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Dynamics of Nitrification and Denitrification in Root-Oxygenated Sediments and Adaptation of Ammonia-Oxidizing Bacteria to Low-Oxygen or Anoxic Habitats†

PAUL L. E. BODELIER,[1]* JACOBUS A. LIBOCHANT,[1] CEES W. P. M. BLOM,[2] and HENDRIKUS J. LAANBROEK[3]

Department of Plant-Microorganism Interactions, Centre for Terrestrial Ecology, Netherlands Institute of Ecology, Heteren,[1] Department of Ecology, University of Nijmegen,[2] and Department of Microbial Ecology, Centre for Limnology, Netherlands Institute of Ecology, Nieuwersluis,[3] The Netherlands

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Oxygen-releasing plants may provide aerobic niches in anoxic sediments and soils for ammonia-oxidizing bacteria. The oxygen-releasing, aerenchymatous emergent macrophyte *Glyceria maxima* had a strong positive effect on numbers and activities of the nitrifying bacteria in its root zone in spring and early summer. The stimulation of the aerobic nitrifying bacteria in the freshwater sediment, ascribed to oxygen release by the roots of *G. maxima*, disappeared in late summer. Numbers and activities of the nitrifying bacteria were positively correlated, and a positive relationship with denitrification activities also was found. To assess possible adaptations of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats, a comparison was made between the freshwater lake sediment and three soils differing in oxicity profiles. Oxygen kinetics and tolerance to anoxia of the ammonia-oxidizing communities from these habitats were determined. The apparent K_m values for oxygen of the ammonia-oxidizing community in the lake sediment were in the range of 5 to 15 μM, which was substantially lower than the range of K_m values for oxygen of the ammonia-oxidizing community from a permanently oxic dune location. Upon anoxic incubation, the ammonia-oxidizing communities of dune, chalk grassland, and calcareous grassland soils lost 99, 95, and 92% of their initial nitrifying capacity, respectively. In contrast, the ammonia-oxidizing community in the lake sediment started to nitrify within 1 h upon exposure to oxygen at the level of the initial capacity. It is argued that the conservation of the nitrifying capacity during anoxic periods and the ability to react instantaneously to the presence of oxygen are important traits of nitrifiers in fluctuating oxic-anoxic environments such as the root zone of aerenchymatous plant species.

Flooded soils and sediments are generally anoxic, except for the upper few millimeters. This is due to a reduced diffusion of oxygen in water compared with air in combination with oxygen-consuming processes (28). The oxidation of ammonia to nitrite and nitrate, by ammonia- and nitrite-oxidizing bacteria, can only occur in the presence of oxygen and will thus be restricted to the marginal oxic parts of flooded soils and sediments. However, a large number of plants which grow in anoxic soils or sediments contain aerenchymatous tissue by which they establish a gas space continuum between the atmosphere and the root tissue (2, 22). The aerenchymatous tissue, which is one of the possible adaptations to anoxic root environments in a vast array of plants (4, 5, 21), provides the roots with oxygen to maintain root respiration. A part of the oxygen, which diffuses to the roots, leaks into the root zone and thus elevates redox conditions preventing the buildup of phytotoxic reduced compounds (Fe^{2+}, Mn^{2+}, S^{2-}) in the rhizosphere (1, 16). Hence, the root zone of aerenchymatous plants may form a niche for the aerobic ammonia- and nitrite-oxidizing bacteria, which oxidize ammonia to nitrite and nitrate. The nitrate produced subsequently can be used by denitrifying bacteria in the adjacent anoxic sediment or soil, which reduce it to molecular nitrogen or nitrous oxide (38), leading to nitrogen loss from the ecosystem. The availability of oxygen for nitrifiers might depend on the developmental stage of the plant in the growing season. It has been established that radial oxygen loss from aerenchymatous roots is linked to the respiratory activity of the roots, which varies with temperature and age of the root (9, 11, 26). Although in situ nitrification in the rhizosphere of aerenchymatous plants has never been reported, a number of studies have indirectly demonstrated a stimulation of the nitrification process by oxygen-releasing plants (7, 12, 15, 18, 30). However, none of these studies gives any information on the survival mechanisms of the nitrifiers in those oxygen-limited environments, where they probably have to compete for the available oxygen with heterotrophic bacteria as well as chemical oxidation processes. The competitive ability for a limiting substrate is defined as the ratio of the maximum consumption capacity (i.e., V_{max}) and the affinity constant (i.e., K_m) (19). Nitrifying bacteria are poor competitors for oxygen due to their low V_{max} and high K_m values, respectively, compared with heterotrophs (23, 24). Thus, nitrifying activity in the rhizosphere of aerenchymatous plants is only possible when there is a surplus of oxygen, assuming that ammonia is not limiting and that there is no spatial separation between nitrifiers and heterotrophs. Another possibility is that nitrifying bacteria have the ability to adapt by yet unknown means to conditions of limited oxygen supply.

The goals of this study were to assess whether oxygen-releasing plants can provide a niche for nitrification and subsequent denitrification and to test whether nitrifying bacteria are adapted to life in low-oxygen or periodically anoxic habitats.

* Corresponding author. Mailing address: Netherlands Institute of Ecology, Centre for Terrestrial Ecology, Department of Plant-Microorganism Interactions, P.O. Box 40, 6666 ZG Heteren, The Netherlands. Phone: (31)-(0)-26 4791306. Fax: (31)-(0)-26 4723227. Electronic mail address: BODELIER@CTO.NIOO.KNAW.NL.

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To this end, the numbers and potential activities of the nitrifying and the denitrifying bacteria inside and outside a *Glyceria maxima* (Hartm.) Holmb. stand were monitored in the littoral zone of a freshwater lake sediment during the course of 1 year. *G. maxima* was chosen because this plant, which is very common in The Netherlands, has been indicated to stimulate nitrifying bacteria in its rhizosphere (7). Moreover, *G. maxima* is highly aerenchymatous (34), and the inability of its roots to survive anoxia predicts continuous oxygenation of the root tissue and oxygen leakage to the rhizosphere (8, 31). The availability of information on the composition of the nitrate-reducing bacterial community in the *G. maxima* root zone (27) was another argument for the choice of this plant species.

Oxygen kinetics and anoxia tolerance of the nitrifying bacteria in the presumably oxygen-limited sediment and in three soil types differing in oxygen availability were determined to assess possible adaptations of the ammonia oxidizers to low-oxygen–anoxic conditions.

MATERIALS AND METHODS

Site description and sampling. For the study of the effect of emergent macrophytes on nitrification and denitrification a *G. maxima* (reed sweet grass) stand in Lake Drontermeer, near the township of Elburg (The Netherlands, 52°58′N, 5°50′E), was selected. This stagnant freshwater lake location was chosen because a stable population of *G. maxima* has been present here for at least 10 years. In the sampled area *G. maxima* represented 100% of the vegetation present. Five intact sediment cores (depth, 25 cm; diameter, 10 cm) were collected inside the *G. maxima* stand, and another five were collected in the bare sediment outside the *G. maxima* stand during the period from May 1994 through May 1995. The cores were transported to the laboratory in polyvinyl chloride cylinders with water from the lake on top. Prior to any further measurements, the top 5 cm of each core was removed to exclude the nitrifying bacteria which might have received oxygen by diffusion from the water layer. From the remaining part of each core, the rooted fraction (15 cm) was separated from the roots, sieved (4-mm mesh), homogenized, and stored (4°C) prior to use for measurements. All measurements performed on the sediment-soil as described below were completed within 3 days after sampling. For dry weight determinations roots were dried for 48 h at 70°C.

To detect possible adaptations of the nitrifying bacteria in the sediment to low-oxygen conditions, a number of soils which differ in oxygen availability were selected for comparison. As a control soil (permanently oxic), sand from the top of a coastal dune (Meijendel, near The Hague, The Netherlands, 52°08′N, 4°19′E) was collected. Two locations were sampled as intermediate soil types (i.e., periodically or partially subjected to low-oxygen conditions). Soil from a chalk grassland in The Gerendal (50°5′N, 5°54′E) in the south of The Netherlands was collected because the resident nitrifying community at that location is partially subjected to anoxia. This soil, containing a high percentage of loess, contains anoxic microsites due to the high moisture percentage (Table 1), which also is indicated by high denitrification enzyme activities (data not shown). A calcareous grassland situated in a river levee near the village of Brummen (The Netherlands) (52°5′N, 6°9′E) was chosen as a periodically anoxic location as it is flooded on an irregular basis. In the winter prior to sampling, this grassland had been flooded for a substantial period of time. From these locations, the top 5 cm of 5 randomly selected plots was sampled by means of a grass-plot sampler. This was done by placing a frame, consisting of 16 squares (225 cm^2 each), randomly at 5 positions on top of the soil. At each position, two sets of 16 cores (depth, 5 cm; diameter, 2.5 cm) were taken, pooled, and regarded as one replicate. The soil collected from these locations was also separated from roots, sieved (4-mm mesh), and homogenized. The moisture content was determined as weight loss after drying (24 h, 105°C), and the organic matter content was determined as loss on ignition (4 h, 550°C). The pH was measured in 1:2.5 (wt/vol) soil or sediment–water slurries after 2 h of shaking. Ammonium, nitrite, and nitrate content of the soils and sediment were determined by extraction with 1 M KCl (1:2.5 [wt/vol]) by using a Technicon Traacs 800 autoanalyzer. Some descriptive parameters, important for nitrification, for all sampled locations are presented in Table 1.

Redox measurements. To get an indication regarding microbial oxidation processes that can occur in the root zone of *G. maxima* and in the bare sediment at Lake Drontermeer, redox potentials were determined as described by Clevering and van der Putten (13). Immediately upon arrival in the laboratory, redox potentials were determined at a 10-cm depth in the intact sediment cores with a platinum electrode, placed in the center of the core, and a calomel reference cell. The potentials were transformed into redox potentials by adding the potential of the reference cell (+242 mV).

Enumeration of nitrifying bacteria. Ammonia- and nitrite-oxidizing bacteria were enumerated by a most probable number (MPN) technique described by Verhagen and Laanbroek (41). Twenty grams of moist sediment or soil was shaken for 2 h (180 rpm, 20°C) in a buffer solution containing the following (grams per liter): NaCl, 8.5; $Na_2HPO_4 \cdot 2H_2O$, 1.34; and NaH_2PO_4, 0.35. Subsamples (0.25 ml) from the suspensions were serially diluted in 10-fold steps in sterile microtiter plates (tissue culture cluster, 24 wells; Costar, Cambridge, United Kingdom) containing growth medium for ammonia- and nitrite-oxidizing bacteria, respectively, as described by Verhagen and Laanbroek (41). Seven replicates were made per dilution; one dilution series was not inoculated with a soil or sediment subsample and was regarded as a negative control. After an incubation period of 8 weeks at 28°C, ammonium oxidation was scored in each well by checking for the presence of nitrite or nitrate by adding Griess Ilosvay reagents (32) and, subsequently, zinc powder. Nitrite oxidation was detected by addition of the Griess Ilosvay reagents to check for the disappearance of the added nitrite. MPN of nitrifying bacteria were obtained from statistical tables by using a computer program as previously described by Verhagen and Laanbroek (41).

Determination of potential ammonium-oxidizing activity and oxygen kinetics. Potential ammonium-oxidizing activity and oxygen kinetics of ammonia-oxidizing bacteria were determined by using sediment or soil suspensions in the presence of excess ammonium and different oxygen concentrations. Flasks (500 ml) containing 20 g of fresh sediment-soil, 0.15 g of $CaCO_3$, and 50 ml of assay medium [(grams per liter) $(NH_4)_2SO_4$, 0.33; K_2HPO_4, 0.14; KH_2PO_4, 0.027 (pH = 7.5)] were flushed with N_2 (purity > 99.9%) to remove all oxygen. Different oxygen concentrations were achieved by introducing oxygen (purity > 99.5%) into the head space of the flasks and subtracting an equivalent amount of gas from the head space to maintain atmospheric pressure. Sediment or soil suspensions from each replicate core were incubated with 11, 7, 5, 3, 2, and 1% (vol/vol) oxygen in the head space of the flasks, which corresponds to oxygen concentrations in the assay medium of ≈100, 70, 50, 40, 15, and 5 μM O_2, respectively. Pilot experiments revealed maximum ammonium oxidation rates at an oxygen concentration of 10% (vol/vol) in the head space of the flasks (data not shown). The flasks were incubated on a rotary shaker (180 rpm, 20°C) in a horizontal position to optimize the transfer of oxygen from the gas to the liquid phase. The production of nitrate and nitrite during 8 h of incubation was monitored by periodic withdrawal of 1-ml samples by means of a syringe. The samples were centrifuged (15,000 × *g*, 15 min) to remove soil-sediment particles and nitrifying bacteria. Centrifugation proved to stop nitrification as efficiently as the addition of 2 M KCl (data not shown). The supernatant was analyzed for nitrate and nitrite content, as described above, within 2 days after sampling. Samples were stored at 4°C prior to analysis. The ammonium-oxidizing activity was calculated from the slope of the linear progression curve of nitrate plus nitrite production versus time. Nitrate plus nitrite production during the first 8 h of incubation was always linear ($R^2 > 0.90$). These activities were stoichiometrically converted to oxygen consumption activities (ammonium oxidation rate × 1.5). The apparent half-saturation constant for oxygen (K_m) and the maximum oxygen consumption rate (V_{max}) were derived from plots of oxygen consumption activities versus oxygen concentration in the liquid phase by means of the computer program Enzpack (version 2.0; P. A. Williams, Bangor, Wales, United Kingdom) by using the direct linear method (14). Oxygen concentration in the head space was determined with a gas chromatograph (Carlo Erba GC 6000) equipped with a hot wire detector and a molsieve 5-Å (1 Å = 0.1 nm) column, operated at 80°C with helium as a carrier gas. Oxygen concentration in the liquid phase was calculated by using the Bunsen absorption coefficient for oxygen (0.0311, 20°C, 1 atm) (39) in combination with the measured oxygen concentrations in the head space of the flasks.

Denitrification activity assay. Denitrification activity assays were performed by the method of Tiedje (39), in flasks (500 ml) containing 20 g of fresh sediment and 50 ml of the following medium (grams per liter): KNO_3, 1.01; K_2HPO_4, 0.14; KH_2PO_4, 0.027; glucose · H_2O, 1.98; and chloramphenicol, 0.1. The flasks were flushed with N_2 (purity > 99.9%). After the addition of 10 kPa of acetylene to inhibit nitrous oxide reduction, the flasks were incubated in a horizontal position on a rotary shaker (180 rpm, 20°C). Nitrous oxide production was measured by injection of head space samples into a gas chromatograph (Carlo Erba GC6000)

TABLE 1. Descriptive parameters of all sampling sites important for nitrification (May 1995)

Sediment/soil type	% Moisture (wt/wt)	Temp (°C)[a]	pH H_2O	Organic matter (%)	Concn[b]		
					NH_4^+	NO_2^-	NO_3^-
Calcareous grassland	0.08	15	7.92	5.32	2.24	ND	4.34
Chalk grassland	0.30	16	7.87	12.53	4.48	ND	5.88
Dune top	0.01	19	7.90	2.50	1.96	ND	2.94
Lake sediment							
Root zone	0.28	19	7.56	2.44	2.31	ND	ND
Bare	0.33	19	7.90	4.11	14.46	ND	ND

[a] Temperature at a depth of 5 cm.
[b] Milligrams of N per kg of dry soil-sediment. ND, not detectable.

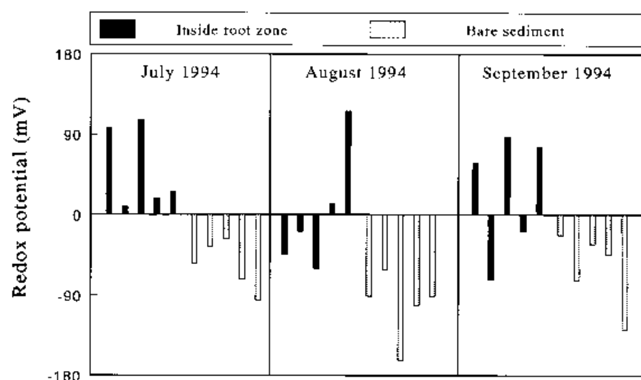

FIG. 1. Redox potentials in the root zone of *G. maxima* and in the bare sediment of Lake Drontermeer at a depth of 5 cm. The presented values are the recordings for all replicate cores of July, August, and September 1994.

equipped with an electron-capture detector and Hayesep Q column (80°C) for gas separation. Denitrification activity was calculated from the slope of the linear progression curve of nitrous oxide concentrations during 8 h of incubation. Accumulation of nitrous oxide in that period was always linear ($R^2 > 0.90$). The applied concentration of chloramphenicol effectively inhibited the de novo enzyme synthesis (data not shown).

Effect of anoxia on nitrification. To investigate the effect of prolonged anoxic conditions on nitrification activity, flasks (100 ml) containing 10 g of fresh sediment or soil and 25 ml of the medium used for the nitrification assay described above were flushed with N_2 (purity > 99.9%) and incubated at 20°C in the dark for 0, 1, 2, 3, and 4 weeks. After anoxic incubation, the flasks were flushed with air and subsequently incubated on a rotary shaker (180 rpm, 20°C). Nitrification activity was determined by monitoring nitrate and nitrite production during an 8-h incubation period.

Statistical analysis. All statistical tests were performed with the aid of the STATISTIX analytical software package (NH Analytical Software, St. Paul, Minn.). All data were checked for normality with the Wilk-Shapiro test. Pairwise comparison of root zone and bare sediment means were performed by using the two-sample *t* test which provides for inequality of variances as described by Snedecor and Cochran (37). Multiple comparisons of means were performed with Tukey's test. In the case of inhomogeneity of variances, determined with Bartlett's test after a one-way analysis of variance test, data were log transformed. In the case of inhomogeneity of variances after transformation, separate *t* tests were performed with a Bonferroni correction of the significance level for the number of comparisons. Significance of correlation coefficients was determined by using the table presented by Snedecor and Cochran (37).

RESULTS

Redox potentials. As can be seen in Fig. 1, the presence of the plant resulted in an elevation of the redox level. Redox potentials in the root zone of *G. maxima* were in the range of −73 to 112 mV and were significantly higher than the values in the bare sediment (−163 to −23 mV). The values found in the root zone were indicative of iron reduction whereas the values observed in the bare sediment indicated the reduction of sulfate (28).

Ammonium content of the sediment. Except for June 1994, the ammonium content was always higher in the bare sediment compared with the root zone (Table 2). Ammonium was never depleted at any sampling date. Ranges found were 1.6 to 4.5 mg of N per kg of dry sediment for the root zone and 6 to 15 mg of N per kg in the bare sediment.

Effect of *G. maxima* on nitrifying bacteria. The numbers of ammonia (Fig. 2A)- and nitrite (Fig. 2B)-oxidizing bacteria were significantly higher in the root zone of *G. maxima* in the spring and summer of 1994 compared with the bare sediment. Over the course of the growing season, the differences in size of the nitrifying community between the root zone and the bare sediment disappeared. As soon as the shoots of the plants started to emerge in the spring of 1995, higher numbers could once again be found in the root zone, except for the numbers of nitrite oxidizers in May 1995. The nitrite-oxidizing bacteria substantially outnumbered the ammonia oxidizers both in the root zone and the bare sediment for all samples. The ammonium-oxidizing activities (Fig. 3) reflected the pattern observed with the MPN.

Oxygen kinetics of the ammonia-oxidizing community. The apparent K_m value of the ammonia-oxidizing community in the Lake Drontermeer sediment varied between 5 and 15 μM and

TABLE 2. Ammonium content of sediment cores sampled in the root zone of *G. maxima* and in the bare sediment at Lake Drontermeer in 1994 and 1995[a]

Mo and yr of sampling	Ammonium content (mg of N · kg^{-1} of dry sediment)	
	Inside root zone	Bare sediment
May 1994	4.54 ± 0.58	14.97 ± 2.59
June 1994	3.53 ± 0.50	6.01 ± 1.65
July 1994	2.50 ± 0.31	7.34 ± 1.79
August 1994	4.07 ± 1.11	8.72 ± 1.12
September 1994	1.58 ± 0.17	13.71 ± 1.61
November 1994	2.18 ± 0.22	12.25 ± 2.20
March 1995	2.93 ± 0.30	13.02 ± 2.83
April 1995	3.89 ± 0.48	13.15 ± 2.43
May 1995	2.31 ± 0.29	14.46 ± 3.15

[a] Values represent means (± standard errors) of 5 replicate cores. Differences in ammonium content between the root zone and the bare sediment were significant for all time periods except June 1994 (two-sample *t* test, $n = 5$, $P < 0.05$).

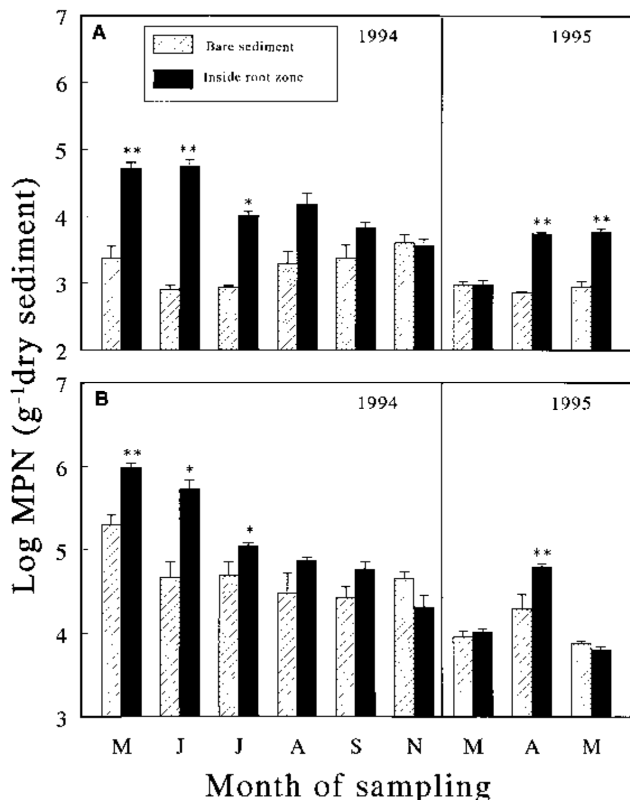

FIG. 2. MPN of the ammonia- (A) and nitrite-oxidizing (B) bacteria in sediment cores sampled inside the root zone of *G. maxima* and in the bare sediment at Lake Drontermeer in 1994 and 1995. Bars represent means (± standard errors) of five replicate cores. Significant differences between the numbers in the root zone and the bare sediment are indicated by asterisks (two-sample *t* test, $n = 5$). **, $P < 0.01$; *, $P < 0.05$.

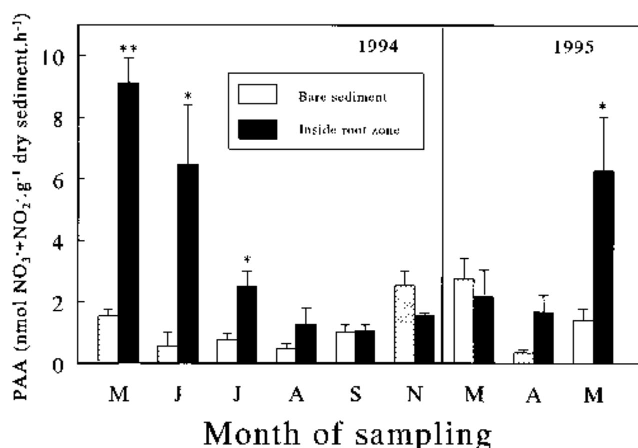

FIG. 3. Potential ammonium-oxidizing activities (PAA) in sediment slurries of cores sampled in the root zone of *G. maxima* and in the bare sediment at Lake Drontermeer in 1994 and 1995. Bars represent means (± standard errors) of 5 replicate cores. Significant differences between the activities in the root zone and in the bare sediment are indicated by asterisks (two-sample *t* test, $n = 5$). **, $P < 0.01$; *, $P < 0.05$.

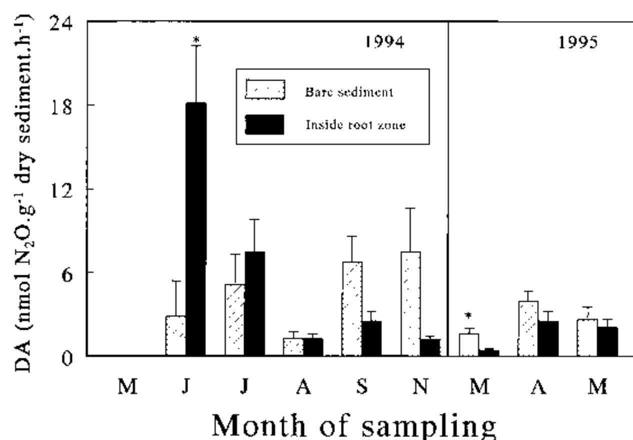

FIG. 4. Denitrification activities (DA) in slurries from sediment cores sampled in the root zone of *G. maxima* and the bare sediment at Lake Drontermeer in 1994 and 1995. Bars represent means (± standard errors) of 5 replicate cores. Significant differences between activities in the root zone and the bare sediment are indicated by asterisks (two-sample *t* test, $n = 5$). **, $P < 0.01$; *, $P < 0.05$.

did not differ significantly between the bare sediment and the sediment from the root zone (Table 3). The calculated specific affinity or substrate-sequestering ability for oxygen of the ammonia-oxidizing community, which is defined as the ratio V_{max}/K_m, is also shown in Table 3. The specific affinity for oxygen of the ammonia-oxidizing community was highest in spring and only differed from the bare sediment in May of 1994 and 1995. The fluctuations in specific affinity in the root zone were the consequence of the differences in maximum oxygen-consuming potentials of the ammonia-oxidizing community rather than changes in apparent K_m for oxygen, which did not change significantly during the sampling period.

Denitrification activity. The results of the denitrifying activity measurements that were determined in the presence of chloramphenicol to inhibit de novo enzyme synthesis are presented in Fig. 4. No measurements were performed in May 1994. The denitrification activity was stimulated by *G. maxima* in early summer. This effect disappeared again as the growing season progressed. In the fall of 1994 and in March 1995, the denitrification activity even tended to be higher in the bare sediment. The pattern in 1994 was very similar to the numbers and activities of the nitrifying bacteria. The stimulation of nitrification by *G. maxima* in the spring of 1995 was, however, not reflected in the denitrifying activity.

Correlations of the measured variables. The correlation coefficients of the measured variables of Lake Drontermeer are presented in Table 4. Nitrifying activities and numbers were significantly correlated with denitrification activities in the root zone of *G. maxima* and in the bare sediment. The correlation between the numbers of ammonia- and nitrite-oxidizing bacteria and denitrification activities was clearly evident. Nitrification activities and numbers were only correlated in the root zone. Numbers of ammonia- and nitrite-oxidizing bacteria were highly correlated in the root zone, whereas this relationship was much weaker in the bare sediment. The ammonium content of the sediment was only significantly correlated with the numbers of ammonia- and nitrite-oxidizing bacteria in the root zone. The root biomass of *G. maxima* showed no significant correlation with any of the measured variables.

Characteristics of the ammonia-oxidizing community of Lake Drontermeer and soils with different oxygen availabilities. (i) Oxygen kinetics. The oxygen kinetics of the ammonia-oxidizing community from the root zone of *G. maxima* and

TABLE 3. Apparent K_ms and specific affinities for oxygen of the ammonia-oxidizing community of sediment cores sampled in the root zone of *G. maxima* and in the bare sediment at Lake Drontermeer in 1994 and 1995[a]

Mo and yr of sampling	Kinetics of oxygen consumption			
	Inside root zone		Bare sediment	
	K_m O_2 (μM)	Sp affinity (ml · g^{-1} · h^{-1})	K_m O_2 (μM)	Sp affinity (ml · g^{-1} · h^{-1})
May 1994	11.18 ± 2.27	1.42 ± 0.27*	7.20 ± 1.65	0.43 ± 0.27
June 1994	12.95 ± 2.72	0.71 ± 0.18	5.34 ± 2.10	0.34 ± 0.13
July 1994	11.24 ± 2.47	0.35 ± 0.06	8.64 ± 1.86	0.18 ± 0.07
August 1994	7.23 ± 1.02	0.45 ± 0.22	10.85 ± 2.75	0.08 ± 0.02
September 1994	8.04 ± 3.36	0.32 ± 0.08	6.05 ± 1.33	0.36 ± 0.15
November 1994	7.36 ± 1.10	0.33 ± 0.06	11.11 ± 4.25	0.68 ± 0.28
March 1995	13.61 ± 5.20	0.28 ± 0.09	14.74 ± 4.94	0.28 ± 0.10
April 1995	6.44 ± 1.92	0.72 ± 0.37	4.50 ± 1.61	0.16 ± 0.03
May 1995	9.06 ± 1.53	1.01 ± 0.23*	8.49 ± 1.31	0.27 ± 0.03

[a] Values represent means (± standard errors) of 5 replicate cores. Asterisks indicate significant differences between the root zone and the bare sediment (two-sample *t* test, $n = 5$, $P < 0.05$).

TABLE 4. Pearson correlation coefficients of measured variables of soil cores sampled in the root zone of *G. maxima* and in the bare sediment (in parentheses)[a]

Variable	PAA[b]	DA[c]	MPN NH_4^+[d]	MPN NO_2^-[e]	Min. NH_4^+[f]
PAA					
DA	0.52** (0.46**)				
MPN NH_4^+	0.47** (0.22)	0.78** (0.37*)			
MPN NO_2^-	0.51** (0.11)	0.84** (0.53**)	0.94** (0.32*)		
Min.NH_4^+	0.22 (0.25)	0.26 (0.19)	0.31** (0.21)	0.32** (−0.11)	
Root biomass (dry weight)	0.15	−0.16	−0.25	−0.23	0.06

[a] df = 43 for PAA, MPN, Min. NH_4^+, and root biomass; df = 38 for DA. *, $P < 0.05$; **, $P < 0.01$.
[b] Potential ammonium-oxidizing activity.
[c] Denitrification activity.
[d] MPN of the ammonia-oxidizing bacteria.
[e] MPN of the nitrite-oxidizing bacteria.
[f] Mineral nitrogen available in the form of NH_4^+.

from soils which differ in oxygen availability are presented in Table 5. The apparent K_m for oxygen of the ammonia-oxidizing community of the dune top was substantially higher than that of all other locations. The maximum oxygen consumption rate of the ammonia-oxidizing community was lowest in the lake sediment, resulting in a specific oxygen affinity value equal to that of the dune top. The specific affinities of the dune top and lake sediment communities appeared to be significantly lower than those of the ammonia-oxidizing communities of the two grassland locations.

(ii) Effect of anoxia. Subjecting the ammonia-oxidizing communities to anoxia for a period of 4 weeks resulted in drastic decreases in ammonium-oxidizing potentials compared with the initial capacity, i.e., 92% reduction in the calcareous grassland, 95% reduction in the chalk grassland, and 99% reduction at the dune top location (data not shown). Loss of nitrifying capacity already occurred after 1 week of anoxic incubation. The ammonium-oxidizing potential of the lake sediment was, however, not affected at all by the anoxic incubation. Subsequent to an anoxic incubation for 4 weeks, the ammonia-oxidizing community from the lake sediment resumed nitrification within 1 h, whereas the ammonia-oxidizing communities of the other locations required a substantial lag period (Table 6). The dune top community tended to reveal the most extreme sensitivity to anoxia both in initial capacity and length of lag time required for reactivation.

TABLE 5. Oxygen kinetics of the ammonia-oxidizing community at locations which differ in oxygen availability[a]

Sediment/soil type	K_m (μM O_2)	V_{max} (nmol $O_2 \cdot g^{-1} \cdot h^{-1}$)	Sp affinity (ml $\cdot g^{-1} \cdot h^{-1}$)[b]
Lake sediment (root zone)	9.06a	9.43a	1.01a
Calcareous grassland	11.29a	65.02b	6.23b
Chalk grassland	11.02a	63.98b	6.14b
Dune top	28.88b	30.70c	1.09a

[a] Different letters indicate significant differences between means ($P < 0.05$, $n = 5$, except for the chalk grassland location where $n = 4$). Apparent K_m and specific affinity data were analyzed by Tukey's test after logarithmic transformation to achieve homogeneity of variances. The V_{max} data were analyzed by two-sample *t* test with Bonferroni correction for the number of comparisons (α/3, $P < 0.017$).
[b] Calculated as V_{max}/K_m.

TABLE 6. Lag times (hours) of the ammonia-oxidizing community after anoxic incubation[a]

Sediment/soil type	Weeks of anoxic incubation			
	1	2	3	4
Calcareous grassland	1.34a	2.39ab	2.46ac	2.20a
Chalk grassland	1.94a	3.26b	3.40a	4.00a
Dune top	2.32a	4.24b	6.15b	7.00c
Lake sediment (root zone)	1.56a	1.33a	0.87c	0.66d

[a] Lag time values are calculated as the *x* axis intercepts of the linear regression equation of nitrifying activity plots versus time. Presented values are means of five replicate sediment cores or soil samples. Different letters, per week of incubation, indicate significant differences between the different locations (Tukey's test, $P < 0.05$).

DISCUSSION

Importance of *G. maxima* for nitrification and denitrification. The emergent macrophyte *G. maxima* had a strong stimulating effect on the numbers of nitrifying bacteria in the Lake Drontermeer sediment in the spring and early summer. The numbers of ammonia- and nitrite-oxidizing bacteria in the root zone were 75 and 50 times as high, respectively, as the numbers of bacteria found in the bare sediment. The root zone/bare sediment ratio for the ammonium-oxidizing activities reached values of up to 9. These observations are in agreement with the only other known report concerning numbers of nitrifiers associated with *G. maxima* roots (7). This other study, however, only sampled the visibly oxidized layer around the roots, whereas the effects we obtained are for complete sediment cores within the vegetation, taking a substantial sediment volume into account which will not directly be influenced by the plant and thereby rendering an even larger overall effect. Hansen reports spring ammonium oxidation rates three times as high in the root zone of *Phragmites australis* compared with the bare sediment (18). These activities, which are within the range we report, are probably overestimations as the authors measured nitrate and nitrite production during a 24-h period, which does not exclude growth of nitrifiers. Wittgren found ammonium oxidation rates in lysimeters planted with *G. maxima* and intermittently supplied with waste water that were four times higher than the highest rates in our study (42). However, the ammonium oxidation rates were lower in the planted plots than in the bare plots, which is explained by ammonium limitation due to plant uptake.

The redox potentials in the root zone indicate oxygen release by the roots which stimulates oxidation processes yielding a stabilization of the redox level. The elevation of numbers and activities of the nitrifiers in this study must be due to this oxygen leakage from the roots. Stimulation of growth and activity of the nitrifying bacteria as the result of higher ammonium availabilities is not very likely, as relatively little nitrification occurs in the bare sediment despite the higher ammonium availability. The decline in number and activity of nitrifiers during the growing season is probably the result of oxygen limitation. Oxygen shortage is most likely due to a decrease in the oxygen leakage from the roots as the plants age (2, 11, 26) in combination with elevated heterotrophic microbial oxygen consumption due to the high summer temperatures. Ammonium limitation due to plant uptake and microbial immobilization cannot be totally ruled out within the scope of

this study. Hence, a combination of an increasing oxygen and/or nitrogen demand in the sediment in concert with a decrease in the amount of oxygen released by the plant may have resulted in inhibition of the nitrifiers over the course of the growing season. Nevertheless, a substantial stimulation of the nitrifying community during a part of the year by *G. maxima* could be of critical importance for survival during the following period of the year when no plants and no oxygen are present.

Most studies on the impact of oxygen-releasing plants on nitrification and denitrification have focused on nitrogen fluxes. They have measured the presence of nitrate in the root zone (12) or the accumulation of nitrogen gas and nitrous oxide after ammonium application (30, 35). Nitrate reductase levels in plants have also been used as an indicator for nitrification (3, 40). However, in this study the emphasis was on the dynamics of the community of nitrifying bacteria during subsequent growing seasons. The dynamic pattern of nitrifier numbers throughout the year would imply high growth and death rates, neither of which are characteristic of nitrifiers. High mortality rate is certainly not likely regarding the presence and activity of the nitrifiers in the oxygen-limited bare sediment during the year, as demonstrated in our study, and long-term survival in anoxic sediment layers as demonstrated in other studies (20, 36). Therefore, the elevated numbers and activities in spring in the root zone probably reflect a high proportion of active or nonresting cells, due to the favorable conditions, which grow in MPN media and are active in short-term assays. The significant correlation found for numbers and activities (Table 4), which has rarely been reported, suggests that a large proportion of the counted bacteria can also be rapidly activated in short-term activity assays.

It is evident that emergent macrophytes can provide temporary conditions, namely oxygen availability, to support the generation of energy by an "activated" nitrifying community.

The activation of the nitrifying community appears to be mirrored by the in situ production of nitrite and nitrate as evident from the high correlation between the numbers of ammonia- and nitrite-oxidizing bacteria (Table 4), with the latter deriving nitrite from the ammonia oxidizers. Interestingly, nitrite-oxidizing bacteria have other means of energy generation, which may explain why they outnumbered the ammonia oxidizers to a substantial degree, as previously discussed by Woldendorp and Laanbroek (43).

The high correlations between the denitrifying activities and the nitrification parameters (Table 4) provide more definite evidence of in situ nitrification. The use of chloramphenicol in the denitrification assays prevents de novo enzyme synthesis. The necessity of nitrate and nitrite for the induction of the denitrification enzymes and the high correlation between nitrification and denitrification activity implies the in situ presence of nitrate or nitrite. The strong relation between nitrification and denitrification was not observed, however, in the spring of 1995. Denitrifying enzyme activity in the root zone was equal to that found in the bare sediment. The higher root biomass in 1995 may have led to a nitrate limitation for the denitrifying bacteria, explaining the inconsistency with the 1994 results.

Oxygen kinetics and adaptive responses of the ammonia-oxidizing community. It is a commonly stated hypothesis that nitrifying bacteria are poor competitors for oxygen compared with heterotrophic bacteria because of their low oxygen affinities (25, 33). However, most published data on oxygen kinetics of nitrifiers to date have been determined by using pure cultures grown in continuous or batch fermenters, measuring oxygen depletion by means of oxygen-specific electrodes. We present here the first determination of oxygen kinetics of ammonium oxidation in sediment, taken directly from the field, by using the stoichiometrical relation between nitrite-nitrate production and oxygen consumption, thereby excluding heterotrophic respiration. The K_m values we found for the ammonia-oxidizing community in the lake sediment varied between 5 and 15 μM O_2, which agrees very well with values found in pure culture studies (23, 24). Although these values permit nitrification activity at low-oxygen concentrations, they are still higher than the range of K_m values (0.02 to 3 μM) reported for heterotrophic bacteria (33). This difference in the half-saturation constants for oxygen between nitrifiers and heterotrophs might explain the repression of nitrification during the growing season. However, when comparing competitive abilities, the maximum consumption capacity at low substrate concentrations must be considered. The specific affinity, defined as V_{max}/K_m and which is equivalent to the substrate-sequestering ability at low-substrate concentrations (19), of the ammonia-oxidizing community in the lake sediment reveals that the spring 1994 and 1995 communities have greater competitive capabilities for oxygen (Table 3). These specific affinity values can only be compared with heterotrophic bacteria by converting them to oxygen conversion rates per cell. For May 1995, we can calculate a specific affinity of 169 $nl \cdot cell^{-1} \cdot h^{-1}$, which is seven times the value found for *Escherichia coli* (10) and 57 times the values found for pure cultures of *Nitrosomonas europaea* (24). Assuming the MPN counts to be representative of the number of active cells present in the nitrification assays, our results indicate that competitive abilities of the ammonia oxidizers on a per cell basis are better than those of heterotrophs. This was also demonstrated by comparing the oxygen kinetics of pure cultures of the heterotroph *Pseudomonas chlororaphis* and the nitrifier *N. europaea*, which were determined by using exactly the same methods and growth conditions (6). However, taking into account the extreme low yields and growth rates of nitrifiers (29) it is very likely that heterotrophic organisms will outnumber nitrifiers when oxygen is limiting and sufficient carbon is available. This would suggest that the stimulation of nitrification was due to sufficient oxygen release by the roots, making both heterotrophic and nitrification activity possible.

There is a large part of the year when plants are not supplying oxygen, and during these periods the nitrifiers will be confronted with anoxic or low-oxygen conditions, making adaptive traits to these conditions very useful. Comparing the ammonia-oxidizing community of the lake sediment to communities from permanently oxic dune sand reveals that the ammonia-oxidizing community in the lake sediment has a higher affinity for oxygen (Table 5). This is also the case with the ammonia-oxidizing communities of two soils in which periodic anoxia and anoxic microsites are present. Apparently, the exposure to anoxia leads to higher oxygen affinity. The specific affinity of the dune community is equal to that found for the lake sediment. This enables them to reach similar conversion rates at low-oxygen concentrations on a soil weight basis. Specific affinities calculated per cell are also lower for the dune community (24 $nl \cdot cell^{-1} \cdot h^{-1}$) compared with the ammonia-oxidizing community in the lake sediment (169 $nl \cdot cell^{-1} \cdot h^{-1}$). Hence, on a per-cell basis the ammonia-oxidizing bacteria from the dune sand, which never have to deal with anoxia, will not be very active in low-oxygen environments.

Apart from functioning at low-oxygen concentrations,

successful survival in anoxic periods might be a very valuable trait in environments that are periodically oxic-anoxic. The ammonia-oxidizing community from the root zone of *G. maxima* was not significantly affected by an anoxic incubation of up to 4 weeks. The capacity for nitrification was maintained, whereas the ammonia-oxidizing communities from dune sand, a calcareous grassland, and a chalk grassland displayed nearly a complete loss of their initial nitrifying capacity and showed substantially longer lag times after 4 weeks of anoxic incubation. This survival of long-term anoxia of nitrifying bacteria has previously been demonstrated by several studies (17, 20, 36). The ammonia-oxidizing community of the lake sediment possesses the ability to react immediately upon the introduction of oxygen at full available capacity, as was also demonstrated with microelectrode studies (20). This ability might be very important for reacting to oxygen released by new roots in the beginning of the growing season, after a period of anoxia of 4 to 5 months when the shoots of the plants have been absent. Moreover, the scavenging of oxygen during the daily fluctuations in oxic-anoxic conditions in the root zone of oxygen-releasing plants might also be facilitated by this trait.

The root zone of emergent macrophytes is an environment with many oxic-anoxic fluctuations, and the adaptations which we have demonstrated may be of prime importance for nitrifier survival in such habitats. Whether these adaptations are due to physiological plasticity or to the presence of genera or species specialized for nitrification at low-oxygen levels or survival in anoxic habitats can now also be addressed with the advent of new molecular detection techniques for these organisms.

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