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

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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 the 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 nitrate 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.

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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.) Holm° 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 aerodynamically (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 nitratereducing 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 at Lake Drontemeer, near the township of Elburg (The Netherlands, 52°58'N, 6°E), was selected. This stagnant freshwater lake location was chosen because a database 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 lids 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 root-free fraction (15 cm) was separated from the roots, sieved (1.6 mm), homogenized, and stored (4°C) prior to use for measurements. Some 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°E) was collected. Two locations were sampled as intermediate soil types (viz., periodically or partially subjected to low-oxygen conditions). Soil 24 wels; at a coastal dune (Meijendel, near The Hague, The Netherlands, 52°08'N, 4°E) was collected. This coastal dune location was chosen because the resident nitrifying community at that location is periodically or partially subjected to anoxia. The soil, containing a high percentage of loamy sand and a high moisture content (Table 3), is well drained on an irregular basis. In the winter prior to sampling, this grassland was flooded for a substantial period of time. From these locations, the top 5 cm of 5 randomly selected plots was sampled as means of a grass-plot sampler. This was done by placing a frame, consisting of 100 squares (225 mm each), randomly at 5 positions on top of the soil. At each position, two sets of 10 cores (depth, 5 cm; diameter, 2.5 cm) were taken, pooled, and regarded as one replicate per treatment. 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 soil:water 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 of 10% (vol/vol) in the head space of the flasks (data not shown). The flasks were incubated on a rotary shaker (192 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 liquid 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 KC1 (data not shown). The supernatant was analyzed for nitrate and nitrite content, as described, 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 regression curve of nitrate plus nitrite consumption rate (µM O2/h) against the added oxygen. The apparent half-saturation constant for oxygen (Km) and the maximum oxygen consumption rate (Vmax) were derived from plots of oxygen consumption activities (ammonium oxidation rate × 1.5). The apparent half-saturation constant for oxygen (Km) and the maximum oxygen consumption rate (Vmax) 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) using the direct linear method (14). Oxygen concentration in the head space of the lake 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.

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

| Sediment/soil type | Moisture (wt/wt) | Temp (°C) | pH | Organic C (%) | NH4+ | NO2- | NO3- | Conc²
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcareous grassland</td>
<td>0.08</td>
<td>15</td>
<td>7.92</td>
<td>5.32</td>
<td>2.24</td>
<td>ND</td>
<td>4.34</td>
<td></td>
</tr>
<tr>
<td>Chalk grassland</td>
<td>0.30</td>
<td>16</td>
<td>7.87</td>
<td>12.53</td>
<td>4.48</td>
<td>ND</td>
<td>5.88</td>
<td></td>
</tr>
<tr>
<td>Dune top</td>
<td>0.01</td>
<td>19</td>
<td>7.90</td>
<td>2.50</td>
<td>1.96</td>
<td>ND</td>
<td>2.94</td>
<td></td>
</tr>
<tr>
<td>Lake sediment</td>
<td>0.28</td>
<td>19</td>
<td>7.56</td>
<td>2.44</td>
<td>2.31</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Bare</td>
<td>0.33</td>
<td>19</td>
<td>7.90</td>
<td>4.11</td>
<td>14.46</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

² Temperature at a depth of 5 cm.

Verhagen and Laanbrough (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; Na2HPO4, 2.1H2O, 1.34; and NaH2PO4, 0.35. Sub-samples (0.25 ml) from the suspensions were serially diluted in 10-fold steps in sterile water plates (drumshaped, 45 mm diameter, 15 mm thick) and 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 consumption rate (µM O2/h) against the added oxygen. The apparent half-saturation constant for oxygen (Km) and the maximum oxygen consumption rate (Vmax) 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) using the direct linear method (14). Oxygen concentration in the head space of the lake 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 the Tiege [39], in blanks (500 ml) containing 20 g of fresh sediment and 50 ml of the following medium (grams per liter): KNO3, 1.01; K2HPO4, 0.14; KH2PO4, 0.027; glucose · H2O, 1.98; and chloramphenicol, 0.1. The flasks were flushed with N2 (purity > 99.9%). After the addition of 10 kPa of acetylene to inhibit nitrous oxide reduction, the flasks were incubated 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 liquid 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 KC1 (data not shown). The supernatant was analyzed for nitrate and nitrite content, as described, 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 regression curve of nitrate plus nitrite production versus time. Nitrate plus nitrite production during the first 8 h of incubation was always linear (R² > 0.90). These activities were stoichiometrically converted to oxygen consumption activities (ammonium oxidation rate × 1.5). The apparent half-saturation constant for oxygen (Km) and the maximum oxygen consumption rate (Vmax) 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) using the direct linear method (14). Oxygen concentration in the head space of the lake 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.
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 Drontemeer sediment varied between 5 and 15 µM and
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

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TABLE 3. Apparent $K_m$s 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.

<table>
<thead>
<tr>
<th>Month of sampling</th>
<th>Inside root zone</th>
<th>Bare sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ (µM)</td>
<td>$Sp$ affinity (ml·g⁻¹·h⁻¹)</td>
</tr>
<tr>
<td>May 1994</td>
<td>11.18 ± 2.27</td>
<td>1.42 ± 0.27*</td>
</tr>
<tr>
<td>June 1994</td>
<td>12.95 ± 2.72</td>
<td>0.71 ± 0.18</td>
</tr>
<tr>
<td>July 1994</td>
<td>11.24 ± 2.47</td>
<td>0.35 ± 0.06</td>
</tr>
<tr>
<td>August 1994</td>
<td>7.23 ± 1.02</td>
<td>0.45 ± 0.22</td>
</tr>
<tr>
<td>September 1994</td>
<td>8.04 ± 3.36</td>
<td>0.32 ± 0.08</td>
</tr>
<tr>
<td>November 1994</td>
<td>7.36 ± 1.10</td>
<td>0.33 ± 0.06</td>
</tr>
<tr>
<td>March 1995</td>
<td>13.61 ± 5.20</td>
<td>0.28 ± 0.09</td>
</tr>
<tr>
<td>April 1995</td>
<td>6.44 ± 1.92</td>
<td>0.72 ± 0.37</td>
</tr>
<tr>
<td>May 1995</td>
<td>9.06 ± 1.53</td>
<td>1.01 ± 0.23*</td>
</tr>
</tbody>
</table>

* 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 5. Oxygen kinetics of the ammonia-oxidizing community at the root zone of G. maxima and in the bare sediment (in parentheses)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>PAA**</th>
<th>DA*</th>
<th>MPN NH₄⁺</th>
<th>MPN NO₂⁻</th>
<th>Min. NH₄⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA</td>
<td>0.52**</td>
<td></td>
<td>0.47**</td>
<td>0.51**</td>
<td>0.22</td>
</tr>
<tr>
<td>DA</td>
<td>(0.46**)</td>
<td></td>
<td>(0.78**)</td>
<td>(0.84**)</td>
<td>(0.53**)</td>
</tr>
<tr>
<td>MPN NH₄⁺</td>
<td></td>
<td></td>
<td>(0.22)</td>
<td>(0.11)</td>
<td>(0.25)</td>
</tr>
<tr>
<td>MPN NO₂⁻</td>
<td></td>
<td></td>
<td>(0.37*)</td>
<td>(0.32*)</td>
<td>(0.19)</td>
</tr>
<tr>
<td>Min. NH₄⁺</td>
<td></td>
<td></td>
<td></td>
<td>(-0.11)</td>
<td>(0.21)</td>
</tr>
<tr>
<td>Root biomass (dry weight)</td>
<td>0.15</td>
<td>-0.16</td>
<td>-0.25</td>
<td>-0.23</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* df = 43 for PAA, MPN, Min. NH₄⁺, and root biomass; df = 38 for DA,*; P < 0.05; ** P < 0.01.

Table 5 presents the oxygen kinetics of the ammonia-oxidizing community at the root zone of G. maxima and in the bare sediment (in parentheses). The apparent Kₐ 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 6. Lag times (hours) of the ammonia-oxidizing community after anoxic incubation*

<table>
<thead>
<tr>
<th>Sediment/soil type</th>
<th>Weeks of anoxic incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Calcareous grassland</td>
<td>1.34a</td>
</tr>
<tr>
<td>Chalk grassland</td>
<td>1.94a</td>
</tr>
<tr>
<td>Dune top</td>
<td>2.32a</td>
</tr>
<tr>
<td>Lake sediment (root zone)</td>
<td>1.56a</td>
</tr>
</tbody>
</table>

* 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 Drontemeer 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 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 over estimations 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 14 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 activity 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
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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 reproducing 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 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* values we found for the ammonia-oxidizing community in the lake sediment varied between 5 and 15 μM O₂, 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* 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* / *K* 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 • cell⁻¹ • 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 • cell⁻¹ • h⁻¹) compared with the ammonia-oxidizing community in the lake sediment (169 nl • cell⁻¹ • 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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