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Emission and oxidation of methane in *Equisetum fluviatile* stands growing on organic sediment and sand bottoms

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Abstract. Methane emission and rhizospheric CH_4 oxidation were studied in stands of *Equisetum fluviatile*, a common cryptogam in boreal lakes. The experiment was performed in mesocosms with organic sediment or sand bottoms under natural variation of temperature and light using the light-oxic – dark-anoxic chamber (LO/DA) technique. Net CH_4 emission from the organic sediment during the growing season varied between 3.4 and 19.0 mg m^{-2} h^{-1}, but from sand the net CH_4 emission was only 3–10% of that measured from the organic sediment. In the organic sediment net CH_4 emission was very significantly correlated with sediment temperature ($r^2 = 0.92$). In the sand mesocosms the variation of net CH_4 emission was better correlated with the shoot biomass than with sediment temperature variation during the growing season, indicating that methanogens were severely limited by substrate availability and were probably dependent on substrates produced by *E. fluviatile*. The proportion of the methane oxidized of the potential CH_4 emission in summer did not differ significantly between the bottom types. The net CH_4 emission during the growing season as a proportion of the seasonal maximum of the shoot biomass was significantly higher in the organic sediment mesocosms (6.5%) than in sand (1.7%). The high CH_4 emissions observed from dense well-established *E. fluviatile* stands in the field appear to be more related to temperature-regulated turnover of detritus in the anaerobic sediment and less to CH_4 oxidation and seasonal variation in plant growth dynamics

Introduction

Emergent and floating-leaved vegetation play a central role in the fluxes of radiatively important gases (CO_2 and CH_4) in the littoral zones of lakes. In the vegetated areas > 90% of the emissions of methane from the anoxic sediment to the atmosphere are mediated by plants (Schütz et al. 1989; van der Nat and Middelburg 1998a). Significant correlation between methane emission and vascular plant production has been reported in wetlands (Whiting and Chanton 1993; Joabsson and Christensen 2001). Vascular plant root systems may be important in transporting fresh carbon compounds to the anoxic layers, thus improving substrate availability for methanogens (van Veen et al. 1989; Chanton et al. 1995). On the other hand, a well-developed root system facilitates the escape of methane from anoxic

layers to the atmosphere (Joabsson et al. 1999). As a result of adaptation to continuous inundation the wetland plants have mechanisms of oxygen transport via aerenchymal tissues to the rhizomes in the anoxic sediment (Allen 1997; Grosse et al. 1996). Thus, oxygen transported by plants to rhizomes supports CH_4 oxidation in the sediment (Epp and Chanton 1993; King 1996). Depending on the growth phase and gas transport mechanism, seasonal variation and interspecific differences in the rhizospheric oxidation have been observed (Calhoun and King 1997; van der Nat and Middelburg 1998b).

Water horsetail (*Equisetum fluviatile* L.) is a common cryptogam in the boreal zone, especially in oligotrophic lakes with varying bottom types. The species often forms large monospecific stands, which extend to the outermost fringe of the helophyte vegetation (Toivonen and Lappalainen 1980; Kairesalo 1983). High emissions of CH_4 were measured from dense stands *E. fluviatile* in the littoral zone of the mesotrophic boreal lake Pääjärvi, S-Finland (Hyvönen et al. 1998), comparable to the highest estimates of emissions of CH_4 from boreal peatlands and beaver ponds (Crill et al. 1992; Bubier and Moore 1994). In the littoral zone of lake Pääjärvi the CH_4 emissions were significantly correlated with temperature, but not with shoot number or plant biomass of *E. fluviatile* (Hyvönen et al. 1998).

In order to determine to what extent plant-mediated CH_4 emissions from *E. fluviatile* stands are due to variation in temperature, sediment quality and rhizospheric CH_4 oxidation, we studied these processes in detail in mesocosms with two homogenous bottom types. *E. fluviatile* stands were established on the sediment of the original growth site, organic silt sediment from the littoral zone of Lake Pääjärvi, and on sand originally very poor in organic matter. The hypotheses of the study were: 1) Sediment quality, especially organic matter content influences CH_4 production and emissions via emergent plants to the atmosphere 2) CH_4 emissions are directly coupled to temperature-dependent methanogenic activity in the sediment and 3) due to the diffusive mode of gas transport (cf. Hyvönen et al. (1998)), the root oxygenation capacity of *E. fluviatile* is weak and the methanotrophs are limited by oxygen availability.

Materials and methods

Experimental design

The experimental mesocosms were established on an open meadow close to lake Pääjärvi (61°04′ N; 25°08′ E), and at the Lammi Biological Station, Southern Finland. Rhizomes of *Equisetum fluviatile*, originating from the littoral zone of lake Pääjärvi, were planted into polypropene plastic buckets (height 0.4 m, surface area 0.086 m^2, vol. 0.03 m^3) with two different bottom types: organic silt sediment from the lake littoral; and sand (grain size 0.1–1 mm) from an esker from the catchment area of Lake Pääjärvi (Table 1). For both bottom types three experimental and three control mesocosms were established. The buckets were partly dug into the ground

Table 1. Properties of the organic sediment and sand (mean ± SE; n = 6) in the experimental mesocosms.

	Org. sediment	Sand
Wet weight (g cm^{-3})	1.36 ± 0.01	1.79 ± 0.02
Water content (% of mass)	47.3 ± 0.9	20.9 ± 1.1
Loss on ignition (% DW)	7.8 ± 0.3	0.9 ± 0.2
TotFe in pore water (μg cm^{-3})	3.87 ± 0.31	36.69 ± 4.70

so that their surfaces were about 15 cm above ground level. The walls of the buckets above ground level were covered with aluminium foil in order to avoid any light penetration and to minimize heating of the sediment. The *E. fluviatile* stands growing on sand were established two growing seasons prior to the experiment. Due to poor growth they were fertilized during the second growing season with a mineral fertilizer for gardens (KEMIRA, Finland: N-P-K 8–4-14), so that 0.275 g NH_4-N, 0.125 g NO_3-N, 0.2 g P and 0.7 g K were added per bucket. The *E. fluviatile* stands on organic sediment were established one growing season prior to the experiment and no fertilizers were added. During the growing seasons the buckets were always filled with water from lake Pääjärvi so that the water level was always at least 5 cm above the sediment surface. In order to measure gas exchange between air and vegetation/water interfaces, the buckets were equipped with gas-tight collars for rapid insertion of chambers.

CH_4 emission and net ecosystem exchange of CO_2 (NEE)

The rates of CH_4 emission and net ecosystem exchange (NEE) of CO_2 in the mesocosms were measured with a closed-chamber technique once a month, from 23 May to 20 September 2000. The chamber (height 1 m, vol. 0.1 m^3) was made of a clear 2-mm thick polycarbonate sheet (light transmission 86–88% at 400–700 nm, 0% at < 390 nm wavelengths) and was equipped with a fan. Prior to CH_4 sampling, NEE was measured as a decrease in the concentration CO_2 in the chamber with a LICOR LI-6252 CO_2 analyser within 2–3 min. The chamber was then ventilated for about 5 minutes and closed again for a 9 or 10 min time series of gas samples taken via silicone tubes, connected with 3-way stopcocks, into 60 ml polypropylene syringes. The increase in CH_4 concentration in the chamber (0, 3, 6 and 9 min) was measured with an HP 5710A gas chromatograph (FID, HayeSepQ column: mesh 80/100), equipped with a 0.5 ml loop in a VALCO 10-port valve, within 12 hours from sampling. On rainy days the LICOR LI-6252 CO_2 analyser could not be used outdoors and then CO_2 was analysed in the laboratory with an infra-red carbon analyser (URAS 3 G, Hartmann & Braun) from gas samples from the same time series as used for CH_4 analyses. The rates of CH_4 emission and NEE were calculated from the slope of the linear regression between the headspace gas concentration and the incubation time. Results with $r^2 > 0.90$ and slopes significantly differing from zero ($p < 0.05$) were accepted. Those measurements in which

the CH_4 concentration remained < 2 ppm and showed no linear increase during 9 min incubation ($p > 0.10$) were regarded as 0 emission. For the organic sediment mesocosms only 2 of 67 CH_4 emission measurements were rejected. For sand the number of rejected measurements was higher (9 of 60), although 5 measurements with slopes differing from zero with $p < 0.10$ were accepted.

The net CH_4 emission estimates for the whole growing season were made by areal integration of LO emission values. Due to the diffuse mode of ventilation by *E. fluviatile* (Hyvönen et al. 1998) no correction for diel CH_4 emission variation was made.

CH_4 oxidation

CH_4 oxidation was estimated by the light-oxic/dark-anoxic (LO/DA) technique, (e.g. Lombardi et al. (1997) and van der Nat and Middelburg (1998b)). CH_4 emission rates from the replicated mesocosms were first measured in light and oxic conditions (LO). Thereafter the mesocosms were covered with polycarbonate chambers (height 1.0 m), darkened by double-layer aluminium foil to avoid light penetration and thus photosynthetic oxygen production. Nitrogen gas (99.5%) was led into the chambers for 12–16 h during the night. Effective circulation of N_2 in the chambers was ensured with fans. In the morning N_2 flushing was stopped and the mesocosms were ventilated for about 5–10 min before measurement of NEE and CH_4 emission rates (DA). The anoxia in the water-sediment interface after the DA period was confirmed with aYSI 55 combined probe (Yellow Springs Instruments). To eliminate possible diurnal variation in LO emissions, adjusted LO values for the experimental mesocosms (Exp_{LOadj}) were calculated. The mean difference between DA and LO emissions in the control (CTRL) mesocosms, measured simultaneously during LO and DA measurements in the experimental mesocosms, was added to the LO values of the experimental mesocosms (Exp_{LO}) as follows:

$$EXP_{LOadj} = EXP_{LO} + (CTRL_{DAmean} - CTRL_{LOmean})$$

The difference between Exp_{DA} and Exp_{LOadj} emission rates was considered to represent the amount of methanotrophic activity in the sediment. For statistical analyses of net CH_4 emission data the Exp_{LO} values from treated mesocosms and all CH_4 emission values from controls were compiled. Exp_{DA} values were regarded as potential emission of CH_4 and were treated separately.

Shoot number and biomass of E. fluviatile

The number of *E. fluviatile* shoots was counted and their total length was measured from each mesocosm within three days after each CH_4 emission and oxidation measurement. Withered shoots or tips of shoots, present in August and September, were not included in the estimates. For biomass estimates randomly chosen shoots from both bottom types were cut on 27 July. The length of the shoots was measured and they were weighed (precision 0.1 mg) after drying at 60 °C (48 h). The

length-dry weight regression equations obtained were applied for shoot weight estimation during the whole growing season. For organic sediment $SW = 0.225^{*}L^{1.811}$ ($n = 30$, $r^2 = 0.893$), and for sand $SW = 1.532^{*}L^{1.322}$ ($n = 24$, $r^2 = 0.923$), where SW = shoot weight (DW mg) and L = length (cm).

After the last LO/DA treatment and gas flux measurements in September, the rhizome biomass was determined for each mesocosm. The rhizomes were carefully washed, dried for 2–4 days at 60 °C and weighed (precision 0.05 g).

Physical and chemical parameters

Solar radiation (W m^{-2}, maximum sensitivity of the probe at 700 nm, > 60% at 450–900 nm) and air temperature (PT 100/RTD sensor) 2 m above ground level and at a distance of < 10 m from the mesocosms was recorded continuously with an automatic data acquisition system (ITU AQUAGuard 100, Hortimic Oy, Finland) at 10 min intervals. Sediment temperature was measured continuously in one mesocosm with organic sediment at a depth of 20 cm below the sediment surface with a TINYTAG Gemini Data Loggers (UK) data logger recording once per hour. During the measurements of CH_4 emissions and NEE the air temperature in the chambers was measured with a PT 100/RTD sensor.

Iron concentration in the sediment pore water was analysed at the end of the experiment. About 1–2 dm^3 organic sediment or sand from the middle of each mesocosm was taken and transported to the laboratory. Pore water from sand was immediately filtered through Whatman 43 ashless filter paper. Organic silt sediment was centrifuged (2000 rpm) and the supernatant was filtered through a Whatman GF/C filter. The samples were preserved in HCl (final concentration 2%) and TotFe was analysed with a Varian 220 Atomic Absorption Spectrometer. Wet weight, water content, dry weight (+105 °C) and organic content (loss on ignition at +550 °C) of the sediments were determined for each mesocosm (see Table 1).

Results

Temperature

The mean temperature from 16 May to 20 September was the same both in the air (+13.3 °C, SD 3.7) and in the sediment of an organic mesocosm at 20 cm depth (+13.3 °C, SD 2.9). However, the diel fluctuation of temperature was lower in the sediment (Figure 1a). The measured night-time minimum (16 September) and afternoon maximum temperature (29 June) were –4.3 and +31.4 °C in the air, and +4.2 and +20.2 °C in the sediment, respectively.

Growth dynamics of Equisetum fluviatile

During the first emission/oxidation measurements (23–26 May) the mean lengths of shoots of *E. fluviatile* were 14 and 18 cm in organic and sand bottom mesocosms, respectively (Figure 1b). The shoots had emerged above the water surface about one week earlier. The mean length and density of the shoots in the stands increased until the end of July. During the growing season the mean length of shoots did not differ significantly between the bottom types, but from June to September the shoot density was higher in the organic sediment. The maximum density in July was 6012 ± 252 and 1791 ± 166 shoots m^{-2} (SE, n = 6) in organic and sand bottoms, respectively. This difference in density caused a 2–4-fold difference in the shoot biomass of the stands (Figure 1c). The maximum shoot biomass in July was 1.19 ± 0.05 and 0.30 ± 0.02 kg DW m^{-2} (SE, n = 6) in organic and sand bottoms, respectively. Based on the C/DW ratio of 0.384 ± 0.007 of *E. fluviatile* shoots sampled on 10 August 1999 (Kankaala, unpublished) the maximum shoot biomasses were thus 0.46 ± 0.18 and 0.11 ± 0.01 kg C m^{-2}, respectively.

In August the stands of *E. fluviatile* started to decline. However, net photosynthesis of plants was still measured on sunny afternoons until 18–20 September, although the minimum air temperature at night was then –3.4 °C. At this time the topmost 1 cm water layer above the sediment and the shoots of plants were frozen in the morning but they thawed before midday.

The rhizome biomass at the end of the growing season was about 3 times higher in the mesocosms with organic sediment (2.73 ± 0.13 kg m^{-2}) than in those with sand (0.87 ± 0.07 kg m^{-2}). However, the ratio of rhizome biomass to maximum shoot biomass was higher in the sand than in organic sediment mesocosms, 3.0 ± 0.1: 1 and 2.3 ± 0.1: 1, respectively (SE, n = 6).

LO/DA treatment

After DA treatment the oxygen concentration measured in the water at the sediment surface of mesocosms did not exceed 0.3 mg O_2 dm^{-3}, which was the limit of detection of the O_2 probe. The only exception was a sand mesocosm in September, when the O_2 concentration was 0.7 mg dm^{-3}. Thus, the DA treatment efficiently depleted oxygen from the sediments. Immediately after the DA treatment the community respiration often exceeded the net CO_2 uptake, or compared with controls, the net CO_2 uptake was lower in the DA-treated mesocosms (data not shown). However, the community metabolism recovered within a few hours. After the first DA treatment (23–24 May), net carbon uptake measured five hours later did not differ significantly between controls and DA-treated organic sediment mesocosms (541.5 ± 71.8 and 677.8 ± 52.4 mg CO_2 m^{-2} h^{-1}, respectively, t-test, t = 1.533, p = 0.200, df = 4). Furthermore the net CH_4 emissions did not differ significantly (DA2 in Figure 2a), being 7.1 ± 0.6 and 7.5 ± 1.1 mg CH_4 m^{-2} h^{-1}, respectively, (t = 0.734, p = 0.504, df = 4). This indicates rapid recovery of the mesocosms after DA treatment. During the whole growing season the LO CH_4 emission values of the experimental mesocosms did not differ significantly from

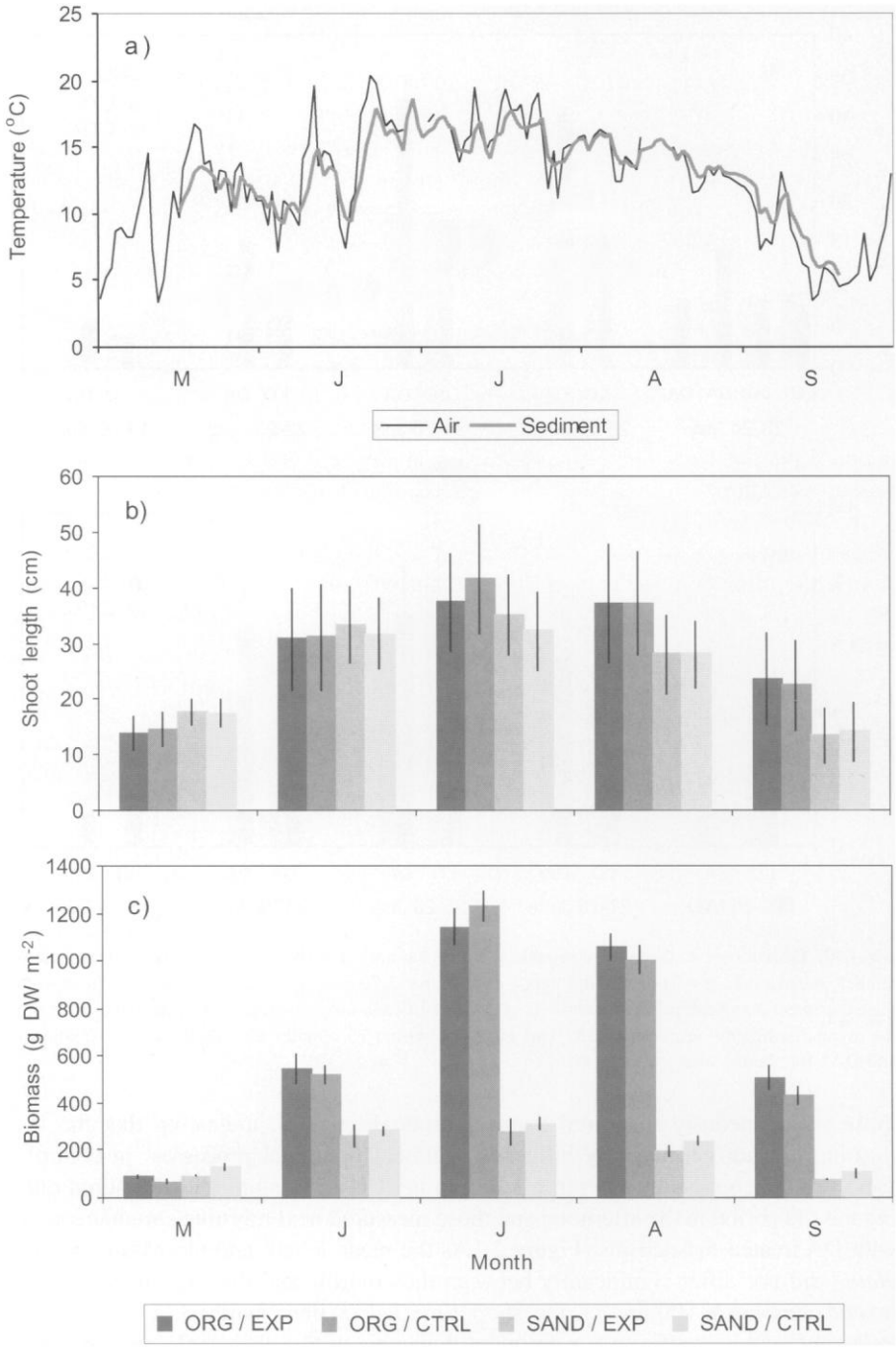

Figure 1. Daily mean of air and sediment temperature (a) and mean length (b) and biomass (c) of shoots in *Equisetum fluviatile* stands (± SE; n = 3) in the experimental and control mesocosms during May – September 2000.

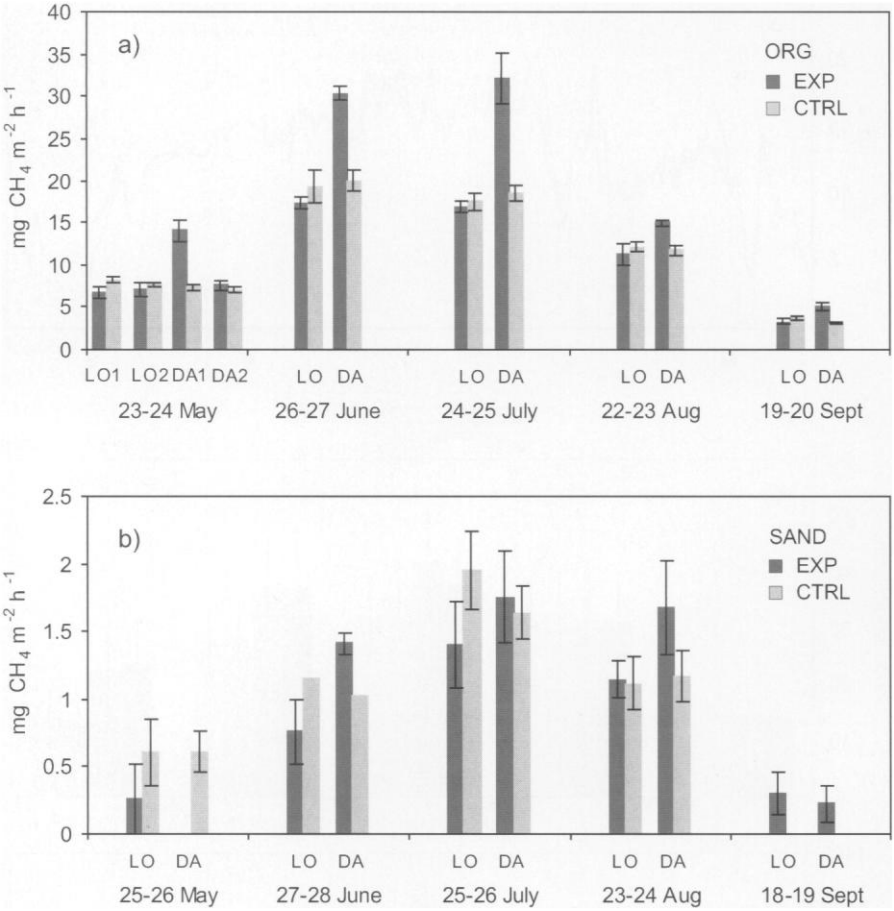

Figure 2. Emission of methane from organic sediment (a) and sand (b) mesocosms during May – September (mean ± SE; n = 3) during the light – oxic phase (LO) and after the dark – anoxic phase from the experimental and control mesocosms (DA). In May LO and DA emission were measured twice from the organic sediment mesocosms; DA1 was measured within 15 minutes after dark – anoxic treatment and DA2 five hours later.

those simultaneously measured from controls (Figure 2), indicating that the N_2 flushing did not permanently influence sediment microbial processes. In controls there was no significant difference between net CH_4 emission rates measured during the LO period in the afternoon and those measured next morning simultaneously with DA treated mesocosms (Figure 2). As the mean length and biomass of *E. fluviatile* did not differ significantly between the controls and the experimental DA-treated mesocosm (Figure 1), the short time lag in photosynthesis after the DA treatment was insignificant for the plant dynamics during the whole growing season.

Table 2. Net emission of methane (mg CH_4 m^{-2} h^{-1}) from *E. fluviatile* stands related to net ecosystem exchange rate (NEE, mg CO_2 m^{-2} h^{-1}), shoot biomass (g DW m^{-2}), instantaneous irradiation (W m^{-2}), daily mean of irradiation (W m^{-2}) and monthly sum of irradiation (kW m^{-2} mo^{-1}) fitted with a linear regression model (y = a + bx). The relationship between methane emission and daily mean of sediment temperature (°C) in the organic sediment was non-linear and was best fitted with the model $y = ae^{bx}$. Regression equations and r^2 values are given only for the relationships with $p < 0.05$.

Bottom type	Independent variable	equation	r^2	p	F	n
Organic sediment	NEE	9.706 – 0.001x	0.090	0.037	4.623	49
	Shoot biomass	6.900 + 0.007x	0.297	< 0.001	20.274	50
	Instantaneous irradiation	14.156–0.012x	0.104	0.022	5.564	50
	Daily mean irradiation			0.866	0.029	50
	Monthly sum of irradiation	4.371 + 0.005x	0.094	0.031	4.968	50
	Sediment temperature	$1.231e^{0.159x}$	0.920	< 0.001	552.011	50
Sand	NEE			0.429	0.192	40
	Shoot biomass	–0.231 + 0.006x	0.626	< 0.001	63.575	40
	Instantaneous irradiation	1.592–0.004x	0.248	0.001	12.538	40
	Daily mean irradiation			0.320	1.015	40
	Monthly sum of irradiation			0.071	3.449	40
	Sediment temperature	–0.696 + 0.126x	0.519	< 0.001	40.963	40

Methane emission related to environmental factors

The mean net emission rate of CH_4 from the *E. fluviatile* stands growing on the organic sediment was 7.7 ± 0.5 mg CH_4 m^{-2} h^{-1} in May, increased to 19.0 ± 0.8 and 17.6 ± 0.5 mg CH_4 m^{-2} h^{-1} in June and July, and decreased again to 3.4 ± 0.2 mg CH_4 m^{-2} h^{-1} towards September (Figure 2a). The net emission rates from the *E. fluviatile* stands growing on sand bottoms were very low, about 3–10% of those measured simultaneously from the organic sediment. (Figure 2b). The highest emissions were measured in July and August (1.1–1.7 mg CH_4 m^{-2} h^{-1}). No net CH_4 emission was detected in two sand mesocosms in May and in four mesocosms in September.

The variation in net CH_4 emission rates in the organic sediment mesocosms was very significantly correlated ($p < 0.001$) with sediment temperature (Table 2). The relationship was best described with an exponential model, which explained 92% of the observed variation. The net CH_4 emission was also significantly correlated ($p < 0.05$) with the shoot biomass of *E. fluviatile* ($r^2 = 0.297$). However, as an additional independent variable in a multiple regression model together with sediment temperature, the shoot biomass had no significant influence ($p = 0.214$). The net emission also showed slight but significant negative correlation with NEE, as well as with instantaneous irradiation, and positive correlation with the monthly sum of irradiation, but these parameters explained only 9–10% of the observed variation.

When plotted against the daily mean of air temperature, the net CH_4 emission from the organic sediment mesocosms fitted into the same range as those measured from the littoral of Lake Pääjärvi from June to September 1994 and 1995 (data

from Hyvönen et al. (1998), Figure 3). The only exception was the low CH_4 emission measured on a warm early summer day (8 June 1995) in Pääjärvi, when the shoots of *E. fluviatile* had just emerged (mean length 9 ± 5 cm) above the water surface. Due to the varying number of replicate measurements (2–9), regression equations describing the relationship between air temperature and net emission were calculated from the mean values for each day. The relationship was best described with exponential models explaining 90.1% of the variation for organic sediment mesocosms and 82.3% of the variation for the littoral of lake Pääjärvi (8 June 1995 excluded). The statistical differences between these two datasets were tested with ANCOVA from $\log_e$ transformed CH_4 emission values. The regression equations did not differ significantly for their slopes ($F = 1.672$, $p = 0.217$) or intercepts ($F = 0.202$, $p = 0.660$, $df = 1$, $n = 17$).

In sand mesocosms 62.6% of the observed variation in net emission was explained by the variation of shoot biomass of *E. fluviatile*. There was also a significant linear correlation between net emission and sediment temperature ($r^2 = 0.519$). However, when included as an additional independent variable in a multiple regression model together with shoot biomass, the impact of sediment temperature was not significant ($p = 0.489$). This was due to the significant correlation between shoot biomass and sediment temperature ($r^2 = 0.568$, $p < 0.001$, $n = 45$). There was also a slight but significant negative correlation between net CH_4 emission and instantaneous irradiation in sand mesocosms ($r^2 = 0.248$, $p = 0.001$).

Potential CH_4 emission rates (emission measured after DA treatment) varied during the study period between 5.0 and 32.2 mg CH_4 m^{-2} h^{-1} in the organic sediment and between 0.2 and 1.8 mg CH_4 m^{-2} h^{-1} in the sand bottom. On both bottom types potential CH_4 emission was significantly correlated with the daily mean of sediment temperature and was best described with exponential models (Table 3). The slopes of the regression equations, calculated from $\log_e$ transformed data, did not differ significantly (ANCOVA, $F = 0.536$, $p = 0.472$), but the difference in intercepts was statistically significant ($F = 524.6$, $p < 0.001$, $df = 1$, $n = 23$). Q_{10} values (acceleration of the reaction rate per 10 °C increase in temperature) between 5 and 17 °C, calculated from the exponential models, were 4.9 and 5.6 for net and potential CH_4 emission in the organic sediment mesocosms and 4.4 for potential CH_4 emission in sand mesocosms. In sand mesocosms potential CH_4 emission was also significantly correlated with shoot biomass, but due to intercorrelation with sediment temperature its influence cannot be treated separately.

The total net emission of methane during the study period (23 May – 19 September) was 36.8 ± 0.8 (SE) g CH_4 m^{-2} from the organic bottom and 2.6 ± 0.4 g CH_4 m^{-2} from sand. Related to the maximum shoot biomass during the growing season, the CH_4 emission expressed as carbon was significantly higher from the organic sediment (6.5 ± 0.4%) than from sand (1.7 ± 0.3%) mesocosms ($t = 8.672$, $p < 0.001$, $df = 10$).

Table 3. Potential emission of methane (mg CH_4 m^{-2} h^{-1}) from *E. fluviatile* stands related to shoot biomass (g DW m^{-2}) and daily mean of sediment temperature (°C). Regression equations and r^2 values are given only for the relationships with $p < 0.05$.

Bottom type	Independent variable	equation	r^2	p	F	n
Organic sediment	Shoot biomass			0.146	2.392	15
	Sediment temperature	$1.627e^{0.172x}$	0.939	< 0.001	201.227	15
Sand	Shoot biomass	0.360 + 0.005x	0.427	0.021	7.440	12
	Sediment temperature	$0.152e^{0.149x}$	0.700	0.001	20.954	11

Table 4. Rate (mg CH_4 m^{-2} h^{-1}) and percentage share of CH_4 oxidized of potential emission in the organic sediment and sand mesocosms during the growing season (mean ± SE). (nd = no data)

Month	Organic sediment Oxidation rate mg CH_4 m^{-2} h^{-1}	%	Sand Oxidation rate mg CH_4 m^{-2} h^{-1}	%
May	7.2 ± 0.5	51.8 ± 2.0	nd	nd
June	11.7 ± 0.02	39.3 ± 1.3	0.6 ± 0.2	38.9 ± 12.5
July	14.4 ± 3.6	43.1 ± 8.1	0.7 ± 0.04	40.6 ± 8.2
August	4.0 ± 1.3	26.9 ± 8.5	0.8 ± 0.2	41.1 ± 8.2
September	2.3 ± 0.1	46.1 ± 2.7	0	0

Methane oxidation

Oxidation of CH_4 was detected throughout the growing season in all *E. fluviatile* stands growing on the organic sediment. The highest CH_4 oxidation rates were measured in June and July (11.7 and 14.4 mg CH_4 m^{-2} h^{-1}, respectively, Table 4). The highest percentages of CH_4 oxidized were in May and September (51.8% and 46.1%) and the lowest in August (26.9%), but the differences between months were not statistically significant (Table 4). In *E. fluviatile* stands growing on sand the oxidation of CH_4 was estimated to vary between 0.0 and 0.8 mg CH_4 m^{-2} h^{-1}. In May the measurements of CH_4 emission after DA treatment were not successful and no estimates of oxidation were made. From June to August ca. 40% of the potential CH_4 emission was oxidized but no oxidation was detected in September.

Discussion

The seasonal plant dynamics had a different influence on CH_4 emissions on the two bottom types studied. In the sand mesocosms the variation of net CH_4 emission was better correlated with the shoot biomass than sediment temperature during the growing season, indicating that methanogens were severely limited by substrate availability and were dependent on substrates produced by *E. fluviatile*. In the organic sediment, net CH_4 emission was more related to temperature-dependent

methanogenic activity in the sediment and was probably less dependent on the seasonal substrate production by plants. The homogeneity of slopes, but the significant difference in the intercepts of the regression equations for potential CH_4 emission in the two bottom types, clearly indicated that the response of the methanogenic activity to temperature was the same, but that the methanogenic community in sand bottoms was more limited by substrate availability than in organic sediments. However, when the variation of shoot biomass of *E. fluviatile* and sediment temperature were intercorrelated, these both factors simultaneously influenced methanogenesis and ventilation of CH_4 out to the atmosphere.

Whiting and Chanton (1993) were able to correlate CH_4 emissions with net ecosystem production (NEP) rate in a wide range of wetlands. They estimated that about 3 per cent of the annual NEP is emitted to the atmosphere as methane. Using shading manipulations of arctic wetland vegetation plots Joabsson and Christensen (2001) demonstrated a direct coupling of vascular plant production and CH_4 emission. This was related to the allocation of recently fixed carbon to below-ground structures, improving substrate availability to methanogens, and also to the well developed rhizome systems of plants, which ventilate CH_4 out from the soil to the atmosphere. In short-term (14–17 days) ^{14}C labelling experiments on wetland plants, recent photosynthates contributed < 1% of CH_4 emission (Megonigal et al. 1999; King and Reeburgh 2002), but in experiments with rice this proportion has been higher (Minoda and Kimura 1996; Dannenberg and Conrad 1999). In our study the measurements of NEE were too scarce to estimate the total CO_2 flux and net primary production (NPP) of *E. fluviatile* during the growing season. The maximum shoot biomass during the growing season generally correlates well with the NPP of above-ground parts of aquatic macrophytes (Westlake 1982). In our experimental mesocosms the proportion of net CH_4 emission of the maximum shoot biomass during the growing season was significantly higher in the organic sediment mesocosms (6.5%) than in sand (1.7%). Thus the hypothesis that sediment quality influences CH_4 production and emissions to the atmosphere in *E. fluviatile* stands was supported in this study.

The role of the alternative electron acceptors such as Fe(III) reducers in giving rise to competing anaerobic organic carbon oxidation and consequent suppression of methanogens, especially in sand mesocosms, cannot be ruled out (cf. Roden and Wetzel (1996)), although this was not studied here. Some indication of this was seen in the concentration of iron in the pore water, which was 10-fold in sand mesocosms compared with organic sediment mesocosms at the end of the experiment (Table 1). As we did not analyze separately Fe(II) and Fe(III) forms, and as the procedure of centrifugation and filtration may have caused precipitation of Fe(II) (see Carignan (1984)), the real difference in soluble Fe(II) concentration between the bottom types might have been lower.

The total net CH_4 emission from organic sediment mesocosms (36.8 g CH_4 m^{-2}) during the study period (23 May – 19 September) was close to that estimated for the period 8 June – 8 November for the littoral of lake Pääjärvi (43.7 g CH_4 m^{-2}, Hyvönen et al. (1998)). The conditions in the mesocosms with organic sediment from lake Pääjärvi littoral zone simulated well those in the dense *E. fluviatile* stands

in the field. This was indicated by the lack of significant difference in the regression models describing the relationship between net CH_4 emission and the daily mean of temperature. The higher variation between replicate measurements in the littoral of lake Pääjärvi was probably due to more variable quality of sediment in the field than in the mesocosms. Although we did not study the accumulation of CH_4 in the sediment, the hypothesis of the direct coupling between temperature-dependent methanogenic activity and CH_4 emissions was supported.

The exponential regression models applied to describe the relationship between the sediment temperature and potential and net emission data (Table 3) predict that Q_{10} is a constant, i.e. the temperature sensitivity does not change with temperature. The Q_{10} value of potential CH_4 emission was slightly higher (5.6) for the organic sediment than for sand (4.4), but the difference was not significant. Q_{10} value for the net CH_4 emission rate in organic sediment was also within the same range (4.9). The values were rather high but the reported variation of the Q_{10} values for CH_4 production is very wide (Segers 1998; van Hulzen et al. 1999). Westermann (1993) reported Q_{10} values as high as 7.5 and 8.5 in the temperature intervals of 5–15 °C and 10–20 °C, respectively, in alder swamp soil slurries. For the net CH_4 emission rates measured *in situ* in the littoral zone of lake Pääjärvi Hyvönen et al. (1998) reported Q_{10} values of 2.3 in the temperature range 10–20 °C and 1.4 in the range 25–35, derived from a linear regression model with ambient chamber temperatures during the CH_4 emission measurements. When the same data was fitted to the exponential model with daily mean temperatures of 6.9–20.6 °C (see Figure 3), a Q_{10} value of 4.8 was obtained. In laboratory experiments methanogenic bacteria have shown decreased temperature dependence as a function of decreasing substrate concentration (Westermann et al. 1989). In our study, the lower organic matter content of the sand meocosms may indicate a smaller amount of substrates available for methanogens than in the organic sediment. Probably both contained methanogenic precursors sufficiently for the existing methanogenic population, but the organic sediment maintained a larger methanogenic population size. This would result in the observed similar temperature response in both types of sediments but higher CH_4 production, and thus higher CH_4 emission rates from the organic sediment.

The oxidation of CH_4 in our study was estimated with the LO/DA technique assuming that oxygen depletion stopped methanotrophy, but did not affect methanogenesis during 12–16 h anoxic treatment during the night. Gerard and Chanton (1993) observed stimulation of methanogenesis under prolonged (1–7 days) dark N_2 treatment of *Sagittaria lancifolia*. With pots of different rice varieties van der Gon and Neue (1996) found 10–190% higher CH_4 emission after N_2 treatment (19–26 h) compared with those treated with methyl fluoride (1.5% vol/vol in the chamber headspace), a specific inhibitor of methanotrophs. In their study the treatments had been made with plants which had been cut 5 cm above water level 1–2 days earlier. Thus, in both studies the plants had clearly suffered from the treatments, which may have promoted anaerobic decomposition and increased substrate availability for methanogens. Furthermore, the above-mentioned studies were performed in greenhouses in Florida (USA) and the Philippines, presumably in higher

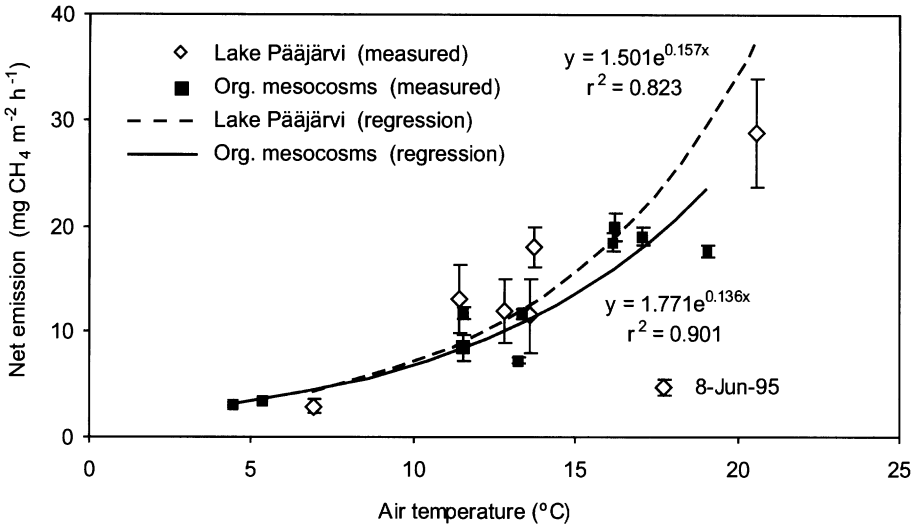

Figure 3. Net CH_4 emission from *Equisetum fluviatile* stands related to daily mean air temperature in the organic sediment mesocosms and in the littoral of lake Pääjärvi in June – September 1994 and 1995 (data from Hyvönen et al. (1998)). The emission value for 8 June 1995 was excluded from the regression equation.

temperatures (data not given) than in our study in the boreal zone. Methanogenesis is known to be very strongly regulated by temperature, the optimal temperature being at 30 °C or even higher (e.g. Zeikus and Winfrey (1976) and Schulz et al. (1997)). In our experiment intact *E. fluviatile* stands did not suffer from the night-time N_2 treatment, and LO emission values recovered within a few hours after the DA treatment. By using night-time DA treatments, comparable to our method, the measured CH_4 oxidation rates in *Pontederia cordata*, *S. langifolia* (Lombardi et al. 1997) and *Scirpus lacustris* stands (van der Nat and Middelburg 1998b) were similar to those obtained by methyl fluoride inhibition. Thus, we believe that the estimated CH_4 oxidation rates for *E. fluviatile* in our study are realistic.

The percentages of CH_4 oxidized in the *E. fluviatile* stands (27–52% in the organic sediment and 0–41% in sand) were within the ranges observed for several emergent aquatic macrophytes (King 1996; Lombardi et al. 1997; van der Nat and Middelburg 1998b). In greenhouse studies based on pot-bound plants with high concentrations of roots (i.e. high root/sediment ratio), misleadingly high CH_4 oxidation percentages (up to 60–100%) have been obtained because of enhanced oxygen availability relative to that in the field (Epp and Chanton 1993; Schipper and Reddy 1996). Although our experiment was performed in mesocosms with limited space, we are confident that the conditions in the mesocosms with sediment from lake Pääjärvi littoral zone simulated well those in the dense *E. fluviatile* stands in the field (cf. Hyvönen et al. (1998)). Our third hypothesis of weak oxidation capacity of the rhizomes of *E. fluviatile* was thus not supported in this study. The good cabability for rhizome oxidation is probably an adaptation of *E. fluviatile* to

colonization of the outermost fringes of the littoral zone. In boreal lakes this species has been found to grow at water depths extending down to 1.2–1.9 m (Kansanen et al. 1974; Toivonen and Lappalainen 1980).

In several studies clear seasonal variation in the oxidation percentage has been detected. The highest percentages have been obtained early in the growing season during the active growth phase of plants and the lowest ones, even down to zero, when the plants were matured (Lombardi et al. 1997; van der Nat and Middelburg 1998b; Popp et al. 2000). In the organic sediment mesocosms no clear difference in the oxidation percentage of *E. fluviatile* stands was detected during the study period from May to September, although the withering and decline of shoot biomass of plants began in August. However, in sand mesocosms no oxidation of CH_4 was detected in September, when emission and production of CH_4 were low. From June to August the percentage of oxidized CH_4 did not vary significantly between the two bottom types. Thus, the higher ratio of rhizome biomass to maximum shoot biomass during the growing season in sand mesocosms appered not to influence the relative oxidation rates.

Our results on emission and oxidation of CH_4 in *E. fluviatile* stands are in accordance with those of van der Nat and Middelburg (1998a), according to which in wetlands dominated by *Phragmites australis* and *Scirpus lacustris* variations in CH_4 production, rather than variations in CH_4 storage and oxidation, largely determined variations in CH_4 emission. The long-term accumulation and/or decomposition of plant detritus on its own growth site influences CH_4 productivity. Compared with many other emergent macrophytes the detritus of *E. fluviatile* is decomposed rapidly (Danell and Sjoberg 1979), which is probably due to its relatively low lignin content (about 5% of DW, Tiina Tulonen pers. comm.). Thus, the high CH_4 emissions from dense *E. fluviatile* stands would be due mainly to temperature-regulated turnover of detritus in the anaerobic sediment and less to CH_4 oxidation and seasonal variation in plant growth dynamics. However, the well-developed rhizome system of *E. fluviatile* is an important pathway for the methane produced to escape from the sediment to the atmosphere via emerged shoots.

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