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Effects of two common macrophytes on methane dynamics in freshwater sediments

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Abstract. The methane cycle in constructed wetlands without plants and with *Phragmites australis* (reed) and *Scirpus lacustris* (bulrush) was investigated. Variations in CH_4 production largely determined variations in CH_4 emission among the systems, rather than variations in CH_4 storage and oxidation. Twofold lower CH_4 production rates in the *Scirpus* system (5.6–13 mmol m^{-2} d^{-1}) relative to the control (16.7–17.6 mmol m^{-2} d^{-1}) were accompanied by a lower contribution of methanogenesis to organic carbon metabolism (∼20% for *Scirpus* vs. ∼80% for control). Sedimentary iron(II) reservoirs were smaller in the *Scirpus* than control sediment (∼300 vs. ∼485 mmol.m^{-2}) and a shuttle role for iron as an intermediate between root O_2 release and carbon oxidation, attenuating the availability of substrate for methanogens, is suggested. Differences in CH_4 production among the *Phragmites* and *Scirpus* systems were controlled by the interspecific variation in sediment oxidation capacities of both plant species. Comparatively, in the *Phragmites* sediment, dissolved iron reservoirs were larger (∼340 mmol.m^{-2}) and methanogenesis was a more important pathway (∼80%). Methane transport was mainly plant mediated in the *Phragmites* and *Scirpus* systems, but ebullition dominated in the non-vegetated control systems as well as in the vegetated systems when plant biomass was low.

Introduction

Methane (CH_4) is an important greenhouse gas, and freshwater wetlands contribute significantly to the atmospheric CH_4 budget (Cicerone & Oremland 1988; Matthews & Fung 1987; Aselmann & Crutzen 1989). Rates of CH_4 emission to the atmosphere are determined by the interplay of production, oxidation and transport processes. Vascular wetland plants affect all three processes by (i) allocation of below-ground labile organic material through root exudation and plant litter production, supporting methanogenesis (Whiting & Chanton 1993; Minoda & Kimura 1994), (ii) input of

atmospheric O_2 to the rhizosphere fuelling CH_4 oxidation (de Bont et al. 1978; King 1994), and (iii) by acting as a conduit allowing CH_4 to 'escape' out of the system (Sebacher et al. 1985; Chanton & Dacey 1991; Whiting & Chanton 1992).

The effect of macrophytes on methane cycling is consequently complex. The oxygen released is not only used for CH_4 oxidation, but also in the oxidation of other reduced components (Fe(II), Mn(II), NH_4, H_2S). Oxidation of dissolved Fe(II) and Mn(II) will result in lower pore-water concentrations of manganese and iron (Burdige 1993) and higher solid phase concentrations of their metal oxides (Roden and Wetzel 1996; Sundby et al. 1997). These Fe(III) and Mn(IV) oxides have been found in relatively large quantities on the root surface of macrophythes and in the adhering sediment (Armstrong 1967; Green & Etherington 1977; Sundby et al. 1997). Methanogens are usually poor competitors with other heterotrophic micro-organisms for limiting amounts of substrate, so that CH_4 production normally commences after reservoirs of alternative electronacceptors are depleted (Achtnich & Rude 1988; Capone & Kiene 1988). As a consequence, activity of heterotrophic microorganisms other than methanogens may suppress CH_4 production rates in substrate limiting environments such as salt and brackish systems with high abundance of sulfate (DeLaune et al. 1983; Lovley & Klug 1986; Bartlett et al. 1987; Middelburg et al. 1996) or Fe(III)-rich freshwater systems (Lovley & Phillips 1986; Burdige 1993; Boon & Mitchell 1995). Recently, Roden and Wetzel (1996) demonstrated the significant suppression of CH_4 production in a vegetated freshwater wetland sediment by microbial Fe(III) reduction compared to a non-plant control sediment.

Because of direct and indirect effects of macrophytes on sediment biogeochemistry and the tight coupling of these processes in the rhizosphere, it is difficult to unravel and quantify these processes in the field. For instance, below-ground allocation of organic matter can stimulate methanogenesis directly by serving as methanogenic substrate or indirectly by improving the suitability of methanogenic circumstances through depletion of the alternative electronacceptors reservoirs or lowering the redox-potential. Furthermore, rhizosphere oxidation may be due to direct O_2 transport by macrophytes, or to the indirect effect of evapotranspiration that result in fluid advection and transport of oxidant rich surface water deeper into the sediment or water-desaturation and air entry (Chanton & Dacey 1991).

In this study we report on CH_4 emission by diffusion, plants and ebullition and sedimentary CH_4 reservoirs in *Phragmites australis*, *Scirpus lacustris* and non-plant (initially) identical sediments. Our experimental set-up made it possible to control potentially important factors in the CH_4 cycle such as temperature (Dunfield et al. 1993), light conditions (King 1990; Whiting et

al. 1991) and water level (Moore & Roulet 1993), and to exclude horizontal transport or drainage of pore-water CH_4 (Kelley et al. 1995). Hence, any temporal variability in plant physiology or plant species effect induce variation in methane dynamics.

These data are combined with reported surface and rhizospheric oxidation rates based on methylfluoride and anoxic/oxic flux chamber inhibition techniques (van der Nat & Middelburg 1998). This allowed us to establish a detailed CH_4 balance that shows that differences in CH_4 storage and oxidation between the vegetated and non-plant systems were rather limited and that CH_4 production is the key factor controlling the emission of methane to ambient air.

Material and methods

Experimental set-up

A detailed description of the set-up is given elsewhere (van der Nat & Middelburg 1998). Briefly, sediment collected at tidal freshwater *Phragmites* and *Scirpus* marsh 'Burcht' was mixed with drainage channel soil and transferred to six identical, fully separated, 1.3 m^3 containers (l×w×h = 1.25×1.25×0.85 m), placed in a temperature (18±0.5 °C), humidity (70±7%) and CO_2 concentration (380±40 ppmv) controlled room. An *in situ* dialysis sampling device (Hesslein 1976) was placed in the centre of each container. The sediments were kept under a water layer of 3 to 4 cm by adding deionized water to compensate water loss. After a 5 month period of settling and accommodation *Phragmites* and *Scirpus* seedlings were planted in two containers each, whilst two containers were left unplanted. Polypropylene collars (∅ = 24 cm) were installed to ascertain consistent placement of the gas collecting chambers and minimise disturbance during successive gas flux measurements. A 16 hours day period with a light intensity of 0.25 $mE.m^{-2}.s^{-1}$ at the sediment surface was set. The non-plant control sediments were covered with floating grains to mimic plant shading and to limit microphytobenthos growth.

The experimental systems have been maintained for more than 3 years including five successive plant growth cycles each lasting about 6 months ('seasons') that were separated by 3 to 4 week periods of darkness. At the end of plant growth cycles dead leave material was removed from the sediment surface. Before each plant growth cycle commercial fertiliser (Barenbrug garden products) was added to the surface-water. Steady-state conditions were present in our experiments as may be inferred from the similarity of carbon dioxide uptake rates, CH_4 fluxes and plant biomass patterns during

growth cycles 2 (van der Nat et al. 1998), 3 (van der Nat & Middelburg 1998) and 4 (this study) and the stability of the dissolved CH_4 reservoirs in the non-plant control system.

Pore- and surface-water

Pore-water samples (10 to 20 ml) were obtained using an *in situ* dialysis sampling device (so-called peeper). One peeper (depth$\times$width = 60$\times$22 cm) contained 25 membrane cells with slits parallel to the sediment-water interface, covered with 0.2 mm biologically inert acrylic co-polymer membrane filter (versapor-200, Gelman Sciences). The upper ten cells (10 ml; width of slit 0.5 cm) covered the first 10 cm of the sediment starting from the sediment surface. The next twelve (20 ml; width of slit 1 cm) covered the next 10 cm to 40 cm and the last three (40 ml; width of slit 2 cm) covered the 40 cm to 55 cm part. At both ends, the cells were connected with tygon tubes to sampling ports at the sediment surface. Pore-water samples were withdrawn with a syringe at one sampling port while nitrogen gas was introduced into the other port. De-oxygenated deionized water was used to refill the compartments after sampling. Pore-water sampling was done with minimal intervals of 6 weeks to allow steady-state conditions between pore and peeper water. Surface-water samples (20 ml) were withdrawn by gas tight syringe directly from the surface-water.

Water samples were divided into two equal portions. One 5 to 10 ml portion went into a 10 or 20 ml glass vial (Chrompack) sealed with a septum crimp-cap, and immediately was acidified by adding 100 to 200 μl 20% H_2SO_4. The other 5 to 10 ml portion went into a 20 ml polypropylene vial and was frozen. The acidified samples were analysed for CH_4 and CO_2 in the headspace using the phase equilibrium technique (McAullife 1971). Gases were analysed with a Carlo Erba high resolution MEGA 5340 gas chromatograph, equipped with a flame ionisation detector. The headspace gas concentrations were recalculated to pore-water concentrations using equations given by Wiesenburg and Guinasso (1979). Earlier experiments showed that addition of H_2SO_4 did not affect CH_4 concentrations, significantly. Acidified samples were analysed for Fe(II) using a graphite furnace atomic absorption spectrometer with Zeeman background correction (Perkin Elmer 3030). Non-acidified samples were analysed for NH_4^+, NO_2^-, NO_3^- and SO_4^{2-} with colorimetric auto analyser techniques.

Sedimentary pool sizes (reservoirs) were calculated from the profiles of pore-water CH_4 and Fe(II) concentration, after correction for sediment porosity. Sediment porosities were determined after the experiment ended using sediment fresh/dry weight ratios and a sediment density of 2.55 g.ml^{-1}. Sediment porosities in the control systems ranged from 0.54$\pm$0.02 at the

surface to 0.46±0.01 at depth. Porosities in the vegetated systems ranged from 0.60±0.02 at the surface to 0.48±0.03 at depth.

Gas fluxes

Fluxes of CH_4 and CO_2 were measured with closed chambers. The CH_4 and CO_2 concentrations in the chamber were measured by circulating chamber air through Teflon tubes between chamber and a multi-gas monitor (type 1302, Brüel & Kjaer, Nærum, Denmark). Gas concentrations were immediately known, providing us with the opportunity to maintain CO_2 levels within the range of ambient values during periods of CO_2 uptake by injecting small amounts of pure CO_2 into the chamber. Care was taken to maintain chamber conditions (temperature and humidity) close to those outside, so that gas fluxes were not affected (Knapp & Yavitt 1992). Between each flux measurements the chamber was vented for 15 minutes by flushing with compressed atmospheric air using the in- and outlet adapters and leaving the chambers in place to minimise disturbance. A detailed description of the techniques used is given elsewhere (van der Nat & Middelburg 1998). The amount of CH_4 transported through ebullition was distinguishable from the total CH_4 flux because bubble events caused a discrete shift in the gas monitor output (Middelburg et al. 1996). The diffusive CH_4 flux in the vegetated systems was determined from surface-water CH_4 concentrations using calculation procedures and constants given by Sebacher et al. (1983) and Jähne et al. (1987). CH_4 concentrations in the surface-water were determined by the headspace equilibration technique (McAullife 1971). The diffusion and bubble fluxes were substracted from total CH_4 flux values to obtain the plant mediated flux. CH_4 emission in the vegetated systems was measured in triplicate. The CH_4 emission rates in the non-plant system are based on long-term measurements, with the first 24 hours of the measurement excluded (Figure 1).

CH_4 oxidation

In situ rates of rhizospheric methane oxidation have been quantified using the methylfluoride inhibition and anoxic/oxic flux chamber techniques (Epp & Chanton 1994; van der Nat & Middelburg 1998). Methylfluoride specifically inhibits methane oxidation without simultaneously blocking methanogenesis, and plants are able to transport the gaseous inhibitor, like atmospheric oxygen, from the atmosphere to the rhizosphere. Methane oxidation is the difference in methane fluxes before and after methylfluoride treatment. Surface oxidation rates were determined from surface-water CH_4 concentrations before and after loading the system with CH_3F. A detailed description of methylfluoride inhibition and anoxic/oxic flux chamber techniques used and

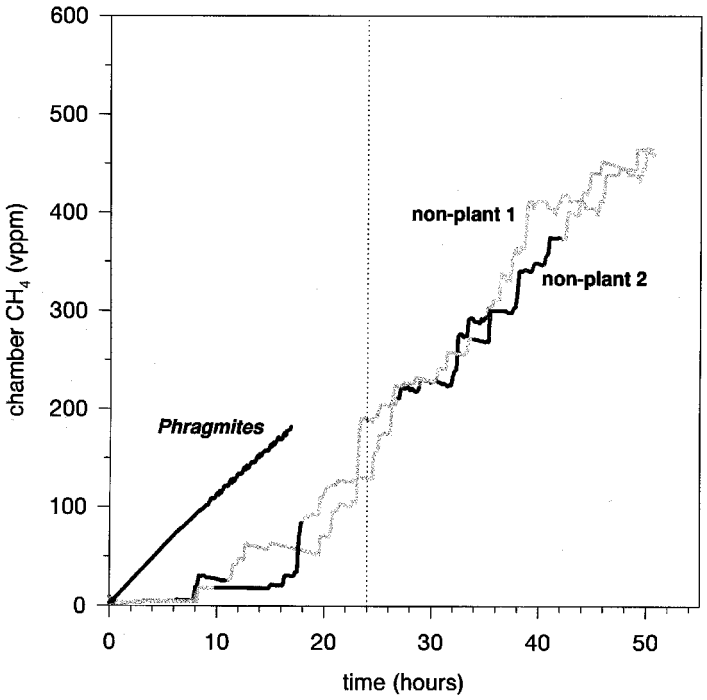

Figure 1. Example of long-term chamber emission measurements. The light regime during the non-plant flux measurement is indicated by grey (light) and black (dark) line parts. The light intensity at the sediment surface of the non-plant system was ~0.12 mE.m^{-2}.s^{-1}. The vertical dotted line indicates the starting point for calculation of the CH_4 flux in the non-plant systems.

results are given elsewhere (van der Nat & Middelburg 1998). Taking into account the similarity in plant growth during the successive cycles, the presence of 'steady-state' conditions and the identical environmental conditions we feel confident about applying the previously measured oxidation rates in the mass balances of this study.

Anaerobic incubations

Sediment cores were collected at day 268 using relatively small acrylic tubes (25 cm long, 3 cm internal ∅) to minimise destruction of the experimental set-up. Slices of equal depth of three cores were thoroughly mixed, put into glass vials, diluted with sterilised water and sealed with screw caps provided with rubber septa. Amendments were given in the form of acetate (final concentration 2.5 mM) and H_2 (7.5% headspace). A detailed description of the method and some control experiments are given elsewhere (van der Nat et al. 1997). Increase of CH_4 and CO_2 in the incubation flask headspace was monitored

by daily analyses of 50 μl headspace samples. This procedure for measuring CH_4 production was chosen mainly by the lack of an reasonable alternative (Yavitt et al. 1988). Unfortunately, the procedure may not yield accurate *in situ* rates due to the coring technique and incubation circumstances such as shaking and exclusion of atmospheric O_2 input (Kelley et al. 1995). Therefore production rates are considered potential rates. Potential CH_4 production in all incubation flasks started without a lag-phase. Rates are calculated for the period when the increase in headspace CH_4 and CO_2 concentration was linear ($R^2 > 0.95$), i.e. the first 3 days of the incubation period.

Biomass

Above-ground biomass could be estimated in a non-destructive way using empirical equations formulated during plant growth cycle 2. Sediment was collected after the experiment had ended using acrylic tubes (55 cm long, 7 cm i.d.). Three cores were sliced and washed in order to obtain below-ground biomass as roots and rhizome material.

Results

Plant growth

Plant growth parameters and associated CO_2 fluxes are shown in Table 1. Variation in the CO_2 fluxes followed the different stages in the plant growth cycle, except for the duplicate *Phragmites* system (*P*2). Other plant dependent processes such as CH_4 emission (Table 2) also deviated significantly from the patterns observed in the other three vegetated systems and during previous growth cycles. Therefore, *Phragmites* 2 is left out for further discussion.

Evapotranspiration rates were the highest in the *Phragmites* system and lowest in the non-plant systems (Table 1). The amount of dry weight root material was higher in the *Phragmites* than in the *Scirpus* systems (Figure 2). Rhizome material was found only in the *Phragmites* system, especially below a depth of 30 cm. The total dry weight of the rhizome material was $\sim$10 times that of total root dry weight. The depth distribution of roots was mirrored by pore-water ammonium profiles. In the non-plant system ammonium concentrations were much higher and increased rapidly with depth (Figure 2).

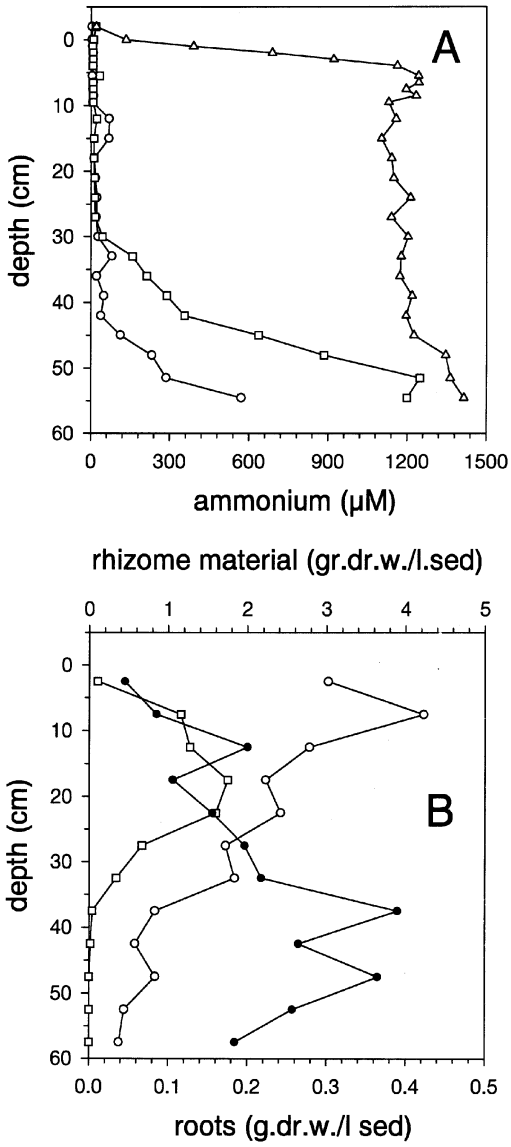

Figure 2. (A) Ammonium concentration versus depth profiles at day 158 (mid-season conditions). (B) Below-ground root biomass (open symbols) and rhizome biomass (solid symbols). The bulrush, reed and control systems are represented by squares, circles and triangles, respectively.

Table 1. Plant growth.

System	Biomass ($g.dr.w.m^{-2}$)	Stems ($nr.m^{-2}$)	CO_2 flux ($mmol.m^{-2}.d^{-1}$)	Evapotr. $l.m^{-2}.seas.^{-1}$
Phragmites 1				
Day 55	194	113	−183	945
Day 158	384	142	−2030	
Day 268	44	26	37.3	
Phragmites 2				
Day 55	226	133	−639	792
Day 158	183	112	−443	
Day 268	24	17	35.5	
Scirpus 1				
Day 55	278	359	−243	474
Day 158	620	535	−1373	
Day 268	25	51	36.0	
Scirpus 2				
Day 55	326	410	−695	390
Day 158	740	741	−2016	
Day 268	37	41	40.4	
Non-plant 1				
Day 55			25.3	165
Day 268			23.1	
Non-plant 2				
Day 55			22.5	160
Day 268			21.9	

CO_2 fluxes were measured under light conditions ($\sim$0.5 $mE.m^{-2}.s^{-1}$, 40 cm below top canopy).

Methane fluxes

The concentration of CH_4 in chambers enclosing vegetated systems increased with no significant sign of saturation even after 18 hours, indicating that CH_4 flux was not affected by chamber conditions (Figure 1), consistent with observations by Chanton et al. (1993). Chamber deployment in the non-plant systems was immediately followed by increased ebullition. This increased ebullition terminated within two hours. Following flushing of the chamber methane concentrations increased only slightly during the first 8 hours, probably due to depletion of sedimentary CH_4 reservoirs. Hereafter, chamber CH_4 concentrations increased by ebullition and diffusion driven methane efflux (Figure 1).

Table 2. Plant CH_4 transport (mmol.m^{-2}.d^{-1} $\pm$ 1 standard error).

System	Light	Dark
Phragmites 1		
Day 55	11.2 $\pm$ 0.62	7.32 $\pm$ 0.66
Day 158	19.4 $\pm$ 1.22	13.3 $\pm$ 1.50
Day 268	3.38 $\pm$ 0.74	3.32 $\pm$ 0.80
Phragmites 2		
Day 55	13.9 $\pm$ 1.35	8.1 $\pm$ 0.89
Day 158	6.5 $\pm$ 1.10	4.4 $\pm$ 0.78
Day 268	1.1 $\pm$ 0.12	1.5 $\pm$ 0.06
Scirpus 1		
Day 55	3.99 $\pm$ 0.29	3.68 $\pm$ 0.72
Day 158	6.09 $\pm$ 0.56	5.82 $\pm$ 0.92
Day 268	1.80 $\pm$ 1.07	0.42 $\pm$ 0.24
Scirpus 2		
Day 55	6.71 $\pm$ 1.08	6.08 $\pm$ 1.23
Day 158	8.21 $\pm$ 0.41	6.96 $\pm$ 0.47
Day 268	2.67 $\pm$ 0.57	2.27 $\pm$ 0.10

Methane efflux was greater in the light than in the dark for the *Phragmites* system, but only during the first and second part of the study period (Table 2). Light versus dark conditions had no significant effect on methane efflux in the *Scirpus* system. An overview of the CH_4 fluxes subdivided into diffusion, ebullition and plant-transport is given in Table 3. Ebullition was the dominant mechanism for CH_4 transport in the non-plant systems. CH_4 fluxes in the *Scirpus* and *Phragmites* systems were dominantly plant-mediated, but ebullition was significant in the *Phragmites* system before and after the growing season. Methane fluxes in the *Phragmites* and non-plant systems were rather similar and higher than those in the *Scirpus* system. CH_4 uptake from the chamber was observed in the non-plant systems in between bubble events (Figure 1).

Sedimentary methane

Dissolved CH_4 depth profiles are shown in Figure 3. Maximum concentrations in the non-plant systems were higher ($\sim$900 μm) than in the vegetated systems, and were already reached at much lower depths ($<$5 cm). Methane reservoirs were much larger in the systems with no plants than with plants

Table 3. Fe(II) and CH_4 reservoirs and the fate of CH_4.

System	Reservoir ($mmol.m^{-2}$)		Oxidations[#] ($mmol.m^{-2}.d^{-1}$)		Flux ($mmol.m^{-2}.d^{-1}$)		
	Fe(II)	CH_4	Rhizosph.	Surface	Plant[*]	Diffusion[#]	Ebullition
Phragmites 1							
Day 55	365	123	3.0 ± 0.13	0.30 ± 0.04	9.9 ± 0.63	0.41 ± 0.27	0.93 ± 1.70
Day 158	313	86	2.8 ± 0.44	0.37 ± 0.04	17.3 ± 1.31	0.43 ± 0.32	0
Day 268	349	150	0.14 ± 0.10	0.45 ± 0.03	3.4 ± 0.76	0.62 ± 0.29	5.9 ± 2.84
Phragmites 2							
Day 55	352	128			12.0 ± 1.20		2.87 ± 5.21
Day 158	252	66			5.8 ± 0.99		0
Day 268	343	147			1.2 ± 0.10		2.09 ± 1.89
Scirpus 1							
Day 55	322	140	4.4 ± 0.15	0.15 ± 0.02	3.9 ± 0.43	0.14 ± 0.18	0.11 ± 0.27
Day 158	253	111	3.3 ± 0.31	0.24 ± 0.03	6.0 ± 0.68	0.17 ± 0.12	0
Day 268	307	158	0.10 ± 0.06	0.66 ± 0.07	1.3 ± 0.79	0.59 ± 0.21	0
Scirpus 2							
Day 55	329	141			6.5 ± 1.13		0
Day 158	264	112			7.8 ± 0.43		0
Day 268	338	134			2.5 ± 0.41		0
Non-plant 1							
Day 55	483	229		3.0 ± 0.13	0	$0.69^{\S} \pm 0.33$	
Day 268	492	226		1.8 ± 0.07	0	$0.47^{\S} \pm 0.18$	13.8 ± 0.27
Non-plant 2							
Day 55	483	221			0		
Day 268	492	224			0		14.6 ± 0.20

$\pm$ represents the combined error calculated using standard error propagation techniques.
[#] Oxidation and diffusion were determined for the #1 systems only. Relative oxidation rates and diffusion in the #2 systems were assumed to be identical.
[*] The light/dark variation of the plant-mediated flux was incorporated in the integration procedures using the light regime set for the climate cell.
[§] The averaged diffusive flux calculated from surface-water CH_4 concentrations in the non-plant systems was 0.41 ± 0.22 $mmol.m^{-2}.d^{-1}$.

(Table 3). The CH_4 profiles in the non-plant systems showed no noticeable variation with sampling day, whereas profiles in the vegetated systems differed. Methane concentrations in the rhizosphere were lowest at day 158 when plants were mature.

In vitro methane production

In general, potential CH_4 production rates increased with depth and were lower for *Scirpus* sediment than control and *Phragmites* sediment (Figure 4). The CH_4 to CO_2 production ratio was nearly 1:1 for the control and *Phrag-*

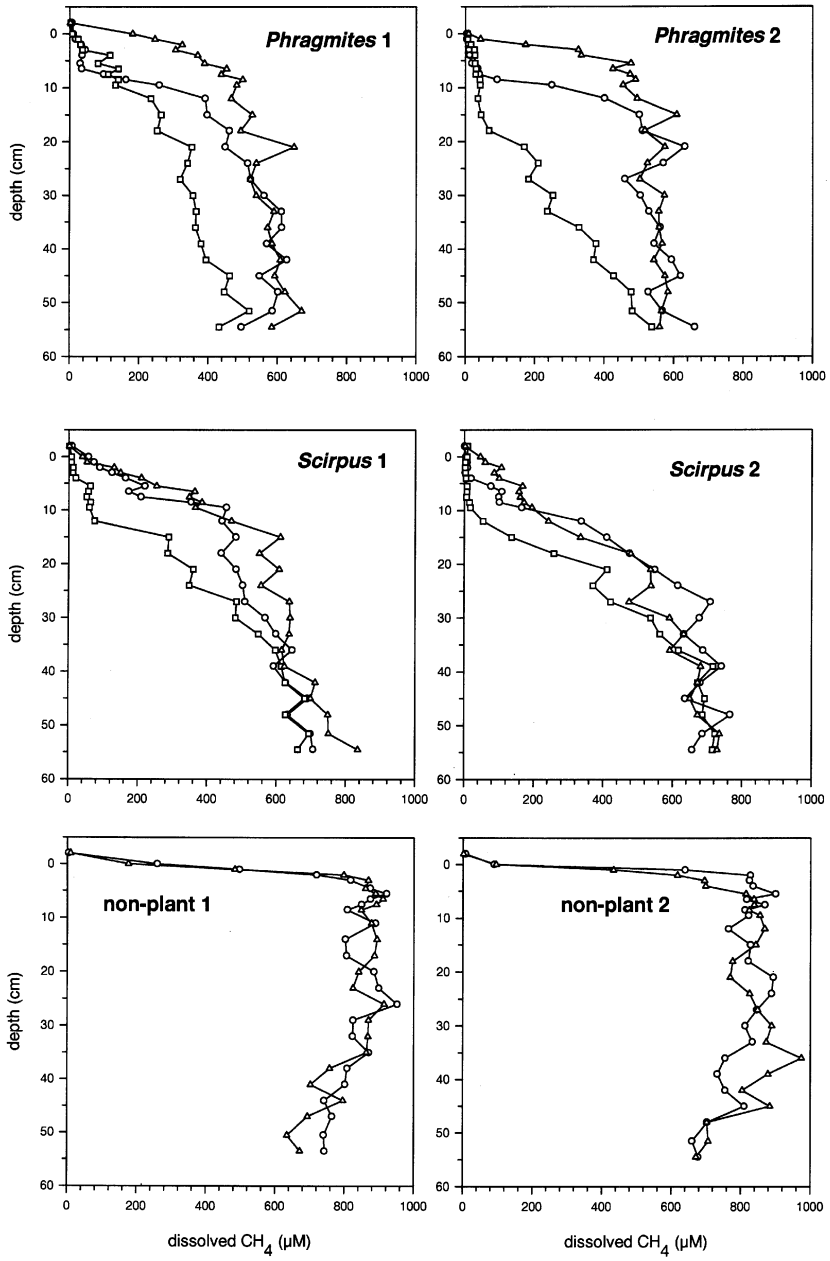

Figure 3. Depth distribution of dissolved CH_4 at day 55 (circles), day 118 (squares) and day 268 (triangles).

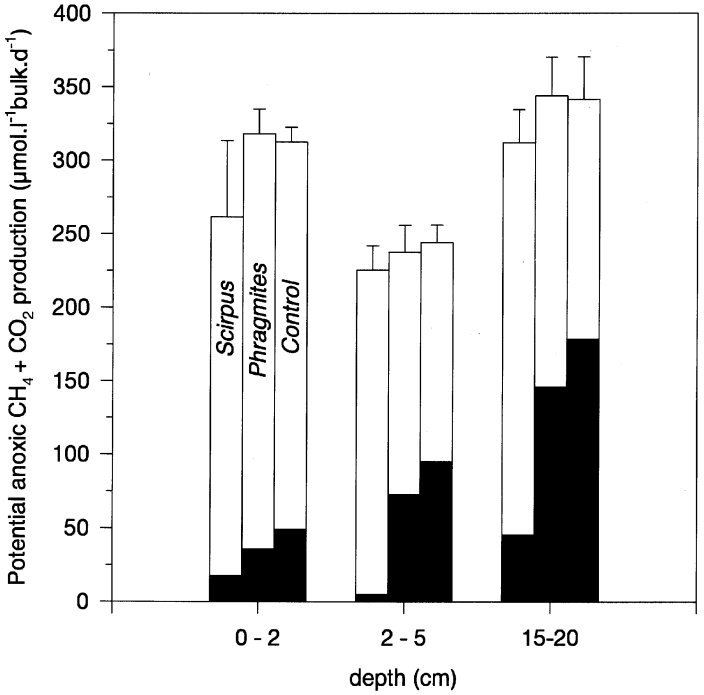

Figure 4. In vitro anoxic CH_4 (black) and CO_2 (white) production with sediment from the *Scirpus*, *Phragmites* and non-plant systems. The error bars represent the combined error of CH_4 and CO_2 production.

mites systems, except at depth interval 0–2 cm. A 1:1 ratio of CH_4 production to CO_2 production is typical for reduced sediment in which no 'free' oxidants are present and methanogenesis is the dominant metabolic process. The 0–2 cm depth interval is likely to contain some oxidants since dissolved oxidants (O_2, NO_3^-) may have diffused from the overlying water. Methanogenesis was in particular stimulated by H_2/acetate addition to slurries with sediment from the *Scirpus* system (Table 4). Stimulation in the *Phragmites* and non-plant slurries were approximately similar. Increments of CO_2 production after addition of H_2/acetate were relative small compared to the increments of CH_4 production.

Methane production

There is no definitive method for obtaining *in situ* methane production rates in vegetated sediments (Yavitt et al. 1988; Kelley et al. 1995). However, production rates may be estimated by difference using the following equation:

$$\text{avg. prod. rate} = \text{avg. flux} + \Delta \text{ reservoir} + \text{avg. oxidation rate} \qquad (1)$$

Table 4. H_2+acetate amended/unamended ratios.

Depth (cm)	*Phragmites*		*Scirpus*		Non-plant	
	CH_4	CO_2	CH_4	CO_2	CH_4	CO_2
0–2	2.05 ± 0.31	1.10 ± 0.15	6.36 ± 1.92	1.01 ± 0.28	2.06 ± 0.62	0.80 ± 0.13
2–5	2.44 ± 0.48	1.34 ± 0.12	8.58 ± 3.52	1.13 ± 0.15	2.27 ± 0.31	1.23 ± 0.13
15–20	2.69 ± 0.72	1.20 ± 0.18	3.24 ± 1.02	1.12 ± 0.16	2.34 ± 0.28	1.38 ± 0.31

$\pm$ represents the combined error calculated using standard error propagation techniques.

in which the average production, flux and oxidation rates during the time between two sampling dates and the change in reservoir size between sampling dates are balanced (Table 5). For the mid-season period of the plant growth cycle (day 55-158), *in situ* production rates were not significantly different between the non-plant and *Phragmites* systems, whereas rates were significantly lower in *Scirpus* systems. Production rates in the control system did not show any temporal variability, in contrast to production in the vegetated systems. The vegetated systems exhibited highest CH_4 production rates when plants were mature (day 158).

Anaerobic metabolism

In situ (dark) CO_2 fluxes in the plant systems during plant growth and after maturation (day 55 and 158, respectively) can not be used in a simple way to estimate mineralisation because of shoot and root respiration associated CO_2 release. In addition, the dark CO_2 fluxes in the plant systems after the growing season (day 268) might still comprise a non-mineralisation contribution since some small newly formed plant biomass of the next plant growth cycle already emerged above the flood-water layer (Table 1). Regrowth of plants was also confirmed by the small but manifest difference between light (35–40 $mmol.m^{-2}.d^{-1}$; Table 1) and dark CO_2 fluxes (73–81 $mmol.m^{-2}.d^{-1}$) at day 268. Nevertheless, the dark CO_2 fluxes in the plant systems measured at day 268 are useful to provide an upper estimate of mineralisation. Dark CO_2 fluxes in the non-plant control sediments (22–25 $mmol.m^{-2}.d^{-1}$) are useful to provide a lower estimate of mineralisation because any mineralisation of plant litter is excluded. Irrespective of the CO_2 flux used, the contribution of methanogenesis to gross carbon mineralisation [CH_4 + CO_2 flux] was lower in the *Scirpus* system than in the *Phragmites* and non-plant system (Table 6). This pattern of lower methanogenic carbon fluxes in the *Scirpus* than in the other two systems was also found *in vitro* (Table 6). The increase of the relative importance of methanogenesis with depth, especially in the control

Table 5. Methane balance (all terms in mmol.m^{-2}.d^{-1}).

System	Δ reservoir	Avg. oxidation	Avg. flux	Avg. prod.
Phragmites 1				
Day 268-55	−0.18	1.94 ± 0.07	10.5 ± 1.73	12.3 ± 1.74
Day 55-158	−0.36	3.24 ± 0.23	14.5 ± 1.14	17.4 ± 1.16
Day 158-268	0.58	1.89 ± 0.23	13.8 ± 1.62	16.3 ± 1.64
Phragmites 2				
Day 268-55	−0.12	2.20 ± 0.08	9.58 ± 2.84	11.66 ± 2.84
Day 55-158	−0.60	2.61 ± 0.11	10.7 ± 2.72	12.74 ± 2.73
Day 158-268	0.73	0.91 ± 0.08	5.09 ± 1.09	6.73 ± 1.05
Scirpus 1				
Day 268-55	−0.12	2.67 ± 0.09	3.03 ± 0.49	5.59 ± 0.50
Day 55-158	−0.29	4.05 ± 0.17	5.15 ± 0.44	8.91 ± 0.47
Day 158-268	0.43	2.14 ± 0.16	4.05 ± 0.53	6.61 ± 0.56
Scirpus 2				
Day 268-55	0.04	4.20 ± 0.14	4.88 ± 0.62	9.12 ± 0.63
Day 55-158	−0.28	6.03 ± 0.24	7.29 ± 0.61	13.0 ± 0.66
Day 158-268	0.20	2.67 ± 0.21	5.54 ± 0.32	8.41 ± 0.38
Non-plant 1				
Day 268-55	0.02	2.38 ± 0.07	14.3 ± 0.27	16.7 ± 0.28
Day 55-268	−0.01	2.38 ± 0.07	14.3 ± 0.27	16.7 ± 0.28
Non-plant 2				
Day 268-55	−0.02	2.38 ± 0.07	15.2 ± 0.23	17.5 ± 0.24
Day 55-268	0.02	2.38 ± 0.07	15.2 ± 0.23	17.6 ± 0.24

± represents the combined error calculated using standard error propagation techniques.

system, is consistent with the supply of dissolved oxidants from the overlying water.

Dissolved iron

Dissolved Fe(II) depth profiles are shown in Figure 5. Dissolved iron concentrations increased with depth as a consequence of solid-phase Fe(III) reduction. Dissolved iron concentrations in the non-plant system reached an asymptote of ∼2 mM at shallow depth (<10 cm) and did not show variability over time. In the vegetated systems, maxima of dissolved iron were lower (<∼1.5 mM) and reached at depth >30 cm, and there was pronounced temporal variability in the upper 30 cm. Reservoirs of dissolved Fe(II) in the vegetated systems were smaller than those in the non-plant system (Table 3).

Table 6. Contribution of methanogenesis to gross mineralisation (%).

System	*In situ*		*In vitro*		
	Minimal	Maximal	0–2 cm	2–5 cm	15–20 cm
Phragmites	24.1 ± 7.92	61.9 ± 31.1	22.5 ± 1.93	61.2 ± 10.9	85.0 ± 14.9
Scirpus	6.43 ± 2.19	19.3 ± 12.1	13.6 ± 4.97	4.60 ± 1.60	38.2 ± 8.11
non-plant	80.4 ± 4.10	80.4 ± 4.10	31.5 ± 6.25	77.9 ± 7.28	104 ± 18.3

± represents the combined error calculated using standard error propagation techniques.

Pore-water pH profiles were very similar for all systems, also when plants were mature, and ranged between 6.7 and 7.4 with highest levels found near the sediment surface (data not shown).

Discussion

Methane transport

More than 90% of CH_4 transport was plant mediated when *Phragmites* and *Scirpus* plants matured (Table 3). The significant light/dark variation in CH_4 efflux in the *Phragmites* system (Table 2) is typical for emergent plants exploiting diffusive transport during periods of low illumination or darkness and additional (pressurised) convective transport during periods of illumination (Sebacher et al. 1985; van der Nat et al. 1998). *Scirpus* uses the diffusive mechanism irrespective of light condition (van der Nat et al. 1998) and there is consequently no light/dark variation in methane efflux (Table 2). The importance of plants for CH_4 transport is further demonstrated by the alternation between plant transport and ebullition in response to temporal changes in plant growth, especially in the *Phragmites* system. It has been suggested that plant transport and ebullition may be mutually exclusive processes (Holzapfel-Pschorn & Seiler 1986; Schütz et al. 1989a, b).

In the non-plant systems, more than 90% of total methane efflux occurred through bubbles. In general ebullition is found a critical mechanism for methane emission in non-vegetated aquatic environments (Chanton et al. 1989; Keller & Stallard 1994). Bubble fluxes are usually difficult to obtain due to the episodic character of ebullition (Chanton et al. 1989). Nevertheless, in this study we could measure ebullition within 5% accuracy, probably as a result of (i) the stability of important factors for ebullition such as hydrostatic pressure and temperature, (ii) the use of permanently installed collars and (iii) the avoidance of physical disturbance effects by introduction of a lag period before measurement. Removal of gas bubbles from soils covered with

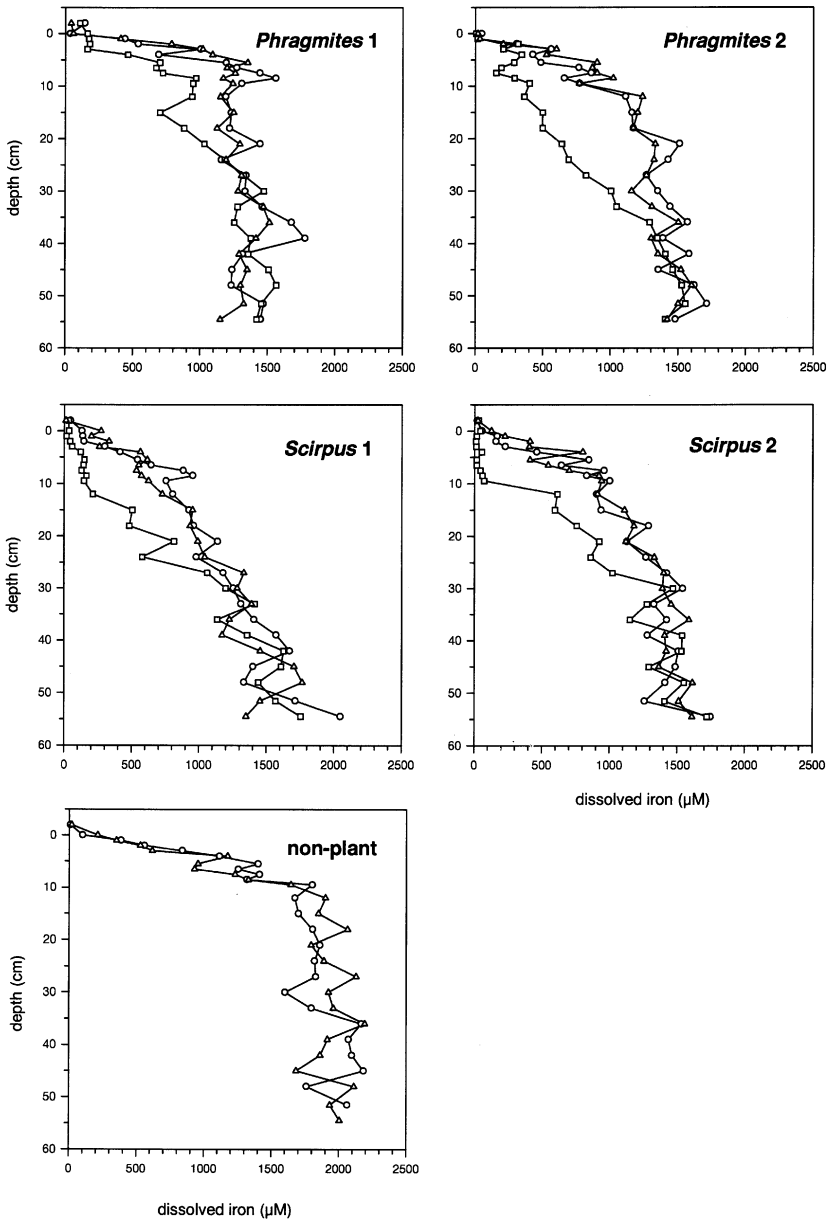

Figure 5. Depth distribution of dissolved Fe(II) at day 55 (circles), day 118 (squares) and day 268 (triangles).

rice resulted in decreased CH_4 flux rates for about 24 hours as well, until the originally observed rates were re-established (Holzapfel-Pschorn et al. 1986). The importance of physical disturbance depends amongst others on bubble reservoir sizes and sediment cohesiveness. No effect of chamber deployment could be noted in the vegetated systems with a more cohesive sediment surface layer (visual observation) and apparently lower bubble reservoirs than the non-plant system.

Diffusion was a relatively insignificant mode of transport in the vegetated and non-plant systems (Table 3). CH_4 fluxes in the non-plant system based on the CH_4 concentration gradient at the sediment surface (Fick's first law modified for sediments, Berner 1980), were much higher (on average 2 $mmol.m^{-2}.d^{-1}$) than those actually measured (0.1–0.7 $mmol.m^{-2}.d^{-1}$; Table 3). This difference between measured and calculated fluxes was probably due to our low depth resolution which may have masked oxidation in the very surface layer.

Rhizosphere oxidation

Oxygen gas circulation in wetland plants is a common adaptation to prevent root anoxia (Armstrong et al. 1994). *Phragmites* and *Scirpus* plants oxidise the sediment as indicated by smaller reservoirs of reduced Fe(II) than non-plant systems. Any O_2 release by the roots of these plants leads to the oxidation of iron with as a result that solid phase Fe(III) oxyhydroxides are formed and dissolved iron decreases. It appears that *Scirpus* has a slightly higher sediment oxidising capacity than *Phragmites*, because dissolved iron is more depleted in the *Scirpus* than *Phragmites* systems (Table 3), in particular in the upper 10 centimetres of the sediment (Figure 5). Moreover, rhizospheric methane oxidation rates in the *Scirpus* system are higher than those in the *Phragmites* system (Table 5; van der Nat & Middelburg 1998). It is also in agreement with the zonation of their habitats, *Scirpus* can tolerate sediments with higher O_2 demand than *Phragmites* and generally grows in deeper water. Differences in oxidation capacity between different plant species have been observed before (Bedford et al. 1991; Wigand 1997; Calhoun & King 1997). These differences may be due to either variation in O_2 transport capacity, or to variation in O_2 consumption by roots and rhizomes. The gas transport capacities of *Phragmites* and *Scirpus* are rather similar on an areal basis (van der Nat & Middelburg 1998), although reed has the capability to exploit diffusive and convective transport mechanisms, whereas diffusive transport dominates in bulrush (van der Nat et al. 1998). Hence, differences in oxygen release are more likely related to differences in root and rhizome respiration than to differences in transport. A significant difference

in root and rhizome respiration between *Phragmites* and *Scirpus* is expected given the large difference in below-ground biomass (Figure 2B).

In vegetated systems the size of sedimentary methane reservoirs is related not only to methane oxidation and production rates, but also to plant ventilation (Chanton & Dacey 1991; Shannon et al. 1996). Dissolved methane inventories of the *Phragmites* systems are lower than those of the *Scirpus* systems (Table 3), despite higher methane production rates and lower oxidation rates in *Phragmites* systems (Table 5). This indicates that plant-mediated methane removal from the *Phragmites* systems is higher than that from *Scirpus* systems, partly because it is proportional to below-ground biomass.

Methane production

Methane production rates based on our detailed and complete mass balance indicate that CH_4 production in the non-plant control system were higher than those in the *Scirpus* system (Table 5). This difference in CH_4 production between the *Scirpus* and non-plant system was also observed in the *in vitro* slurry incubations (Figure 4). Relatively low CH_4 production in the *Scirpus* system was accompanied by a limited importance of methanogenesis as metabolic process in the breakdown of organic matter (Table 6). Substrate availability does not seem likely considering that CH_4 production rates in the *Scirpus* system were low, even with the (potential) extra carbon input due to plant CO_2 fixation. Moreover, potential gross mineralisation rates were fairly similar after the period of active vegetation indicating a similar base-line gross mineralisation for both systems (Figure 4). Other metabolic processes probably competed with methanogens for substrate considering the relatively large increase of CH_4 production in the *Scirpus* slurries after addition of methanogenic substrates (Table 4). It is interesting to note that *Scirpus* slurries (2–5 cm depth) with lowest rates of CH_4 production (Figure 4) exhibited the largest amended/unamended ratios (Table 4).

The correlation between dissolved Fe(II) reservoirs and CH_4 production imply an important role for microbial Fe(III) reduction as the alternative metabolic process. Microbial Fe(III)-oxyhydroxide reduction may account for a considerable fraction of organic carbon oxidation and significantly suppress CH_4 production in the *Scirpus* system, as has been proposed for systems with the freshwater rush *Juncus effusus* (Roden & Wetzel 1996). The iron cycle may act as an intermediate shuttle between O_2 released at the root and sediment surface and carbon mineralisation: O_2 consumption is then coupled to iron oxidation, while carbon oxidation is coupled to iron reduction. Consequently less electron donors are available for methanogens. A similar shuttle role has been proposed for manganese in bioturbated marine sediments by Aller (1994).

Iron (III) reduction rates can be estimated assuming that it is the only alternative process for methanogenesis, a stoichiometry of Fe(III) reduction to CO_2 production of 4:1, and a stoichiometry of CH_4 production to CO_2 production of 1:1. Using the maximal values for the *in situ* methanogenic contribution (Table 6), the remaining non-methanogenic CO_2 flux in the *Scirpus* system would require Fe(III) reduction rates of more than 75 mmol Fe(III).m^{-2}.d^{-1}. For the control system this rate was estimated less than 30 mmol Fe(III).m^{-2}.d^{-1}. Iron(III) reduction rates for the first 0–30 cm depth interval based on *in vitro* CH_4/CO_2 rates are: ∼237 and ∼47 mmol.Fe(III).m^{-2}.d^{-1} for *Scirpus* and control, respectively. These relatively high *in vitro* rates, are probably partly the result of the (short) exposure of the sediment samples to atmospheric air after coring, which has resulted in the additional formation of Fe(III) oxyhydroxides.

The maximal difference in the dissolved Fe(II) reservoirs between the *Scirpus* and control systems was less than 250 mmol.m^{-2}, which would be sufficient to maintain the calculated *in situ* Fe(III) reduction rates in the *Scirpus* system for ∼4 days. This indicates either that (i) Fe(III) oxyhydroxides were rapidly renewed by fast Fe(II) oxidation, or that (ii) a much larger Fe(III) reservoir was present in the sediment than could be estimated from the dissolved Fe(II) pool sizes. Alternatively, (iii) other respiration processes than Fe(III) reduction may have been important. High rates of iron cycling have been reported for heavily bioturbated marine sediments (Canfield et al. 1993; Aller 1994) and vegetated wetland sediments (Roden & Wetzel 1996). Iron(III)-oxyhydroxide renewal is obviously coupled to rhizospheric O_2 input. Continuation of O_2 input by emergent, but very small, regrown plants and dead culms is confirmed by the presence of rhizospheric oxidation, although at a much lower rate, throughout the year (van der Nat & Middelburg 1998; Figure 5). Input of atmospheric O_2 was excluded in the anaerobic incubation experiment whereas differences in CH_4 production still occurred, indicating that continuous O_2 input is not a prerequisite for suppression of methanogenesis. Similarly, *in vitro* studies with rice paddy soil showed continuous suppression of CH_4 production by Fe(III) reduction (Achtnich & Rude 1988). More evidence has been reported for paddy soil studies in which CH_4 production started a few days to several weeks after submerged conditions had been established (Mayer & Conrad 1990). A large period of continuous suppression without additional O_2 input, suggests a large Fe(III) reservoir and supports explanation (ii). Support for the third explanation are the dissolved manganese reservoirs which are ∼10% of the dissolved iron reservoirs, and exhibit a pattern similar to that of iron (van der Nat & Middelburg, unpublished data). Manganese cycling has been shown important for mineralisation in the same manner as iron cycling (Canfield et al.

1993; Aller 1994). Aerobic breakdown, denitrification and sulfate reduction were probably insignificant metabolic processes, because surface-water concentrations of nitrate and sulphate were very low (a few μM).

Higher CH_4 production rates in the *Phragmites* than *Scirpus* system are consistent with the difference in sediment oxidation capacity and correlated variation in the Fe(II) reservoir sizes (Table 3; Figure 5) and the contribution of methanogenesis to gross mineralisation (Table 6). However, CH_4 production in the *Phragmites* system equalled production in the control system (Table 5), eventhough the potential for suppression, based on dissolved iron concentrations (Figure 5), was larger in the *Phragmites* system. We hypothesise that this apparent discrepancy is the result of carbon addition by *Phragmites* plants. Carbon addition may not only limit the effect of substrate competition between methanogens and Fe(III) reducers but may also stimulate methanogenesis directly. Carbon addition may also explain temporal variability in CH_4 production over the course of a plant growth cycle (Table 5). CH_4 production coupled to plant growth and photosynthesis via exudation of labile organic carbon has been suggested before (Schütz et al. 1989a; Kimura et al. 1991; Whiting & Chanton 1992; Nouchi et al. 1994; Kelley et al. 1995; Minoda & Kimura 1996; Shannon et al. 1996; Sigren et al. 1997). We have few data to support the hypothesis of carbon addition by *Phragmites* plants. In an incubation experiment to estimate potential CH_4 oxidation rates conducted during plant growth cycle 3 (van der Nat & Middelburg 1998), potential oxic sedimentary CO_2 production rates were measured as well (Figure 6). When plants were not active (post-season), rates were not significantly different in the three different systems. However, when plants were active (mid-seasonally), potential CO_2 production rates in *Phragmites* sediments were significantly higher than those in non-plant and *Scirpus* sediments below depth 2 cm, whereas the non-plant and *Scirpus* rates remained not significantly different. Hence, aquatic macrophytes may control sediment biogeochemistry not only by oxidising the sediment, but also by input of labile organic carbon. These processes may interact and future studies should consequently focus on both processes simultaneously.

Finally, rhizospheric iron cycling may also increase CH_4 fluxes since iron oxidation competes with methanotrophs for O_2 in the rhizosphere. Nevertheless, suppression of CH_4 production seems a more important mechanism for attenuating CH_4 fluxes than rhizospheric CH_4 oxidation, because even complete inhibition of rhizospheric CH_4 oxidation in the *Scirpus* system would not increase CH_4 fluxes to the level observed for the non-plant system. So, the effect of rhizosphere iron cycling is stronger on CH_4 production for attenuating CH_4 fluxes than it is on rhizospheric CH_4 oxidation for increasing CH_4 fluxes. However, rhizospheric CH_4 oxidation remains an important

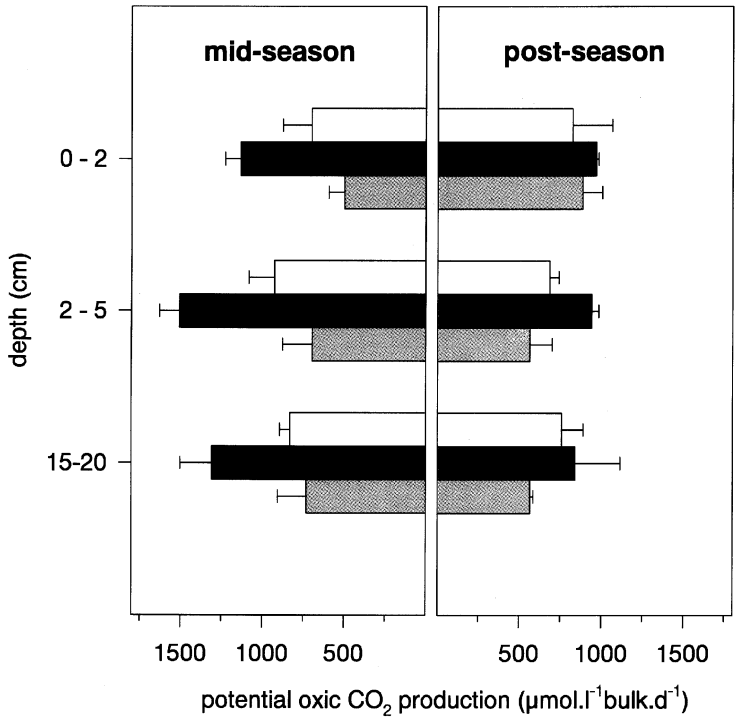

Figure 6. *In vitro* oxic CO_2 production with sediment from the non-plant (open bars), *Phragmites* (black bars) and *Scirpus* (grey bars) systems. The error bars represent the standard error of the mean (n = 3). CO_2 production rates were collected during an *in vitro* CH_4 oxidation incubation (van der Nat & Middelburg, 1998), and have not been published before. Rates presented are corrected for the (small) contribution of CO_2 formed during CH_4 oxidation.

process for attenuating CH_4fluxes, because without rhizospheric oxidation, CH_4 fluxes in the mature *Scirpus* system would have been almost 2 times higher, all other factors being equal.

Conclusions

1. CH_4 transport to the atmosphere occurs predominantly through ebullition when plant biomass is low; when plant biomass increases, transport is predominantly plant mediated.
2. CH_4 fluxes from freshwater marshes are primarily controlled by CH_4 production, oxidation and sedimentary storage are of minor importance.
3. Sediments hosting plant species with a potential large capacity for sediment oxidation show lower CH_4 fluxes than non-vegetated sediments or sediments hosting plant species with a relatively small oxidation capacity.

4. Low CH_4 fluxes from relatively oxidised sediments are coupled to the suppression of CH_4 production, probably as a result of substrate competition between metal oxide reducing bacteria and methanogens.

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