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 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.

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7.56
0.33
7.87
0.08
19
0.30
0.28
2.50
12.53
15
2.44
5.32
NO,
ND
7.90
7.92
0.01
no
7.90
CO
ight loss after drying (24 h, 105°C), and the organic matter content was
side (4-nm mesh), and homogenized. The moisture content was determined as
ndering and van der Putten (13). Immediately upon arrival in the laboratory, redox
 COLORS
unum, nitrite, and nitrate were determined by a most probable number (MPN) technique described by
s turbidimetry. Controls were always included for assay blanks for aeration and inhibition by 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, 0.135 g of CaCO3, and 50 ml of assay medium ([grams per liter] (NH4)2SO4, 0.33; K2HPO4, 0.14; KH2PO4, 0.027 (pH = 7.5)] were flushed with N2 (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 O2, 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 the presence of periodic withdrawals of 1-mI 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, 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 (R2 > 0.90). These activities were stoichiometrically converted to oxygen consumption activities (ammonium oxidation rate × 1.5). The apparent half-saturation constant for oxygen (Ko2) 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) 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 molisev 5-A (1 A = 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 Bunson 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 Tiedje (39), in flasks (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, 0.01. The flask were flushed with N2 (purity > 99.9%). After the addition of 10 K of acetylene to inhibit denitrification, the oxide removal was measured by a gas chromatograph (Carlo Erba GC 6000).
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.

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**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**

<table>
<thead>
<tr>
<th>Mo and yr of sampling</th>
<th>Ammonium content (mg of N • kg⁻¹ of dry sediment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inside root zone</td>
</tr>
<tr>
<td>May 1994</td>
<td>4.54 ± 0.58</td>
</tr>
<tr>
<td>June 1994</td>
<td>3.53 ± 0.50</td>
</tr>
<tr>
<td>July 1994</td>
<td>2.50 ± 0.31</td>
</tr>
<tr>
<td>August 1994</td>
<td>4.07 ± 1.11</td>
</tr>
<tr>
<td>September 1994</td>
<td>1.58 ± 0.17</td>
</tr>
<tr>
<td>November 1994</td>
<td>1.58 ± 0.22</td>
</tr>
<tr>
<td>March 1995</td>
<td>2.93 ± 0.30</td>
</tr>
<tr>
<td>April 1995</td>
<td>3.89 ± 0.48</td>
</tr>
<tr>
<td>May 1995</td>
<td>2.31 ± 0.29</td>
</tr>
</tbody>
</table>

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).

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**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 sulfite (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...
ADAPTATION OF AMMONIA-OXIDIZING BACTERIA

6.05 ± 1.33
0.33 ± 0.06
0.45 ± 0.22
0.68
0.34 ± 0.13
7.23 ± 1.02
M
0.35 ± 0.06
J
0.36 ±0.15
Inside root zone
7.20 ± 1.65
9.06 ± 1.53
M
J
1.01 ± 0.23
12.95 ± 2.72
Bare sediment
0.16 ± 0.03
10.85 ± 2.75
14.74 ± 4.94
Bare sediment
0.43 ± 0.27
Sp affinity
7.36 ± 1.10
1.42 ± 0.27*
Km
0.18 ± 0.07
8.04 ± 3.36
11.18 ± 2.27
0.28 ± 0.09
4.50 ±1.61
J
5.34 ±2.10
8.49 ± 1.31
11.11 ± 4.25
8.64 ± 1.86
13.61 ± 5.20
J
0.32 ± 0.08
0.27 ± 0.03

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 am-
onia-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 activ-
ity measurements that were determined in the presence of
chloramphenicol to inhibit de novo enzyme synthesis are pre-
sented 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 co-
efficients 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 bac-
teria and denitrification activities was clearly evident. Nitrifi-
cation 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 relation-
ship 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 signifi-
cant correlation with any of the measured variables.

**Characteristics of the ammonia-oxidizing community of
Lake Drontermeer and soils with different oxygen availabili-
ties. (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 and yr of sampling</th>
<th>Inside root zone</th>
<th>Bare sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ O$_2$ ($\mu$M)</td>
<td>Sp affinity $K_m$ O$_2$ ($\mu$M)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ml \cdot g$^{-1}$ \cdot 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 test, $n = 5$, $P < 0.05$).
from soils which differ in oxygen availability are presented in Table 5. The apparent $K_n$ 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 hour, 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 Drontemer 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-hour 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 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

### TABLE 5. Oxygen kinetics of the ammonia-oxidizing community at different sediment/soil types

<table>
<thead>
<tr>
<th>Sediment/soil type</th>
<th>$K_n$ (µM O$_2$)</th>
<th>$V_{max}$ (nmol O$_2$ g$^{-1}$ h$^{-1}$)</th>
<th>$Sp$ affinity (ml g$^{-1}$ h$^{-1}$)$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake sediment (root zone)</td>
<td>9.06a</td>
<td>9.43a</td>
<td>1.01a</td>
</tr>
<tr>
<td>Calcareous grassland</td>
<td>11.29a</td>
<td>65.02b</td>
<td>6.23b</td>
</tr>
<tr>
<td>Chalk grassland</td>
<td>11.02a</td>
<td>63.98b</td>
<td>6.14b</td>
</tr>
<tr>
<td>Dune top</td>
<td>28.88b</td>
<td>30.70c</td>
<td>1.09a</td>
</tr>
</tbody>
</table>

* Different letters indicate significant differences between means $(P < 0.05, n = 5)$, except for the chalk grassland location where $n = 4$. Apparent $K_n$ 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 (3/2, $P < 0.017$).

* Calculated as $V_{max}/K_n$. 

### 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)

<table>
<thead>
<tr>
<th>Variable</th>
<th>PAA$^b$</th>
<th>DA$^b$</th>
<th>MPN NH$_4^+$</th>
<th>MPN NO$_3^-$</th>
<th>Min. NH$_4^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA</td>
<td>0.52**</td>
<td>0.46**</td>
<td>(0.78**)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td></td>
<td>0.78**</td>
<td>(0.37**)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPN NH$_4^+$</td>
<td>0.47**</td>
<td>0.22</td>
<td>(0.32**)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPN NO$_3^-$</td>
<td>0.51**</td>
<td>0.26</td>
<td>(0.21)</td>
<td></td>
<td>(0.11)</td>
</tr>
<tr>
<td>Min. NH$_4^+$</td>
<td>0.22</td>
<td>0.31**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root biomass</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. NH$_4^+$, and root biomass; df = 38 for DA.$^a$, $^b$ 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$_4^+$. 

$^a$ Significant at $P < 0.05$; **. Significant at $P < 0.01$. 

$^b$ 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 (3/2, $P < 0.017$).
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 unextinguished 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 denovo 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 and 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 *Km* 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 *Km* 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 *Vmax/Km* and which is equivalent to the substrate-queering 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 log 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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