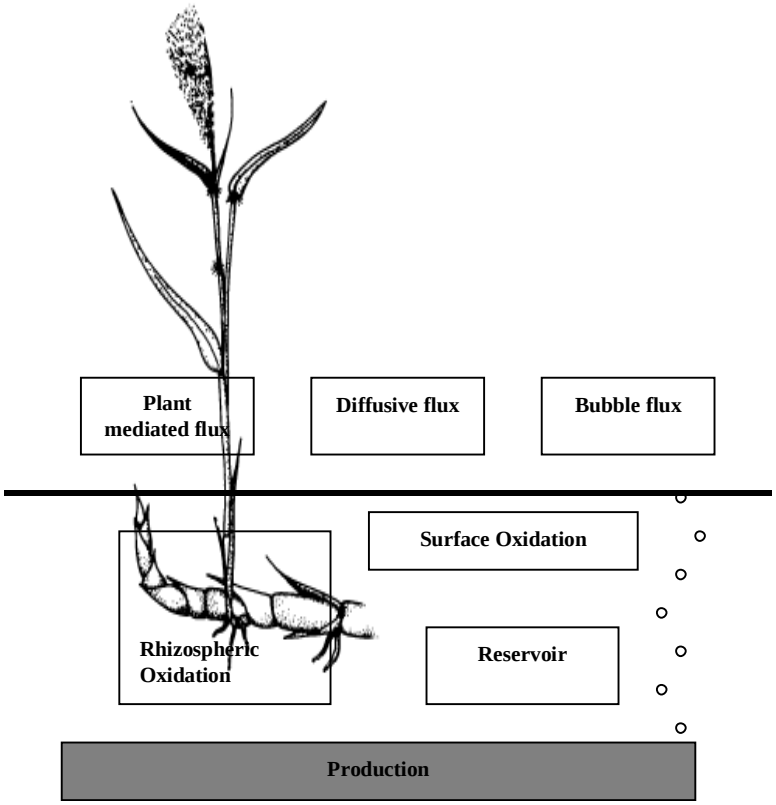
Plant mediated flux
Diffusive flux
Bubble flux
Surface Oxidation
Rhizospheric Oxidation
Reservoir
Production

Methane Emission from Freshwater Marshes

Methane Emission from Freshwater Marshes

Een wetenschappelijke proeve op het gebied
van de Natuurwetenschappen

Proefschrift

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Frans-Jaco Willy Anthony van der Nat

geboren 19 september 1965 te Tiel

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Voor mijn eerste kind en zijn/haar moeder, mijn vriendin.

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Contents

Introduction

The greenhouse effect

The chemical composition of the atmosphere determines the balance between transmission, absorption, and reflection of incoming solar radiation and outgoing terrestrial radiation. Part of the outgoing infrared radiation is absorbed in the troposphere by radiatively active gases whereas these gases have little effect on the incoming solar radiation. Absorption changes the global heat balance and this process is therefore generally referred to as the greenhouse effect. Without it, the Earth would be one big ice-ball with a mean global surface temperature of about -15°C. Instead, it averages about +12°C. The gases involved, generally referred to as greenhouse gases, are water vapour (H_2O), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), ozone (O_3) and the man-made chlorofluorocarbons (CFC's) (Schlesinger 1991; Charlson 1992).

Human activity has caused an increase of the atmospheric concentration of CO_2, CH_4, N_2O and CFC's (Bouwman 1990). These increases have enhanced the greenhouse effect. Recently the intergovernmental panel on climate change (IPCC) summarized evidence for global warming as a result of this. The Earth's climate is a very sensitive function of temperature such that even a small increase (< 1°C), if persisted for a few decades, will cause significant changes. Predicted effects include changed weather patterns and accelerated sea level rise. However, in the complex atmospheric (climate) system any increase in greenhouse gas concentration and temperature change will induce feedback effects such as changes in cloudiness and cloud formation. The nature of these feedback effects, positive or negative, is as yet highly uncertain, but crucial to the net effect of increasing concentrations of greenhouse gases in the atmosphere. (Schlesinger 1991; Charlson 1992).

Of the greenhouse gases showing dramatic increases in recent years, CO_2 is the most important one contributing about 50% to the greenhouse effect. CH_4 comes second contributing about 15% (IPCC 1994). However, each molecule of CH_4 in the atmosphere contributes 20-fold to the Earth's greenhouse effect relative to each molecule of CO_2 and the mean residence time of CH_4 in the atmosphere (~10 y) is longer than that of CO_2 (~3 y) (Cicerone & Oremland 1988). Thus, the increase of methane in the atmosphere has great potential consequences for global warming in the future. Moreover CH_4 affects atmospheric O_3 concentration , and when this effect is included, CH_4 may be even more important than CO_2.

Concern about the steadily increasing atmospheric methane concentrations has initiated and stimulated research to quantify its sources and sinks. Methane production (methanogenesis) is exclusively accomplished by CH_4 producing bacteria (methanogens) in the strict absence of oxygen or other alternative oxidants such as nitrate (NO_3^-), ferric

iron (Fe^{3+}), manganese (Mn^{4+}), and sulfate (SO_4^{2-}) (Oremland 1988; Conrad 1989). Therefore, methanogenesis and CH_4 emission can be expected in freshwater and terrestrial environments where anoxic conditions develop due to O_2 consumption by respiration and limitation of O_2 diffusion from the atmosphere. Indeed, global methane budgets presented by the IPCC identify natural wetlands and cultivated wetlands (rice paddies) as major sources of atmospheric methane. Global annual emission estimates for these wetlands, according to the IPCC, vary from 75 to 250 Tg ($=10^{12}$ g) per year, accounting for roughly 14% to 45% of the sources of CH_4 to the atmosphere .

Methane emission from wetlands

Methane emission from a particular wetland is controlled by the rate of methanogenesis, the rate of CH_4 oxidation (methanotrophy), and the pathway of CH_4 transport from the sediment into the atmosphere. As soon as conditions for methanogenesis are favourable, its rate is highly dependent on the availability of degradable organic matter (Conrad 1989). The CH_4 oxidizing bacteria (methanotrophs) require CH_4 and O_2 for their metabolism. In predominantly anoxic wetlands methanotrophy is therefore usually controlled by the availability of O_2. Methanotrophs and methanotrophy are usually found in the oxic sediment (surface) layers (King 1990; Bosse 1993). Because of methanotrophy the flux of CH_4 from the surface is significantly less than the rate of production at depth.

Vegetated wetlands, such as marshes, resemble uplands, but with high rates of CH_4 production. Freshwater marshes are found at the interface between upland and lakes or upland and (tidal) rivers. Virtually all emergent wetland plants have, in order to survive the anoxic conditions of their habitat, developed extensive air spaces, called aerenchyma, in their roots and stems that facilitate O_2 transport from the atmosphere to the roots (Justin 1987; Armstrong 1994). As a result of bringing O_2 down to their submerged roots, they transport as well CH_4 from the sediment to the atmosphere (Sebacher 1985).

The emergent vegetation can both enhance and attenuate CH_4 emission compared to unvegetated wetlands. Enhancement occurs through production of litter from leaves and roots and root exudates stimulating methanogenic activity (Whiting and Chanton 1993; Minoda and Kimura 1994), as well as by transport of CH_4 through the aerenchyma of the plant stems (Chanton and Dacey 1991; Whiting and Chanton 1992). Attenuation occurs by the release of O_2 from the roots into the sediment, extending the anoxic-oxic interface from the surface layer to the rhizosphere and stimulating methanotrophic activity (de Bont et al. 1978; King 1994).

The final effect of vegetation on CH_4 emission depends upon the delicate balance between the three mechanisms: organic matter input, O_2 input and CH_4 transport. For example, CH_4 emission from freshwater lakes is almost restricted to shallow littoral marshes where the wetland plants facilitate CH_4 transport and protect CH_4 from oxidation in the surface layer (Shannon and White 1994). However, other systems may behave differently. The relative contribution of each mechanism depends upon the plant species

involved. For example, *Phragmites australis* (reed) and *Scirpus lacustris* (bulrush) differ dramatically with respect to their ability of aerating the sediment (this thesis).

Besides the plant community structure, CH_4 emission is also controlled by abiotic environmental factors such as light (King 1990), temperature (King and Wiebe 1978), type and amount of organic matter (Kelley and Chynoweth 1981), flooding, tidal cycling and moisture saturation (Fechner and Hemond 1992; Roulet et al. 1992; Moore et al. 1994). These factors may exert their influence directly or indirectly and they may interact as well. Temperature controls CH_4 emission directly through its effect on the metabolic activity of methanogens and methanotrophs and indirectly through its effect on the photosynthetic activity of the plants.

Outline of this thesis

Regulation of CH_4 emission is very complex, especially in marshes. This complexity leads to a striking individuality of *in situ* emission rates on various time scales (hour, diurnal, seasonal), and at spatial scales of less than 1 m^2, even within the same habitat (King and Wiebe 1978; Delaune et al. 1983; Bartlett et al. 1987; Wilson et al. 1989; Pulliam 1993). In order to reduce this variation in today's global annual emission budget calculations, CH_4 emission and its seasonal variation from a larger number of wetlands have to be measured. But most of all, we have to increase our knowledge about the processes determining CH_4 cycling so that models predicting CH_4 emission rates from wetlands can be constrained more accurately.

This thesis describes the results of a four-year study (1992 - 1996) into the CH_4 cycle of freshwater marshes dominated by reed and bulrush. This research was conducted in the framework of the research theme "carbon and nutrient dynamics in vegetated littoral systems" of the department of Littoral Vegetation of the Centre for Estuarine and Coastal Ecology (Netherlands Institute of Ecology, Yerseke). Emphasis is given to the biogeochemical processes involved in the production, oxidation and transport of CH_4. Reed and bulrush were selected as model plants on the basis of the pioneering work of Gerard Klaver in which he found significant differences in CH_4 emission rates from reed and bulrush marshes in the Scheldt estuary. Moreover, reed and bulrush are used regularly in biological wastewater treatment facilities and the fundamental results of our study are therefore of great use to civil and environmental engineers designing and optimizing such constructed wetland filter beds.

The approach chosen includes detailed field observations on CH_4 stocks and fluxes at the tidal marsh Burcht (Scheldt estuary), and laboratorium experiments. Furthermore, replicate reed, bulrush and non-vegetated permanently flooded experimental ecosystems of 1.5 m^2 each were installed in the mesocosmos facility of the institute enabling us to control important factors in the CH_4 cycle. These experimental systems allowed us to compare directly, at the ecosystem level, the biogeochemical processes and CH_4 cycle in vegetated and non-vegetated sediments, since the initial sediment composition and external forcings were identical.

The first two chapters focus on CH_4 oxidation. Chapter 1 describes the diurnal and seasonal variation of CH_4 oxidation in reed and bulrush vegetated sediments. The methane oxidation rates described in this chapter have been quantified *in situ* using a relatively new technique. Chapter 2 deals with the results of a laboratorium study in which the kinetics of CH_4 oxidation has been measured as a function of depth in sediment, type of vegetation and concentrations of CH_4 and ammonium (NH_4^+). The importance of plant roots for CH_4 oxidation and inhibition of CH_4 oxidation by NH_4^+ in tidal marshes are discussed. In chapter 3, the effect of O_2 release by roots on the sedimentary biogeochemical cycle of components other than CH_4 such as iron, manganese, uranium and arsenic is discussed. Chapter 4 focuses on CH_4 transport by reed and bulrush plants. Results demonstrate unequivocally the dominant role of plant transport in diel CH_4 emission patterns. The last two chapters each deal with the whole CH_4 cycle and how it is affected by plant growth. In chapter 5, a detailed experimentally derived CH_4 balance is presented that provide us with insight concerning the relative contribution of CH_4 production, oxidation and transport to CH_4 emission. It is argued that supression of methanogenesis due to rhizosphere oxidation is a major term in the CH_4 cycle. Chapter 6 includes the results of a two-year field study in the tidal marsh Burcht. The CH_4 cycle in tidal marshes is, besides vegetation, additionally controlled by flooding. The consequences and relative importance of both factors are discussed.

Chapter 1

Seasonal variation in methane oxidation by the rhizosphere of *Phragmites australis* and *Scirpus lacustris*

Frans-Jaco W.A. van der Nat and Jack J. Middelburg

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Abstract

Methane oxidation in the rhizosphere of two common wetland plants, reed (*Phragmites australis* (Cav.) Trin. Ex Steud.) and bulrush (*Scirpus lacustris* L.), was quantified using the methylfluoride (CH_3F) inhibition and anoxic/oxic flux chamber techniques. The similarity of rhizospheric CH_4 oxidation rates determined with the two techniques and the absence of an adverse effect of CH_3F on plant metabolism indicated that the CH_3F inhibition flux chamber technique was a useful tool for measuring rhizospheric CH_4 oxidation in freshwater wetlands. A significant seasonal pattern for both plant species as well as a significant difference between the plant species were observed. Light or dark conditions had no significant effect on rhizospheric CH_4 oxidation. When averaged over the growing season, CH_4 oxidation in the rhizosphere of bulrush and reed reduced the potential CH_4 flux by 34.7% $\pm$ 20.3 and 16.1% $\pm$ 7.86, respectively. Highest CH_4 oxidation rates were noted early in the plant growth cycle with more than 55% of the generated methane being oxidized in the bulrush system. Methane oxidation rates were lowest after plants matured. The difference in rhizospheric CH_4 oxidation capacity between reed and bulrush and the seasonal variation for reed were confirmed by a series of slurry incubations. Slurry incubations also showed a significant decrease of potential CH_4 oxidation as a function of depth in sediment.

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Introduction

In wetlands the net CH_4 flux is determined by the balance of its production, oxidation and transport. Oxidation has only recently been recognised as a key factor regulating CH_4 fluxes (King 1992). The regulation of aerobic CH_4 oxidation by bacteria is obviously related to the requirement for O_2 and CH_4. As a consequence, methanotrophic activity in submerged freshwater marshes is predominantly found in the oxic rhizosphere of macrophytes (de Bont et al. 1978; Gerard and Chanton 1993; King 1994), and in the oxic surface layer of the sediment (King et al. 1990; Bosse et al. 1993).

Occurrence of rhizospheric CH_4 oxidation has been demonstrated using indirect and more direct methods. Indirect methods include slurry and rinsed root incubations (King et al. 1990; King 1994) and mass balances approaches in which oxidation is estimated by difference from (potential) production and emission (Holzapfel-Pschorn et al. 1986; Sass et al. 1990). Direct methods are based on a comparison of *in situ* CH_4 fluxes under anoxic dark conditions and oxic light conditions (Frenzel et al. 1992; Gerard and Chanton 1993; Denier van der Gon and Neue 1996) or *in situ* CH_4 fluxes under ambient conditions with and without CH_4 oxidation inhibitors like acetylene (de Bont et al. 1978; Holzapfel-Pschorn et al. 1985 1986; Gilbert and Frenzel 1995; King 1996) and methylfluoride (CH_3F) (Oremland and Culbertson 1992; Epp and Chanton 1994; Denier van der Gon and Neue 1996; Schipper and Reddy 1996; King 1996). It has been argued that the indirect methods are less accurate and appear to overestimate rates of rhizospheric CH_4 oxidation (Gerard and Chanton 1993; Epp and Chanton 1994; Denier van der Gon and Neue 1996).

Seasonal variability of rhizospheric CH_4 oxidation rates has been demonstrated (Holzapfel-Pschorn et al. 1985; Epp and Chanton 1994; Denier van der Gon and Neue 1996; Schipper and Reddy 1996; King 1996; Lombardi et al. 1997). However, in these studies (a) short-term variability in light and temperature conditions in field and outdoor conditions and the use of small pots might have induced changes in CH_4 fluxes and oxidation rates, (b) conclusions are based upon a relatively small number of sampling dates (Denier van der Gon and Neue 1996) or (c) measurements cover only a restricted part of the plant growth cycle (King 1996).

We examined the diurnal and seasonal variation of rhizospheric CH_4 oxidation in *Phragmites australis* (Cav.) Trin. Ex Steud. (reed) and *Scirpus lacustris* L. (bulrush) stands under controlled environmental conditions. Three different methods were applied namely the CH_3F inhibition flux chamber technique, the anoxic/oxic flux chamber technique, and slurry incubations. We concluded that significantly higher oxidation rates for both plant species occurred while plants were growing and that diurnal changes did not affect rhizospheric CH_4 oxidation rates for these species. Furthermore, rhizospheric CH_4 oxidation differs among both tested plant species, with bulrush having higher rates of rhizospheric oxidation than reed.

Methods

Experimental set-up

Measurements have been made in large constructed wetlands kept in a light, temperature, humidity and carbon dioxide controlled room. Reed and bulrush sediment were collected at an intertidal freshwater marsh in the Schelde estuary near Burcht ($4^{o}23'70''$ E, $50^{o}13'60''$ N; van der Nat et al. 1997). Sediments were air-dried and material that passed a 1.5 cm sieve was mixed with drainage channel soil (4:1 v/v) and root and rhizome material which remained after sieving. Approximately 0.9 m^3 of this mixture was transferred to six identical, fully-separated, 1.3 m^3 large containers (l×w×h = 1.25×1.25×0.85 m). While transferring, 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. Water loss from this layer was compensated for by adding de-ionised water. The sediment was allowed to settle for a period of 5 months. After this period sediment height was about 65 cm and did not decrease further in the next 2 years. Reed and bulrush seedlings with shoots of 5 to 8 cm height, grown from seed collected at our field station, were planted in a density of 100 seedlings/m^2 in two containers each. After the first plant growth cycle polypropylene collars with a diameter of 24 cm were installed in the centre of each container to ascertain consistent placement of the gas collecting chambers and minimise disturbance during successive CH_4 and CO_2 flux measurements. A 16 h day period with a light intensity of ~0.3 mE m^{-2} s^{-1} at the sediment surface (about 2 m below lamps) was set. Temperature, relative humidity and CO_2 gas 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 relative 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 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. Particulate organic carbon contents of the sediment ranged between 4 and 5 wt%. Pore water pH values varied between 6.9 and 7.3 with highest values near the sediment surface.

Plant growth cycles were terminated by a 3 to 4 week period of darkness. At the end of a plant growth cycle dead leave material was removed from the sediment surface, but dead shoots were retained. Before each plant growth cycle commercial fertiliser (Barenbrug garden products) was added to the flood water. Steady-state conditions were present in our experimental systems as can be inferred from the similarity of carbon dioxide uptake rates, methane fluxes and plant biomass patterns for plant growth cycles 2, 3 and 4 (van der Nat et al. 1998; van der Nat and Middelburg 1998). Results are presented for plant growth cycle 3 which started in January 1995 and lasted until July 1995. Three containers (no-plant, reed, bulrush) were used for oxidation rate measurements, whereas their replicates were only used for methane flux measurements (controls).

Biomass

Mature bulrush and reed plants from the second plant growth cycle were clipped to water level. Height and diameter as well as fresh and ash free dry weight were determined. Only green. healthy plants were selected. From the fit between the natural logarithm of biomass with plant height (R^2 >0.95; n=50 for both plant species), biomass could be estimated in a non-destructive way during the following plant growth cycles. The estimated biomass has been corrected for systematic bias due to back transformation from logarithmic to arithmetic units (Baskerville 1972). Prior to termination of the experimental set-up, sediment was collected using acrylic tubes (55 cm long and a diameter of 7 cm). Three cores were sliced and washed for obtaining below-ground biomass. Biomass was separated in roots and rhizome material.

Gas fluxes

CH_4 and CO_2 flux measurements were conducted using a closed chamber technique (van der Nat et al. 1998). Transparent (90% PAR transmission) acrylic plastic chambers with a height of 40 cm, a diameter of 23 cm and an open bottom were placed over the intact vegetation with the rim of the chamber below the water surface and fitted into a groove in the collar. Chambers could be stapled and sealed gastight in order to keep up with plant growth. Chamber gas was mixed by a 12V fan fixed to the cover of the upper chamber. 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). This analyser is a Fourier transform infra red spectrometer based on photo-acoustic detection that allows measurement of CH_4 and CO_2 at atmospheric levels and below (Middelburg et al. 1996a). Gas concentrations were measured each 1.5 minutes for a period of 1.5 h. 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. 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. 1996a). The CH_4 and CO_2 fluxes were calculated by linear regression analysis from change of CH_4 and CO_2 concentration in the chamber with time. Fluxes with a regression coefficient <0.98 (n>8) were discarded. Between flux measurements the chamber was vented for 15 minutes by flushing with compressed atmospheric air or N_2, depending on treatment. Flushing was done via the in- and outlet adapters so that the chambers were left in place and any potential disturbance was minimised. All measurements were conducted in the same system, except for a series of control measurements which were performed in the control non-treated systems.

The diffusive CH_4 flux from the flood water layer enclosed by the collar was determined from the flood 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 flood water were determined by the headspace equilibration technique (McAullife 1971). Water samples were obtained just prior to loading the sediment with CH_3F and directly after. The diffusion and

bubble fluxes were subtracted from total flux values to obtain the plant mediated flux. Diffusive fluxes were measured when plants were mature and senescent.

Methane oxidation

Methane oxidation has been estimated from the difference between methane fluxes before and after incubation with CH_3F or N_2. Each oxidation determination started with replicate methane flux measurements which were then followed by an overnight incubation period before another series of replicate flux measurements was performed. The CH_3F inhibition flux chamber technique used is a slightly modified version of that of Epp and Chanton (1994). In short, CH_3F was injected into the chamber to a final concentration of 2%. Hereafter the chamber was sealed and left for 18 h during which the CH_3F and CO_2 concentrations were monitored. Normal light conditions were maintained during this loading period and CO_2 uptake by photosynthesis was compensated. After the loading period and before the first MF-oxic flux measurement was started, the chamber was flushed for 1 h with compressed atmospheric air. The CH_4 concentrations in the chamber during the MF-oxic flux measurements were also determined with a FID equipped gas chromatograph (Carlo-Erba high resolution MEGA 5340), because of CH_3F interference with the gasanalyzer CH_4 measurement. Eight gas samples (2.5 ml; <0.005% of chamber volume) were collected at 6-8 minutes intervals using gastight syringes through a rubber stopper installed halfway the chamber. Interference of CH_3F with the gasanalyzer CH_4 measurement allowed us to monitor CH_3F concentrations in the chamber during the loading period. Obviously, chamber CH_4 increase during measurement interfered with this measurement, however this effect was minimal due to much larger CH_3F than chamber CH_4 concentrations (~four orders of magnitude difference). The decrease in chamber CH_3F concentrations during the loading period was used to calculate an effective CH_3F diffusivity constant (D_{eff}) according to Rolston et al. (1991). The pore water CH_3F concentrations in the no-plant sediment after the loading period were calculated using (Carslaw and Jaeger, 1959; p. 306):

$$C(z,t) = \phi.\ \alpha.C_0 \exp\left(\frac{z}{a} + \frac{D_{\text{eff}}t}{a^2}\right)\text{erfc}\left(\frac{z}{2\sqrt{D_{\text{eff}}t}} + \frac{\sqrt{D_{\text{eff}}t}}{a}\right)$$

where C is the concentration of CH_3F in the sediment, ϕ is porosity (determined previously in sediment surface: 0.52 ± 0.02, n=3), α is the Bunsen coefficient (1.398; T=18°C, Frenzel and Bosse 1996), z is the distance from the sediment surface, t is time after CH_3F injection, a is volume of the chamber per unit area of the collar and D_{eff} is the effective CH_3F diffusivity constant.

Anoxic conditions, required for the anoxic/oxic flux chamber technique, were attained by flushing with N_2. The O_2 content of the chamber was monitored by an O_2 electrode installed inside the chamber. Anoxic conditions were reached within 1 h and maintained for

18 h before the first anoxic flux measurement was started. To prevent formation of O_2 by photosynthesis, CO_2 was not injected during anoxic-flux measurements. Flux measurements were also conducted with a chamber headspace composition of pure O_2 (hyperoxic-flux). The hyperoxic treatment was conducted similar to the anoxic treatment, but with CO_2 injection.

Slurry incubations

Sediment cores were collected using acrylic tubes (25 cm long and a diameter of 3 cm). The cores were sliced, and slices of equal depth were thoroughly mixed, put into glass vials, diluted with sterilised water and sealed with screw caps provided with rubber septa. CH_4 was added to the headspace to a final concentration of 15 µmol l^{-1}. Slurries inhibited with CH_3F (2% headspace) were used as a control. A detailed description of the method used is given elsewhere (van der Nat et al. 1997).

Statistics

Statistical analysis was performed 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

Plant growth

Plant growth and associated CO_2 uptake rates are shown in Figure 1.1. In both systems the CO_2 uptake rate varied significantly ($P<0.001$) over the course of the plant growth cycle and correlated strongly with the amount of green plant biomass. In the reed system, 3 months after beginning of the experiment the plants reached their maximum height (2 m), due to climate cell height limitations, and green plant biomass and CO_2 uptake rates gradually diminished. Green biomass and CO_2 uptake rates of the bulrush plants, for which maximum height remained within limits of the set-up, did not diminish until after about 4.5 months. Maximum shoot density in the reed and bulrush system was 142 and 710 stems m^{-2}, respectively. In the mature vegetation stands at our field station Burcht, above-ground biomass weight was ~700 to ~1000 g [dr wt] m^{-2} whereas shoot densities were ~121 stems m^{-2} (reed) and ~625 stems m^{-2} (bulrush). The amount of dry weight root material was higher in the *Phragmites* than in the *Scirpus* systems (Figure 1.1). 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 ~10 times that of total root dry weight.

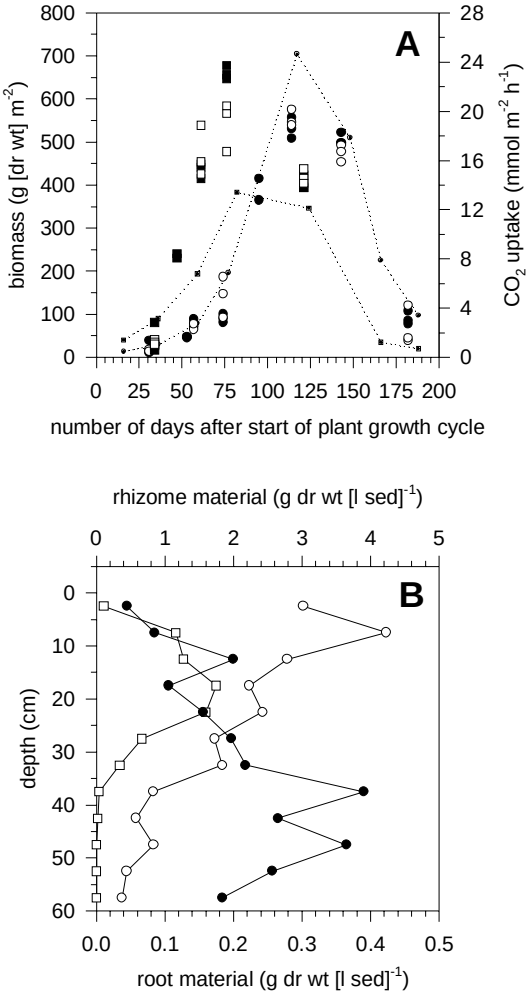

Figure 1.1 (A) Above-ground plant biomass (dotted lines) and CO_2 uptake rates before (open symbols) and after (filled symbols) CH_3F treatment. (B) Below-ground root biomass (open symbols) and rhizome biomass (filled symbols). The bulrush system is represented by circles and the reed system by squares. Only CO_2 uptake rates in excess of the CO_2 uptake rates measured in the no-plant system under light conditions (~0.9 mmol m^{-2} h^{-1}) are included.

Control experiments

The oxic-CO_2 uptake rates in the bulrush and reed systems were not significantly different from the MF-oxic CO_2 uptake rates (Figure 1.1). Furthermore, oxic CH_4-flux measurements preceding and following (after an overnight adjustment period) day 48 anoxic-CH_4 flux measurements were statistically not different (illuminated 226 ± 3.12 and 221 ± 5.63 µmol m^{-2} h^{-1} and dark 187 ± 23.1 and 194 ± 17.5 µmol m^{-2} h^{-1}). The oxic-CH_4 fluxes in the treatment reed system were not significantly different from those in the control reed system. In the treatment bulrush system oxic CH_4 fluxes were consistently ~20% lower than those in the control bulrush system. However, the bulrush systems were not identical, because plant biomass in the control bulrush system exceeded plant biomass in the bulrush treatment system by more than 30%.

Transport of CH_3F

The decline of CH_3F in the chamber during the 18 h loading period is given in Figure 1.2. Apparently, CH_3F disappeared relatively fast from the chamber when plants were growing and after they matured. One exception was noted: on day 74 in the reed system disappearance of CH_3F was unexpectedly slow. At the end of the plant growth cycles when the plants senesced, CH_3F disappeared relatively slowly. The total amount of CH_3F transported back from the sediment during the period of MF-oxic flux measurements was less than 10% of the amount transported into the sediment. In the no-plant system CH_3F declined by 3% during the 18 h loading period, whereas sealed chambers always lost less than 0.1% over this time period. This CH_3F decline was less than the lowest values reported for the vegetated systems. The corresponding sedimentary CH_3F concentration profile in the no-plant system was calculated assuming CH_3F was not consumed and homogeneously mixed in the overlying flood water layer. The outcome of the calculations showed that CH_3F penetrated the no-plant sediment up to 7 to 10 cm and that CH_3F pore water concentrations were ~900 µmol l^{-1} in the surface layer.

Table 1.1 Diffusive CH_4 fluxes and surface oxidation rates

System	Season	Flux[a] (µmol m^{-2} h^{-1})		Oxidation[b]	
		Before CH_3F treatment	After CH_3F treatment	Absolute (µmol m^{-2} h^{-1})	Relative (%)
No-plant	Mid	25.6 ± 12.4	116 ± 28.1	90.4 ± 30.8	77.9 ± 42.2
	Post	44.4 ± 17.1	234 ± 57.9	190 ± 60.4	81.0 ± 37.1
Bulrush	Mid	11.8 ± 4.08	21.9 ± 12.3	10.1 ± 12.9	46.1 ± 30.4
	Post	19.4 ± 7.10	33.4 ± 10.0	14.0 ± 12.2	41.9 ± 19.8
Reed	Mid	18.1 ± 7.13	44.1 ± 13.1	26.0 ± 14.9	58.9 ± 29.1
	Post	29.4 ± 17.8	62.6 ± 16.3	33.2 ± 24.1	53.0 ± 34.9

[a] ± represents the standard error of the mean (*n*=3).

[b] ± represents the combined error calculated using standard error propagation techniques.

Methane oxidation

Diffusive oxic CH_4 fluxes and surface oxidation rates are shown in Table 1.1. Highest efficiency levels of surface oxidation were observed in the no-plant system, probably as a result of a steeper CH_4 concentration gradient at the sediment surface in this particular system. Ebullition was the dominant CH_4 transport mechanism in the no-plant systems (van der Nat and Middelburg 1998). In the plant systems bubbles were observed at the start and end of the plant growth cycle, especially in the reed system. In the reed system ebullition contributed 188 to 375 µmol m^{-2} h^{-1} CH_4 to the total CH_4 flux. Ebullition was not significantly affected by CH_3F treatment. Probably bubble methane escaped from oxidation processes.

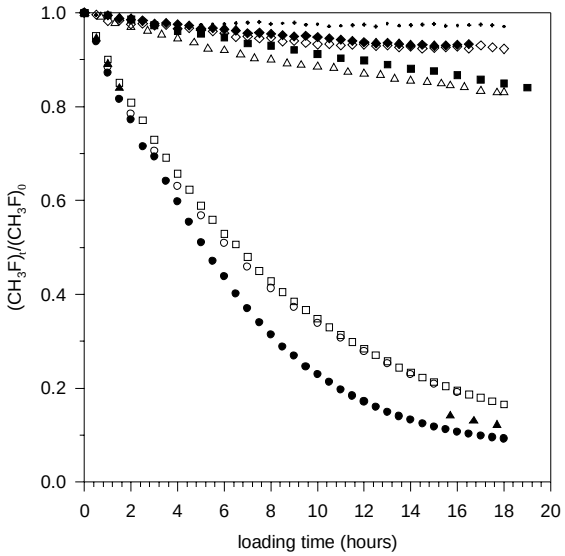

Figure 1.2 Decline of the $(CH_3F)_t/(CH_3F)_0$ ratio in the chamber during the loading period of 18 h in the bulrush system on day 74 (○), day 114 (□), day 143 (Δ), day 182 (◊); in the reed system on day 57 (●), day 74 (■), day 114 (▲), day 182 (♦); and in the no-plant system (+).

In the bulrush and reed systems the plant mediated MF-oxic and anoxic CH_4 fluxes were higher than the preceding plant mediated oxic fluxes (Figure 1.3), except for the last day of sampling in the reed system when dead leaves were present inside the collar. In both systems oxidation rates were not significantly affected by light and dark conditions (Table 1.2). Relative oxidation percentages (reduction of potential CH_4 flux) followed the same pattern as the absolute oxidation rates with highest reduction levels when plants grew and relatively lower after plants matured. The observed seasonal pattern was proven significant in both plant systems (bulrush: $P<0.001$ and reed: $P<0.02$) as well was the difference between both plant species ($P<0.01$). Hyperoxic conditions diminished the oxic

flux by 16.6% ± 3.9 in the bulrush system and by 5.8% ± 3.1 in the reed system. The effect of hyperoxic treatment was only significant (P<0.001) in the bulrush system.

Table 1.2 Rhizospheric oxidation rates

Day[a]	Method[b]	Light condition	Absolute (µmol m^{-2} h^{-1})		Relative (%)	
			Bulrush	Reed	Bulrush	Reed
16	CH_3F	Light	30.1 ± 14.8	18.8 ± 17.7	17.7 ± 1.55	7.96 ± 1.86
		Dark	19.5 ± 7.95	31.1 ± 8.83	12.7 ± 1.60	12.4 ± 3.56
31	CH_3F	Light	225 ± 26.9	121 ± 52.5	53.0 ± 4.11	20.4 ± 1.27
		Dark	156 ± 31.1	105 ± 66.3	43.3 ± 3.73	19.9 ± 0.91
48	N_2	Light	170 ± 23.8	188 ± 30.2	42.9 ± 2.62	22.3 ± 0.97
		Dark	138 ± 26.5	243 ± 55.8	42.5 ± 5.57	32.3 ± 3.48
57	CH_3F	Light	289 ± 39.4	170 ± 73.0	56.9 ± 6.64	20.8 ± 2.17
		Dark	249 ± 14.0	188 ± 54.7	57.0 ± 5.67	26.4 ± 2.31
74	CH_3F	Light	312 ± 30.6	122 ± 71.2	58.6 ± 12.4	16.4 ± 2.57
		Dark	85.3 ± 40.5	97.6 ± 57.8	22.7 ± 4.09	14.4 ± 1.48
96	N_2	Light	171 ± 53.5		38.7 ± 6.68	
		Dark	166 ± 65.3		41.2 ± 7.63	
114	CH_3F	Light	144 ± 48.1	61.4 ± 96.4	34.8 ± 2.08	9.50 ± 0.76
		Dark	148 ± 46.2	126 ± 57.2	37.7 ± 5.88	21.3 ± 2.80
143	CH_3F	Light	128 ± 38.6		31.7 ± 5.75	
		Dark	126 ± 58.7		36.5 ± 6.51	
182	CH_3F	Light	13.9 ± 52.7	<0	5.51 ± 0.97	<0
		Dark	59.0 ± 53.4	47.2 ± 99.7	17.8 ± 3.85	7.46 ± 3.99

[a] number of days after start of plant growth cycle.

[b] CH_3F: methylfluoride inhibtion flux chamber technique; N_2: anoxic/oxic flux chamber technique.

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

Slurry incubations

In the headspace of incubation flasks filled with sediment from the no-plant system a significant decrease of CH_4 with time was found only at depths 0-2 cm, whereas in the headspace of the flasks filled with sediment from the vegetated systems a significant decrease was observed at all tested depth intervals (Table 1.3). This decrease was probably mediated by biological oxidation since we did not observe a decrease of CH_4 in the headspace in flasks treated with CH_3F or following autoclaving (data not shown). A significant difference (P<0.006) between mid- and post-seasonal potential CH_4 oxidation rates was only observed in the reed slurries. Averaged over the three depth intervals, the potential CH_4 oxidation rates in the bulrush slurries were significantly higher than in the reed slurries, namely 18% (P<0.03) mid-seasonally and 32% (P<0.002) post-seasonally.

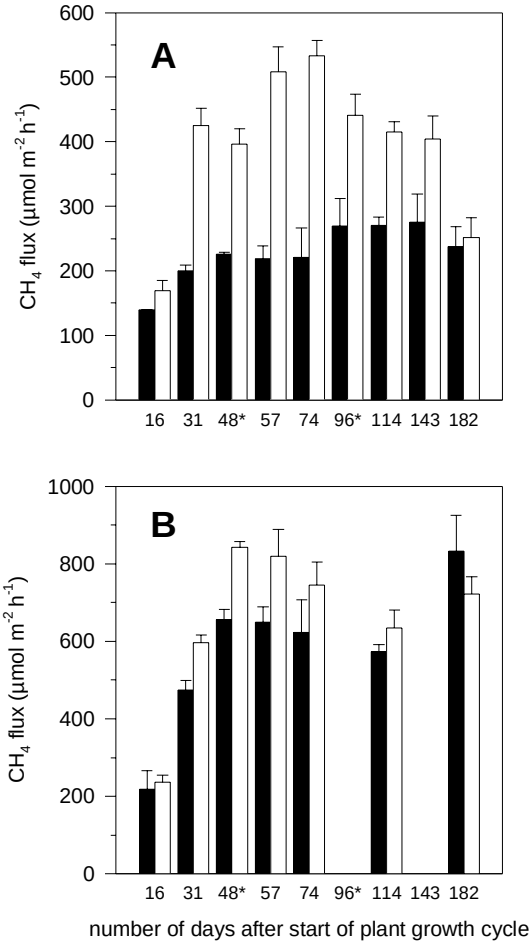

Figure 1.3 Plant-mediated CH_4 fluxes before (solid bars) and after CH_3F or anoxic treatment (open bars) under light conditions in the bulrush system (A) and reed system (B). Please note the difference in scale. ***** denotes anoxic treatment. Error bars represent the standard error of the mean (*n*=3).

Discussion

The CH_3F inhibition flux chamber technique

Basic assumptions of the inhibition flux chamber technique are: (i) the inhibitor is transported to the active sites of CH_4 oxidation in the sediment in a sufficient concentration, (ii) the inhibitor specifically inhibits CH_4 oxidation without affecting CH_4 availability, and (iii) the inhibitor and the closed chamber technique do not affect the metabolism of the plant. Moreover, if seasonal variation in rhizospheric CH_4 oxidation will be studied, it is also necessary that the inhibitor is reversible, thereby allowing repeated measurements on the same plant-bacteria system.

(i). In CH_4-rich submerged sediments availability of molecular O_2 is usually the determining factor for localisation of numbers and activity of CH_4 oxidizing bacteria (King 1992; Epp and Chanton 1994). The decrease of the hyperoxic CH_4-flux compared to the oxic-CH_4 flux indicate that O_2 is the rate limiting factor for CH_4 oxidation in our type of sediment as well. The CH_3F inhibition flux chamber method is based on the assumption that CH_3F is transported and released from the plant roots similar to and simultaneously with O_2. For a given concentration of CH_3F in the chamber, the effectiveness of CH_3F to inhibit CH_4 oxidation is determined by its rate of transport through the plants and to the actual sites of CH_4 oxidation resulting in a concentration sufficient to inhibit CH_4 oxidation. The net amount of CH_3F transported into the sediment while plants were fully green exceeded by more than ten-fold the net amount transported while plants senesced or had died as well as the amount transported in the no-plant system. Hence, healthy plants transport CH_3F into the sediment faster than dying plants and than in the absence of plants. The sediment layer where CH_4 oxidation occurred was less than 2 cm thick in the no-plant system. CH_3F concentrations calculated for this layer were sufficient for complete inhibition of CH_4 oxidation.

Table 1.3 Slurry CH_4 oxidation rates

System	Season	CH_4 oxidation rate (nmol g^{-1} dry wt h^{-1})		
		0-2 cm	2-5 cm	15-20 cm
No-plant	Mid	3.95 ± 0.78	<0.1	<0.1
	Post	4.91 ± 0.80	<0.1	<0.1
Bulrush	Mid	4.54 ± 0.31	4.27 ± 0.18	2.91 ± 0.60
	Post	4.85 ± 0.29	3.41 ± 0.86	2.15 ± 0.26
Reed	Mid	5.53 ± 0.48	3.15 ± 0.35	0.97 ± 0.38
	Post	4.86 ± 0.49	1.59 ± 0.29	0.65 ± 0.13

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

Directly after the chamber was vented, CH_3F concentrations in the pore water decreased due to transportation back to the atmosphere as we observed. This reverse transport might also comprise CH_3F which was still present in the plant aerenchymatic

tissue although the chamber was flushed for 1 h before flux measurements were started. Other processes responsible for CH_3F decrease are bacterial uptake, diffusion away from the rhizoplane into the sediment and escape through buried roots and rhizomes of the plants that are connected to plants outside the chamber. However, the relatively low variation of the plant mediated MF-oxic CH_4 flux measurements within each triplicate series indicated that any possible decrease of CH_3F concentration at the active sites of CH_4 oxidation did not result in a consistent variation of CH_4 oxidation rates during the period of completing one series of measurements.

(ii). The ideal inhibitor for CH_4 oxidation should effectively block CH_4 oxidation, but should not affect methanogenesis. Direct inhibition of methanogenesis by CH_3F in slurry incubation experiments has been demonstrated for a number of wetland sediments (e.g. Frenzel and Bosse 1996; King 1996), but some of these inhibitions effects were not significant (Schipper and Reddy 1996; Lombardi et al. 1997), consistent with our experience (data not shown). Direct inhibition of methanogenesis by CH_3F may depend on the substrate (Janssen and Frenzel 1997).

It is important to distinguish between an effect of CH_3F on methanogenesis and on CH_4 emission (King 1996). The supply of CH_4 for plant mediated fluxes is probably not depending on short-term fluctuations in CH_4 production when a large standing stock of CH_4 is present in the sediment. In our sediments standing stocks of dissolved CH_4 were 5 to 900 µmol l^{-1}, depending on depth (van der Nat and Middelburg 1998). Moreover, the CH_4 oxidation rates determined by the anoxic/oxic flux chamber technique were similar to the CH_4 oxidation rates determined by the CH_3F inhibition flux chamber technique. Apparently, methane fluxes in our sediments did not depend directly on short-term fluctuations in methane production and any potential inhibition of methanogenesis would not affect the oxidation rate estimates.

(iii). The physiological status of the plants, as far as indicated by the CO_2 uptake rate, appears to be unaffected by CH_3F (Figure 1.1). Similarly, Epp and Chanton (1994) and King (1996) did not observe any effect of CH_3F on photosynthesis, nor on stomatal conductance. The anoxic treatment did not cause major changes in the plant-bacteria systems, because oxic-CH_4 fluxes before and directly after anoxic treatment were similar. There were also no conspicuous effects of enclosure on methane fluxes, which is consistent with the results of Schipper and Reddy (1996). They observed no effect of 18 h enclosure on mature bulltongue plants.

The similarity between oxic-CH_4 fluxes in the control reed and treatment reed systems indicate that the period of at least 12 days between CH_3F measurements was sufficient to minimise a possible 'memory' effect of CH_3F treatment. The difference in oxic CH_4 fluxes between the control bulrush and treatment bulrush system was probably the result of plant biomass differences. The relationship between vegetation cover and methane release seemed to be positive, as revealed by strong correlations between plant production and (or) biomass and methane emission (Whiting and Chanton 1993). The similarity between the control and treatment systems also indicated that inhibition of CH_4 oxidation by CH_3F was reversible.

Rhizospheric CH_4 oxidation

Slurry incubations showed that the potential for CH_4 oxidation below the sediment surface layer was restricted to the planted sediments. Previous studies have found that methanotrophs at greater depth live near or even within the roots of macrophyte species (de Bont et al. 1978; Gerard and Chanton 1993; King 1994; van der Nat et al. 1997). Emergent bulrush and reed plants accounted for 90-95% of total CH_4 emission. As a consequence, rhizospheric oxidation is a much more important process for the consumption of CH_4 than surface oxidation, despite differences in oxidation efficiency (42 to 81% for surface oxidation versus <59% for rhizosphere oxidation). During the seasonal period of emergent plants, rhizospheric oxidation contributed more than 80% of total oxidation in the plant systems.

Two periods with respect to the degree of rhizospheric oxidation can be distinguished while plants are present. A period while plants grow and oxidation rates were relatively high and a period after the plants matured and oxidation rates were relatively low. Obviously, the relatively low rates at the start and at the end of the plant growth cycle are related to the absence of green emergent plant biomass. After newly formed stems of the bulrush and reed plants emerged above the water, oxidation rates increased drastically. The higher CH_4 flux measured on the last day of sampling compared to the first day was probably caused by enhanced CH_4 production through the input of leaf litter, since litter removal resulted in lowering of the fluxes with a factor 2.

The seasonal variation of CH_4 oxidation rates was reflected in the reed slurry incubations, but not in the bulrush slurry incubations. One of the reasons for this discrepancy might be that the methanotrophs inhabiting vegetated sediments, where anoxic and oxic periods alternate continuously, are able to survive long periods of anoxia and become active without a lag-phase when incubated (King 1992; van der Nat et al. 1997). Overall, the potential CH_4 oxidation rates in sediment of our experimental containers were similar to those in sediment from our field station (van der Nat et al. 1997). This is another indication that the experimental sediments in their third plant growth cycle represented field circumstances with respect to the depth distribution and activity of methanotrophs.

The rather smooth seasonal rhizospheric oxidation patterns observed in this study are a consequence of the well controlled environmental conditions. Temperature, light intensity and water level changes have been reported to affect CH_4 emission and oxidation (Cicerone and Oremland 1988; Whiting and Chanton 1996). In our experimental set-up, temperature, light and water level conditions remained constant over the entire plant growth cycle. Accordingly, any seasonality in methane oxidation rates must be due to seasonality in the functioning or physiology of the plants.

Seasonal variation in rhizosphere methane oxidation is probably due to seasonality in rhizosphere oxygen concentrations. Availability of O_2 for methanotrophs is determined by the delicate balance between the amount transported and the amount consumed by root respiration and (non-methanotrophic) oxygen demanding processes in the rhizosphere. On the basis of similar CH_3F transport rates we argue that the amount of O_2 transported by

growing and mature plants was not dramatically different. Therefore, seasonality in rhizospheric oxidation was probably associated with seasonal variations in rhizospheric O_2 demand. Seasonal variation in root respiration and intensity of rhizospheric O_2 consuming processes have been reported previously (Dunbabin et al. 1988; Bedford et al. 1991; Caffrey and Kemp 1991).

Various studies have reported effects of light on rhizospheric oxygen concentrations, through its control on photosynthetic activity and O_2 transport (Armstrong and Armstrong 1991; Oremland and Taylor 1977; Caffrey and Kemp 1991). However, the relevance of light/dark effects on rhizospheric oxidation in bulrush and reed was limited under the conditions set, despite differences in gas transport mechanisms when illuminated (van der Nat et al. 1998). Similar observations were made by Frenzel et al. (1992) for rice, a plant species demonstrated to posses only a non-active gas transport mechanism.

Methane oxidation rates in the rhizosphere of bulrush are higher than those in the rhizosphere of reed, irrespective whether they are based on the CH_3F inhibition and anoxic/oxic flux chamber technique or slurry incubations. Higher rates of rhizospheric oxidation in the bulrush than reed system are in agreement with the zonation of their habitats, *Scirpus* can tolerate sediments with higher O_2 demand than *Phragmites*. It is also consistent with our parallel study showing enhanced suppression of methane production and more extensive oxidation of reduced iron in bulrush than in reed systems (van der Nat & Middelburg 1998a). This difference between bulrush and reed is also in agreement with several applied research studies in which constructed wetlands with bulrush plants proved to be more efficient in NH_4^+ and biological oxygen demand (BOD) removal than constructed wetlands with reed plants (Gersberg et al. 1986; Wathugala et al. 1987). 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 rhizospheric O_2 demand due to differences in labile organic carbon loss and root respiration activity. The CH_3F uptake data indicated that the gas transport capacity of reed and bulrush were similar on an area basis, although reed had the capability to exploit diffusive and convective transport mechanisms, whereas diffusive transport dominate in bulrush (van der Nat et al. 1998). Hence, differences in oxygen availability for methanotrophs are more likely related to differences in root and rhizome respiration and oxygen demand than to differences in transport. A significant difference in root and rhizome respiration between reed and bulrush is expected given the large difference in below-ground biomass.

Chapter 2

Spatial distribution and inhibition by ammonium of methane oxidation in intertidal freshwater marshes

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Abstract

In two intertidal marshes, the vertical distribution in the sediment and inhibition by ammonium of methane oxidation were investigated by slurry incubation experiments. The two sites differ in their dominant vegetation type, i.e. reed and bulrush, and in their height above sea level. The reed site was elevated with respect to the bulrush site resulting in a lower frequency and duration of flooding and, consequently, a higher potential for methane oxidation. Methane oxidation decreased with depth in the bulrush and reed slurries, although methane oxidation associated with root material from the bulrush plants increased with depth. Reed root material had a limited capacity for methane oxidation and showed no significant increase with depth. Inhibition of methane oxidation by ammonium was observed in all samples and depended on methane and ammonium concentrations. Increasing ammonium concentrations resulted in greater inhibition and increasing methane concentrations in less. Ammonium concentrations had to exceed methane concentrations by at least 30-fold to become effective for inhibition. This ratio was found only in the surface layer of the sediment. Hence, the ecological relevance for ammonium inhibition of methane oxidation in intertidal marshes is rather limited and is restricted to the surface layer. Nitrate production was restricted to the 0- to 5-cm-depth slurries.

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Introduction

Methane is an important greenhouse gas (Bouwman 1990; Cicerone & Oremland 1988; Conrad 1989). Emissions from wetlands contribute significantly to the atmospheric methane budget (Aselman & Crutzen 1989; Matthews & Fung 1987). Net methane flux is determined by the balance of its production, oxidation and transport. Oxidation has been recognized as a key factor regulating methane fluxes (Galchenko et al. 1989; King 1992). Methane oxidation in freshwater sediments depends in a complex manner on hydrology and vegetation (Epp & Chanton 1994; King 1992; Moosavi et al. 1996; Oremland & Culbertson 1992b; Roslev & King 1996).

The control of aerobic methane oxidation is obviously related to the requirement for oxygen and methane (King 1992; Sundh et al. 1995). As a consequence, maximum oxidation rates are found where diffusion of oxygen from above and of methane from below is optimal for methanotrophs, as has already been demonstrated in sediments of Lake Washington and Lake Constance (Frenzel et al. 1990; Kuivila et al. 1988). In intertidal vegetated sediments, atmospheric oxygen may enter sediments directly by diffusion at low tide and indirectly via plants at low and high tide. As a consequence, intertidal vegetated sediments have a high methane oxidation potential (Kelley et al. 1995).

Ammonium has been reported as an additional factor, controlling methane oxidation in pure-culture studies (King & Schnell 1994a; O'Neill & Wilkinson 1977), landfill-cover soil (Boeckx & Cleemput 1996), terrestrial soils (Bender & Conrad 1994; Dunfield & Knowles 1995; Schnell & King 1994) and aquatic sediments (Bosse et al. 1993; Jones & Morita 1983). The inhibition mechanism seems to be rather complex, including not only competitive inhibition of the methane monooxygenase enzyme by ammonium (O'Neill & Wilkinson 1977) but also noncompetitive (toxic) inhibition by hydroxylamine and nitrite produced by the oxidation of ammonium (Dunfield & Knowles 1995; King & Schnell 1994a). Nitrate, another product of ammonium oxidation, does not seem to be important (Adamsen & King 1993; Boeckx & Cleemput 1996; Dunfield & Knowles 1995). Moreover, both methanotrophs and ammonium oxidizers are capable of oxidizing ammonium and methane (Bédard & Knowles 1989; Jones & Morita 1983; O'Neill & Wilkinson 1977). In natural samples, it is rather difficult to estimate the relative contributions of methanotrophs and ammonium oxidizers to ammonium and methane oxidation, respectively (Roy & Knowles 1995).

In the present study, we have determined the potential for methane oxidation in bulrush- and reed-vegetated intertidal sediment from different depth horizons and have studied the effect of ammonium on this capacity. Our experiments had a full factorial design so that interactions between methane oxidation, plant species, depth, and ammonium as well as those between ammonium, depth, and methane could be observed. The experiments allowed us not only to detect ammonium inhibition of methane oxidation but also to constrain its importance under natural conditions.

Material and methods

Station description

Samples were taken at an intertidal marsh (Burcht) located in the Schelde Estuary just opposite of Antwerp, Belgium. The vegetation at this site shows a clear zonation pattern, with a 10-m-broad *Scirpus lacustris* (bulrush) site closest to the river (2.5 m above sea level) and a 20-m-broad *Phragmites australis* (reed) site (3.2 m above sea level). This 0.7-m difference in altitude results in large differences in flooding frequency, duration of submergence, and flood height. In 1995, the reed site was flooded on 184 days (twice a day on 116 occasions), whereas the bulrush site was flooded 331 days in that year (twice a day on 321 occasions). Total periods of submergence of the reed and bulrush sites in 1995 were 210 and 1,027 h, respectively, whereas the averaged periods of submergence per flooding were 0.7 and 1.6 h, respectively. The average heights of the flood water above the sediment surface during submergence were 16.6 cm (reed site) and 43.4 cm (bulrush site). The annual average concentrations of ammonium, nitrite, and nitrate in the flood water were 160, 20 and 210 µM, respectively.

Pore water sampling

Pore water was sampled by *in situ* dialysis (Hesslein 1976), using a sampling device known as a peeper (height by width, 60 by 10 cm) which contains 25 membrane cells that are in contact with sediments via a 0.2-mm-thick biologically inert acrylic copolymer membrane filter (Versapor-200; Gelman Sciences). At both ends, the cells were connected with Tygon tubes to sampling ports at the sediment surface. The samplers were installed January 1994, and pore water samples were withdrawn in August 1995 with a syringe at one sampling port while nitrogen gas was introduced via the other port. Deoxygenated deionized water was used to refill the compartments after sampling. Samples were analyzed for methane, ammonium, nitrite, and nitrate concentrations.

Slurry incubations

In July and September 1995, sediment cores (50 cm long) were collected by using acrylic tubes (60-cm length, 7-cm internal diameter). Sediment compression, based on a comparison between the surface level inside and outside the cylinder, was about 3 to 5 cm and was taken into account during the slicing of the cores. Samples from three depth intervals were selected and put in plastic bags, which were sealed and transported to the laboratory within 3 h. Thirty-gram portions of the moist field sediment (including roots) were put into 70-ml incubation flasks and diluted with sterilized water to give slurries of approximately 50 g. This resulted in a slurry-to-headspace ratio of 40:30 (vol/vol). Headspaces were backflushed with atmospheric air before and after an overnight preincubation period to ensure equivalent aerobic conditions at the start of the experiments. Subsamples of the slices were used to obtain root material. Treatment of the root material was executed according to the procedure of King (1994). In short, the roots and rhizomes were sorted to remove nonliving matter and other foreign material and then blotted to

remove excess water. Washed roots and rhizomes from one plant species and from the same depth were pooled before use in incubations. About 1 g of fresh root material was put into a 20-ml acrylic cylinder with a diameter of 6 cm. Roots were spread out over the bottom surface to ensure extensive contact between them and headspace atmosphere. The cylinders were sealed with screw caps containing rubber septa. Sterilized water (0.5 ml) was added to prevent drying of the roots.

Two experiments were set-up. In the July experiment, methane was added to the headspace of each of the incubation flasks and slurries of one series were amended with ammonium chloride. Methane concentrations in the slurries were calculated by using Henry's law and the Bunsen solubility coefficient for methane in freshwater at a temperature of 20°C. Root-associated consumption was tested by adding methane to the headspace of the cylinders containing root or rhizome material. In the September experiment, methane and ammonium were each added in three different concentrations. Zero-time samples were taken 10 min after the addition of these substances to allow their uniform distribution. After the incubation period, slurries and root material were dried and weighed. Samples for dissolved inorganic nitrogen analysis (5 ml) were collected from flasks treated identically to those used for gas analysis. The samples were centrifuged at -5°C for 10 min at 33,000×*g*. After measurement of its pH, the supernatant was frozen (completed within 10 min after centrifugation). Methyl fluoride (2.5% of headspace) treatments were used as a control, and possible production of methane under oxic conditions was tested by addition of bromoethanesulfonic acid (BES: 1 ml of a 10% solution). Flasks were simultaneously and continuously rotated and gently shaked at 20°C. Consequently, not only was the slurry thoroughly mixed; the thinness of the slurry layer (~2mm) also ensured optimal contact of the slurry with the headspace methane. The absence of phase transfer limitation in slurries containing different amounts of sediment was examined.

Additional experiments in the form of methane production measurements were performed. Slurries used for methane production were treated by the same procedure as that used for methane consumption but instead of backflushing with air, nitrogen gas was used. Slurries of one series were amended with acetate (final concentration, 2,500 µM), and 3 ml of hydrogen gas and 1 ml of carbon dioxide gas were added to the headspace. BES-treated slurry specimens were used as controls.

Analysis

Methane was measured with a Carlo Erba high-resolution MEGA 5340 gas chromatograph equipped with a flame ionization detector and a 3-m Haysep-Q and 2-m Molsieve column. Chromatograms were analyzed with the Maxima 820 software package. Ammonium, nitrite, and nitrate were measured colorimetrically with an automated analyzer system.

Data Analysis

Because log [CH_4] decreased linearly as a function of time (see Figure 2.1), methane consumption could be expressed with first-order kinetics: d[CH_4]/dt = k[CH_4]. From the slope of this curve, the first-order consumption rate constant (k) was calculated (per h) by linear regression. Initial methane production was calculated by linear regression analysis over the first 4 days, when the increase was linear (R^2 >0.96). Nitrate production was stimulated by higher initial ammonium concentrations (see Table 2.4), but since 40 to 70% of the added ammonium was not recoverable shortly after addition, correction of nitrate production for initial ammonium concentration was difficult. Therefore, we chose to express nitrate production with zero-order kinetics: d[NO_3^-]/dt = k_0 (in nanomolar units per h). The increase in nitrate concentration during incubation was nearly linear (R^2 >0.95).

All measurements were done in triplicate. Methane consumption results of the July and September experiments were tested by three-way analysis of variance (ANOVA) with site, ammonium, and depth in sediment, and with methane, ammonium, and depth in sediment as independent factors, respectively. In both, k was used as a dependent variable. Nitrate production was tested by two-way ANOVA, for each ammonium concentration separately, with methane and depth in sediment as independent factors and nitrate production as a dependent variable. Multiple comparisons are based on post-hoc Tukey contrasts.

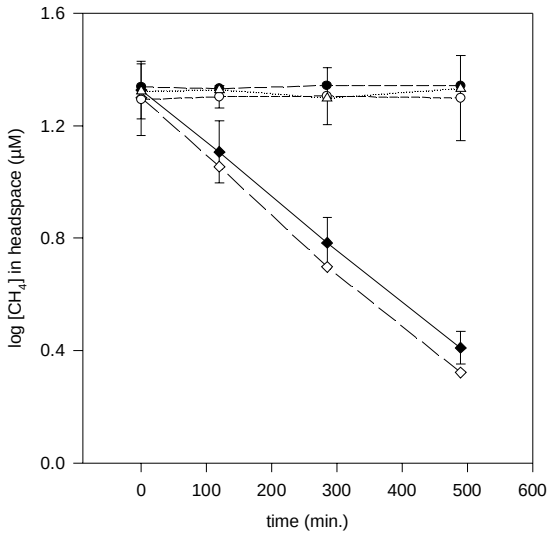

Figure 2.1 Logarithms of methane concentrations in the headspaces of oxic slurry incubation flasks. The initial methane concentration was 1.25 µM. Untreated, ♦; 5% headspace methyl fluoride, •; autoclaved prior to incubation, o; water only, Δ; 3 mM BES, ◊. The error bars represent the standard errors of the means (n=3). Presented are data for bulrush 15- to 20-cm-depth slurries.

Results

Methodology

Methane concentrations in the headspaces of flasks filled with deionized water or with slurries treated with methyl fluoride or autoclaved remained constant during incubation, whereas methane concentrations in untreated slurries and slurries treated with BES decreased according to a first-order process (Figure 2.1). Methane consumption increased linearly with increasing amounts of sediment in the slurries (Figure 2.2). Nitrate production, like methane consumption, was effectively blocked by methyl fluoride and autoclaving, whereas the ammonium concentration still decreased (data not shown).

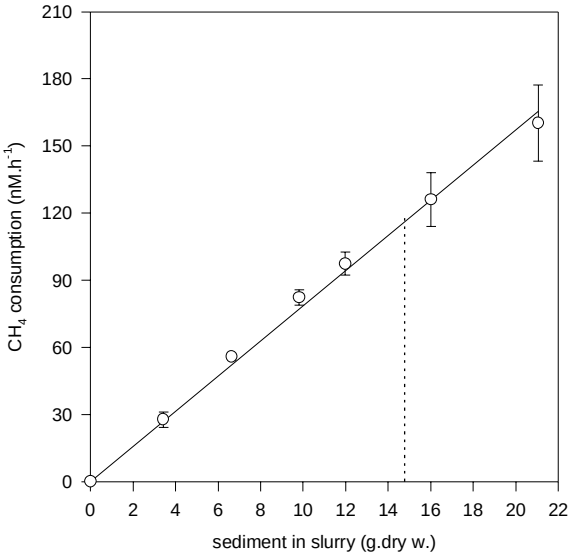

Figure 2.2 Methane consumption relative to the amount of sediment (in grams dry weight [g dry wt.]) in the slurries (bulrush, 15- to 20-cm depth). The initial methane concentration was 1.25 µM. Solid line, linear fit with R^2 >0.99; dashed line, amount of slurry (dry weight) used in the experiments. The error bars represent the standard errors of the means (n=3).

Effect of site, depth in sediment, and ammonium concentration

The results of the July experiment showed that the site, depth in sediment, and ammonium concentration had significant effects and that site and depth in sediment were a significant interaction term (Figure 2.3; Table 2.1). Consequently, the effect of the site on k depended on the

depth in the sediment and, conversely, the effect of the depth in the sediment depended on the site. For example, methane oxidation in reed slurries was 20% higher than that in bulrush slurries at a depth of 0- to 5-cm (P<0.001), nearly 53% higher at 15- to 20-cm (P<0.001), and 15% higher at 40- to 45-cm (not significant [P >0.905] (Figure 2.3). Addition of ammonium resulted in about 20% lower k values (Figure 2.3). Methane consumption associated with root material increased with depth for bulrush plants, but not for reed plants (Table 2.2). Methane consumption associated with bulrush roots from the 40- to 45-cm-depth interval was more than 16 times higher than that associated with reed roots from the same depth. Reed rhizome material was not important for consumption of methane.

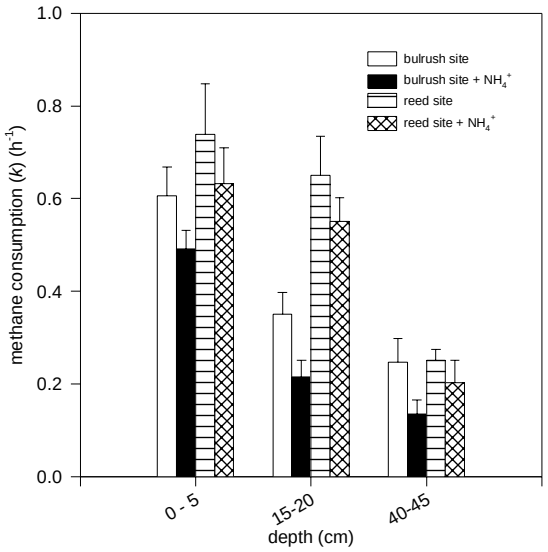

Figure 2.3 Effect of site, depth in sediment, and ammonium concentration on methane consumption. Initial methane and ammonium concentrations were 1.25 and 300 µM, respectively. The error bars represent the standard errors of the means (n=3).

The results from the September experiment (bulrush sediment only) showed that depth, ammonium addition, and methane concentration had significant effects on k (Table 2.1). The decrease in k values between the depths 15 to 20 cm and 0 to 5 cm (51%) were similar to those observed in the July experiment (49%). The decrease in k between the 40- to 45-cm and the 15- to 20-cm slurries was 20%, again, like that
seen in the July experiment. The interaction term methane by ammonium proved to be significant (Table 2.1). Consequently, the inhibitory effect of ammonium depended on the initial ammonium and methane concentrations. Inhibition was strong with relatively low methane and

relatively high ammonium concentrations, and it was when weak when these were reversed (Table 2.3). The pH varied little during incubation. With the highest concentration of ammonium added, the pH decreased from approximately 8.2 to 7.9, whereas the control remained at a pH of approximately 8.1.

Table 2.1 ANOVA tables of the July and September experiments

Statistical parameters for[a]:									
Methane consumption (3-way ANOVA)						Nitrate production (2-way ANOVA)			
July			September			September			
Factor	F-ratio	*P*	Factor	F-ratio	*P*	$[NH_4^+]$	Factor	F-ratio	*P*
D*	138	<0.0001	D*	274	<0.0001	30	D*	112	<0.0001
S*	65.8	<0.0001	M*	116	<0.0001		M	0.61	
N*	26.1	<0.0001	N*	77.7	<0.0001		D×M	0.30	
D×S*	16.9	<0.0001	D×M	3.41		300	D*	144	<0.0001
D×N	0.32		D×N	1.95			M	2.24	
S×N	0.83		M×N*	14.3	<0.0001		D×M	1.20	
D×S× N	0.17		D×M×N	1.72		1,500	D*	999	<0.0001
							M	6.97	
							D×M*	5.18	<0.05[b]

[a] Factors with significant effects are indicated with asterisks. Abbreviations: D, depth in sediment; S, site; N, concentration of ammonium; M, concentration of methane; $[NH_4^+]$. initial ammonium concentration (in micromolar units).

[b] The significant effect observed stimulates the rate of the tested variable.

The *k* values of slurries starting with 0.02 µM methane were significantly lower than those of slurries starting with 1.25 and 10 µM concentrations, whereas the difference between the values for the last two was insignificant. To determine if this observation was reproducible, we incubated freshly collected bulrush sediment with six different concentrations of methane. Again, the *k* value with 0.02 µM was significantly lower than the other ones except at the 15- to 20-cm depth, whereas the others were not significantly different (Figure 2.4).

Significant accumulation of nitrite in the slurries could not be detected (nitrite production rates were below 10 nmol g [dry weight] of sediment^{-1} h^{-1}). Nitrate production appeared to occur mainly in the 0- to 5-cm-depth slurries, with little production in the 15- to 20-cm and 40- to 45-cm slurries (Table 2.4). This is quite different from what was found with respect to methane consumption, which also occurred, although at lower rates, in the 15- to 20-cm and 40- to 45-cm slurries. Methane concentration did not have a significant effect on the rate of nitrate production in the 0- to 5-cm slurries (Table 2.1).

Table 2.2 Methane consumption rates by root material[a]

	Amt of methane consumed (nmol g [dry wt] of sediment^{-1} h^{-1})[b]:	
Depth (cm)	Reed	Bulrush
0-5	10.9 ± 2.30	4.23 ± 3.31
15-20	34.6 ± 11.9	65.2 ± 10.4
40-45	8.08 ± 3.65	146 ± 9.70
40-45[c]	0.09 ± 0.04	Not determined

[a] The initial methane concentration in the cylinders was 25 µmol l^{-1} of headspace^{-1}.

[b] Values are means ± standard errors of the means.

[c] Only consumption by rhizomes was measured.

Methane production

Production of methane without a lag-phase was observed only in the 40- to 45-cm-depth slurries. In the 0- to 5-cm and 15- to 20-cm slurries, methane production started after a lag phase of 2 to 3 days. Mean methane production measurements in the 40- to 45-cm slurries ± standard errors of the mean were 4.55 ± 0.25 and 1.16 ± 0.09 nmol g [dr.w] of sediment^{-1} h^{-1} for reed and bulrush, respectively. Addition of methanogenic precursors did not induce methanogenic activity in the 0- to 5-cm and 15- to 20-cm slurries but increased production dramatically (about threefold) in the 40-45 cm slurries. Methane production was not observed in the slurries treated with BES. The absence of methanogenic activity coincided with the omnipresence of oxidized iron plaques in the sediment of the corresponding horizons (data not shown).

Table 2.3 Methane oxidation inhibition percentages in bulrush site slurries from different depth horizons with different initial ammonium concentrations

Initial conc (µM) of:		% Inhibition of methane oxidation at depth (cm) of[a]:		
NH_4^+	CH_4	0-5	15-20	40-45
300	0.2	28	59	68
	1.25	8	23	17
	10	2	0	0
1,500	0.2	90	94	100
	1.25	17	46	45
	10	14	33	10

[a] Inhibition percentages for the 300 and 1,500 µM treatments are relative to the 30 µM treatment values.

In situ methane and ammonium pore water concentrations

The vertical profile of methane showed distinct patterns for the bulrush and the reed sites (Figure 2.5). In the surface layer, methane concentrations were between 0.1 and 5 µM (the concentration in equilibrium with atmospheric methane is ~0.003 µM) and methane levels approached the methane saturation concentration at depths >50 cm (approximately 800 µM at ambient temperature and salinity). At both sites, ammonium concentrations never exceeded methane concentrations except in the upper 4 cm of the sediment, where ammonium concentrations reached levels of more than 70 µM. Concentrations of dissolved nitrite and nitrate were measurable only in the top few centimeters (1.5 and 40 µM, respectively). The *in situ* pore water pH values ranged between 7.4 and 7.7, with the highest values being at the sediment surface.

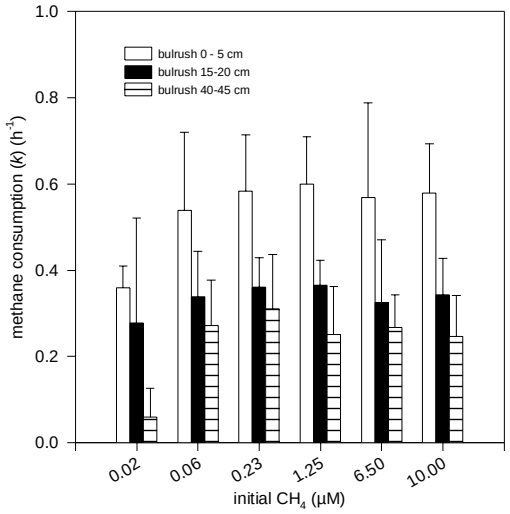

Figure 2.4 Effect of methane concentration on methane consumption. The error bars represent the standard errors of the means (*n*=3).

Discussion

Methodological considerations

Methane consumption was not attributable to leakage, and methane consumption rates were proportional to the amount of sediment, indicating that gas transfer between gas phase and the slurry phase was not rate limiting (Figure 2.2). The absence of methane consumption and nitrate production in the methyl fluoride-treated and autoclaved water specimens points out that methane consumption and nitrate production in our slurries were biologically mediated aerobic oxidation processes. Methyl fluoride specifically inhibits methane and ammonium oxidation (Miller et al. 1993; Oremland & Culbertson 1992a). The fact that the methane uptake capacity of the BES-treated samples was almost identical to that of the controls indicates that methane was not produced during oxic incubation. Ammonium loss, as revealed by the low-level recovery of ammonium shortly after addition, was not effectively blocked by methyl fluoride or autoclaving; hence, processes other than oxidation of ammonium (i.e. nonbiological processes) influenced ammonium consumption, such as adsorption of ammonium to exchange sites in the slurry. Oxic incubation periods were always less than 9 h, leaving little time for substantial growth of microorganism.

Table 2.4 Nitrate production rates in bulrush site slurries with different amounts of ammonium added

	Nitrate production rate (nmol g [dry wt] of sediment^{-1} h^{-1}) at initial [NH_4^+] (μM) of [a]:		
Depth (cm)	30	300	1500
0-5	38.9± 5.52	186 ± 10.6	597 ± 17.9
15-20	15.0± 4.37	33.9 ± 4.70	50.2 ± 4.35
40-45	0.96± 0.47	1.92 ± 0.59	0.50 ± 0.44

[a] Values are means ± standard errors of the means.

Observations of both *in vitro* anaerobic methanogenic and *in vitro* aerobic methanotrophic activities in the same samples (as were made for the 40- to 45-cm-depth slurries in the present study) have been reported before (King 1990; Roslev & King 1996). The methanotrophic population present at this depth in the sediment probably comprises types that are able to survive longer periods of anoxia. These anoxia-tolerant methanotrophs occur favorably in vegetated and intertidal sediments, where anoxic and oxic periods alternate continuously (King 1992). One of the features of these types of methanotrophs, also observed in our slurries, is the absence of a lag phase during incubation (Jones & Morita 1983; King 1992). The potential for methane oxidation measured in our slurries is reproducible, and representative (for longer periods of time) *k* values in the July and September experiments were similar.

Initial concentrations of dissolved methane concentrations in our experiments (0.02 to 10 μM) are below or in the lower range of K_m values reported in literature for wetland sediment and

methanotrophs associated with aquatic plant roots (King 1992, 1994). The similarity of *k* values, except when very small amounts of methane were added, indicates that methane concentrations used were indeed lower than or similar to the K_m value for these sediments. The apparent decrease in *k* at very low levels of added methane might have been the result of the biphasic kinetics of high-affinity activity (dominant at relatively low levels of added methane) and low-affinity activity (dominant at relatively high levels of added methane). It has been suggested that biphasic kinetics arises from mixed methanotrophic bacteria populations (Bender & Conrad 1992) and specific properties of the monooxygenase enzyme complex (Dalton et al. 1990).

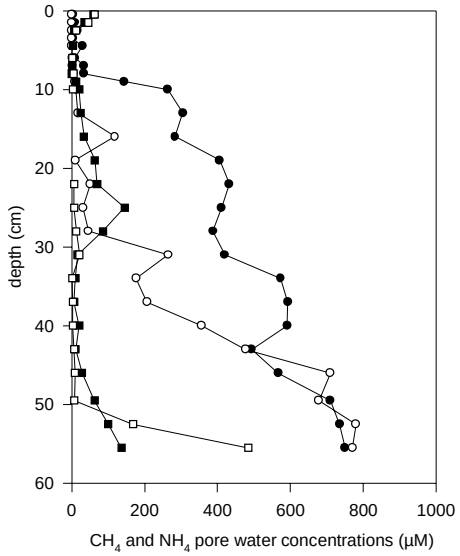

Figure 2.5 Pore water methane (circles) and ammonium (squares) concentrations at the bulrush (open symbols) and reed sites (solid symbols).

Effect of site and depth in sediment

Methane-oxidizing bacteria are generally oxygen limited and methane saturated in the deeper parts of the sediment and possibly methane limited and oxygen saturated in the uppermost layer of the sediment (King 1992, 1994; Sundh et al. 1995). Oxidation rates in our sediments are probably determined by oxygen availability. Pore water methane concentrations increase with depth (Figure 2.5), whereas the potential for methane oxidation decreases with depth (Figure 2.3 and 4). However, in the 0- to 5-cm-horizon pore water, methane concentrations are relatively low, and it is not clear whether oxygen or methane confines upper limits for oxidation.

Because of the rinsing of the root material prior to incubation, only methanotrophs tightly attached to the roots are included; hence, root-associated methane oxidation may have been underestimated. Nevertheless, the strong increase of methane oxidation with depth associated with bulrush roots indicates the importance of these plants in methane oxidation at greater depths. Apparently methanotrophs at greater depth live near or even within the roots of the bulrush plants, as demonstrated previously for several other macrophyte species (Gerard & Chanton 1993; King 1994). The increase in root-associated methane oxidation with depth was not observed for root material from reed plants, suggesting that they are not as important as bulrush plants for methane oxidation.

It appears that methane oxidation in bulrush slurries decreases with depth (Figure 2.3; Table 2.1), whereas that associated with roots increases with depth (Table 2.2). Moreover, methane oxidation levels in reed slurries are higher than those of bulrush slurries, although reed root material has a limited methane oxidation capacity. This discrepancy cannot be resolved by invoking differences in root biomass. In a parallel study, we investigated *in situ* rates of methane oxidation in the rhizosphere of bulrush and reed plants growing in permanently flooded sediment by using the methyl fluoride flux chamber inhibition technique (van der Nat & Middelburg 1998b). In that study, we observed that the methane oxidation rate in the rhizosphere of the bulrush was two to three times higher than that in the rhizosphere of the reed.

The bulrush and reed sites differ not only in their dominant plant species but also in their heights above sea level. At both sites, methane production was not observed up to at least 20 cm in depth and ferric iron was omnipresent in the sediment. Moreover, during summer, large cracks in the sediment down to a depth of 25 cm were observed at low tide. So, despite regular flooding, the upper part of the site is not permanently saturated with water, as evidenced by a lower dialysis cell yield. Because of the difference in the altitudes of the sites and consequent differences in time of exposure to atmospheric air and duration of low-tide periods, we suggest that oxygen directly penetrates into the sediment more frequently and in larger amounts at the reed site than at the bulrush site. The lower water content measured in the upper 50 cm of the sediment at the reed site (mean ± standard error, 45% [wt/wt] ± 1.8) compared to the bulrush site (54% [wt/wt] ± 1.7) supports this suggestion.

Effect of ammonium

Inhibition by ammonium was found to occur in all sediment samples, and its level depended on the methane and ammonium concentrations. Various studies (Boeckx & Cleemput 1996; Bosse et al. 1993; Dunfield & Knowles 1995; King & Schnell 1994b; Schnell & King 1994) using different types of soil and sediment have reported that the degree of ammonium inhibition of methane oxidation depends on the concentrations of methane and ammonium. Because of the low specificity of the methane monooxygenase enzyme for ammonium and methane (Bédard & Knowles 1989), as well as the production of hydroxylamine and nitrite through ammonium oxidation by methanotrophs (methanotrophic ammonium oxidation) and ammonium oxidizers, a combination of bot a competitive mechanism and a toxic mechanism might explain the increase in methane oxidation inhibition observed with increasing amounts of ammonium. The contribution of either mechanism to total inhibition is difficult to establish (Boeckx &

Cleemput 1996; Bosse et al. 1993; Dunfield & Knowles 1995). Nevertheless, competitive inhibition is probably the most important mechanism in our slurries, as indicated by a significant interaction between methane and ammonium (Table 2.1) and by the decrease in methane oxidation to zero with increasing ammonium concentrations (Table 2.3). We found no effect of methane on nitrate production (Table 2.1), as has been reported before (Boeckx & Cleemput 1996; Bosse et al. 1993; Dunfield & Knowles 1995). Ammonium is probably the more aggressive substrate, so even moderate concentrations can exclude methane from the active site on the monooxygenase enzyme, but not vice versa.

Initial methane and ammonium concentrations in the slurries were chosen to range from concentrations found in the surface layer to ten times more for ammonium (Figure 2.5). This range was chosen because we expected inhibition of methane oxidation by ammonium to be more important in the surface layer than in deeper parts of the sediment, because the ratio of methane to ammonium increases with depth.

Our incubation results indicate that ammonium concentrations have to exceed methane concentrations by at least 30-fold to be effective for methane oxidation inhibition. Ratios of *in situ* concentrations of ammonium and methane in the pore water are usually far below 30 except in the surface layer (Figure 2.5). The sediments get flooded with ammonium-rich water (>150 µM), resulting in relatively high pore water ammonium concentrations in the surface layer while methane concentrations in this layer are low (Figure 2.5). Therefore, inhibition might be important in the surface layer, especially directly following flooding. The ecological relevance of ammonium inhibition of methane oxidation at greater depths is limited because of the relatively low ammonium concentrations there, probably caused by high-level uptake by plants and relatively high methane concentrations.

It has been postulated that NH_3 rather than ammonium is the inhibitor for methane oxidation (O'Neill & Wilkinson 1977). Slurry NH_3 concentrations are less than ~5% of the added ammonium concentration given measured slurry pH values and the fairly large amount of added ammonium readily immobilized by sediment adsorption. As such, NH_3 would be a more effective inhibitor of methane oxidation than ammonium, and thus the pH might be important in the process of ammonium inhibition. When NH_3 is the inhibiting species, the calculated ammonium/methane ratio for effective inhibition represents an minimum estimate for *in situ* conditions because *in vitro* slurry pH values were higher than *in situ* values.

Finally, we observed very low rates of nitrate production in the 15- to 20-cm and 40- to 45-cm-depth slurries (Table 2.4), whereas methane oxidation still occurred. Ammonium oxidation is therefore not an important process at depths of >15 cm. Ammonium oxidation might be outcompeted for oxygen by methane oxidation and other oxygen-consuming processes, like ferrous iron oxidation, as indicated by the presence of iron plaques, or it might be limited due to a lack of ammonium.

In conclusion

The vertical distribution of methane oxidation in intertidal marshes is probably determined more by hydrological conditions that change with distance from the tidal river rather than by the dominant type of vegetation present. Inhibition of methane oxidation by ammonium may occur in

vegetated intertidal sediments. However, due to relatively high methane and relatively low ammonium concentrations, inhibition by ammonium is not as important as has been reported for agricultural and forest soils.

Chapter 3

Oxidation in the rhizosphere of macrophytes: consequences for elemental cycles

Abstract

The pore-water chemistry of sediments covered with *Phragmites australis* (common reed) and *Scirpus lacustris* (bulrush) are compared with those of identical sediments without plants. Plants affect the biogeochemical cycles in sediments primarily through uptake of essential nutrients and release of oxygen. Oxygen release results in the mobilisation of uranium and formation of iron and manganese (oxy)hydroxides following oxidation of reduced iron and manganese. These newly formed metal(oxy)hydroxides scavenge nutrients (silica), trace elements (arsenic) and dissolved organic matter from solution and consequently limit the mobility of these elements. Bulrush appears to have a larger capacity to oxidize the rhizosphere than reed.

Introduction

Emergent aquatic macrophytes such as *Phragmites australis* (common reed) and *Scirpus lacustris* (bulrush) are dominant features of many littoral systems in temperate zones. Despite their importance in terms of biomass, productivity and areal coverage, the biogeochemistry of their hosting sediments has received rather limited attention. Studies of sediment and pore-water chemistry have traditionally been focused on non-vegetated sediments, that usually appear zoned with respect to the dominant organic matter decomposition process (Berner 1980). Sediments hosting a large and active population of benthic animals may not show such a simple biogeochemical zonation because of intense pore-water irrigation and bioturbation (Boudreau 1996).

Similarly, sediment covered with macrophytes are characterised by a complex three dimensional, time-dependent, distribution pattern of biogeochemical processes, because the root system of these plants affects the biogeochemistry of their hosting sediments in various ways (Vale & Sundby 1997). They may directly affect sedimentary cycles (1) through uptake of water, nutrients and other essential elements, (2) through exudation of organic compounds that may fuel respiration processes or solubilize nutrients from otherwise difficult to assess pools and (3) through release of oxygen from the roots into the sediments (Saleque & Kirk 1995). They may also indirectly affect sedimentary cycles through enhanced transport of soluble, and in particular gaseous, components (Chanton & Dacey 1991), through stabilisation of sediments and canopy friction induced particle and organic matter trapping (Leonard & Luther 1995).

Many of these macrophyte effects on sediment biogeochemistry are tightly coupled and it is therefore instructive to distinguish between primary, secondary and tertiary effects (Figure 3.1). Primary effects include plant uptake of nutrients such as ammonium and phosphate and plant release of oxygen and organic compounds. Secondary effects as a result of oxygen release to the rhizosphere include chemical and microbial oxidation processes such as nitrification, methane oxidation and oxidation of reduced manganese, iron and sulphur compounds (Reddy et al. 1989; Roden & Wetzel 1996; Sundby et al. 1997). Secondary interactions also comprise root exudate supported methanogenesis (Minoda & Kimura 1994) and sulphate reduction (Isaksen & Finster 1996). Tertiary effects include denitrification of the nitrate resulting from nitrification (Reddy et al. 1989), co-precipitation and sorption of trace elements, phosphate and dissolved organic matter following the formation of iron(oxy)hydroxides (Sposito 1984), suppression of methanogenesis because of enhanced iron-oxide reduction (Roden & Wetzel 1996; van der Nat & Middelburg 1998a), and dissolution of sedimentary carbonate as a result of acid generation (Middelburg et al. 1996b).

Because of these direct and indirect macrophyte effects 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, enhanced rates of sulphate reduction or methane production in the rhizosphere may be due to exudate release, result from enhanced particulate organic matter inputs due to below-ground allocation of organic matter (direct effects) or from canopy friction induced trapping of particulate organic matter

(an indirect effect). Similarly, rhizosphere oxidation may result from root oxygen release (a direct effect) or from sediment water desaturation caused by enhanced rate of evapotranspiration (an indirect effect). The most direct approach to establish the effect of macrophytes on the sediment biogeochemistry is to compare the pore-water chemistry of non-vegetated sediments with identical sediments hosting macrophytes. Any difference in pore-water composition between the control (non-vegetated system) and vegetated system can then be ascribed to the macrophyte, because differences in sediment composition are excluded. This comparative approach has been applied in studies that employed either artificial substrates such as hydrocultures or confined compartments, or pot experiments with non-representative root/sediment ratios (Saleque & Kirk 1995; Reddy et al. 1989; Sheppard & Evenden 1991). Moreover, these studies were mostly limited to young plants (seedlings or first growing season).

In this study we will present results from a large scale mesocosm experiment in which we have grown reed and bulrush for four plant growth cycles under controlled conditions. Pore-water data for iron, manganese, ammonium, silicate, dissolved organic carbon, arsenic and uranium in mesocosm sediment with and without plants are compared. This comparison provides not only useful information on macrophyte effects at the ecosystem level, but may also help in the evaluation and design of constructed wetlands for waste water treatment. Constructed wetlands and macrophyte filter beds with reed and bulrush are regularly used to remove nitrogen and phosphorus from waste water systems (e.g. Gersberg et al. 1986; Wathugala et al. 1987), yet their effect on trace element mobilisation is poorly known (Sheppard & Evenden 1991; Sundby et al. 1997).

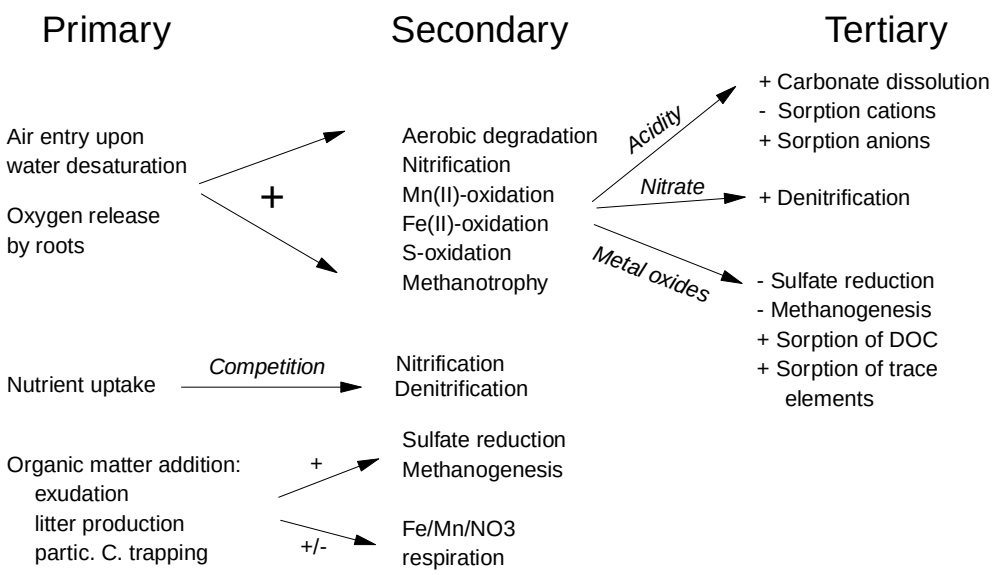

Figure 3.1 Effect of plants on biogeochemical processes in sediments.

Material and methods

A detailed description of the mesocosm experiment is given elsewhere (van der Nat & Middelburg 1998b) and a brief description is given below. Six identical, fully separated, 1.3 m^2 large containers (l×w×h = 1.25×1.25×0.85 m) were each filled with about 0.9 m^3 sediment and were placed in a temperature (18 ± 0.5 ^oC), humidity (70 ± 7%) and CO_2 concentration (380 ± 40 ppmv) controlled room. The sediment was obtained by thorough mixing of air dried and sieved tidal freshwater, reed and bulrush dominated, marsh sediments with drainage channel mud. During the 5 month period of settling and accommodation, the sediment was wetted with tap-water to ascertain similar waterlogged conditions. Hereafter, sediments were maintained under a water layer of 3 to 4 cm by adding deionized water to compensate water loss. Reed and bulrush seedlings of 5 to 8 cm height, grown from seed, were planted in a density of 100 seedling m^{-2} in two containers each. A 16 h day period with a light intensity of 0.25 mE m^{-2} s^{-1} at the sediment surface was set for the reed and bulrush containers. The control sediment was covered with floating grains to reduce light and so limit microphytobenthos growth. Typical maximum above-ground biomass values obtained are 700 and 400 g dr w m^{-2} for bulrush and reed, respectively, though growth of the latter was limited by the height of our experimental set up (2 meter maximum). Successive plant growth cycles (about 6 months) were separated by 3-week periods of darkness. Results are presented for mid-season of plant growth cycle 4 and for single containers only since replicate systems gave similar results for pore-water profiles of ΣCO_2, CH_4, ammonium, nitrate, phosphate and silicate.

Pore-water samples were obtained by *in situ* dialysis, using a peeper (Hesslein 1976) that contains 25 membrane cells that are in contact with the sediments via a 0.2 mm inert acrylic co-polymer membrane filter (Versapor-200, Gelman Sciences). 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 via the other port. De-oxygenated de-ionized water was used to refill the compartments after sampling. Acidified pore-water were analysed for iron and manganese using a graphite furnace atomic absorption spectrometer with Zeeman background correction (Perkin Elmer 3030), and for uranium-238 and arsenic-75 using a Fisons Plasma VG-PlasmaQuad II inductively coupled plasma mass spectrometer. Silicate and ammonium were measured with colorimetric auto-analyser techniques. Dissolved organic carbon was measured with an UV-wet chemical oxidation technique. A selection of four samples from each profile (depths 3.5, 15.5, 24.5, 45.5 cm) were freeze-dried, then acidified and the C/N, $\delta^{13}C$, $\delta^{15}N$ characteristics of the dissolved organic matter were

measured using a Carlo Erba Elemental analyser coupled via a con-flow 2 interface with a Finnigan Delta S mass spectrometer. Leaf and stem material was analysed for organic C, and N using a Carlo Erba Elemental analyser and for P and Si by colorimetry.

Results

Control sediments

Concentrations of dissolved iron first increase with depth and then seem to reach an asymptote of about 2 mM (Figure 3.2a). Interstitial water concentrations of manganese first increase to a maximum of about 150 μM at a depth of 12 cm and then decrease to about 75 μM at depths of 20 cm and more (Figure 3.2b). These asymptotes at depth can be attributed to either solubility or exchange equilibrium with a variety of (authigenic) solid phases or to exhaustion of the reactive iron and manganese pools with depth (Middelburg et al. 1987; Burdige 1993). Interstitial water concentrations of uranium are very low at all depths (<1.5 nM; Figure 3.2d), whereas those of arsenic increase with depth and reach levels up to 3-6 μM (Figure 3.2c). Dissolved organic matter, ammonium and silicate increase rapidly with depth and reach their asymptotes of about 3, 1.2 and 0.75 mM at depths of 3, 15 and 7 cm, respectively (Figures 3.2e,f,g).

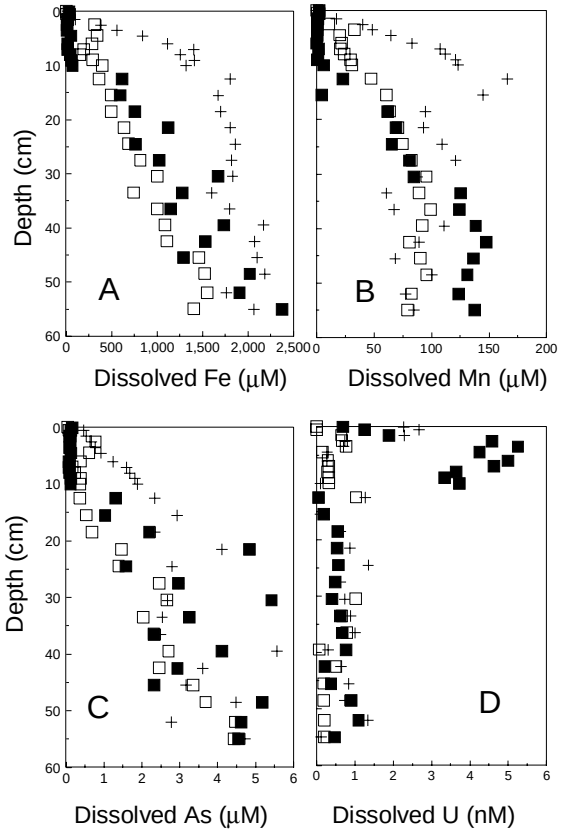

Figure 3.2 Concentration versus depth profiles. Control: crosses; Solid squares: bulrush; Open squares: reed. A: Fe (μM); B: Mn (μM); C: As (μM); D: U (nM); E: DOC (μM); F: NH4 (μM); G: Si (μM).

Vegetated sediments

Although the asymptotic values of the control and vegetated systems are rather similar, there are pronounced differences in the upper 10 cm's (Figure 3.2). Dissolved ammonium concentrations are strongly depleted relative to the control down to depths of 30 and 45 cm in bulrush and reed sediments, respectively. In the upper 10 cm of vegetated sediments, dissolved iron, manganese, arsenic, silica and organic carbon concentrations are significantly lower than those in control sediments without vegetation. In contrast, dissolved uranium concentrations in the upper 10 cm's of especially the bulrush vegetated sediments are higher than those of control sediments. Moreover, it seems that dissolved

iron, manganese and arsenic are lower in the upper 10 cm's of bulrush systems, whereas dissolved organic carbon and silicate are lower in the reed systems (Figures 3.2e,g,h). However, the characteristics of dissolved organic matter are not significantly different between control and vegetated sediments or between reed and bulrush systems (Table 3.1), and do not show changes with depth (data not shown). It should be realized that any ammonium in the pore-water not being volatilized during the sample handling and pre-treatments may have affected our $\delta^{15}N$ values and C/N ratios. pH values in the reed (7.0 ± 0.1) and bulrush (7.0 ± 0.1) sediments are similar to those in the control sediments (7.0 ± 0.3).

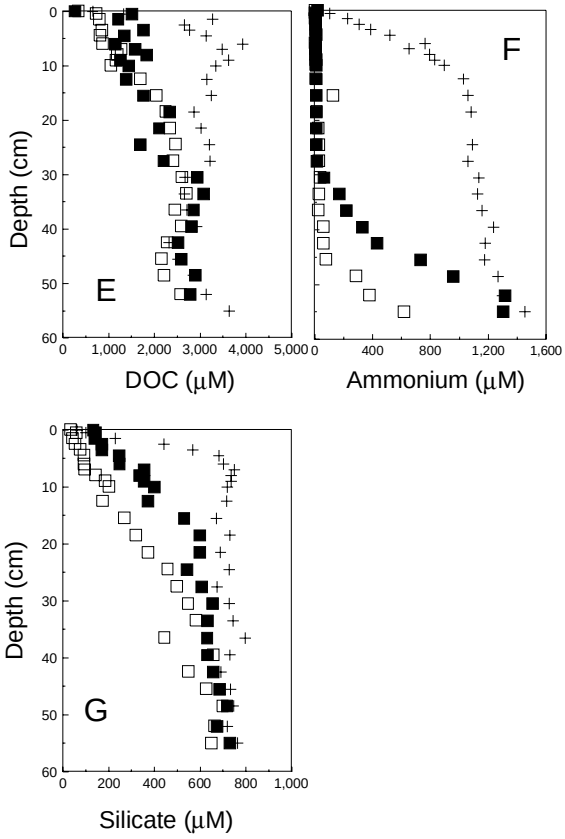

Figure 3.2 continued

Discussion

Our pore-water data can be used to put some constraints on the relative importance of various plant effects on highly anaerobic sediments. Our reasoning is based on the assumption that the microbial and chemical processes resulting in liberation of iron, manganese, silicate, arsenic and dissolved organic carbon from the solid-phase to the pore-waters in the vegetated sediments were, at least initially, similar to those in the control sediments.

The absence of any difference in dissolved organic matter characteristics (Table 3.1) and the lack of higher dissolved organic matter concentrations in the vegetated systems relative to the control (Figure 3.2c) indicate that exudation, might it occur, is not of significant importance for the standing stock of dissolved organic matter. Our mesocosm study shows that reed and bulrush systems have lower concentrations for most pore-water constituents in the upper 10 cm's of sediment relative to the control, except for uranium (Figure 3.2). Lower pore-water concentrations may result from either root uptake (a primary effect) or from secondary and higher order effects induced by rhizosphere oxidation. Oxygenation resulting from air entry upon water desaturation due to high rates of evapotranspiration can probably be excluded since a water layer of 3 to 4 cm was maintained above the sediments.

Table 3.1 Dissolved organic matter composition

	C/N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Control	17.4 ± 2.4	-26.6 ± 0.6	-6.0 ± 1.1
Reed	17.1 ± 2.1	-26.2 ± 0.1	-4.6 ± 2.1
Bulrush	21.9 ± 9.6	-26.7 ± 0.8	-5.1 ± 2.4
ANOVA			
F value	0.636	0.764	0.511
P	0.562	0.494	0.616
N	9	12	12

The control sediments are characterised by high pore-water levels of iron, manganese (Figure 3.2) and methane (~800 μM; van der Nat & Middelburg 1998a). Any oxygen lost from the roots of reed and bulrush, and entering the anoxic sediments, will rapidly be consumed by oxidation of these reduced components. Oxidation of iron(II) and manganese(II) results in lower pore-water concentrations, because their oxidized forms are readily precipitated as iron- and manganese(oxy)hydroxides (Burdige 1993; Sundby et al. 1997). Iron-oxide rich root precipitates have been reported for a number of wetland plants, including reed (Snowden & Wheelers 1995). These newly formed metal-(oxy)hydroxides have very high specific surface areas and act as efficient scavengers for many trace elements, dissolved organic matter, phosphate and silicate (Sposito 1984). The decrease of dissolved arsenic in the upper 10 cm's of vegetated sediment relative to the control can

be attributed to sorption and co-precipitation of arsenic with Fe(oxy)hydroxides (Sullivan & Aller 1996). Removal of arsenic from the pore-water can be considered a tertiary effect since the primary effect (oxygen release) acts on arsenic via oxidation and precipitation of iron (secondary effect). Similarly, the consumption of dissolved organic carbon in the rhizosphere might also be related to interaction of dissolved organic carbon with newly formed metal oxides (Tipping 1981).

Table 3.2 Elemental composition plants (wt %)

	C	N	P	Si
Phragmites				
Fresh leaves	44.45	3	0.27	0.9
Senescence leaves	41.83	2.56	0.27	1.24
Stem	44.63	0.78	0.15	0.72
Scirpus				
Leaves/Stem	38.74	2.17	0.31	0.80
Flower	47.41	1.63		1.19

Concentrations of dissolved uranium in the control sediments and at depth in the vegetated sediments are very low (<1.5 nM), consistent with observations in the literature that uranium is removed from solution in anoxic environments (Klinkhammer & Palmer 1991). These studies have also reported that mobilisation of uranium may occur upon oxidation. The enhanced pore-water concentrations of uranium in especially the rhizosphere of bulrush (up to 5-6 nM) can therefore be used as further evidence for rhizosphere oxidation.

The enhanced levels of uranium and lower levels of iron and manganese in the rhizosphere of bulrush relative to reed (Figure 3.2) indicate that bulrush has a larger capacity to oxidize the rhizosphere than reed. This is consistent with methane oxidation estimates based on the methylfluoride flux inhibition technique showing that bulrush and reed oxidize 35 ± 20% and 16 ± 8% of the methane produced, respectively (van der Nat & Middelburg 1998b). That study also showed pronounced variability of rhizosphere oxidation over the course of a plant growth cycle. Hence, our one time observations may not necessarily be representative for the whole growth season.

Nitrogen is a major component of plant tissue and therefore ammonium is strongly depleted in the rhizosphere (Figure 3.2f). The difference in depletion depths (bulrush about 30 cm and reed about 45 cm) is related to a difference in the distribution of active roots (van der Nat & Middelburg 1998a). However, ammonium may also be oxidized in the rhizosphere (Reddy et al. 1989). The relative importance of plant uptake and nitrification as ammonium sinks can not be determined with the data at hand.

The consumption of silica within the rhizosphere of reed and bulrush can be attributed to interaction with newly formed iron(oxy)hydroxides (e.g. Loder et al. 1978; Mayer & Jarrel 1996;) and to root uptake, as silica may be an essential nutrient (Herman et al. 1996;

Alexandre et al. 1997) and reed and bulrush contain about 0.7-1.2 wt% Si (Table 3.2). Separation of these two processes requires detailed knowledge of the Fe/Si ratio of the precipitate forming in the rhizosphere or on silica uptake by roots of reed and bulrush, neither of which is available.

In conclusion, it appears that bulrush and reed have a large potential to oxidize the rhizosphere, with bulrush having a larger oxidizing capacity than reed. Rhizosphere oxidation result in the formation of metal(oxy)hydroxides that scavenge nutrients, trace elements and dissolved organic matter. As such, the oxidized rhizosphere limits escape of pore-water components from the reduced sediments to the overlying water and may therefore limit the mobility of many compounds. However, some other elements such as uranium and technetium (Sheppard & Evenden 1991) that are more soluble under more oxidized conditions may be mobilised. These processes, and the seasonality of rhizosphere oxidation, should be taken into account when macrophytes are used to remove nitrogen and phosphorus from sewage and wastewater.

Chapter 4

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

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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.

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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. 1989; 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 1998b).

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 h 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×w×h = 1×0.8×0.7 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-h 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 1998b). 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≥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 min) 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 h 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 h 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):

$$D_{ij} = 0.0026280 \frac{\sqrt{T^3 (M_i + M_j)/(2M_i M_j)}}{p\sigma^2{}_{ij}\Omega_{ij}}$$

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_{CH4-He} 0.621 $cm^2.s^{-1}$ and D_{CH4-N2} 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 h, 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.

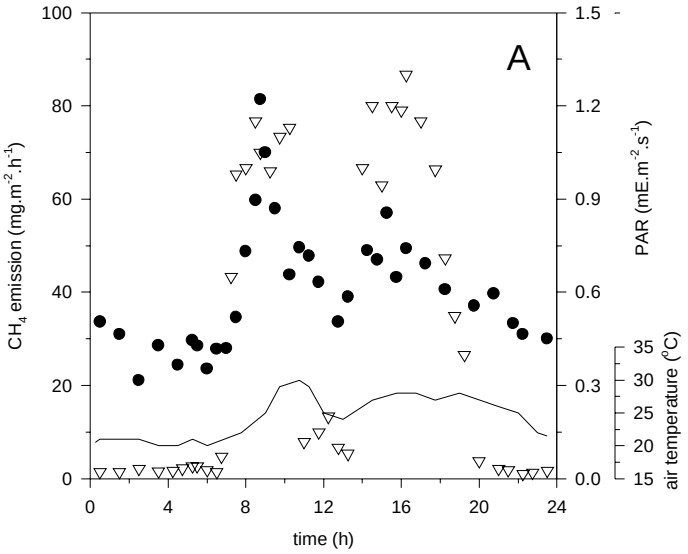

Figure 4.1 Diel variation of methane emission (solid circles) from *Phragmites* (panel A), *Scirpus* (panel B) and non-vegetated sediment (panel C) under naturally varying light (open inverted triangles), air temperature (solid line) and sediment temperature (dotted line) conditions. The decrease in 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.

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 4.1a). 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 of the day (Figure 4.1b). 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 4.1c). 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.

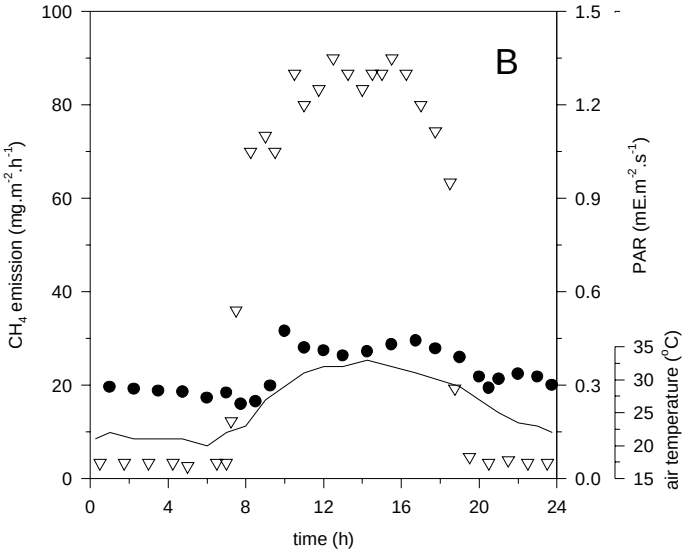

Figure 4.1 continued

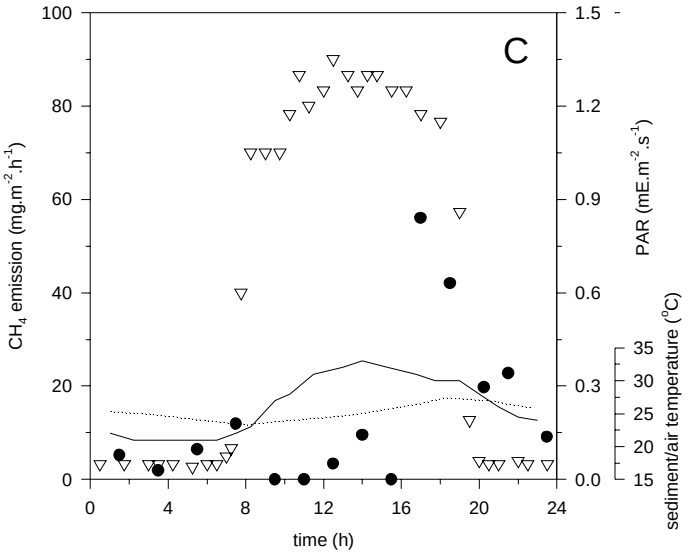

Figure 4.1 continued

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 4.2). The dark to light transition resulted in emission peaks for *Scirpus* and *Phragmites* similar to those observed outdoors following 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 4.1). Unlike methane emission, changes in illumination were not immediately followed by changes in chamber humidity levels. The first changes were observed more than 1 h 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 4.3).

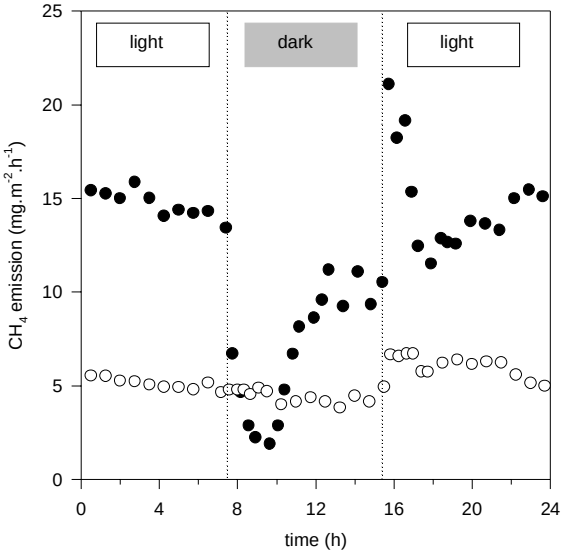

Figure 4.2 Effect of light and dark periods and light/dark transitions on methane emission from *Phragmites* (solid circles) and *Scirpus* (open circles) under controlled conditions. CO_2 uptake during light was not compensated for.

Lacunal methane (outdoors)

Methane concentrations within *Phragmites* and *Scirpus* stems decreased rapidly relative to stem height, starting at the stem base (Figure 4.4). The stem height at which methane was found in excess of ambient levels (± 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 4.4). The volume of the first 30 cm of *Phragmites* and *Scirpus* stems averaged 1.92 ± 0.65 and 9.32 ± 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 4.2). 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.

Table 4.1 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 errors of the means (*n*=3).

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 (Figures 4.5a,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.

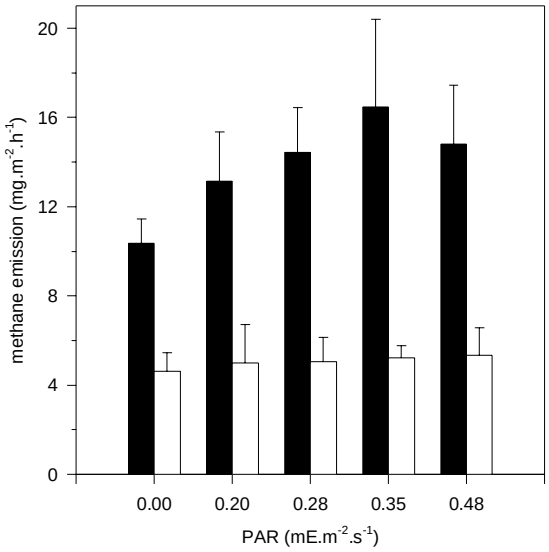

Figure 4.3 Effect of increasing light on methane emission from *Phragmites* (black bars) and *Scirpus* (open bars) under controlled conditions. The error bars represent standard errors of the means (*n*=3).

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 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 (Figures 4.1a,b) and temperature controlled diel cycles (Figure 4.2), which suggested that variations in air and sediment temperature did not play an important role in controlling the *Phragmites* and *Scirpus* diel emission pattern. 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 4.1) 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).

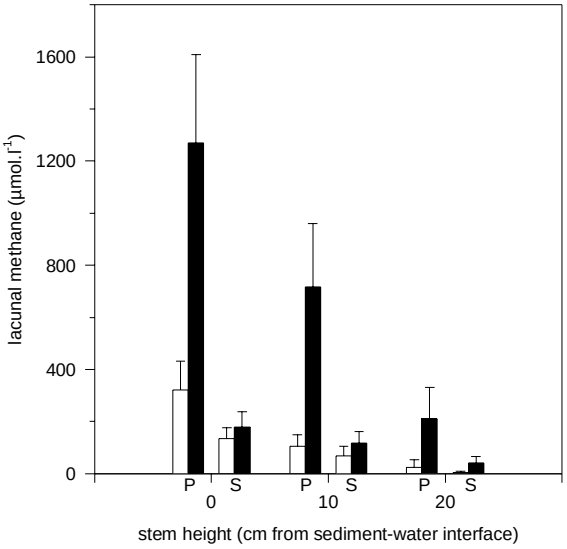

Figure 4.4 Methane concentrations within *Phragmites* (P) and *Scirpus* (S) stems at daytime (open bars) and night-time (black bars) under naturally varying conditions. At daytime, maximum PAR, air temperature and sediment temperature were 1 $mE.m^{-2}$ s^{-1}, 25°C and 22°C, respectively. At night-time: 0.04 $mE.m^{-2}$ s^{-1}, 18.3°C and 20.9°C, respectively. The error bars represent standard errors of the means (*n*=15).

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 4.1a). Diel differences in emission (Figures 4.1a; 4.2) and lacunal methane concentrations (Figure 4.4) 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 4.4) when diffusion dominated gas transport. Similarly, the very low fluxes following darkening (Figure 4.2) 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.

Table 4.2 Methane emission in air, nitrogen and helium

		Emission (mg m^{-2} h^{-1})			Ratio He/N_2	Diffusion (%) $(He-N_2)/2.13$	Oxidation (%) $1-air/N_2$
		Air	N_2	He			
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	L/D				3.13		

Measurements were conducted under controlled conditions. CO_2 uptake during light was not compensated for. ± represents the standard errors of the means (*n*=3).

It has been shown that the degree of pressurisation in *Phragmites* depends on 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 4.3) and indicates that convective transport 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. 1989; Chanton et al. 1993; Denier van der Gon & Breemen van 1993; 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. 4.1b; 4.2), and lacunal concentrations (Figure 4.4) 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 4.3) 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*.

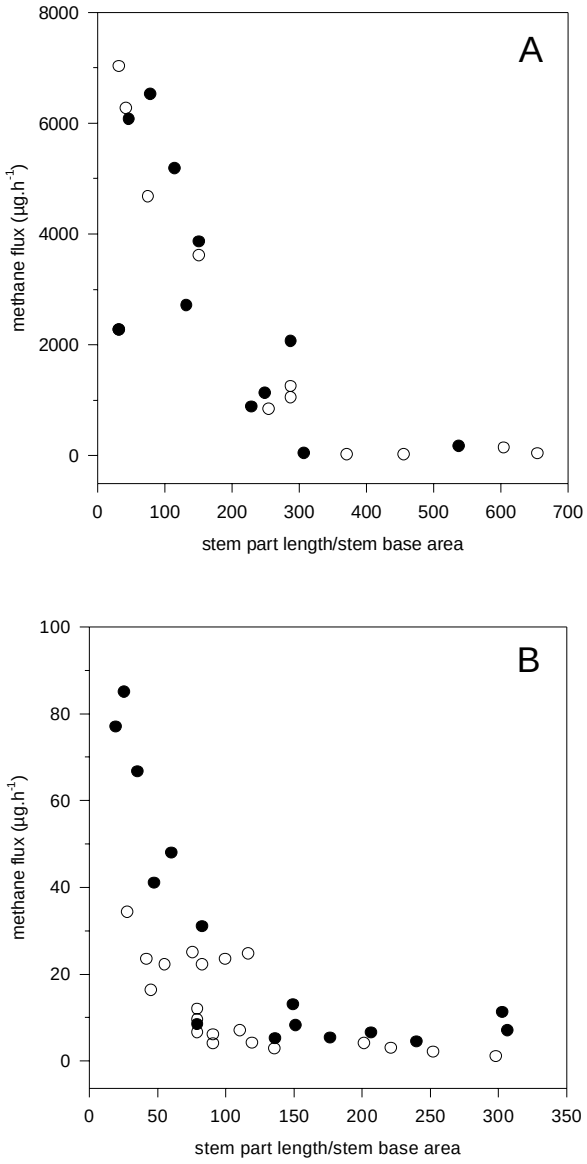

Figure 4.5 *In vitro* flux rates of methane under light (open circles) and dark (solid circles) conditions through stem parts, varying in length and stem base area, of *Phragmites* (panel A) and *Scirpus* (panel B). Illumination levels were in between 0.7 and 0.9 mE m^{-2} s^{-1}. The variation in temperature under dark and light conditions was less than 2°C.

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 4.2). 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 4.2) were less than those measured in the same system with the methylfluoride flux chamber inhibition technique (van der Nat & Middelburg 1998b). 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 h 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 4.5a,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 4.5a). 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 4.1b; 4.2) and lacunal concentrations (Figure 4.4), 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 4.2) 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 4.5b). 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.

Chapter 5

Effects of two common macrophytes on methane dynamics in freshwater sediments

Frans-Jaco W.A. van der Nat and Jack J. Middelburg

Biogeochemistry 43: 79-104, 1998

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* versus ~80% for control). Sedimentary iron(II) reservoirs were smaller in the *Scirpus* than control sediment (~300 versus ~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.

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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. 1996a) 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 1998b). 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 1998b). Briefly, sediment collected at tidal freshwater marsh Burcht (dominated by *Phragmites* and *Scirpus*) 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 (Ø=24 cm) were installed to ascertain consistent placement of the gas collecting chambers and minimise disturbance during successive gas flux measurements. A 16 h 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 cycle 2 (van der Nat et al. 1998), 3 (van der Nat & Middelburg 1998b) 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×width=60×22 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 ± 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 1998b). 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. 1996a). 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 h of the measurement excluded (Figure 5.1).

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 and 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 results are given elsewhere (van der Nat & Middelburg 1998b). 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 Ø) 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.

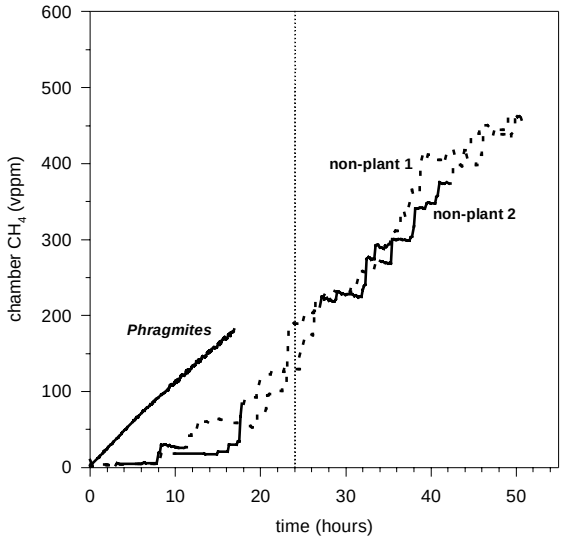

Figure 5.1 Example of long-term chamber emission measurements. The light regime during the non-plant flux measurement is indicated by dashed (light) and continued (dark) line parts. The light intensity at the sediment surface of the non-plant system was ~0.12 mE m^{-2} s^{-1}. The vertical dotted line indicates the starting point for calculation of the CH_4 flux in the non-plant systems.

Results

Plant growth

Plant growth parameters and associated CO_2 fluxes are shown in Table 5.1. Variation in the CO_2 fluxes followed the different stages in the plant growth cycle, except for the duplicate *Phragmites* system. Other plant dependent processes such as CH_4 emission (Table 5.2) 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 5.1). The amount of dry weight root material was higher in the *Phragmites* than in the *Scirpus* systems (Figure 5.2). Rhizome material was found in the *Phragmites* system only, especially below a depth of 30 cm. The total dry weight of the rhizome material was ~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 5.2).

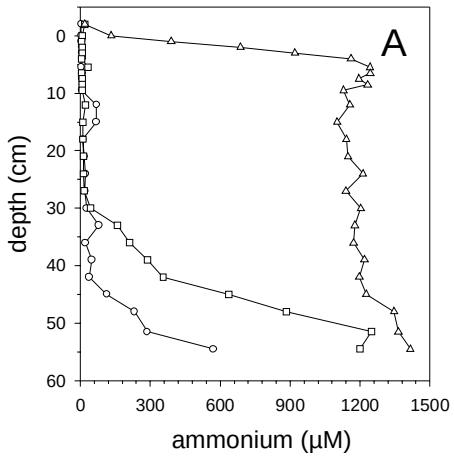

Figure 5.2 (A) Ammonium concentration versus depth profiles at day 158 (mid-season conditions). (B) Below-ground root biomass (open symbols) and rhizome biomass (solid symbols). The bulrush, reed and control systems are represented by squares, circles and triangles, respectively.

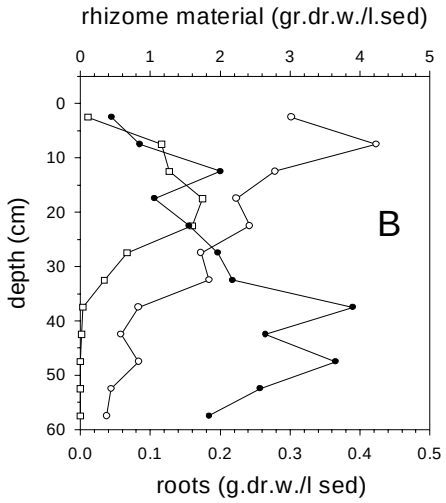

Figure 5.2 continued

Methane fluxes

The concentration of CH_4 in chambers enclosing vegetated systems increased with no significant sign of saturation even after 18 h, indicating that the CH_4 flux was not affected by chamber conditions (Figure 5.1), 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 5.1).

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 period studied (Table 5.2). 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 5.3. 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 5.1).

Table 5.1 Plant growth

System	Biomass (g dr wt 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 (~0.5 mE m^{-2} s^{-1}, 40 cm below top canopy).

Sedimentary methane

Dissolved CH_4 depth profiles are shown in Figure 5.3. Maximum concentrations in the non-plant systems were higher (~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 5.3). 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.

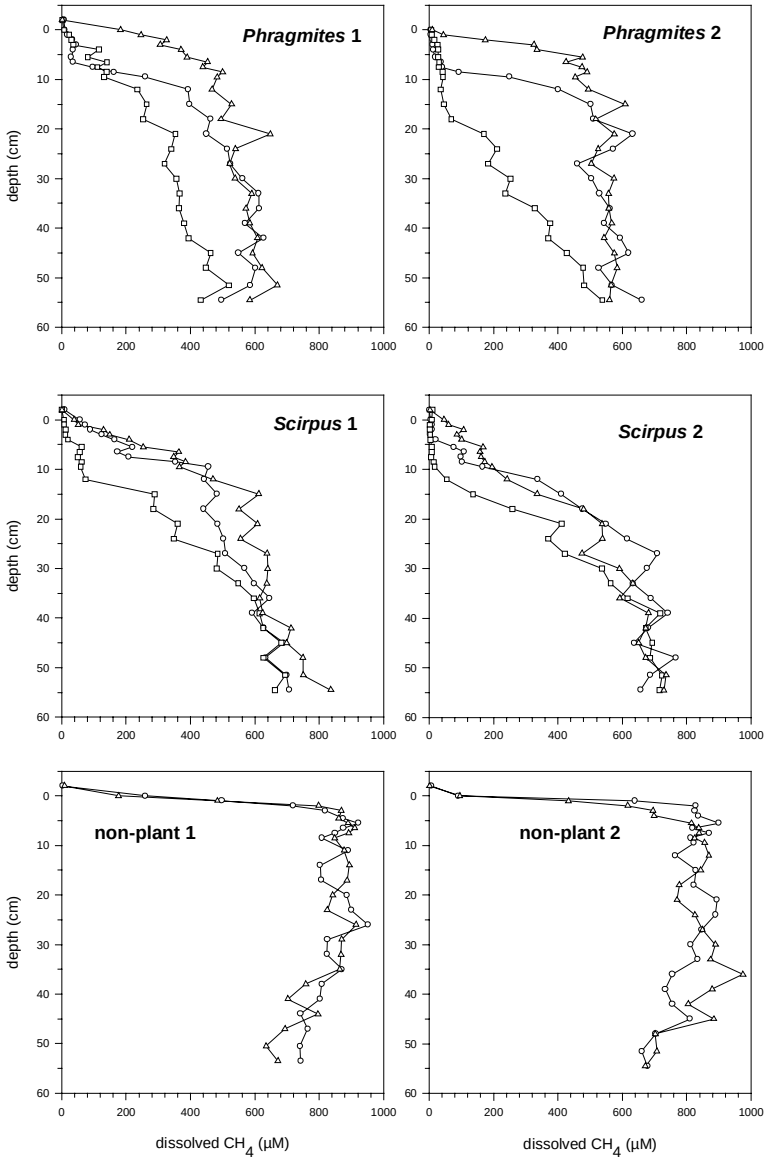

Figure 5.3 Depth distribution of dissolved CH_4 at day 55 (circles), day 118 (squares) and day 268 (triangles).

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 5.4). The CH_4 to CO_2 production ratio was nearly 1:1 for the control and *Phragmites* systems, except at the 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 5.4). 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.

Table 5.2 Plant CH_4 transport (mmol m^{-2} d^{-1} ± 1 standard error)

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

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:

- avg. prod. rate = avg. flux + Δ reservoir + avg. oxidation rate

in which the average production, flux and oxidation rates between and the change in reservoir size between sampling dates are balanced (Table 5.5). 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).

Table 5.3 Fe(II) and CH_4 reservoirs and the fate of CH_4

System	Reservoir (mmol m^{-2})		Oxidation[#] (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[§] ± 0.33	
Day 268	492	226		1.8 ± 0.07	0	0.47[§] ± 0.18	13.8 ± 0.27
Non-plant 2							
Day 55	483	221			0		
Day 268	492	224			0		14.6 ± 0.20

± 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}.

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 5.1). Regrowth of plants was also confirmed by the small but manifest difference between light (35-40 mmol m^{-2} d^{-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 5.6). This pattern of lower methanogenic carbon fluxes in the *Scirpus* than in the other two systems was also found *in vitro* (Table 5.6). The increase of the relative importance of methanogenesis with depth, especially in the control system, is consistent with the supply of dissolved oxidants from the overlying water.

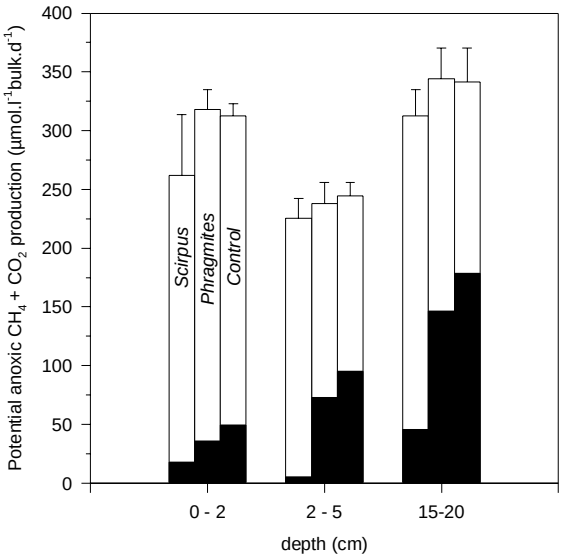

Figure 5.4 *In vitro* anoxic CH_4 (black) and CO_2 (white) production with sediment from the *Scirpus*, *Phragmites* and non-plant systems. The error bars represent the combined error of CH_4 and CO_2 production.

Dissolved iron

Dissolved Fe(II) depth profiles are shown in Figure 5.5. 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 5.3). Pore-water pH profiles were 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).

Table 5.4 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

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

Discussion

Methane transport

More than 90% of CH_4 transport was plant mediated when *Phragmites* and *Scirpus* plants matured (Table 5.3). The significant light/dark variation in CH_4 efflux in the *Phragmites* system (Table 5.2) 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 5.2). 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. 1989).

Table 5.5 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.

In the non-plant systems, more than 90% of total methane efflux occurred through bubbles. 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 rice resulted in decreased CH_4 flux rates for about 24 h as well, before 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 was observed 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. 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 (~2 mmol m^{-2} d^{-1}) than those actually measured (0.1-0.7 mmol m^{-2} d^{-1}). 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.

Table 5.6 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.

Rhizosphere oxidation

Oxygen gas circulation in wetland plants is a common adaptation to prevent root anoxia (Armstrong et al. 1994). *Phragmites* and *Scirpus* plants oxidize 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 higher sediment oxidizing capacity than *Phragmites,* because dissolved iron is more depleted in the *Scirpus* than *Phragmites* systems (Table 5.3), in particular in the upper 10 centimetres of the sediment (Figure 5.5). Moreover, rhizospheric methane oxidation rates in the *Scirpus* system are higher than those in the *Phragmites* system (Table 5.5; van der Nat & Middelburg 1998b). 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 area basis (van der Nat & Middelburg 1998b), despite the difference in gas transport mechanism usage between both plant species. 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 5.2B).

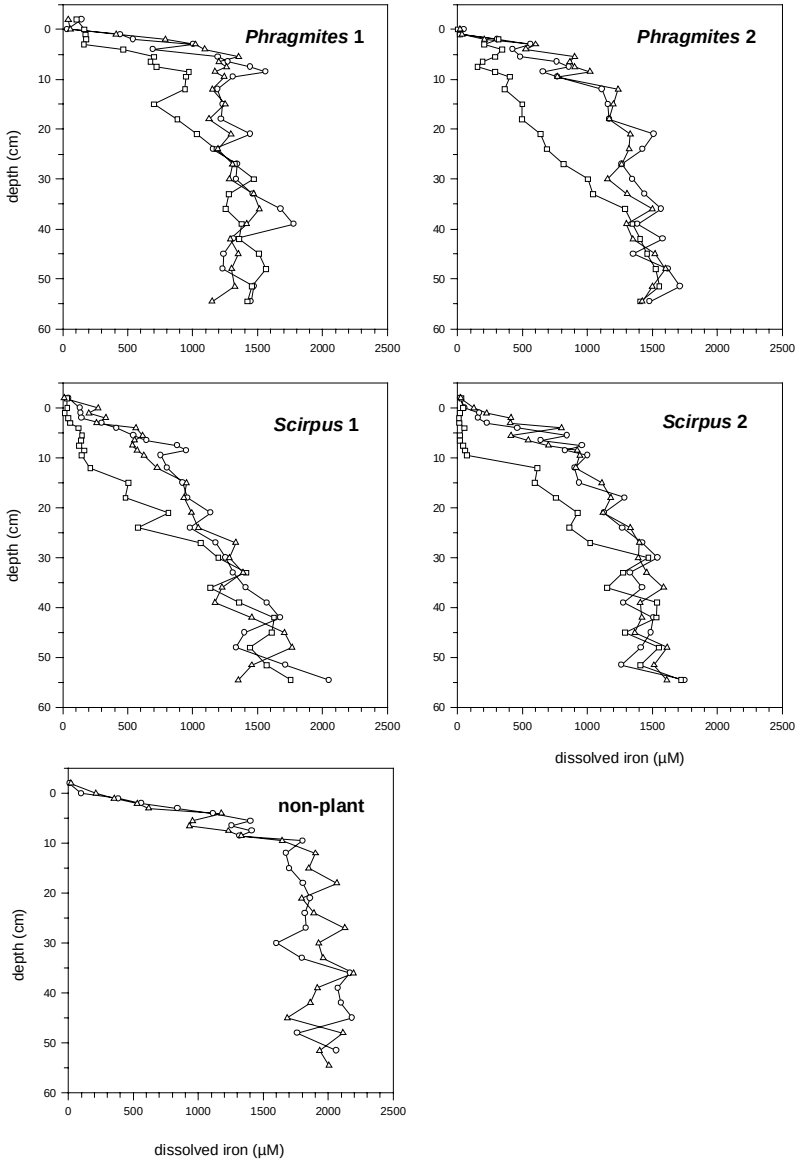

Figure 5.5 Depth distribution of dissolved Fe(II) at day 55 (circles), day 118 (squares) and day 268 (triangles).

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 5.3), despite higher methane production rates and lower oxidation rates in the *Phragmites* systems (Table 5.5). 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 5.5). This difference in CH_4 production between the *Scirpus* and non-plant system was also observed in the *in vitro* slurry incubations (Figure 5.4). 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 5.6). Less substrate availability in the *Scirpus* system seems unlikely considering that CH_4 production rates in the *Scirpus* system were low, even with the (potential) extra carbon input as a result of plant CO_2 fixation. And, potential gross mineralisation rates were fairly similar after the period of active vegetation indicating a similar base-line gross mineralisation for both systems (Figure 5.4). Probably, other metabolic processes competed with methanogens for substrate in the *Scirpus* system, as evidenced by the relatively large increase of CH_4 production in the *Scirpus* slurries with added methanogenic substrates (Table 5.4). It is interesting to note that *Scirpus* slurries (2-5 cm depth) with lowest rates of CH_4 production exhibited the largest amended/unamended ratios.

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).

Fe(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 5.6), 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, were probably partly the result of the (short) exposure of the sediment samples to atmospheric air after coring, which 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 implies that either (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 1998b; Figure 5.5). Nevertheless, continuous O_2 input seems not a prerequisite for suppression of methanogenesis. Input of atmospheric O_2 was excluded in the anaerobic incubation experiment whereas differences in CH_4 production still occurred. 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: surface-water nitrate and sulphate concentrations were 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 and the contribution of methanogenesis to gross mineralisation. However, CH_4 production in the *Phragmites* system equalled production in the control system, eventhough the potential for suppression, based on dissolved iron concentrations, 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 5.5). CH_4 production coupled to plant growth and photosynthesis via exudation of labile organic carbon has been suggested before (Schütz et al. 1989; 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, potential oxic sedimentary CO_2 production rates were measured as well (*see* Figure 5.6). When plants were not active (post-season), rates were not significantly different in the three different systems. But, 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 oxidizing the sediment, but also by input of labile organic carbon. These processes may interact and future studies should consequently focus on both processes simultaneously.

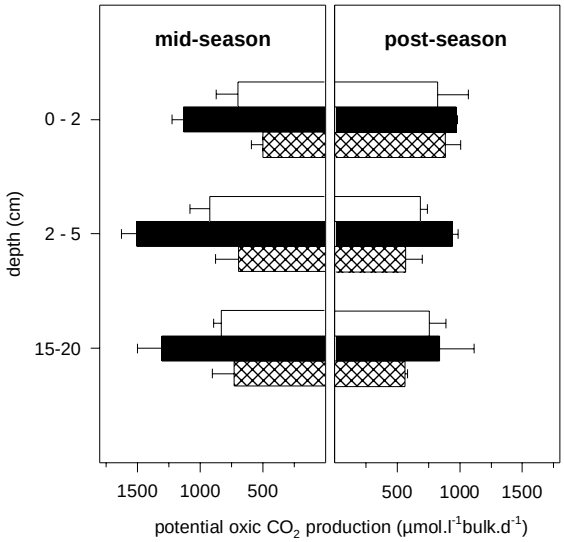

Figure 5.6 *In vitro* oxic CO_2 production with sediment from the non-plant (open bars), *Phragmites* (black bars) and *Scirpus* (patterned bars) systems. The error bars represent the standard error of the mean (n=3). CO_2 production rates were collected during an *in vitro* CH_4 oxidation incubation (van der Nat & Middelburg 1998b), and have not been published before. Rates presented are corrected for the (small) contribution of CO_2 formed during CH_4 oxidation.

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. Rhizospheric CH_4 oxidation remains an important process for attenuating CH_4 fluxes. Without it, CH_4 fluxes in the mature *Scirpus* system would have been almost two 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 oxidized sediments are coupled to the suppression of CH_4 production, probably as a result of substrate competition between metal oxide reducing bacteria and methanogens.

Chapter 6

Methane emission from tidal freshwater marshes

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Abstract

In two tidal freshwater marshes, methane emission, production and accumulation in the pore-water have been studied. The two sites differ in their dominant vegetation, i.e. reed and bulrush, and in their heights above sea level. The reed site was elevated in relation to the bulrush site and had higher rates of methane emission and production. It is argued that this difference in methane emission between sites was primarily due to a different effect of reed and bulrush plants on methane dynamics rather than methane oxidation related to tidal elevation. Methane emission showed strong seasonality related primarily to plant physiology and only secondarily to temperature. Two control sites at which vegetation was removed systematically had lower emission rates indicating an overall stimulating effect of plants on methane emission from tidal marshes. Flooding reduced methane emission, probably by blocking the primary sites of methane release in the lower part of the plant stems.

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Introduction

Natural wetlands are thought to be a major source of atmospheric methane (Cicerone & Oremland 1988). Quantification of this source is difficult, because individual wetlands often show great spatial and temporal variations in methane emission. This variability in time and space is related to environmental factors that affect methane production, oxidation or transport. These factors include the type and amount of organic matter (Kelly & Chynoweth 1981), the dominant organic matter decomposition pathway (DeLaune et al. 1983; Bartlett et al. 1987; Middelburg et al. 1996a), temperature (King & Wiebe 1978; Bartlett et al. 1987), hydrological conditions (Roulet et. al. 1992; Fechner & Hemond 1992; Kelley et al. 1995) and vegetation (Dacey & Klug 1979; Sebacher et al. 1985; Wilson et al. 1989, Schütz et al. 1991; Whiting et al. 1991; Chanton et al. 1992).

In tidal freshwater marshes plants and daily fluctuations in water level may affect methane emission by controlling its production, oxidation and transport. Plants may support methanogenesis directly through root exudation (Raimbault et al. 1977; Whiting & Chanton 1992) and indirectly through enhancing carbon input by (1) litter accumulation (Whiting & Chanton 1993) or (2) canopy-friction induced trapping of suspended organic matter (Middelburg et al. 1997). Oxygen may enter intertidal vegetated sediments directly by diffusion at low tide and indirectly via the aerenchyma of the plants, at low and high tide (King 1994). Oxygen in the rhizosphere attenuates methane emission directly through methane oxidation (King 1994; van der Nat & Middelburg 1998b) and indirectly through suppression of methanogenesis (Roden & Wetzel 1996; van der Nat and Middelburg 1998a). Moreover, wetland plants serve as direct conduits to the atmosphere facilitating vertical methane transport into the atmosphere (Sebacher et al. 1985; Chanton & Dacey 1991; Whiting & Chanton 1992; van der Nat et al. 1998). Finally, lateral transport of dissolved methane through drainage, driven by tidal flushing, may transport a significant portion of dissolved methane horizontally before release into the atmosphere (Kelley et al. 1995).

This paper reports the spatial and temporal variability in methane emission and pore water methane concentrations from a tidal marsh in the Scheldt estuary. Two sites, differing in plant community structure and flooding frequency and duration, were monitored regularly over a period of two years and the processes involved in methane cycling are discussed. An experimental container subject to water level fluctuations was constructed to determine the effect of tide on methane emission.

Material and methods

Study area

Station Burcht is an oligohaline tidal marsh located near Antwerp in the Scheldt Estuary. The salinity of the river Scheldt water off Burcht averaged ~2.5 ‰, with limited seasonal variability. Vegetation at Burcht shows a clear zonation pattern with a 10 m broad

dense *Scirpus lacustris* (bulrush) stand closest to the river and a 20 m broad dense *Phragmites australis* (reed) stand. The two sites have been designated SCR (*Scirpus* Close to River) and PFR (*Phragmites* Far from River). SCR and PFR have tidal elevations of ~2.5 m and ~3.2 m above sea level, respectively. This 0.7 m difference in tidal elevation results in large differences with respect to flooding frequencies, duration of submergence and flood height. Total period of submergence in 1995 of PFR and SCR was 210 and 1027 h, respectively, whereas the averaged period of submergence per flooding was 0.7 and 1.6 h, respectively. Averaged height of the flood water above the sediment surface during high tide was 16.6 cm (PFR) and 43.4 cm (SCR). In two 2×2 m plots (SCR-P and PFR-P) the newly emergent vegetation was cut weekly directly above the sediment. Plots were shrouded for darkness to mimic plant shading and limit microphytobenthos growth by installation of a 40% light transparent cloth ~20 cm above the sediment. Cloth could be easily removed before sampling.

Methane emission

The enclosed chamber technique used to measure methane emission has been described previously (van der Nat et al. 1998). Methane emission was calculated by linear regression analysis from the change of methane concentration in the mixed chamber with time over a 30 to 45 min. period (n=20-30). The methane accumulating in the chamber is transported by three mechanisms: molecular diffusion, gas bubble ebullition and by transport through plants. The amount of methane transported through ebullition was calculated using a partitioning approach (Middelburg et al. 1996a; van der Nat & Middelburg 1998b). Measurements with an initial chamber methane concentration exceeding ambient levels > two times and emissions with a regression coefficient <0.90 (n ≥20) were discarded. Molecular diffusion rates were calculated according to Fick's first law modified for sediments (Berner 1980), using the methane concentration gradient at the sediment surface. The diffusion and bubble fluxes were subtracted from total emission values to obtain the plant transport rates. Emission measurements were always conducted during spring tide and air exposure. Flux measurements could only be performed at low tide because of logistic constraints (i.e. >4 m tidal range). Fluxes were determined under light and dark conditions as well as after clipping the plants close to the sediment surface. CO_2 levels within the chamber were maintained at or above ambient levels during periods of CO_2 uptake by injecting small amounts of CO_2 into the chamber. The chamber was shrouded for dark measurements with a black-out cloth.

Pore water sampling

Pore water was sampled by *in situ* dialysis (Hesslein 1976), using a sampling device known as a peeper (height×width = 60×10 cm) which contained 25 membrane cells that were in contact with sediments via a 0.2 mm biologically inert acrylic copolymer membrane filter (Versapor-200, Gelman Sciences). At both ends, the cells were connected with Tygon tubes to sampling ports at the sediment surface. The samplers were installed

January 1994. Pore water samples were withdrawn with a syringe at one sampling port while nitrogen gas was introduced via the other port. Deoxygenated de-ionised water was used to refill the compartments after sampling. The sample was transported into 5, 10 or 20 ml glass vial sealed with a septum crimp-cap (Chrompack) and immediately acidified by adding 50 to 200 µl 20% H_2SO_4. The acidified samples were analysed for methane in the headspace using the phase equilibrium technique (McAullife 1971). Methane was 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 methane concentrations significantly. Sedimentary pool sizes (reservoirs) were calculated from the profiles of pore-water methane concentration, after correction for sediment porosity. Porosity was calculated from water content (weight loss on drying at 105°C) assuming a dry density of 2.55 g ml^{-1}. Sediment porosity in the PFR system ranged from 0.66 ± 0.03 at the surface to 0.63 ± 0.03 at depth. Porosity in the SCR system ranged from 0.76 ± 0.04 at the surface to 0.73 ± 0.02 at depth.

Anaerobic incubations

Sediment cores were collected in July 1995 using acrylic tubes (55 cm long, 7 cm internal diameter). Sediment of equal depth of three cores was 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 and CO_2 production was chosen mainly by the lack of a reasonable alternative in vegetated systems (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. Rates are calculated for the period when increases in headspace concentrations were linear (R^2 >0.95), i.e. the first 3 days of the incubation period.

Biomass

Above-ground biomass was estimated by averaging the weight of two vegetation samples that have been dried for 3 days at 60°C. Only green and healthy looking plants were included. The CO_2 fixation rates were measured similar to methane emission by linear regression analysis from the change of chamber CO_2 concentration with time. An initial CO_2 concentration of ~1000 ppmv was used. Rates were linear (R^2 >0.90) in the 700 to 400 ppmv range. For estimating below-ground biomass, cores (n=5) were collected in September 1994, using acrylic tubes (55 cm long, 7 cm internal diameter). Cores were

sliced and washed and the obtained biomass was separated in live roots and rhizome material.

Sediment and environmental parameters

Each month the organic carbon content of the sediment, stripped from foreign material and root biomass, was determined according to Nieuwenhuize et al. (1994). No distinct temporal pattern could be detected and the results were pooled (*n*=21). 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. Simple mercury glass thermometers measured air and sediment temperature. Sediment temperature was measured ~1 cm below the sediment-air 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 and was usually within 25% of ambient conditions.

Experimental intertidal marsh

A mixture (1:4) of garden soil (Barenburg products) and Burcht sediment was mixed and transferred to a container (l×w×h = 2.0×0.5×0.8 m). The container was placed in a temperature (18 ± 0.5°C), humidity (70 ± 7%) and CO_2 concentration (380 ± 40 ppmv) controlled room. The sediment was allowed to settle for a period of 4 months. After this period sediment height was ~35 cm. *Scirpus* plants were collected in the field and planted carefully in a similar density as found in the field. Two polypropylene collars with a diameter of 24 cm were installed in the centre of the container to ascertain consistent placement of the gas collecting chambers and minimise disturbance during successive emission measurements. A 16 h day period with a light intensity of ~0.4 mE m^{-2} s^{-1} at the sediment surface (~1.6 m below lamps) was set, except during the measurements when light was continuously supplied to avoid interference of the light regime with methane emission (van der Nat et al. 1998). Two times a day the tide changed resulting in 4 h periods of low and high tide separated by 2 h of pumping time. Pumping rate was ~0.4 cm min^{-1}. The tidal range was ~33 cm. A large basin (~500 l) was used as a reservoir for the overlying water. Experiments were conducted on plants of the second generation. To avoid pressure problems with the changing water level the flux chambers were equipped with a hollow capillair needle sticking through the chamber's side. Chamber methane concentrations were recalculated to absolute methane content in order to incorporate the change in chamber volume during floodwater pumping.

Integration and statistical analysis

Annual flux estimates were calculated by integration of the curves connecting averages of replicate measurements. Pore-water reservoir sizes were obtained by integrating pore-water data to 55 cm and taking into account porosity. For each plant species the difference between light, dark and clipped emission was tested with the t-test using pooled variances. Other statistical analysis was done with one- or multi-way ANOVA tests, depending on the number of factors, so possible interactions between factors would be revealed. In all analysis where $P<0.05$, the factor tested was considered statistically significant.

Results

Methane emission

Methane emission rates, sediment temperature and above-ground biomass values are presented in Figure 6.1. Emission rates were variable, even when measurements were conducted consecutively on the same day and collar. There are nevertheless consistent trends on a (i) spatial basis, (ii) temporal basis, and (iii) with respect to the immediate effect of light.

(i) Methane emission was much lower at SCR than PFR (Table 6.1). Maximum differences were observed mid-seasonally when plants were mature and sediment temperature had reached its highest levels. In 1994 annual emission rates differed by more than 10 times (0.28 versus 4.7 mol CH_4 m^{-2} yr^{-1} for SCR and PFR, respectively, Table 6.1). Continual removal of the vegetation at the adjacent corresponding plots reduced emission to 0.13-0.15 and 0.54-0.56 mol CH_4 m^{-2} yr^{-1} for SCR-P and PFR-P respectively (Table 6.1), indicating a net positive effect of *Phragmites* and *Scirpus* plants on methane emission. CO_2 fixation rates in July 1994 on a clear and sunny day were 2.9 ± 0.23 and 2.0 ± 0.21 mol m^{-2} d^{-1} for PFR and SCR, respectively.

(ii) Methane emission showed strong seasonality. Emission started during spring and increased until maxima were reached in mid- (SCR) and late-summer (PFR). Emission rates declined again during late summer (SCR) and autumn (PFR). Seasonal patterns were reproducible for at least 2 years. Increment of sediment temperature levels in the plots due to the absence of plant shading was not apparent. No difference in sediment temperature between SCR and PFR was observed.

(iii) Methane emission rates under light conditions were usually higher than under dark conditions, especially at PFR (Figure 6.1; Table 6.1). Highest light/dark emission ratios were noted when plants were mature and fully green and lowest ratios when plants were small or senescing. In addition, the ratio varied with the amplitude in light intensity. Relative high ratios were measured on clear sunny days with light intensities exceeding 1.3 mmol m^{-2} s^{-1}. The effect of light was comparatively small at the non-vegetated plots. Clipping the reed plants significantly lowered methane emission compared to rates measured with intact plants under light conditions ($P=0.036$), but not under dark

conditions. Clipping the bulrush plants did not significantly affect the outcome of the measurements.

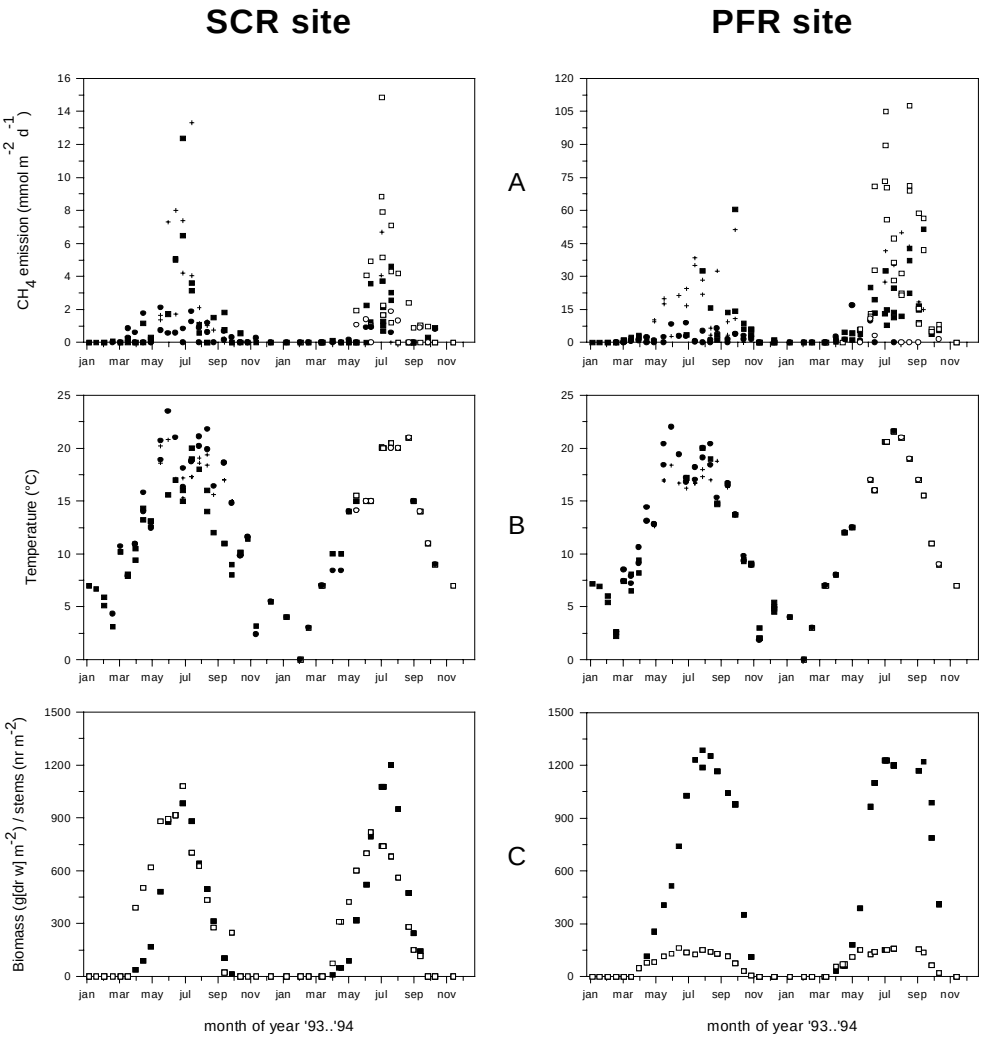

Figure 6.1 Time course of methane emission (A), sediment temperature (B) in clipped (+), intact vegetation (squares) and non-vegetated plots (circles) under light (open symbols) and dark conditions (closed symbols) and (C) above-ground biomass dry weight (closed squares) and number of stems.m^{-2} (open squares) at SCR (left) and PFR (right). Please note the differences in scale in panel A.

Episodic increases of chamber methane concentrations, indicating occurrence of ebullition was only observed occasionally at SCR when plants were senescing and contributed less than 10% to total methane emission at that time. The contribution of molecular diffusion in the sediment contributed less than 5% to total emission.

Table 6.1 Annual methane fluxes (mol CH_4 m^{-2} yr^{-1})

Station	Period	Vegetation	Condition	Flux
SCR	1993	Clipped	Dark	0.51
SCR	1993	Intact	Dark	0.38
SCR-P	1993	Removed	Dark	0.13
PFR	1993	Clipped	Dark	3.11
PFR	1993	Intact	Dark	2.30
PFR-P	1993	Removed	Dark	0.56
SCR	1994	Intact	Dark	0.17
SCR	1994	Intact	Light	0.36
SCR-P	1994	Removed	Dark/Light*	0.15
SCR	1994	Intact	Dark/Light*	0.28
PFR	1994	Intact	Dark	3.02
PFR	1994	Intact	Light	5.91
PFR-P	1994	Removed	Dark/Light*	0.54
PFR	1994	Intact	Dark/Light*	4.71

* Integration based on 14h light and 10h dark periods.

Dissolved methane

Dissolved methane pore-water depth profiles are shown in Figure 6.2. In the surface layer methane concentrations were between 0.1 and 5 µM, i.e. similar to that in river Scheldt water, but they were above those in equilibrium with the atmosphere (~0.003 µM). Methane reservoirs were much larger at PFR than SCR (Table 6.2). The winter methane reservoir sizes were about double those of spring/early-summer. The seasonal decrease of the methane reservoir started in March/April whereas the subsequent increase started in August. Relatively low dissolved reservoir sizes at PFR during the months May-July were accompanied by a shift towards concave methane versus depth profiles, whereas profiles at SCR were concave over the whole season. Concave profiles indicate that methane oxidation losses due to oxidation and venting dominate over methane gains from production or lateral inputs.

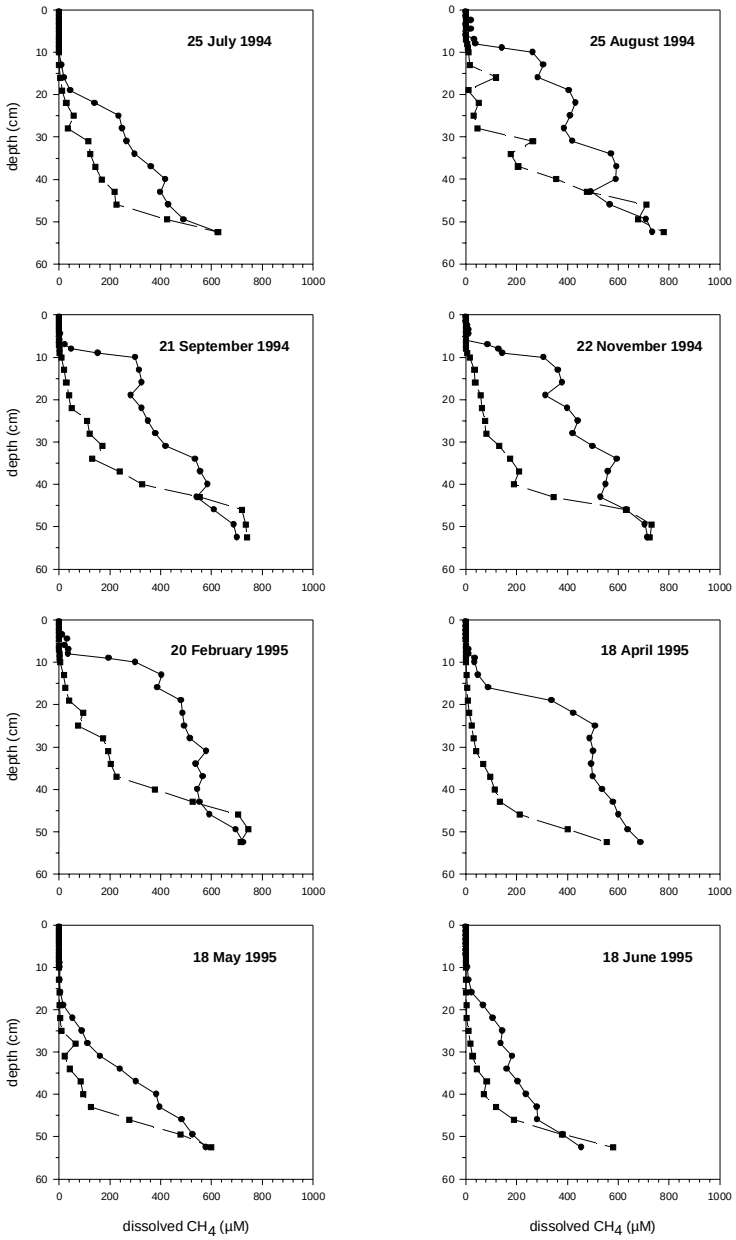

Figure 6.2 Depth distribution of dissolved pore-water methane at SCR (squares) and PFR (circles). The dates refer to the days when pore-water samples were recovered.

Table 6.2 Dissolved methane reservoir (mmol m^{-2})

Date	PFR	SCR
25 Jul 1994	77.9	49.1
25 Aug 1994	137.6	88.3
21 Sep 1994	132.2	89.7
22 Nov 1994	142.8	79.1
20 Feb 1995	151.1	92.6
18 Apr 1995	125.9	38.7
18 May 1995	65.8	41.1
18 Jun 1995	52.6	34.7
Annual average	111	64

Methane and carbon dioxide production

Potential rates of methane and carbon dioxide production were measured in July 1995. Methane production without a lag-phase was observed only in the surface layer slurries (0- to 2-cm) and 40- to 45-cm slurries (Table 6.3). In the 2- to 5-cm and 15- to 20-cm slurries, methane production started after a lag phase of >3 days. Methane production was not observed in the slurries treated with BES, a specific inhibitor of methanogenesis. Addition of methanogenic precursors stimulated methane production 2 to 3 times at SCR and 7 to 9 times at PFR. The depth integrated production rate of methane at PFR (16.4 ± 2.2 mmol m^{-2} d^{-1}) was significantly higher than that at SCR (6.4 ± 0.9 mmol m^{-2} d^{-1}), whereas gross mineralisation (depth integrated CO_2 + CH_4 production) at PFR (118 ± 5 mmol m^{-2} d^{-1}) was lower than that at SCR (130 ± 9 mmol m^{-2} d^{-1}). This difference depended however on depth (interaction term significant at P<0.0001) with mineralisation rates at SCR in the surface layer being higher than those at depth (Table 6.3). The contribution of methanogenesis to carbon mineralisation at depth was consequently higher at PFR (71%) than SCR (37%).

Table 6.3 Methane and carbon dioxide production

Site	Depth interval (cm)	CH_4 production (µmol l^{-1} h^{-1})	CO_2 production (µmol l^{-1} h^{-1})	CO_2 + CH_4 (µmol l^{-1} h^{-1})	Contribution of Methanog. (%)
PFR	0-2	0.27 ± 0.08	11.7 ± 1.2	12.0 ± 1.2	4.5 ± 1.4
	2-5	n.s.	9.70 ± 1.10	9.7 ± 1.1	
	15-20	n.s.	10.0 ± 0.9	10.0 ± 0.9	
	40-45	3.38 ± 0.46	6.2 ± 0.2	9.6 ± 0.5	71 ± 10
SCR	0-2	0.73 ± 0.12	18.5 ± 1.4	19.2 ± 1.4	7.6 ± 1.3
	2-5	n.s.	16.2 ± 1.5	16.2 ± 1.5	
	15-20	n.s.	10.4 ± 1.9	10.4 ± 1.9	
	40-45	1.26 ± 0.18	5.6 ± 0.2	6.9 ± 0.3	37 ± 6

n.s.= no significant production during first three days.

Organic carbon and below-ground biomass

The concentration of organic carbon at PFR (4.23 ± 0.22 mmol cm^{-3}) was independent of depth, whereas that at SCR depended significantly on depth (2.68 ± 0.18 mmol cm^{-3} in the surface layer to 1.84 ± 0.17 mmol cm^{-3} at depth). In general, organic carbon contents tended to be lower in the non-vegetated plots than in the adjacent vegetated sites: PFR-P and SCR-P pool sizes were 3.66 ± 0.40 and 1.86 ± 0.16 mmol cm^{-3}, respectively.

Phragmites roots penetrated the sediment to greater depth than *Scirpus* roots and the amount of root dry weight was also higher for *Phragmites* (Figure 6.3). Rhizome material was found only in the *Phragmites* system, especially below a depth of 35 cm. Rhizome material was also found at depths >90 cm (data not shown). The total weight of dry rhizome material exceeded the total weight of dry roots more than 10 times

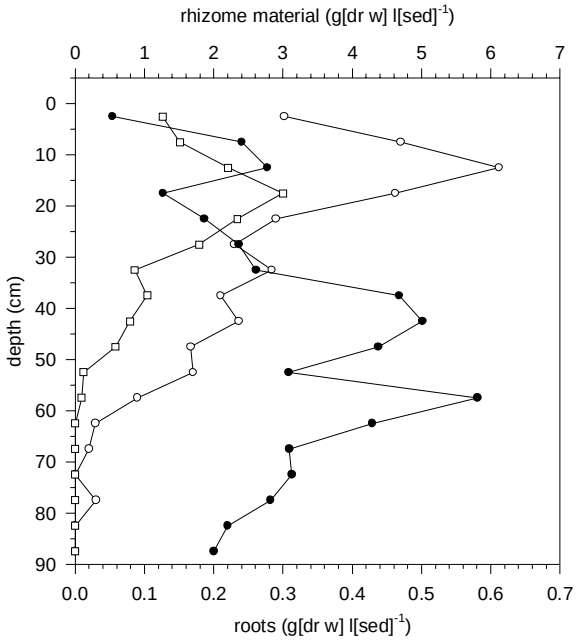

Figure 6.3 Depth distribution of live root and rhizome material at SCR (squares) and PFR (circles). Rhizome material is represented by filled symbols. Standard error bars are not included for reasons of clarity.

Tidal experimental container

Methane emission appeared to be a function of tidal height (Figure 6.4). The low-tide and high-tide emissions averaged 28.2 ± 2.6 and 17.6 ± 1.7 mmol m^{-2} d^{-1}, respectively (*n*=3). Especially when the flood water level dropped below about 8 cm height, the accumulation of methane in the chamber increased considerably. Base low-tide emission rates were again present after flood water height levelled the sediment surface. Ebullition was observed occasionally during the shift from high to low tide and contributed less than 20% to total methane emission. The time period selected for presentation in figure 6.4 was free of bubble methane release.

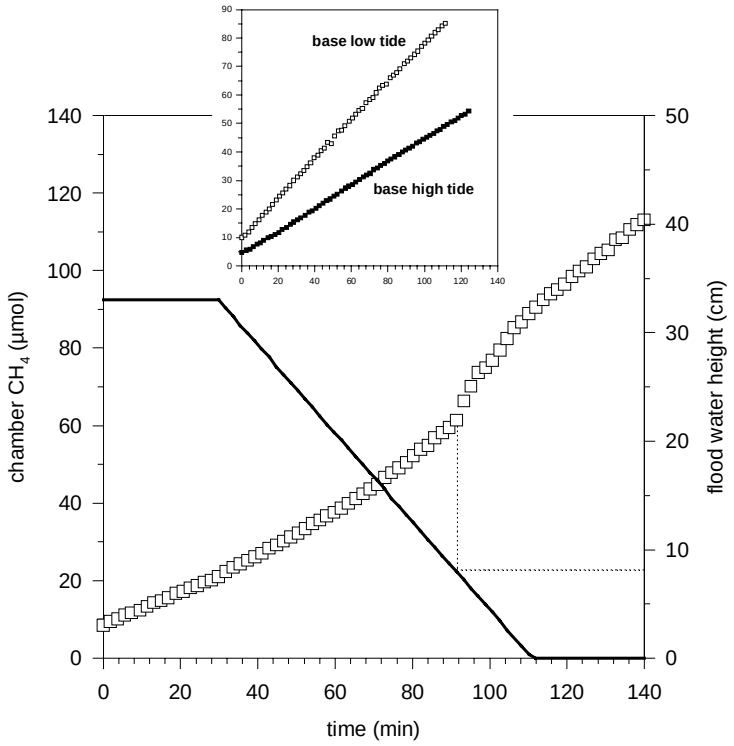

Figure 6.4 The effect of water level on methane accumulation in flux chamber. The inset shows low and high tide measurements conducted when pumps were shut off. The dashed line relates the break in the accumulation of methane with a water level of 8 cm.

Discussion

Methane emission from the permanently clipped plots (SCR-P and PFR-P) was significantly lower than that from vegetated sites of similar setting (Figure 6.1; Table 6.1). Apparently, the processes attenuating emission (stimulation of rhizospheric methane oxidation and suppression of methanogenesis) were outweighed by those enhancing emission (transport and stimulation of methanogenesis).

Methane transport through aerenchymatic plants is usually much greater than diffusion in sediments and ebullitive transport (Sebacher et al. 1985; Chanton & Dacey 1991; Whiting & Chanton 1992; van der Nat et al. 1998; van der Nat and Middelburg 1998). It has been suggested that plant transport and ebullition may be mutually exclusive processes (Holzapfel-Pschorn & Seiler 1986; Schütz et al. 1989). The presence of methane-transporting plants may enhance methane emission even further by allowing methane to bypass zones of methane oxidation (Shannon & White 1994). In our study more than 85% of net methane emission was a result of methane venting to the atmosphere through the *Scirpus* and *Phragmites* system. This escape route seems especially important at our sites because fluctuating water tables (due to tides and water uptake by plants) and air-exposure support intensive methanotrophy to greater depth (van der Nat et al. 1997).

The significant light/dark difference in methane emission by *Phragmites* (P=0.01; Figure 6.1) is typical for emergent plants exploiting diffusive transport during periods of low illumination or in the dark and additional convective transport during periods of illumination (Sebacher et al. 1985; van der Nat et al. 1998). *Scirpus* uses a diffusive mechanism irrespective of light condition (van der Nat et al. 1998) and there is consequently no significant light-dark variation in methane flux. It is interesting to note that the different effect of clipping *Phragmites* and *Scirpus* plants on methane emission (Figure 6.1) is in accordance with the usage of the different gas transport mechanisms. Clipping a plant that exploits diffusive and convective transport such as *Phragmites* under light conditions should decrease methane emission because the capacity for pressurised transport is aborted. Clipping does not affect steady-state methane emission from plants employing diffusive transport such as *Scirpus* (Figure 6.1), rice (Denier van der Gon & van Breemen 1993) *Carex* (Kelker & Chanton 1997), and plants with light dependent transport modes such as *Phragmites* under dark conditions.

Seasonal variability

An important factor controlling seasonal methane emission is plant growth (Schütz et al. 1989, 1991; Wilson et al. 1989; Sass et al. 1990; Conrad 1989; van der Nat et al. 1998; van der Nat & Middelburg 1998a). The dissolved methane versus depth profiles reflect the balance of processes governing the production, oxidation and transport of methane. Although profiles alone do not allow one to distinguish between these processes, changes in profiles over time together with changes in emissions can provide clues about how plant growth controls seasonal emissions of methane. The sharp decrease in the dissolved

methane reservoir size and simultaneous increase in emissions during springtime indicate the importance of transport. An increase in oxidation or a decrease in methane production might explain the decrease in pore-water methane, but would not have led to the observed increment in methane emission. The residence time of methane in pore-water with respect to ventilation (reservoir size/methane emission) at SCR varied from 42 days in May, 7 to 8 days in June- July, to 53 and 230 days in August and September, respectively. At PFR the residence time was much lower and decreased from 11 days in May to 1-2 days in June-August and rose again to 4 days in September. Other research also showed that methane-transporting plants may deplete the pool of dissolved pore-water methane rapidly (Chanton & Dacey 1991; Chanton et al. 1992; Shannon et al. 1996).

The changes in emission and reservoir size closely followed the emergence of new green plant parts. Consistently, when *Scirpus* plants started to senesce in August methane emissions decreased (Figure 6.1) and the size of the methane reservoir increased (Table 6.2). However, at PFR the increase in reservoir size was not accompanied by a decrease in emission and did not follow the dying of the *Phragmites* plants (October). An explanation might be that methane production is stimulated through addition of methanogenic substrates and their rate of supply is closely linked to the plant's growth cycle. Correlation between the plant growth cycle and methane production has been reported before for *Phragmites* (van der Nat & Middelburg 1998a) and other plants (Sass et al. 1990; Whiting & Chanton 1992; Kelley et al. 1995; Minoda & Kimura 1996).

The difference in time for emission to decline at PFR and SCR indicate that the effect of sediment temperature was less important than that of plant physiology. The effect of temperature on methane emission is not straightforward. (i) Increasing temperatures will not only enhance methane production rates (Kelly & Chynoweth 1981), but also methane oxidation rates (King & Adamsen 1992). (ii) The effect of temperature on methane production in a substrate limited environment is inherently small (Kelley et al. 1995) and the stimulation of methane production by the addition of methanogenic substrate indicates that methanogenesis was probably substrate limited (in July 1995). (iii) Temperature was not measured in the methanogenic zone (>40 cm depth) of the sediment, but temperature fluctuations are normally dampened with depth.

Spatial variability

The observed difference in methane emission between SCR and PFR could be related to a difference in tidal elevation (i.e. flooding frequency and duration) or to a difference in plant communities. Several studies already demonstrated a correlation between the toposequence of a particular site and methane emission (Morrissey & Livingston 1992; Moore et al. 1994; Kelley et al. 1995). In general, higher elevated sites are likely to emit less methane than less elevated sites due to differences in oxidizing capacity and lateral transport. Indeed, bulk sediment oxidation rates were significantly higher at PFR than SCR (van der Nat et al. 1997). Moreover, SCR is flooded more regularly than PFR with the likely result that more labile organic matter is being trapped in the canopy. Mineralisation rates in the surface layer at SCR are indeed significantly higher than at PFR, but most of the

deposited labile organic matter does not reach the zone of active methanogenesis at depth (Table 6.3). Flooding can also affect methane emission directly as evidenced by our observations with the tidal experimental container. Methane is released primarily from the lower 20 to 30 cm of the *Phragmites* and *Scirpus* stems emerging above the sediment (van der Nat et al. 1998). Hence, at high tide when the lower part of the plant stems are under water, methane emission to the atmosphere should decrease (Figure 6.4). The extent of a tidal effect would differ between PFR and SCR as a result of flooding differences and so contribute to spatial differences. In the long run, this direct effect of flooding on methane emission would only matter when methane is oxidized during high tide and not merely delayed from escaping the sediment until low-tide conditions are again present. Accordingly, on the basis of tidal elevation we would expect higher emission at SCR due to lower bulk sediment oxidation rates (van der Nat et al. 1997) and higher allochthonous inputs of labile carbon at the surface (Table 6.3). However, observed methane emissions are higher at PFR suggesting that spatial zonation in methane emission is due to zonation of plant species rather than tidal elevation.

In a parallel study, we have studied the effect of *Phragmites* and *Scirpus* on methane dynamics in constructed wetlands (van der Nat & Middelburg 1998a) so that we could eliminate non-plant effects. We concluded that methane fluxes from freshwater marshes are primarily controlled by methane production with methane oxidation and transport being of secondary importance. Moreover, methane emission and production in *Phragmites* systems were higher than those in *Scirpus* systems due to (1) a higher contribution of methanogenesis to mineralisation, (2) lower rates of rhizospheric methane oxidation and (3) carbon addition by *Phragmites*.

Similar observations have been made in this study. Methane production rates and the contribution of methanogenesis to mineralisation were higher at PFR than SCR (Table 6.3). Carbon addition by *Phragmites* was likely higher than that by *Scirpus* given higher rates of carbon dioxide fixation (up to 2.9 versus 2.0 mol m^{-2} d^{-1} at PFR and SCR, respectively), higher sedimentary organic carbon contents at depth (4.26 versus 1.84 mmol cm^{-3} at PFR and SCR, respectively) and higher mineralisation rates at depth (Table 6.3). Moreover, *Phragmites* has its roots and especially its rhizomes extended too much greater (i.e. methanogenic) depths and in much larger quantity than *Scirpus* (Figure 6.3). Supportive evidence for a difference in carbon addition also comes from the typical convex pore-water methane profile suggesting net production during spring, autumn and winter at PFR (Figure 6.2). The variation in residence time of methane in pore-water with respect to production (~8 for PFR and ~15 for SCR, =reservoir size/methane production) further indicates the difference in production at both sites.

Observed higher rates of rhizospheric oxidation and methane production suppression by *Scirpus* compared to *Phragmites* (van der Nat et al. 1998) possibly also contributed to the observed spatial variability. Rhizospheric methane oxidation can effectively limit methane emission even when potential bulk sediment oxidation rates are already relatively high (due to tidal elevation) because oxygen transported by plants is injected deep in the sediment, where methanotrophs around the root constitute an efficient sink for methane before it can enter the roots (King 1994; van der Nat et al. 1997). Moreover, methane

consumption rates associated with root material from *Scirpus* are higher than those associated with *Phragmites* roots (van der Nat et al. 1997). In conclusion, spatial variability in methane emission at our tidal marsh was more controlled by the dominant plant species than flooding of the sediment.

References

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Summary

Methane (CH_4) is an important greenhouse gas. Wetlands, both natural and agricultural (rice fields), have been identified as major sources of CH_4 to the atmosphere. Towards the goal of refining the global budget, it is essential to quantify CH_4 emission from vegetated wetlands and its temporal variation, as well as to understand the factors and processes controlling such variations. 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, (ii) input of atmospheric oxygen (O_2) to the rhizosphere fuelling CH_4 oxidation, and (iii) by acting as a conduit allowing CH_4 to 'escape' out of the system. This thesis presents the results of a four-year research project on the effect of two wetland plants, *Phragmites australis* (reed) and *Scirpus lacustris* (bulrush), on CH_4 fluxes in freshwater marshes.

A slurry incubation experiment using sediment collected from the permanently flooded experimental ecosystems showed different distribution patterns for CH_4 oxidation in the vegetated and unvegetated systems (chapter 1). CH_4 oxidation was restricted to the surface layer of the unvegetated sediment whereas in the vegetated sediment CH_4 oxidation was observed at depth as well. In addition, CH_4 oxidation levels in the bulrush slurries were higher than those of the reed slurries. The *in situ* rates of rhizospheric CH_4 oxidation in the experimental ecosystems, measured using the methylfluoride inhibition technique, showed a significant seasonal pattern for reed and bulrush and a significant difference between the plant species, similar as observed with the slurry incubations. When averaged over the growing season, CH_4 oxidation in the rhizospheres of bulrush and reed reduced the potential CH_4 flux by 34% and 16%, respectively. Highest CH_4 oxidation rates were noted early in the plant growth cycle with more than 55% of the generated methane being oxidized in the bulrush system.

For the slurry and rinsed root incubation experiments described in chapter 2 the sediment was collected from the intertidal marsh near Burcht. Rates of CH_4 oxidation decreased with depth in the bulrush and reed slurries, although methane oxidation rates associated with root material from the bulrush plants increased with depth. Apparently, methanotrophs at greater depth live near or even within the roots of the bulrush plants. Reed root material had a limited capacity for methane oxidation and showed no significant increase with depth, which is in agreement with the rhizospheric CH_4 oxidation results described in chapter 1. However, despite the limited capacity of reed root material for CH_4 oxidation, oxidation levels in reed slurries were higher than those of bulrush slurries. Because the reed site was elevated with respect to the bulrush site, it experienced a lower

frequency and duration of flooding and, consequently, a longer time of exposure to atmospheric air. Hence, in intertidal marshes, bulk sediment CH_4 oxidation is probably determined by hydrological conditions that change with distance from the tidal river rather than by the dominant type of vegetation present.

Inhibition of methane oxidation by ammonium was observed in all Burcht samples. Increasing ammonium concentrations resulted in greater inhibition and increasing methane concentrations in less. Ammonium concentrations had to exceed methane concentrations by at least 30-fold to become effective for inhibition. This ratio was found only in the surface layer of the sediment. Hence, the ecological relevance for ammonium inhibition of methane oxidation in intertidal marshes is rather limited and restricted to the surface layer.

The effect of O_2 release by plants on other components than CH_4 has been investigated by comparing the pore-water chemistry of iron, manganese, arsenic, uranium, dissolved organic carbon, ammonium and silicate in matured reed, matured bulrush and unvegetated experimental ecosystems (chapter 3). Reed and bulrush have lower concentrations for most pore-water constituents relative to the control, except for uranium. Probably, O_2 release by the plants resulted in the formation of iron and manganese (oxy)hydroxides following oxidation of reduced iron and manganese. These newly formed metal(oxy)hydroxides scavenge nutrients (silica), trace elements (arsenic) and dissolved organic matter from solution and consequently limit the mobility of these elements. Uranium mobility is known to increase upon oxidation. This study confirmed the larger capacity for bulrush to oxidize the rhizosphere than reed.

In the intertidal marsh and the experimental ecosystems more than 85% of net CH_4 emission was a result of CH_4 venting to the atmosphere through the reed and bulrush roots and stems (chapters 4, 5 and 6). The importance of plants for CH_4 emission was also demonstrated by the alteration between plant transport and ebullition in response to temporal changes in plant growth. Several experiments including measurement of CH_4 emission in a helium atmosphere confirmed the exploitation of a two-way gas transport mechanism for reed (diffusive at night-time and additional (pressurised) convective at daytime), and an all day diffusive mechanism for bulrush. The observed difference in diel CH_4 emission patterns for both plant species is consistent with the difference in gas transport mechanism (chapter 4).

Sediments hosting plant species with a potential large capacity for sediment oxidation (bulrush) show lower CH_4 emission rates than unvegetated sediments or sediments hosting plant species with a relatively small oxidation capacity (reed). From the detailed experimentally derived CH_4 balance presented in chapter 5 we concluded that suppression of CH_4 production is quantitative more important than CH_4 oxidation in attenuating CH_4 emission. The relatively large capacity of bulrush plants for rhizosphere oxidation apparently stimulated active rhizosphere iron cycling, sedimentary Fe(II) reservoirs were more than 1.6 fold smaller in the bulrush system than unvegetated system and a shuttle

role for iron as intermediate between O_2 release and carbon oxidation, attenuating the availability of substrate for methanogens, is suggested.

The results of the two-year field study in the tidal marsh Burcht revealed a typical seasonal pattern in CH_4 emission which correlated strongly with the bulrush and reed plant growth cycles (chapter 6). Especially plant transport appeared a controlling factor for the observed seasonality as indicated by the temporal changes in the dissolved sedimentary CH_4 reservoirs and emission rates. The two unvegetated control sites showed significantly lower CH_4 emission rates than in the vegetated sites of similar setting. Probably, the presence of plants enhanced CH_4 emission by allowing CH_4 to bypass the zones of extensive CH_4 oxidation at the intertidal site where fluctuating water tables and air-exposure cause intensive methanotrophy to greater depth. In the permanently flooded experimental ecosystems where the oxidized surface layer remained small, CH_4 emission rates were on average higher from the unvegetated than vegetated systems (chapter 5).

In addition to the seasonal pattern, the field study at Burcht also revealed a typical spatial pattern in CH_4 emission with higher CH_4 emission rates from the reed site than bulrush site (chapter 6). This spatial pattern was controlled by the different effect of reed and bulrush plants on CH_4 production, CH_4 production suppression, rhizospheric CH_4 oxidation and CH_4 transport rather than CH_4 oxidation related to tidal elevation. CH_4 emission rates from flooded vegetated sites were significant lower compared to non-flooded rates, even at relatively low water levels (< 20 cm). Probably the primary sites of CH_4 release were blocked during ‘high-tide’ since most of the transported CH_4 is released into the atmosphere within the first 20 cm of the plant stems (chapter 4).

Samenvatting

Methaan (CH_4) is een belangrijk broeikasgas. Zowel de natuurlijke waterrijke gebieden als de waterrijke gebieden die voor de landbouw worden gebruikt (natte rijstbouw) zijn aanzienlijke bronnen van atmosferisch CH_4. In het streven naar een betrouwbare balans van bronnen en putten van atmosferisch CH_4, is het essentieel om de emissie van CH_4 in de verschillende waterrijke gebieden en de variatie in de tijd te kwantificeren, alsmede de achterliggende processen en factoren te begrijpen die de variatie in ruimte en tijd controleren. De snelheid van CH_4 emissie wordt bepaald door de interactie tussen CH_4 productie, CH_4 oxidatie en CH_4 transport processen. Emergente waterplanten beinvloeden deze drie processen middels (i) toevoeging van labiele organische koolstofverbindingen aan het sediment via wortelexudatie en het afsterven van plantmateriaal (stimulatie van CH_4 productie), (ii) afgifte van atmospherische zuurstof (O_2) aan de rhizosfeer (stimulatie van CH_4 oxidatie), en (iii) faciliteren van gastransport via de kanalen in wortel en stengel waardoor CH_4 uit de bodem kan ontsnappen (stimulatie van CH_4 transport). Dit proefschrift presenteert de resultaten van een onderzoek van vier jaar naar het effect van twee emergente waterplanten, *Phragmites australis* (riet) and *Scirpus lacustris* (bies), op CH_4 emissie in zoetwater moerassen.

De uitkomsten van een slurrie incubatie experiment met sediment bemonsterd in de permanent onder water staande experimentele ecosystemen (mesocosmos) lieten verschillende verdelingspatronen zien voor CH_4 oxidatie in de begroeide en onbegroeide sedimenten (hoofdstuk 1). De oxidatie van CH_4 bleef beperkt tot de oppervlaktelaag van het onbegroeide sediment, in tegenstelling tot het begroeide sediment waar CH_4 oxidatie ook optrad op grotere diepten. Veder bleek dat de oxidate van CH_4 sneller verliep in de bies slurries dan in de riet slurries. De *in situ* snelheden van CH_4 oxidatie in de rhizosfeer, gemeten met de methylfluoride inhibitie techniek, vertoonde een significant seizoens-patroon voor zowel riet als bies. Eveneens werd een significant verschil tussen beide plantensoorten gemeten (gelijk aan het resultaat met de slurrie incubaties). Gemiddeld over het groeiseizoen reduceerde CH_4 oxidatie in de rhizosfeer de emissie van CH_4 met 34% in het bies systeem en met 16% in het riet systeem. De hoogste CH_4 oxidatie snelheden werden gemeten in het bies systeem relatief vroeg in het seizoen. De potentiële emissie van CH_4 werd daarbij met meer dan 55% teruggebracht.

In hoofdstuk 2 worden de slurrie en gewassen wortel incubatie experimenten beschreven. Het benodigde sediment werd bemonsterd in het intergetijde moerasgebied vlakbij Burcht (België). De CH_4 oxidatie snelheid nam af met de diepte in de bies en riet slurries. Echter in de incubaties met het gewassen wortel materiaal van de bies planten nam de snelheid met de diepte toe. Blijkbaar leven de CH_4 oxideerders (methanotrofen) op

grotere diepte nabij of zelfs binnen de wortels van de biezen. De rietwortels bleken een beperkte capaciteit te hebben voor CH_4 oxidatie en lieten geen significante toename met de diepte zien wat in overeenstemming is met de CH_4 oxidatie resultaten van de rhizosfeer beschreven in hoofdstuk 1. Echter, ondanks de beperkte capaciteit van rietwortels voor CH_4 oxidatie waren de oxidatie snelheden in de riet slurries groter dan in de bies slurries. Omdat de riet zone hoger lag dan de bies zone, was de overspoelings-duur en frequentie lager, en daarmee de tijd dat het sediment aan de atmosferische lucht was blootgesteld langer, voor de riet zone dan voor de bies zone. Kortom, in intergetijde moerasgebieden wordt de snelheid van CH_4 oxidatie in het bulk sediment waarschijnlijk meer bepaald door de hydrologische omstandigheden welke veranderen met de afstand tot de rivier dan door de variatie in plantensoorten.

In alle Burcht monsters is de inhibitie van van CH_4 oxidatie door ammonium waargenomen. Hogere concentraties ammonium resulteerde in meer inhibitie en hogere CH_4 concentraties in minder. De ammonium concentratie diende minimaal 30 maal hoger te zijn dan de CH_4 concentratie voor inhibitie. Deze ratio is alleen gevonden in de meest oppervlakkige laag van het sediment. Dientengevolge lijkt de ecologische relevantie van CH_4 oxidatie inhibitie door ammonium in intergetijde moerassen beperkt en gelimiteerd tot de bovenste lagen van het sediment.

Het effect van O_2 afgifte door planten op andere elementen dan CH_4 is onderzocht door het vergelijken van de porie-water chemie van ijzer, mangaan, arseen, uranium, opgelost organisch koolstof en silicaat in de onbegroeide en met volwassen riet en bies planten begroeide experimentele ecosystemen (hoofdstuk 3). Met uitzondering van uranium waren de gemeten stoffen lager in het poriewater van de riet en bies systemen vergeleken met het onbegroeide controle systeem. Waarschijnlijk heeft de O_2 afgifte van de planten geresulteerd in de oxidatie van gereduceerd ijzer en mangaan en de vorming van ijzer en mangaan (oxy)hydroxiden. Deze nieuw gevormde metaal(oxy)hydroxiden vangen nutriënten (silicaat), sporenelementen (arseen) en opgelost organische stof in uit het poriewater en beperken zo de mobiliteit van deze elementen. Het is bekend dat de mobiliteit van uranium toeneemt na oxidatie. Deze studie bevestigt de grotere capaciteit van bies planten om de rhizosfeer te oxideren dan riet planten.

In het intergetijde moeras en de experimentele ecosystemen verliep meer dan 85% van de netto CH_4 emissie naar de atmosfeer via de riet en bies wortels en stengels (hoofdstuk 4, 5 en 6). Het belang van de planten voor de emissie van CH_4 wordt ook duidelijk uit de afwisseling tussen het transport van CH_4 via de planten en via bellen als functie van de groeicyclus van de plant. Meedere experimenten, waaronder de meting van CH_4 emissie in een helium atmosfeer, bevestigden de exploitatie van een twee-weg gastransport mechanisme voor riet (diffusie gedurende de nacht en een combinatie van diffusie en convectie gedurende de dag), en een één-weg mechanisme voor bies (diffusie gedurende de dag en de nacht). Het waargenomen verschil in het dagelijkse CH_4 emissie patroon van beide plantensoorten is consistent met het verschil in transport mechanisme (hoofdstuk 4).

Sedimenten begroeid met planten die een potentieel grote capaciteit hebben voor oxidatie van het sediment (bies) hebben lagere CH_4 emissie snelheden dan onbegroeide sedimenten of sedimenten begroeid met planten met een relatief kleine oxidatie capaciteit (riet). Uit de gedetailleerde experimenteel verkregen CH_4 balans (hoofdstuk 5) hebben we geconcludeerd dat het onderdrukken van CH_4 productie kwantitatief belangrijker is voor de vermindering van CH_4 emissie dan CH_4 oxidatie. De relatief grote capaciteit van bies om de rhizosfeer te oxideren heeft blijkbaar de oxidatie van ijzer in de rhizosfeer gestimuleerd, Fe(II) reservoirs in het sediment waren meer dan 1.6 maal kleiner in het bies systeem dan in het onbegroeide systeem en een rol voor ijzer als pendeldienst tussen de O_2 afgifte en de oxidatie van organisch materiaal, wat weer de beschikbaarheid van substraat voor methanogenen beperkt, is geopperd.

De resultaten van een twee-jaar durende veldstudie in het intergetijde moeras Burcht lieten een typisch CH_4 emissie patroon zien dat sterk correleerde met de groeicycli van bies en riet (hoofdstuk 6). Speciaal het transport van CH_4 door de planten bleek een controlerende factor te zijn voor het waargenomen seizoens-patroon zoals duidelijk werd uit de fluctuaties in de hoeveelheid opgelost sedimentair CH_4 en CH_4 emissie snelheden. De CH_4 emissie snelheid in de onbegroeide controle plots was significant lager dan in de begroeide plots. Waarschijnlijk faciliteerde de planten de emissie van CH_4 door een alternatieve route te bieden voor het sedimentaire CH_4 waarbij de zone van extensieve CH_4 oxidatie in het intergetijde gebied, waar de fluctuerende water spiegels en blootstelling aan de atmosfeer intensieve methanotrofie tot grotere diepte tot gevolg heeft, wordt vermeden. In de permanent onder water staande experimentele ecosystemen blijft de geoxideerde oppervlaktelaag relatief klein en de CH_4 emissie snelheden waren dan ook gemiddeld hoger in de onbegroeide systemen dan in de begroeide systemen (hoofdstuk 5).

Naast het typische seizoens-patroon liet de veldstudie op Burcht ook een typisch ruimtelijk patroon zien in CH_4 emissie met hogere CH_4 emissie snelheden in de riet zone dan in de bies zone (hoofdstuk 6). Dit ruimtelijke patroon werd meer gecontroleerd door het verschillende effect van riet en bies op CH_4 productie, onderdrukking van CH_4 productie, CH_4 oxidatie in de rhizosfeer en CH_4 transport dan door het effect van de verschillende getij hoogte op CH_4 oxidatie. De CH_4 emissie snelheden in de begroeide plots waren significant lager wanneer de plots waren ondergelopen dan wanneer niet, zelfs bij relative lage waterhoogtes (< 20 cm). Waarschijnlijk werden de primaire loci voor CH_4 afgifte in de plantenstengels geblokkeerd bij ‘vloed’. Het merendeel van het getransporteerde CH_4 komt binnen de eerste 20 cm van de plantstengel vrij (hoofdstuk 4).

Nawoord

"Ik zou ontzaglijk geleerd willen zijn – en er niets mee doen". (J.C. Bloem, *Aforismen*).

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