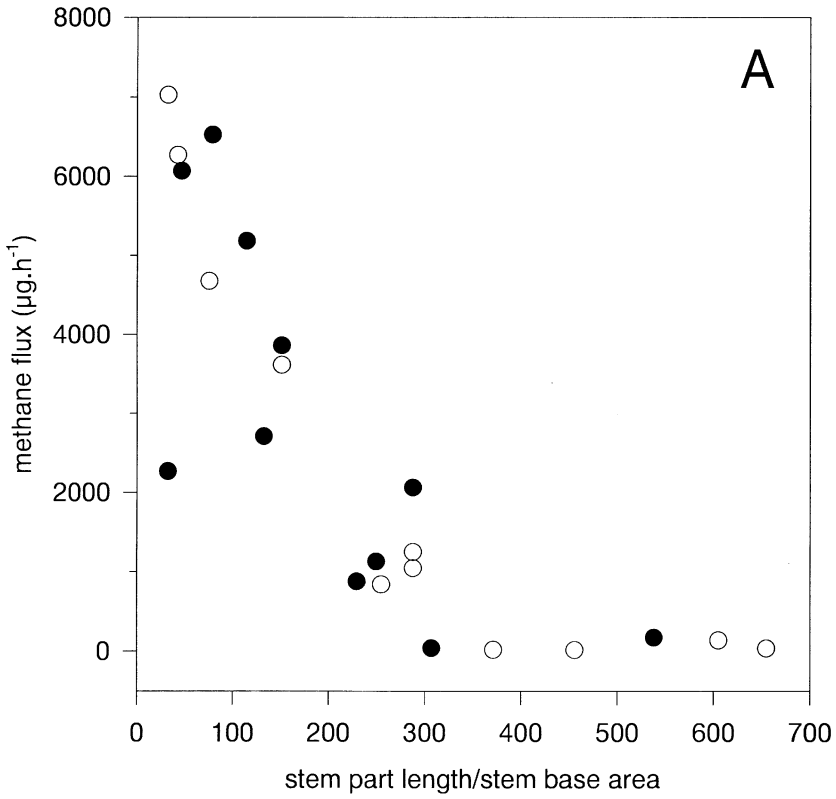

Figure 5. *In vitro* flux rates of methane under light (open circles) and dark (solid circles) conditions through stem parts, varying in length and stem base area, of *Phragmites* (panel A) and *Scirpus* (panel B). Illumination levels were in between 0.7 and 0.9 mE.m^{-2}.s^{-1}. The variation in temperature under dark and light conditions was less than 2 °C.

is not an on/off mechanism but a gradual one, with the rate depending on light intensity. Stimulation of methane emission from *Phragmites* by light is also observed in the field (van der Nat, unpublished data). It can be inferred that gradually changing light conditions, for example on a cloudy day, would result in a gradually changing emission pattern for *Phragmites* and less pronounced peaks. Light dependence of pressurisation has been observed for humidity or thermal induced convection (Brix et al. 1992). Unfortunately, we only have temperature and humidity data for chamber air and not for plant leaf and stem lacunal air. Therefore no attempt has been made to distinguish the contribution of thermal and humidity induced convection.

The emission pattern observed for *Scirpus* is similar to the pattern observed for plants exploiting diffusive transport all day long (Seiler et al. 1984; Schütz et al. 1989a; Chanton et al. 1993; Denier van der Gon & Breemen van 1993;

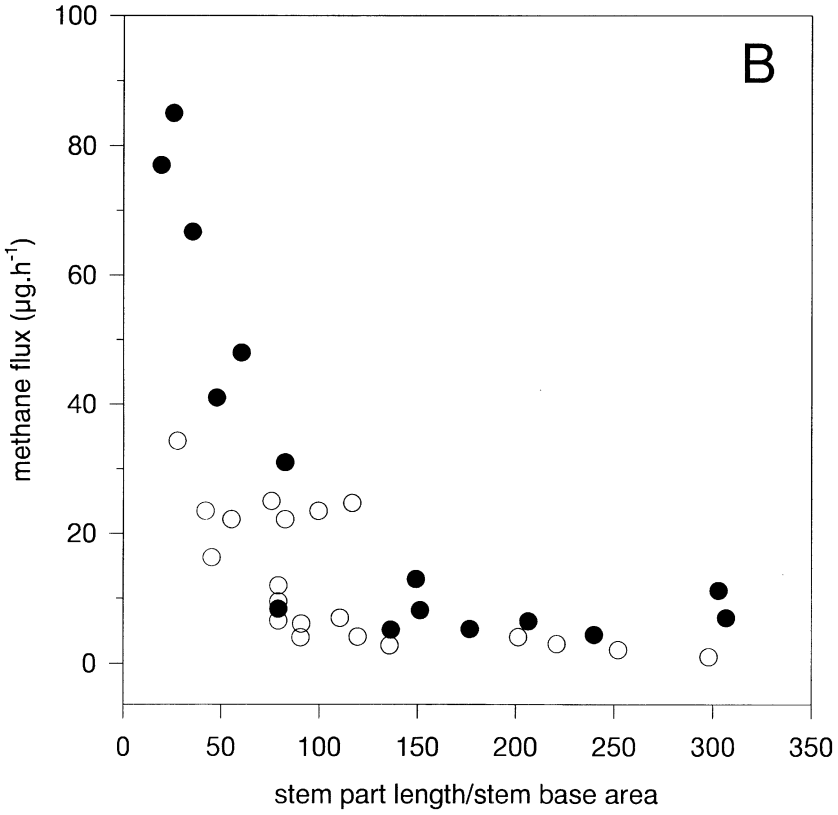

Figure 5. Continued.

Shannon et al. 1996; Whiting & Chanton 1996). For such one-way (diffusive) transport plant species, shifts in illumination are accompanied by small or no emission peaks, and diel differences in emission (Figures 1b, 2), and lacunal concentrations (Figure 4) are relatively small compared to two-way transport plant species. The observed emission patterns and the difference between the two plant species in their emission response to increasing light (Figure 3) show that emission from *Scirpus* is much less sensitive to changing light than emission from *Phragmites*, probably because light regulated convective transport does not occur in *Scirpus*.

The gas kinetic experiment confirms the exploitation of different methane transport mechanisms by *Phragmites* and *Scirpus*. If the experimentally determined He/N_2 ratio is similar to that predicted by diffusion theory, a diffusive transport mechanism is operating (Denier van der Gon & Breemen van 1993). Similarly, an experimental He/N_2 ratio lower than the theoretical value is indicative of a convective transport mechanism. Accordingly, molecular

diffusion dominates methane transport in *Scirpus* and there is a non-diffusive component in methane transport by *Phragmites* (Table 2). The relatively large change of the He/N_2 ratios for *Phragmites* when light conditions alter also supports a convective transport mechanism. The decrease of the light/dark variation of emission in helium compared to air and nitrogen might be the result of the specific properties of helium gas. The oxidation percentages obtained by the anoxic/oxic flux chamber technique (Table 2) were less than those measured in the same system with the methylfluoride flux chamber inhibition technique (van der Nat & Middelburg 1998). This difference might be inherent to the complexity of the processes determining oxidation, e.g. seasonal variation. It might also have been the result of the difference in methodology, because the preincubation flushing time of 5 to 6 hours with nitrogen in the gas kinetic experiment might have been too short to reach full anoxic conditions at the sites of methane oxidation. This is not a problem if nitrogen and helium exhibit the same transport kinetics. If not, and helium reaches the sites of methane oxidation faster than nitrogen, then the He/N_2 ratios are overestimated. Using the seasonally averaged oxidation percentages of the methylfluoride inhibition technique to re-estimate the emission in nitrogen, the dark and illuminated He/N_2 ratios become 3.05 and 2.82, respectively for *Scirpus* and 1.76 and 1.17, respectively for *Phragmites*, not changing the conclusions drawn.

A convective transport mechanism for oxygen from the atmosphere into the root system of *Phragmites* has been demonstrated (Armstrong & Armstrong 1990, 1991; Brix et al. 1992, 1996). From these studies it was clear that the typical morphological and anatomical characteristics of *Phragmites*, such as the cylindrical culm and linear leaves, the presence of old dead culms and live shoots using the same rhizome system, the presence of old brittle and green leaves attached to the same stem, and the absence of meristematic tissue, facilitate and endorse the pressurised transport mechanism. When comparing the morphological and anatomical characteristics of *Scirpus* and *Phragmites*, the absence of a pressurised system for *Scirpus* may not come as a surprise. *Scirpus* only has full green stalks with no ‘leaves’ attached and offers a much higher resistance to gas flow than *Phragmites* (Figure 5a,b), probably due to the presence of meristematic tissue inside the stems. *Scirpus* appears not to be able to carry out humidity- or temperature-induced pressurisation.

Stomatal aperture may offer an alternative explanation for the observed diel patterns. Emission peaks from *Phragmites* would in this case be caused by the initial opening of the stomata following illumination. However, the importance of stomatal control of methane emission is ambiguously discussed in the literature for several plant species including *Peltandra* (Chanton et al.

1992; Frye et al. 1994; Whiting & Chanton 1996) and *Typha* (Knapp & Yavitt 1992; Whiting & Chanton 1996). Evaluation of its importance is not easy because large differences in stomatal conductance do not necessarily indicate the presence of stomatal control (Whiting & Chanton 1996). Armstrong et al. (1992) concluded that internal pressurisation during the day is influenced by stomatal aperture, which may affect methane emission since the dominant transport mechanism during the day for *Phragmites* is convective.

We argue that stomatal aperture is not an important mechanism for *Phragmites* to control methane emission because: (1) the loci of methane release are predominantly in the lower 30 cm of the plant stem, which is lignified and apparently not rich in stomata. Recent studies of Nouchi et al. (1990) and Harden et al. (1994) showed that the lower parts of *Oryza*, *Pontederia* and *Sagittaria* stems were responsible for methane release. The authors excluded possible contribution of stomatal aperture and emphasised the importance of micropores for methane release. (2) *In vitro* methane fluxes through *Phragmites* stem parts under light and dark conditions were similar (Figure 5a). If stomata are important, one would expect lower fluxes under light (stomata opened, decreased radial resistance) than under dark conditions (stomata closed, increased radial resistance).

The (small) diel variation in methane emission (Figures 1b, 2) and lacunal concentrations (Figure 4), and (small) emission peak following incident light for *Scirpus* are not accounted for by a shift in transport mechanism like in *Phragmites*. An explanation of the variation in *Scirpus* might be related to heating of plant tissue by light and subsequently higher methane diffusion rates. However, light control of diffusion seems unlikely (*see above*) and the nearly instantaneous response of methane emission to the dark/light transitions (Figure 2) suggests a mechanism capable of a very rapid response to changing light conditions. Stomatal aperture is such a rapid mechanism (Morrissey et al. 1993; Frye et al. 1994; Whiting & Chanton 1996). Further evidence for stomatal control: (1) the lower, important, part for methane release of *Scirpus* stems is fully green, not lignified, and apparently rich in stomata. (2) *Scirpus* showed a noticable variation in gas transport in response to changing light (Figure 5b). Unfortunately, the number of measurements were too small to make conclusive statements. The amount of light reaching the lower parts of stems in a dense *Scirpus* vegetation is probably less than was present in the *in vitro* experiment and the difference between light and dark fluxes might consequently have been overestimated. It is clear that more work needs to be done on the subject of stomatal control of methane emission by *Scirpus*.

Finally, it is interesting to note that at night-time when both plant species, apparently, operate a diffusive transport mechanism and stomatal aperture is

minimal, the absolute methane contents in the stems of *Scirpus* and *Phragmites* were similar, while methane emission still differed. This difference in night-time emission is probably the result of the difference in number of stems present in the respective containers.

In conclusion, diel variation of methane emission from wetlands is important, especially when plants present are capable of exploiting more than one transport mechanism. Accordingly, sampling strategies for estimating the amount of methane emitted from wetlands have to be carefully designed in order to include this variation and favourably make use of continuous measurement or equipment using both light transparent and darkened chambers.

Acknowledgements

We thank Hugo Denier van der Gon, Riks Laanbroek and Mathieu Starink for critically reading an earlier version of this manuscript, three anonymous reviewer for constructive comments and Joop Nieuwenhuize and Yvonne Maas for help with and maintenance of the GC-equipment used. This is publication 2295 of the Netherlands Institute of Ecology.

References

Armstrong J & Armstrong W (1990) Light-enhanced convective throughflow increases oxygenation in rhizomes and rhizosphere of *Phragmites australis* (Cav.) Trin. ex Steud. New Phytol. 114: 121–128

Armstrong J & Armstrong W (1991) A convective through-flow of gases in *Phragmites australis* (Cav.) Trin. ex Steud. Aquat. Bot. 39: 75–88

Armstrong J, Armstrong W & Beckett PM (1992) *Phragmites australis*: Venturi- and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. New Phytol. 120: 197–207

Aselman I & Crutzen PJ (1989) Global distribution of natural freshwater wetlands and rice paddies, their net primary productivity, seasonality and possible methane emissions. J. Atmos. Chem. 8: 307–358

Bendix M, Tornbjerg T & Brix H (1994) Internal gas transport in *Typha latifolia* L and *Typha angustifolia* L. 1. Humidity-induced pressurisation and convective throughflow. Aquat. Bot. 49: 75–89

Brix H, Sorrell BK & Orr PT (1992) Internal pressurisation and convective gas flow in some emergent freshwater macrophytes. Limnol. Oceanogr. 37: 1420–1433

Brix H, Sorrell BK & Schierup H (1996) Gas fluxes achieved by in situ convective flow in *Phragmites australis*. Aquat. Bot. 54: 151–163

Chanton JP & Dacey JWH (1991) Effects of vegetation on methane flux, reservoirs, and carbon isotopic composition. In: Sharkey T, Holland E & Mooney H (Eds) Trace Gas Emissions by Plants (pp 65–92). Academic Press, San Diego

Chanton JP & Whiting GJ (1996) Methane stable isotopic distributions as indicators of gas transport mechanisms in emergent aquatic plants. Aquat. Bot. 54: 227–236

Chanton JP, Whiting GJ, Showres WJ & Crill PM (1992) Methane flux from *Peltandra virginica*: Stable isotope tracing and chamber effects. Global Biogeochem. Cycles 6: 15–31

Chanton JP, Whiting GJ, Happell JD & Gerard G (1993) Contrasting rates and diurnal patterns of methane emission from emergent macrophytes. Aquat. Bot. 46: 111–128

Chanton JP, Bauer JE, Glaser PA, et al. (1995) Radiocarbon evidence for the substrates supporting methane formation within northern Minnesota peatlands. Geochim. Cosmochim. Ac. 59: 3663–3668

Chanton JP, Whiting GJ, Blair NE, Lindau CW & Bollich PK (1997) Methane emission from rice: Stable isotopes, diurnal variations, and CO_2 exchange. Global Biogeochem. Cycles 11: 15–27

Conrad R (1989) Control of methane production in terrestrial ecosystems. In: Andrea MO & Schimmel DS (Eds) Exchange of Trace Gases Between Terrestrial Ecosystems and the Atmosphere (pp 39–58). John Wiley & Sons, New York

Dacey JWH & Klug MJ (1979) Methane emission from lake sediment through water lelies. Science 203: 1253–1255

Denier van der Gon HAC & Breemen van N (1993) Diffusion-controlled transport of methane from soil to atmosphere as mediated by rice plants. Biogeochemistry 21: 177–190

Denier van der Gon HAC & Neue HU (1996) Oxidation of methane in the rhizosphere of rice plants. Biol. Fertil. Soils 22: 359–366

Fechner-Levy EJ & Hemond HF (1996) Trapped methane volume and potential effects on methane ebullition in a northern peatland. Limnol. & Oceanogr. 41(7): 1375–1383

Frenzel P, Rothfuss F & Conrad R (1992) Oxygen profiles and methane turnover in a flooded rice microcosm. Biol. Fertil. Soils 14: 84–89

Frye JP, Mills AL & Odum WE (1994) Methane flux in *Peltandra virginica* (Araceae) wetlands – Comparison of field data with a mathematical model. American Journal of Botany 81: 407–413

Gerard G & Chanton J (1993) Quantification of methane oxidation in the rhizosphere of emergent aquatic macrophytes – Defining upper limits. Biogeochemistry 23: 79–97

Grosse W, Buchel HB & Tiebel H (1991) Pressurised ventilation in wetland plants. Aquat. Bot. 39: 89–98

Harden HS & Chanton JP (1994) Locus of Methane Release and Mass-Dependent Fractionation from two Wetland Macrophytes. Limnol. Oceanogr. 39: 148–154

Hirschfelder JO, Curtiss CF & Bird RB (1964) Transport phenomena of dilute gases. In: Molecular Theory of Gases and Liquids (pp 514–543). John Wiley & sons, New York

Knapp AK & Yavitt JB (1992) Evaluation of a closed-chamber method for estimating methane emissions from aquatic plants. Tellus 44B: 63–71

Koncalová H, Pokorný J & Kvét, J (1988) Root ventilation in *Carex gracilis* curt.: Diffusion or mass flow? Aquat. Bot. 30: 149–155

Leffelaar PA (1987) Dynamic simulation of multinary diffusion problems related to soils. Soil Science 143: 43–55

Mevi-Schutz J & Grosse W (1988) A two-way gas transport system in *Nelumbo nucifera*. Plant Cell and Environment 11: 27–35

Mikkela C, Sundh I, Svensson BH & Nilsson M (1995) Diurnal variation in methane emission in relation to the water table, soil temperature, climate and vegetation cover in a Swedish acid mire. Biogeochemistry 28: 93–114

Minoda T & Kimura M (1994) Contribution of photosynthesised carbon to the methane emitted from paddy fields. Geophys. Res. Lett. 21: 2007–2010

Morrissey LA, Zobel DB & Livingston GP (1993) Significance of stomatal control on methane release from *Carex*-dominated wetlands. Chemosphere 26: 339–355

Nouchi I, Mariko S & Aoki K (1990) Mechanism of methane transport from the rhizosphere to the atmosphere through rice plants. Plant Physiology 94: 59–66

Schütz H, Holzapfel-Pschorn A, Conrad R, Rennenberg H & Seiler W (1989a) A 3-year continuous record on the influence of daytime, season, and fertiliser treatment on methane emission rates from an Italian rice paddy. J. Geophys. Res. 94: 16,405–16,416

Schütz H, Seiler W & Conrad R (1989b) Processes involved in formation and emission of methane in rice paddies. Biogeochemistry 7: 33–53

Schütz H, Schröder P & Rennenberg H (1991) Role of plants in regulating the methane flux to the atmosphere. In: Sharkey T, Holland E & Mooney H (Eds) Trace Gas Emissions by Plants (pp 29–63). Academic Press, San Diego

Sebacher D, Harriss RC & Bartlett KB (1985) Methane emissions to the atmosphere through aquatic plants. J. Environ. Qual. 14: 40–46

Seiler WA, Holzapfel-Pschorn A, Conrad R & Scharffe D (1984) Methane emission from rice paddies. J. Atmos. Chem. 1: 241–268

Shannon RD, White JR, Lawson JE & Gilmour BS (1996) Methane efflux from emergent vegetation in peatlands. J. Ecol. 84: 239–246

Sorrell BK & Boon PI (1994) Convective gas flow in *Eleocharis sphacelata* R. Br.: methane transport and release from wetlands. Aquat. Bot. 47: 197–212

van der Nat FJWA & Middelburg JJ (1998) Seasonal variation of methane oxidation in the rhizosphere of *Phragmites australis* and *Scirpus lacustris*: A mesocosm study Aquatic Botany (submitted)

Whiting GJ & Chanton JP (1993) Primary production control of methane emission from wetlands. Nature 364: 794–795

Whiting GJ & Chanton JP (1996) Control of the diurnal pattern of methane emission from emergent aquatic macrophytes by gas transport mechanism. Aquat. Bot. 54: 237–253