MICROBIAL PRODUCTION AND CONSUMPTION OF NITRATE IN AN ANNUAL GRASSLAND

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Abstract. Gross nitrification rates (calculated by $^{15}$N pool dilution) ranged from 12 to 46% of gross mineralization rates during the growing season of annual grasses. Pools of $\text{NH}_4^+$ and $\text{NO}_3^-$ (measured as N) remained below 7 and 4 µg/g soil, respectively, but turned over about once a day. Microbial assimilation of $\text{NO}_3^-$ occurred at rates similar to previous estimates of plant uptake. Hence two common assumptions, that nitrifying bacteria are poor competitors for $\text{NH}_4^+$ and that microbial immobilization of $\text{NO}_3^-$ is insignificant, are not correct for this grassland system. Soil heterogeneity probably results in $\text{NH}_4^+$ availability to $\text{NH}_4^+$ oxidizers at some microsites, while $\text{NO}_3^-$ assimilation by heterotrophic microorganisms occurs at other microsites where $\text{NH}_4^+$ is not available. Relatively high rates of $\text{NO}_3^-$ production and consumption in an ecosystem with an annual mean hydrologic loss of NO$_3$-N of only 3.3 kg/ha indicate the importance of NO$_3^-$ in the internal N cycle of this ecosystem.

Nitrification potential rates, which are an index of population size, declined during the dry season. However, a significant population remained viable when soil water potential was below $-9\text{ MPa}$, indicating that nitrifying bacteria can tolerate severe desiccation. A simple diffusion model demonstrates the dependence of $\text{NH}_4^+$ availability on soil moisture. Population decline during the dry season may result from both desiccation stress and a lack of substrate availability for maintenance energy of the population. Spatial compartmentalization of sites of production and consumption of inorganic-N, along with diffusional constraints among such microsites, appear to be critical factors affecting N-cycling characteristics of the ecosystem.

Key words: annual grassland; California; immobilization; mineralization; $^{15}$N; nitrate assimilation; nitrification; soil nitrogen.

INTRODUCTION

Ammonium-oxidizing bacteria generally have been considered to be poor competitors for $\text{NH}_4^+$ relative to plants and heterotrophic microorganisms (e.g., Vitousek et al. 1982, Gosz and White 1986, Robertson 1989). When $\text{NO}_3^-$ is produced, it is often assumed that plant uptake, leaching, and possibly denitrification are the major fates of $\text{NO}_3^-$, and that microbial immobilization of $\text{NO}_3^-$ is insignificant. The most commonly cited reference supporting these two assumptions is a laboratory study of mixed soil samples from an N-deficient, sandy, lateritic podzol of Australia (Jones and Richards 1977). Results from that study have been extrapolated from extremely nutrient-poor soils to soil processes in general, and from a well-mixed laboratory soil sample to heterogeneous soil environments in situ. However, spatial heterogeneity of substrate availability could result in more nitrification and more microbial assimilation of $\text{NO}_3^-$ than might be predicted from studies of mixed soils. Furthermore, an ecosystem that is N-limited but more productive than a sandy, lateritic podzol might provide a better test of competition for $\text{NH}_4^+$ and $\text{NO}_3^-$. The silt loam soil of the grassland site studied here is N-limited, has low $\text{NH}_4^+$ and $\text{NO}_3^-$ pool sizes, and is a well-structured alfisol in which microsite heterogeneity may be important. One of the purposes of the present study is to critically examine the assumptions of poor competitive ability of $\text{NH}_4^+$-oxidizing bacteria and insignificant microbial assimilation of $\text{NO}_3^-$ by using in situ measurement techniques in unmixed soil.

Because microbial assimilation of $\text{NO}_3^-$ is usually assumed to be negligible, measures of net nitrification are often reported simply as “nitrification rates.” Indeed, nearly all of our understanding of ecosystem-level regulation of “nitrification” is based on measures of net nitrification in buried bags or in laboratory incubations. Ecosystem properties that affect net nitrification rates have been well characterized (Robertson 1982a, b, 1989). This approach has revealed mechanisms that help explain differences in $\text{NO}_3^-$ leaching losses following disturbance of different ecosystem types (Vitousek et al. 1982). By distinguishing between regulation of $\text{NO}_3^-$ production and $\text{NO}_3^-$ consumption, further insight into the dynamics of the internal N cycle of an undisturbed ecosystem may be gained. The second purpose of the present study is to investigate seasonal and spatial variation of $\text{NO}_3^-$ production and consumption within an annual grassland of California.
To this end, we have employed a variety of techniques, each of which provides different information about NO₃⁻ production and consumption processes.

**MATERIALS AND METHODS**

**Study Site**

The study site at the University of California Sierra Foothills Range Field Station is located at 200 m elevation in the central valley of California (39°15' N, 121°17' W). The vegetation, described by Jackson et al. (1988), includes primarily annual grasses with a blue oak (Quercus douglasii H. & A.) and live oak (Q. wislizenii A.) overstory. The soil is an Argonaut silt loam (Mollic Haploxeralfs). Selected soil properties obtained from a 10 × 10 m sampling grid in a previous study (P. Zinke, personal communication) are given in Table 1. Differences between the open-grass and under-oak communities regarding species composition and phenomenology of grasses and forbs have also been noted (Jackson et al. 1988). In the present study, six study plots (2 × 2 m) were stratified by vegetation type, with three under canopies of oak trees and three in open grassy areas.

**¹⁵N pool dilution method for gross rates**

In October, January, and March (1986–1987 growing season), four plastic cylinders (4 cm diameter × 9 cm deep) were driven into the soil at each plot. A larger metal cylinder (8 cm diameter × 9 cm deep) was then driven into the soil around each small cylinder so that the two formed concentric circles. The pair was removed, and the soil between the cylinders was placed in plastic bags, mixed, and immediately subsampled for extraction in 2 mol/L KCl (≈ 15 g dry mass equivalent in 75 mL). The remaining mixed soil was later used for gravimetric moisture determination. Two of the small cores received (¹⁵NH₄)₂SO₄ injections and two received K¹⁵NO₃ injections. Spinal needles were used to make six 1-mL injections per core. The solutions contained N at 30 mg/L, thus providing N at −2 μg/g dry soil. Solution ¹⁵N enrichments ranged from 65 to 99%.

Following injection of solutions, the cores were capped and buried for 24 h in the holes from which they came. The soil was then removed, mixed, and subsampled for immediate extraction in 2 mol/L KCl. The remaining soil was kept on ice and returned to the laboratory for nitrification potential assays and gravimetric moisture determination.

The KCl solutions were filtered through Whatman Number 1 filters that had been rinsed with KCl. Analysis for NH₄⁺ and NO₃⁻ was conducted colorimetrically (Keeney and Nelson 1982) using a Lachat flow injection autoanalyzer. A diffusion procedure (Brooks et al. 1989) was used to prepare samples for ¹⁵N analysis. In brief, NO₃⁻ was reduced to NH₄⁺ with Devarda’s alloy; NH₃ was volatilized in a base solution with added MgO; NH₃ vapor was captured on an acidified glass fiber filter; and the filters were sent to Isotope Services, Los Alamos, New Mexico, for ¹⁵N analysis.

Gross mineralization rates were calculated for cores that received ¹⁴NH₄⁺ using the model of Kirkham and Bartholomew (1954). Initial ¹⁴NH₄⁺ pool sizes were estimated from the KCl extract of soil collected between the plastic and metal cylinders. The initial ¹⁴NH₄⁺ pool was known from the amount injected. Final pool sizes were determined from the KCl extracts of soil from plastic cores after 24 h incubation. Gross nitrification rates were calculated from the same model, substituting NO₃⁻ values for NH₄⁺ values. Gross NH₄⁺ and NO₃⁻ immobilization rates calculated from this model include other possible consumptive fates of inorganic-N (e.g., NH₄⁺ fixation, denitrification) and therefore may be somewhat overestimated. The assumptions involved in the calculation of NH₄⁺ and NO₃⁻ production and consumption from the changes in the ¹⁵N enrichment of NH₄⁺ and NO₃⁻ pools are discussed elsewhere (E. A. Davidson et al., unpublished manuscript).

Use of the ¹⁵N pool dilution method could not be continued during the summer because the soil became too dry and hard to take intact cores, and because addition of ¹⁵N solutions would have caused artifactual effects from wetting dry soil.

**Nitrification potentials**

The chlorate inhibition method of Belser and Mays (1980) was slightly modified. On dates when ¹⁵N labelling was conducted, subsamples for nitrification potential assays were taken from the same cores used for gross nitrification measurements. During the summer dry months, bulk soil samples from each plot were excavated to 9 cm depth with a chisel. Subsamples (≈ 15 g dry mass equivalent) of soil were placed in 250-mL Erlenmeyer flasks with 100 mL of the buffered (NH₄)₂SO₄ solution described by Belser and Mays (1980). The flasks were continuously shaken and aliquots were removed after 2, 6, 12, and 24 h. The aliquots were either filtered through Whatman Number 40 paper or were centrifuged for 10 min at 13 000 g. Concentrations of NO₂⁻ and NO₃⁻ were determined colorimetrically using a Lachat flow injection auto-

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**Table 1.** Selected properties of soils (0–10 cm) on a 10 × 10 m sampling grid. N = 12 for open grass; N = 8 for under oak.

<table>
<thead>
<tr>
<th></th>
<th>Open grass</th>
<th>Under oak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SE</td>
</tr>
<tr>
<td>Bulk density (g/cm³)</td>
<td>1.38</td>
<td>0.03</td>
</tr>
<tr>
<td>Acidity (pH)</td>
<td>5.62</td>
<td>0.06</td>
</tr>
<tr>
<td>Carbon (%)</td>
<td>2.11</td>
<td>0.13</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>0.19</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**NS** = not significant; *P ≤ .05 (one-way ANOVA comparing vegetation types).
of the model. Expected mean squares of this model given by SAS indicated that the F test for vegetation type was not exact, due to unequal cell sizes. However, the differences in expected mean squares overestimated the error term, thus making the F test more conservative. Hence, any significant vegetation type effect observed would probably also be significant had a balanced design permitted an exact F test.

**RESULTS AND DISCUSSION**

**Variation of nitrification rates**

We define net nitrification as net accumulation of NO\textsubscript{3}\textsuperscript{-} (production minus consumption in buried bags), gross nitrification as actual production of NO\textsubscript{3}\textsuperscript{-} (\textsuperscript{15}N pool dilution), and potential nitrification as production of NO\textsubscript{2}\textsuperscript{-} and NO\textsubscript{3}\textsuperscript{-} when NH\textsubscript{4}\textsuperscript{+} is added in excess (slurries).

Gross nitrification and potential nitrification rates were higher under the oak canopy than in open-grass plots (Fig. 1). Soil under the oak canopy exhibited higher total-C content (Table 1), higher inorganic-N pool sizes (Fig. 2), and higher rates of mineralization and immobilization (Table 2). Gross, net, and potential nitrification rates increased from fall to spring in both plot types (Fig. 1). This increase corresponds to changes in soil moisture (Fig. 3). Rainfall begins in the autumn, peaks during the winter, and rapidly declines in the spring. Growth of grasses in this Mediterranean-type climate is restricted to the rainy period. Plant demand for N decreases soil inorganic-N pool sizes from autumn to spring (Fig. 2; Jackson et al. 1988). Low winter temperatures temporarily depress plant uptake rates of N (Jackson et al. 1988) and probably also influence nitrification. However, the gross nitrification rates we observed in January were intermediate between October and March observations (Fig. 1). Although several factors may influence seasonal variation of nitrification rates at this site, soil moisture clearly covaries with nitrification rates. The mechanisms by which soil moisture could affect nitrification are complex (see below, Nitrification and soil moisture).

**Nitrification and competition for NH\textsubscript{4}\textsuperscript{+}**

Nitrogen is generally the most common limiting nutrient in soils of California annual grasslands (George et al. 1985). An increase in net primary production of annual grasses in response to N fertilization has been observed (Jones and Winans 1967). Observed microbial immobilization of NO\textsubscript{3}\textsuperscript{-} (Table 2) will be discussed in more detail below, but is relevant here to demonstrate NH\textsubscript{4}\textsuperscript{+} limitation to microorganisms in this ecosystem. Microbial assimilation of NO\textsubscript{3}\textsuperscript{-} probably occurs only at NH\textsubscript{4}\textsuperscript{+}-free microsites (Rice and Tiedje 1989). Despite NH\textsubscript{4}\textsuperscript{+} limitation at some microsites, we observed that NH\textsubscript{4}\textsuperscript{+} oxidizers were able to obtain a significant proportion of the NH\textsubscript{4}\textsuperscript{+} produced in this soil. Gross rates of mineralization and
nitrification are given in Table 2 and ratios of these rates are given in Table 3. Between 12 and 46% of the N mineralized was nitrified during the January and March sampling dates (Table 3).

Microsite heterogeneity of NH₄⁺ availability may partially explain the success of NH₄⁺ oxidizing bacteria in obtaining significant amounts of NH₄⁺ in an N-limited system. If mineralization occurs at localized “hot spots,” then the NH₄⁺ produced is not equally available to all organisms. The organisms closest to the site of production have the greatest probability of obtaining the substrate as it diffuses from its microsite of production. Rather than invoking a poorly defined concept of competition for substrate among soil organisms, the fate of NH₄⁺ may be best described as a stochastic process of diffusion of substrate among microsites colonized by a variety of organisms.

While microsite heterogeneity may explain how NH₄⁺ oxidizers obtain a significant piece of the NH₄⁺ pie, the amount of N available for nitrification is also affected by the size of the pie. However, the NH₄⁺ pie available for consumption is not simply the NH₄⁺ pool size, but rather the amount of NH₄⁺ produced over time. Although the N in KCl-extractable NH₄⁺ amounted to <7 μg/g soil during the wet season (Fig. 2), 1-d mineralization rates were similar in magnitude to the size of the NH₄⁺ pool (Table 2), indicating that this relatively small NH₄⁺ pool turned over approximately daily during the wet season. While NH₄⁺ availability regulates nitrification in a general way at the ecosystem scale (Robertson 1989), spatial heterogeneity and gross NH₄⁺ production rates are critical controllers of NH₄⁺ availability at a microsite scale.

Michaelis-Menten kinetics and nitrification rates

The nitrification potential assay indicates the maximum rate (V_max) of NH₄⁺ oxidation by the NH₄⁺ oxidizer population present in a soil sample when assayed in the laboratory at a given temperature with excess NH₄⁺ substrate. Under these conditions, the factor presumably limiting nitrification is the population size of ammonium-oxidizing bacteria. Hence, the nitrification potential assay can be used as an index of NH₄⁺-oxidizer population size (Belser 1979). The ratio of gross nitrification to nitrification potential indicates how fast the field process is occurring relative to the V_max of the existing NH₄⁺-oxidizer population. The gross rates ranged from 21 to 48% of the potential rates (Table 3).

Gross nitrification rates approaching 50% of potential nitrification suggest that the NH₄⁺-oxidizing population was experiencing NH₄⁺ concentrations near its apparent k_M. According to kinetic theory, a population

![Figure 1. Nitrification rate (measured as N) for open-grass plots and plots under oak canopy (means ± se). The effect of month and the interaction of month and vegetation type are significant at P = .01 for net, gross, and potential nitrification. The vegetation type effect (open vs. canopy) is not significant for net nitrification, is significant at P = .01 for gross nitrification, and is significant at P = .05 for nitrification potential.](image)

![Figure 2. Ammonium and nitrate pool means (± se) for open-grass plots and plots under oak canopy. Means of NH₄⁺ and NO₃⁻ are summed to plot total inorganic-N. The effect of month and the interaction of month and vegetation type are significant at P = .01 for NH₄⁺, NO₃⁻, and total inorganic-N. The vegetation type effect (open vs. canopy) is not significant for NO₃⁻; is significant at P = .01 for NH₄⁺, and is significant at P = .05 for total inorganic-N.](image)
or enzyme system experiencing substrate concentrations at or below its apparent \( k_M \) functions in a first-order response range, and is well positioned to respond to changes in resource availability (Cleland 1967). Although KCl-extractable \( \text{NH}_4^+ \)-N never varied by more than a factor of 3 (Fig. 2), \( \text{NH}_4^+ \) availability may be more dynamic at a microsite scale, and first-order response to changing availability may be an important adaptive mechanism.

Ratios of gross-to-potential nitrification may not always occur in the range we observed. A high gross rate relative to \( V_{\text{max}} \) might indicate a recent increase in resource availability, but is unlikely to be sustained. If \( \text{NH}_4^+ \) availability is the primary factor limiting nitrification, the existing population would respond quickly to an increase in \( \text{NH}_4^+ \) by increasing its gross rate. Using the energy from this enhanced activity, the population could grow, causing \( V_{\text{max}} \) to increase and the ratio of gross nitrification rate to \( V_{\text{max}} \) to decrease. Similarly, if a recent decline in resource availability caused gross rates to drop very low relative to \( V_{\text{max}} \), then maintenance energy requirements of the existing population might not be met. The population size and \( V_{\text{max}} \) would decline, and the ratio of gross rate to \( V_{\text{max}} \) would increase. In short, changes in resource availability could cause temporary changes in the ratio of gross rate to \( V_{\text{max}} \), but very high or very low ratios would not be sustained.

Gross-to-potential nitrification ratios did not differ between under-canopy and open-grass plots (Table 3), despite significant differences in rates of N cycling processes and N pool sizes between these two soil habitats (Tables 1 and 2, Figs. 1 and 2). Although population sizes and gross nitrification rates may be affected by ecosystem characteristics, this comparison of gross rate-to-\( V_{\text{max}} \) ratios in two soil habitats suggest that the ratio may be independent of ecosystem characteristics. Perhaps homeostasis of gross rate-to-\( V_{\text{max}} \) ratios, such that responses to changing resources are generally first order, is a fundamental characteristic of microbial processes in heterogeneous soil environments.

**Nitrification and soil moisture**

Nitrification potentials and net nitrification rates declined as the soil dried during the spring and summer, but a lag occurred between the onset of drying and the decline in nitrification potentials (Figs. 1 and 3). The soil water potential had declined to \(-1.5 \text{ MPa}\) in May, when plant anthesis was complete (Jackson et al. 1988), but the nitrification potential had not yet declined (Fig. 1). This low matric water potential may be more stressful for plants than for soil microorganisms. Occasional dew formation has been observed in May

### Table 2. Comparison of process rates, measured as N. Means (and se) of gross mineralization, gross nitrification, and microbial immobilization determined by \(^{15}\text{N}\) pool dilution in January and March 1987 of the present study. Plant uptake rates from Jackson et al. (1989) are from nearby open grassy plots in February and April 1985.

<table>
<thead>
<tr>
<th>Season</th>
<th>Gross Mineralization</th>
<th>Gross Nitrification</th>
<th>Microbial immobilization</th>
<th>Plant uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu g \cdot (g \text{ dry soil})^{-1} \cdot d^{-1})</td>
<td>(\mu g \cdot (g \text{ dry soil})^{-1} \cdot d^{-1})</td>
<td>(\mu g \cdot (g \text{ dry soil})^{-1} \cdot d^{-1})</td>
<td>(\mu g \cdot (g \text{ dry soil})^{-1} \cdot d^{-1})</td>
</tr>
<tr>
<td><strong>Open grass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>5.01 (0.38)</td>
<td>0.59 (0.13)</td>
<td>6.60 (0.35)</td>
<td>0.58 (0.06)</td>
</tr>
<tr>
<td>Spring</td>
<td>4.90 (0.77)</td>
<td>0.81 (0.11)</td>
<td>7.29 (0.50)</td>
<td>0.79 (0.17)</td>
</tr>
<tr>
<td><strong>Under canopy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>6.96 (1.28)</td>
<td>1.38 (0.29)</td>
<td>8.12 (1.05)</td>
<td>1.49 (0.27)</td>
</tr>
<tr>
<td>Spring</td>
<td>9.14 (2.47)</td>
<td>3.47 (0.52)</td>
<td>11.67 (2.98)</td>
<td>3.69 (0.51)</td>
</tr>
</tbody>
</table>

**Significant effects**

<table>
<thead>
<tr>
<th></th>
<th>Open vs. canopy</th>
<th>Winter vs. spring</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>(P \leq 0.05)</td>
<td>(P \leq 0.01)</td>
<td>(P = 0.06)</td>
</tr>
</tbody>
</table>

\( \text{NS} \) = not significant; \( \ast \ P \leq 0.05; \ast \ast \ P \leq 0.01 \) (ANOVA). See Materials and methods: Statistical analyses for discussion of F tests.
and June (T. Clark, Sierra Field Station, and J. Stark, 
personal observations), which has been hypothesized 
to relieve water stress temporarily for microorganisms 
in the top few millimetres of soil (Schimel and Parton 
1986). Apparently, the NH$_4^+$-oxidizer population sur-
vived and may have expanded during the transition 
from wet to dry seasons.

Soil water potential declined to levels well below $-9$
MPa by midsummer. Some microsites of less severe 
water stress may have existed, but all microsites were 
clearly very dry in the summer. Survival of a significant 
proportion of the population when the soil water po-
tential fell well below $-9$ MPa indicates that mecha-
nisms exist for tolerating severe desiccation. Attrib-
uting population decline entirely to desiccation stress 
may be an oversimplification of process-level dynam-
ics. Diffusion of NH$_4^+$ in thin water films of dry soil 
may limit NH$_4^+$ availability to NH$_4^+$ oxidizers, as the 
following simple diffusion model of Papendick and 
Campbell (1981) illustrates:

$$J = \frac{(c_s - c_b)D_jkq^3}{s},$$

where $J$ is the rate of NH$_4^+$ diffusion; $c_s$ and $c_b$ are the concentrations of NH$_4^+$ at the surface of an ammonium-oxidizing bacterium cell and in the bulk soil, respectively; $D_j$ is the diffusion coefficient for NH$_4^+$-N in H$_2$O ($2 \times 10^{-9}$ m$^2$/s); $k$ is a constant (2.8); $\theta$ is the volumetric soil water content; and $s$ is the diameter of a bacterial cell ($0.5 \times 10^{-6}$ m). For simplicity, bulk soil solution concentration of NH$_4^+$ was assumed to equal total KCl-extractable NH$_4^+$ divided by the gravime-
tric moisture content, and the NH$_4^+$ concentration 
at the cell surface was assumed to be zero. These two 
assumptions are almost certainly false, resulting in 
overestimation of the diffusional gradient, and provid-
ing an estimate of the maximum rate of diffusion that 
might occur under the observed moisture and NH$_4^+$ levels.

As the soil dried, the calculated NH$_4^+$ in bulk soil 
solution (and thus the diffusional gradient) increased 
dramatically, but the diffusion rate declined (Table 4). 
Because $\theta$ is cubed, the diffusion rate is most strongly 
influenced by soil moisture. Availability of NH$_4^+$ at 
the microsite scale clearly cannot be assessed by the 
KCl-extractable pool, especially when diffusion limits 
substrate supply in dry soil. Hence, NH$_4^+$ availability, 
as affected by diffusional limitation, could contribute 
to NH$_4^+$-oxidizer population decline as the soil dries.

**Microbial immobilization of NO$_3^-$**

The highest rates of gross nitrification were observed 
in March (Fig. 1), when NO$_3^-$ pool sizes were lowest 
(Fig. 2) and when rapidly growing grasses were sinks 
of inorganic-N (Jackson et al. 1988). These data in-
dicate that movement of N through the NO$_3^-$ pool was

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### Table 3. Ratios of N process rates (gross nitrification, gross mineralization, nitrification potential, net nitrification, and NO$_3^-$ immobilization) for January and March sample dates (rates were too low in October for reliable estimates of ratios). Data are means (and se) across plots for ratios of plot means of each process.

<table>
<thead>
<tr>
<th>Month</th>
<th>Gross nit.</th>
<th>Gross nit.</th>
<th>Net nit.</th>
<th>NO$_3^-$ imm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>0.12 (0.02)</td>
<td>0.25 (0.07)</td>
<td>0.56 (0.17)</td>
<td>1.67 (0.37)</td>
</tr>
<tr>
<td>Mar</td>
<td>0.18 (0.04)</td>
<td>0.35 (0.10)</td>
<td>0.27 (0.02)</td>
<td>2.32 (0.34)</td>
</tr>
</tbody>
</table>

#### Significant effects

- Open vs. canopy: NS NS NS *
- Jan vs. Mar: NS NS * NS
- Interaction: NS NS NS NS

NS = not significant; * $P \leq .05$; ** $P \leq .01$ (ANOVA). See Materials and methods: Statistical analyses for discussion of $F$ tests.

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### Table 4. Maximum diffusion rates calculated from Eq. 1 (see text, Results and discussion: Nitrification and soil moisture).

<table>
<thead>
<tr>
<th>Month</th>
<th>Volumetric moisture (cm$^3$/cm$^3$)</th>
<th>[NH$_4^+$-N] (μg/g)</th>
<th>[NH$_4^+$-N] (mg/L)</th>
<th>NH$_3^+$-N diffusion rate (mg m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct</td>
<td>0.207</td>
<td>5.77</td>
<td>38.7</td>
<td>3.84</td>
</tr>
<tr>
<td>Jan</td>
<td>0.396</td>
<td>4.59</td>
<td>16.1</td>
<td>11.17</td>
</tr>
<tr>
<td>May</td>
<td>0.507</td>
<td>3.65</td>
<td>10.0</td>
<td>14.56</td>
</tr>
<tr>
<td>May</td>
<td>0.124</td>
<td>1.55</td>
<td>17.4</td>
<td>0.37</td>
</tr>
<tr>
<td>Jun</td>
<td>0.056</td>
<td>2.60</td>
<td>65.0</td>
<td>0.12</td>
</tr>
<tr>
<td>Jul</td>
<td>0.044</td>
<td>3.89</td>
<td>121.6</td>
<td>0.12</td>
</tr>
<tr>
<td>Sep</td>
<td>0.050</td>
<td>3.71</td>
<td>103.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Oct</td>
<td>0.182</td>
<td>5.76</td>
<td>38.1</td>
<td>2.57</td>
</tr>
<tr>
<td>Jan</td>
<td>0.329</td>
<td>6.71</td>
<td>24.6</td>
<td>9.80</td>
</tr>
<tr>
<td>May</td>
<td>0.452</td>
<td>6.55</td>
<td>17.5</td>
<td>18.05</td>
</tr>
<tr>
<td>Jun</td>
<td>0.155</td>
<td>4.73</td>
<td>36.7</td>
<td>1.54</td>
</tr>
<tr>
<td>Jul</td>
<td>0.076</td>
<td>5.20</td>
<td>65.0</td>
<td>0.65</td>
</tr>
<tr>
<td>Sep</td>
<td>0.064</td>
<td>5.54</td>
<td>104.5</td>
<td>0.51</td>
</tr>
</tbody>
</table>

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Microbial assimilation of NO$_3^-$ is usually assumed to be insignificant, but evidence for the importance of a microbial assimilatory sink for NO$_3^-$ is growing (Schimel 1986, Jackson et al. 1989, Rice and Tiedje 1989). In the present study, rates of microbial immobilization of NO$_3^-$ were higher than were previous estimates for NO$_3^-$ uptake by plants (Table 2). Our method may overestimate NO$_3^-$ immobilization because addition of NO$_3^-$-N to 2 $\mu$g/g soil significantly increased the ambient NO$_3^-$ pool and may have stimulated microbial assimilation of NO$_3^-$.

Plant uptake is well recognized as an important sink for NO$_3^-$; microbial assimilation of NO$_3^-$ is usually assumed to be insignificant, but evidence for the importance of a microbial assimilatory sink for NO$_3^-$ is growing (Schimel 1986, Jackson et al. 1989, Rice and Tiedje 1989, Schimel et al. 1989). In the present study, rates of microbial immobilization of NO$_3^-$ were higher than were previous estimates for NO$_3^-$ uptake by plants (Table 2). Our method may overestimate NO$_3^-$ immobilization because addition of NO$_3^-$-N to 2 $\mu$g/g soil significantly increased the ambient NO$_3^-$ pool and may have stimulated microbial assimilation of NO$_3^-$.

Indeed, ratios of gross NO$_3^-$ immobilization to gross nitrification (both estimated by $^{15}$N pool dilution) were consistently > 1.0 (Table 3), indicating that NO$_3^-$ immobilization had been stimulated. Nevertheless, the microbial sink for NO$_3^-$ is clearly of the same order of magnitude as the plant sink (Table 2). Although heterotrophic microorganisms may exhibit a preference for NH$_4^+$ when both NH$_4^+$ and NO$_3^-$ are available in a well-mixed soil (Jansson et al. 1955, Jones and Richards 1977), spatial heterogeneity of substrate availability in an undisturbed soil may result in NO$_3^-$ assimilation in microsites where NH$_4^+$ availability is insufficient to meet microbial demand (Jackson et al. 1989, Rice and Tiedje 1989, Schimel et al. 1989).

Although net nitrification rates followed a seasonal trend similar to the gross rates (Fig. 1), the ratio of net-to-gross rate decreased from winter to spring (Table 3). Changes in this ratio suggest that the NO$_3^-$ production and consumption processes did not vary commensurately across seasons. Because gross nitrification and microbial immobilization of NO$_3^-$ are confined within buried bags and may vary independently, net nitrification estimates cannot be used to reveal the importance of NO$_3^-$ in the internal N cycle of an ecosystem.

We observed a peak in capture of NO$_3^-$ by resin bags in January (Fig. 4), when plant and microbial uptake had not yet reduced NO$_3^-$ pool sizes to the spring minimum, and precipitation was sufficient to cause leaching. However, when converted to common units, the amount of NO$_3^-$ captured in resin bags during January was three orders of magnitude lower than gross nitrification rates. Major pulses of NO$_3^-$ occur in the stream draining this site after autumn rains, but decrease to low levels during the spring growing season (W. Parton, personal communication). Annual NO$_3^-$-N losses via hydrologic export between 1980 and 1986 for the watershed on which our study site is located ranged in amounts of N from 1.0 to 6.3 kg/ha and averaged 3.3 kg/ha. Hence, N losses via leaching are relatively small for a system in which NO$_3^-$ production rates are relatively high. Because NO$_3^-$ can play an important role in the internal N cycle of a system, high rates of NO$_3^-$ production do not necessarily result in commensurately high rates of N loss.

**CONCLUSIONS**

Ammonium-oxidizing bacteria utilized a significant fraction of the N mineralized in an N-limited system during the growing season of grasses. Soil heterogeneity and rapid turnover of the NH$_4^+$ pool affect availability of NH$_4^+$ to NH$_4^+$ oxidizers at a microsite scale. Similarly, absence of NH$_4^+$ at some microsites may result in immobilization of NO$_3^-$ by heterotrophic microorganisms. An important assimilatory microbial sink for NO$_3^-$ has been demonstrated in this grassland soil. By measuring gross rates, we have demonstrated that rapid production and consumption of NO$_3^-$ result in rapid turnover of a small NO$_3^-$ pool.

Ammonium-oxidizing bacteria survived severe desiccation stress. A diffusion model demonstrated how constraints in thin water films of dry soil can affect NH$_4^+$ availability. Hence, the observed gradual decline in NH$_4^+$-oxidizer population during the dry season may have resulted from both desiccation stress and
reduced availability of substrate for maintenance energy.

Microsite heterogeneity of inorganic-N availability, which results from spatial compartmentalization of production and consumption processes and diffusional constraints among microsites, is a critical factor affecting N cycling processes of this grassland ecosystem.

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