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Spatial Distribution and Inhibition by Ammonium of Methane Oxidation in Intertidal Freshwater Marshes†

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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 heights 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 resulted 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.

Methane is an important greenhouse gas (8, 9, 10). Emissions from wetlands contribute significantly to the atmospheric methane budget (2, 26). Net methane flux is determined by the balance of its production, oxidation, and transport. Oxidation has been recognized as a key factor in the regulation of methane fluxes (15, 21). Methane oxidation in freshwater sediments depends in a complex manner on hydrology and vegetation (13, 21, 28, 31, 32).

The control of aerobic methane oxidation is obviously related to the requirement for oxygen and methane (21, 35, 36). 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 (14, 25). 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 (19).

Ammonium has been reported as an additional factor, controlling methane oxidation in pure-culture studies (23, 29), landfill cover soil (6), terrestrial soils (5, 12, 34), and aquatic sediments (7, 18). The inhibition mechanism seems to be rather complex, including not only competitive inhibition of the methane monooxygenase enzyme by ammonium (29) but also noncompetitive (toxic) inhibition by hydroxylamine and nitrite produced by the oxidation of ammonium (12, 23, 24). Nitrate, another product of ammonium oxidation, does not seem to be important (1, 6, 12). Moreover, both methanotrophs and ammonium oxidizers are capable of oxidizing ammonium and methane (3, 18, 29). In natural samples, it is rather difficult to estimate the relative contributions of methanotrophs and ammonium oxidizers to ammonium and methane oxidation, respectively (33).

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 *Scripus 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 average periods of submergence per flooding were 0.7 and 1.6 h, respectively. The average heights of the floodwater 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 floodwater were 160, 20, and 210 μM, respectively.

Pore water sampling. Pore water was sampled by in situ dialysis (17), 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 in 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

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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 levels 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 (22). 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 the 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 each 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 shaken at 20°C. Consequently, not only was the slurry thoroughly mixed; the thinness of the slurry layer (~2 mm) 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 them 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 Fig. 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 hour) 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 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 hour). 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.

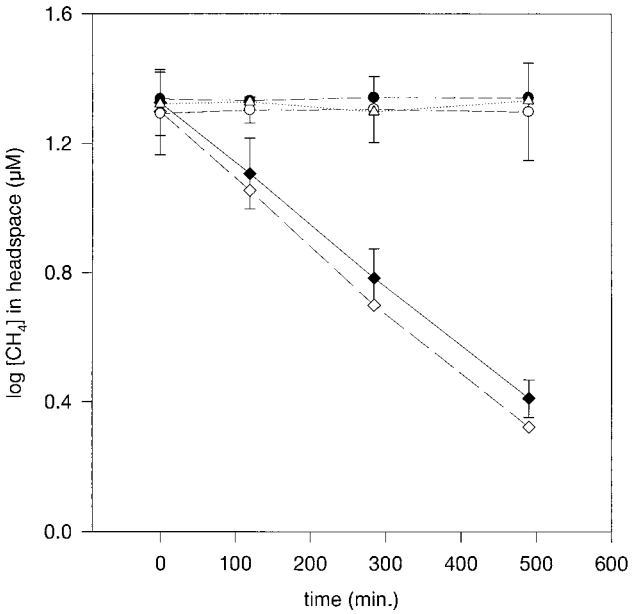

FIG. 1. Logarithms of methane concentrations in the headspaces of oxic-slurry incubation flasks. The initial methane concentration was 1.25 μM. Untreated, ◆; 5% headspace methyl fluoride, ●; autoclaved prior to incubation, ○; water only, Δ; 3 mM BES, ◇. The error bars represent the standard errors of 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 (Fig. 1). Methane consumption increased linearly with increasing amounts of sediment in the slurries (Fig. 2). Nitrate production, like methane consumption, was effectively blocked by methyl fluoride and autoclaving, whereas the ammonium concentration still decreased (data not shown).

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 (Fig. 3; Table 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 [$P > 0.905$] (Fig. 3). Addition of ammonium resulted in about 20% lower k values (Fig. 3). Methane consumption associated with root material increased with depth for bulrush plants, but not for reed plants (Table 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.

The results from the September experiment (bulrush sediment only) showed that depth, ammonium addition, and methane concentration had significant effects on k (Table 1). The decrease in k values between the depths of 15 to 20 cm and 0 to 5 cm (51%) were similar to those observed in the July

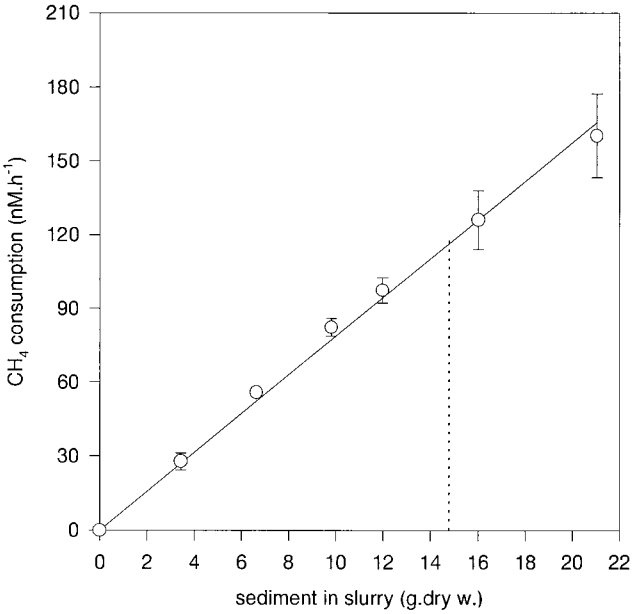

FIG. 2. Methane consumption relative to the amount of sediment (in grams dry weight [g.dry wt.]) in the slurries (bulrush, 15- to 20-cm depth). The initial methane concentration was 1.25 μM. Solid line, linear fit with $r^2 > 0.99$; dashed line, amount of slurry (dry weight) used in the experiments.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

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 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 weak when these were reversed (Table 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.

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 treatment was significantly lower than the other ones except at the 15- to 20-cm depth, whereas the others were not significantly different (Fig. 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 occurring in the 15- to 20-cm and 40- to 45-cm slurries (Table 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 1).

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 (dry weight) of sediment^{-1} · h^{-1} for reed and bulrush, respectively. Addition of methanogenic precursors did not induce methanogenic activity in 0- to 5-cm and 15- to 20-cm slurries but increased production dramatically (about threefold) in 40- to 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).

In situ methane and ammonium pore water concentrations. The vertical profile of methane showed distinct patterns for the bulrush and the reed sites (Fig. 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 saturation concentration at depths of >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.

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 the gas phase and the slurry phase was not rate limiting (Fig. 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 specif-

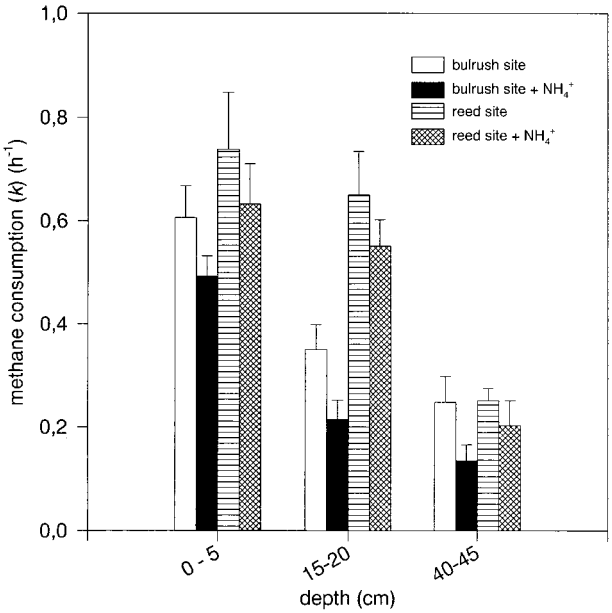

FIG. 3. Effect of site, depth in sediment, and ammonium concentration on methane consumption. Initial methane and ammonium concentrations were 1.25 and 300 μM, respectively. The error bars represent the standard errors of the means ($n = 3$).

TABLE 1. ANOVA tables for 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.

ically inhibits methane and ammonium oxidation (27, 30). 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 microorganisms.

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 (20, 32). 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 (21). One of the features of these types of methanotrophs, also observed in our slurries, is the absence of a lag phase during incubation (18, 21). 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 in our experiments (0.02 to 10 μM) are below or in the lower range of K_m values reported in the literature for wetland sediment and methanotrophs associated with aquatic plant roots (21, 22). The similarity of *k* values, except when very small amounts of methane were added, indicates that the methane concentrations used were indeed lower than or similar to the K_m values 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 (4) and specific properties of the mono-oxygenase enzyme complex (11).

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 are possibly methane limited and oxygen saturated in the uppermost layer of the sediment (21, 22, 35, 36). Oxidation rates in our sediments are probably determined by oxygen availability. Pore water methane concentrations increase with depth (Fig. 5), whereas the potential for methane oxidation decreases with depth (Fig. 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 the upper limits of 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 depths live near or even within the roots of the bulrush plants, as demonstrated previously for several other macrophyte species (16, 22). 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 (Fig. 3; Table 1) whereas that associated with roots increases with depth (Table 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

TABLE 2. Methane consumption rates by root material[a]

Depth (cm)	Amt of methane consumed (nmol · g [dry wt] of sediment^{-1} · h^{-1}) in[b]:	
	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 · liter of headspace^{-1}.
[b] Values are means ± standard errors of the means.
[c] Only consumption by rhizomes was measured.

TABLE 3. Methane oxidation inhibition percentages in bulrush site slurries from different depth horizons with different initial ammonium concentrations

Initial concn (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.

inhibition technique (37). 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.

TABLE 4. Nitrate production rates in bulrush site slurries with different amounts of ammonium added

Depth (cm)	Nitrate production rate (nmol · g [dry wt]$^{-1}$ · h^{-1}) at initial [NH_4^+] (μM) of[a]:		
	30	300	1,500
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.

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 (6, 7, 12, 23, 24, 34) 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 (3), as well as the production of hydroxylamine and nitrite through ammonium oxidation by methanotrophs (methanotrophic ammonium oxidation) and ammonium oxidizers, a combination of both 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 (6, 7, 12). Nevertheless, competitive inhibition is probably the most important mechanism in our slurries, as indicated by a significant interaction between methane and ammonium (Table 1) and by the decrease in methane oxidation to zero with increasing ammonium concentrations (Table 3). We found no effect of methane on concentration nitrate production (Table 1), as has been reported before (6, 7, 12). Ammonium is probably the more aggressive substrate, so even moderate concen-

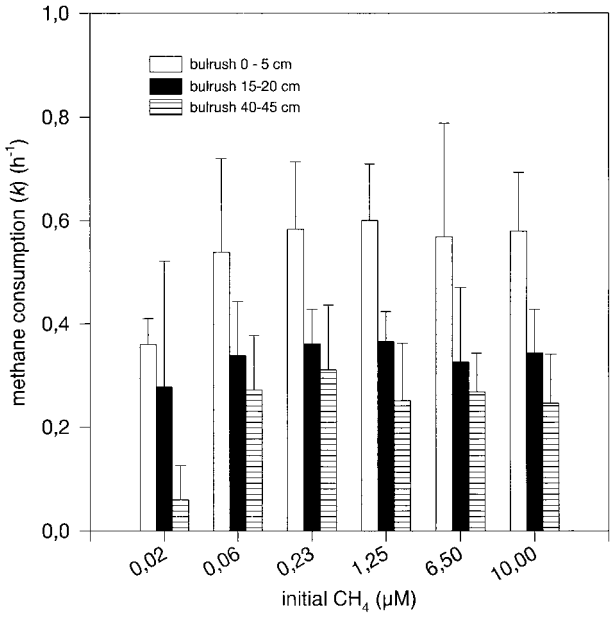

FIG. 4. Effect of methane concentration on methane consumption. The error bars represent the standard errors of the means (n = 3).

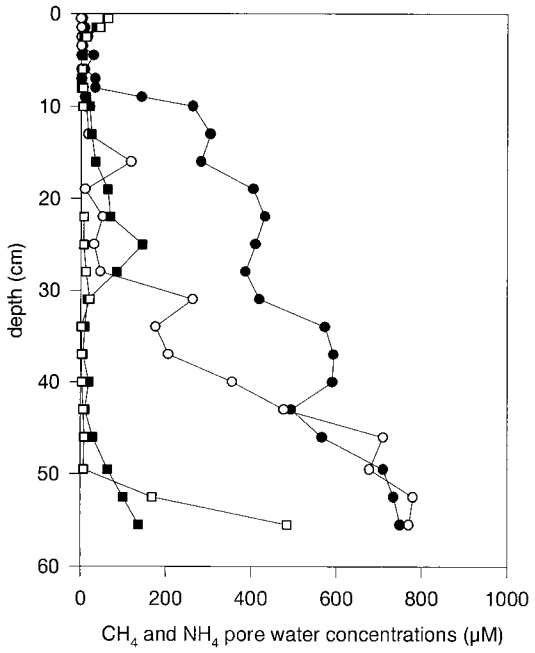

FIG. 5. Pore water methane (circles) and ammonium (squares) concentrations at the bulrush (open symbols) and reed (closed symbols) sites.

trations 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 10 times more for ammonium (Fig. 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 (Fig. 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 (Fig. 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 of methane oxidation (29). 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 a 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 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.

ACKNOWLEDGMENTS

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