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MICROBIAL PRODUCTION AND CONSUMPTION OF NITRATE IN AN ANNUAL GRASSLAND[1]

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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 NH_4^+ and NO_3^- (measured as N) remained below 7 and 4 μg/g soil, respectively, but turned over about once a day. Microbial assimilation of NO_3^- occurred at rates similar to previous estimates of plant uptake. Hence two common assumptions, that nitrifying bacteria are poor competitors for NH_4^+ and that microbial immobilization of NO_3^- is insignificant, are not correct for this grassland system. Soil heterogeneity probably results in NH_4^+ availability to NH_4^+ oxidizers at some microsites, while NO_3^- assimilation by heterotrophic microorganisms occurs at other microsites where NH_4^+ is not available. Relatively high rates of 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 MPa, indicating that nitrifying bacteria can tolerate severe desiccation. A simple diffusion model demonstrates the dependence of 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 NH_4^+ relative to plants and heterotrophic microorganisms (e.g., Vitousek et al. 1982, Gosz and White 1986, Robertson 1989). When NO_3^- is produced, it is often assumed that plant uptake, leaching, and possibly denitrification are the major fates of NO_3^-, and that microbial assimilation of 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 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 NH_4^+ and NO_3^-. The silt loam soil of the grassland site studied here is N-limited, has low NH_4^+ and 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 NH_4^+-oxidizing bacteria and insignificant microbial assimilation of NO_3^- by using in situ measurement techniques in unmixed soil.

Because microbial assimilation of 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 1982*a*, *b*, 1989). This approach has revealed mechanisms that help explain differences in NO_3^- leaching losses following disturbance of different ecosystem types (Vitousek et al. 1982). By distinguishing between regulation of NO_3^- production and 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 NO_3^- production and consumption within an annual grassland of California.

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To this end, we have employed a variety of techniques, each of which provides different information about NO_3^- 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 douglassii* 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 phenology 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.

^{15}N 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 two 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 $(^{15}NH_4)_2SO_4$ injections and two received $K^{15}NO_3$ 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 ^{15}N 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 NH_4^+ and NO_3^- 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 ^{15}N analysis. In brief, NO_3^- was reduced to NH_4^+ with Devarda's alloy; NH_3 was volatilized in a base solution with added MgO; NH_3 vapor was captured on an acidified glass fiber filter; and the filters were sent to Isotope Services, Los Alamos, New Mexico, for ^{15}N analysis.

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.

	Open grass		Under oak		
	$\bar{X}$	SE	$\bar{X}$	SE	
Bulk density (g/cm³)	1.38	0.03	1.32	0.08	NS
Acidity (pH)	5.62	0.06	5.74	0.17	NS
Carbon (%)	2.11	0.13	3.03	0.51	*
Nitrogen (%)	0.19	0.01	0.25	0.04	NS

NS = not significant; *$P \leq .05$ (one-way ANOVA comparing vegetation types).

Gross mineralization rates were calculated for cores that received $^{15}NH_4^+$ using the model of Kirkham and Bartholomew (1954). Initial $^{14}NH_4^+$ pool sizes were estimated from the KCl extract of soil collected between the plastic and metal cylinders. The initial $^{15}NH_4^+$ 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 NO_3^- values for NH_4^+ values. Gross NH_4^+ and NO_3^- immobilization rates calculated from this model include other possible consumptive fates of inorganic-N (e.g., NH_4^+ fixation, denitrification) and therefore may be somewhat overestimated. The assumptions involved in the calculation of NH_4^+ and NO_3^- production and consumption from the changes in the ^{15}N enrichment of NH_4^+ and NO_3^- pools are discussed elsewhere (E. A. Davidson et al., *unpublished manuscript*).

Use of the ^{15}N 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 ^{15}N 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 ^{15}N 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 $(NH_4)_2SO_4$ 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 NO_2^- and NO_3^- were determined colorimetrically using a Lachat flow injection auto-

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analyzer. Although chlorate is intended to block oxidation of NO_2^- to NO_3^-, we observed NO_3^- accumulation throughout the incubation period. Therefore, we summed NO_2^- and NO_3^- production and regressed this sum against time to estimate nitrification potential rates. It is assumed that excess NH_4^+ in a well-mixed system represses NO_3^- assimilation (Rice and Tiedje 1989).

Buried bag method for net nitrification

When the soil was wet enough to take intact cores, four more cores in plastic cylinders were sampled from each plot, placed in plastic bags (38 μm [1.5 mil] thickness), and buried in their holes. The cores were retrieved after 30 d; the soil was mixed, and subsamples were immediately extracted in KCl. The initial NO_3^- pool size was estimated from the plot mean for the initial pool sizes determined for the ^{15}N pool dilution work. Net nitrification was calculated from the change in NO_3^- content of the soil during the 30-d buried bag incubation. When the soil was too dry and hard for coring, bulk soil samples were excavated and mixed, and four subsamples of ≈100 g each were placed in plastic bags and buried for 30 d.

Resin bags

The use of bags containing ion-exchange resins, as described by Binkley and Matson (1983), was slightly modified. Mixed bed resins (J T Baker M-614) were washed in 1 mol/L NaCl. Approximately 3 g (dry mass equivalent) of resins were placed in small bags made of nylon stockings. A plastic-coated wire ring (4 cm diameter) was placed inside the bag to provide support and shape. Four short cylinders (4 cm diameter × 5 cm deep) were driven into each plot on the October, January, March, and May dates. The cylinders were removed, the bottom 0.5 cm of soil was replaced by a resin bag, and the core with resin bag was put back into the hole from which it came. This procedure placed the resin bags at 4.5 cm depth at the bottom of a core, which is the midpoint of the cores used for gross and net nitrification measurements. The resin bags were retrieved 30 d later, and the resins were extracted in 75 mL 2 mol/L KCl.

Statistical analyses

Analysis of variance was performed using PROC GLM of SAS (SAS 1985). Differences in soil properties reported in Table 1 were determined by one-way ANOVA. For data reported in Tables 2 and 3 and Figs. 1–4, Type III sums of squares were computed for effects of month, vegetation type (canopy vs. open), month × vegetation type interaction, and study plot nested within vegetation type. The study plot effect was random, and all other effects were fixed. The F test for vegetation type effects used the Type III mean square for the study plot-within-type effect as an error term; all other F tests used the Type III mean square error 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_3^- (production minus consumption in buried bags), gross nitrification as actual production of NO_3^- (^{15}N pool dilution), and potential nitrification as production of NO_2^- and NO_3^- when NH_4^+ 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_4^+

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_3^- (Table 2) will be discussed in more detail below, but is relevant here to demonstrate NH_4^+ limitation to microorganisms in this ecosystem. Microbial assimilation of NO_3^- probably occurs only at NH_4^+-free microsites (Rice and Tiedje 1989). Despite NH_4^+ limitation at some microsites, we observed that NH_4^+ oxidizers were able to obtain a significant proportion of the NH_4^+ produced in this soil. Gross rates of mineralization and

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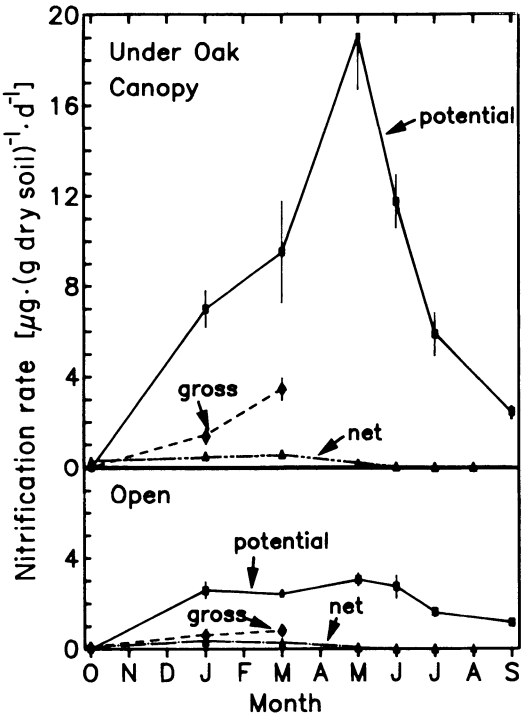

FIG. 1. Nitrification rate (measured as N) for open-grass plots and plots under oak canopy (means ± SE). The effect of month and the interaction of month and vegetation type are significant at $P = .01$ for net, gross, and potential nitrification. The vegetation type effect (open vs. canopy) is not significant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

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 NH_4^+ availability may partially explain the success of NH_4^+ oxidizing bacteria in obtaining significant amounts of NH_4^+ in an N-limited system. If mineralization occurs at localized "hot spots," then the NH_4^+ 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 NH_4^+ 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 NH_4^+ oxidizers obtain a significant piece of the NH_4^+ pie, the amount of N available for nitrification is also affected by the size of the pie. However, the NH_4^+ pie available for consumption is not simply the NH_4^+ pool size, but rather the amount of NH_4^+ produced over time. Although the N in KCl-extractable NH_4^+ 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 NH_4^+ pool (Table 2), indicating that this relatively small NH_4^+ pool turned over approximately daily during the wet season. While NH_4^+ availability regulates nitrification in a general way at the ecosystem scale (Robertson 1989), spatial heterogeneity and gross NH_4^+ production rates are critical controllers of NH_4^+ availability at a microsite scale.

Michaelis-Menten kinetics and nitrification rates

The nitrification potential assay indicates the maximum rate (V_{max}) of NH_4^+ oxidation by the NH_4^+ oxidizer population present in a soil sample when assayed in the laboratory at a given temperature with excess NH_4^+ 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 NH_4^+-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 NH_4^+-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 NH_4^+-oxidizing population was experiencing NH_4^+ concentrations near its apparent k_M. According to kinetic theory, a population

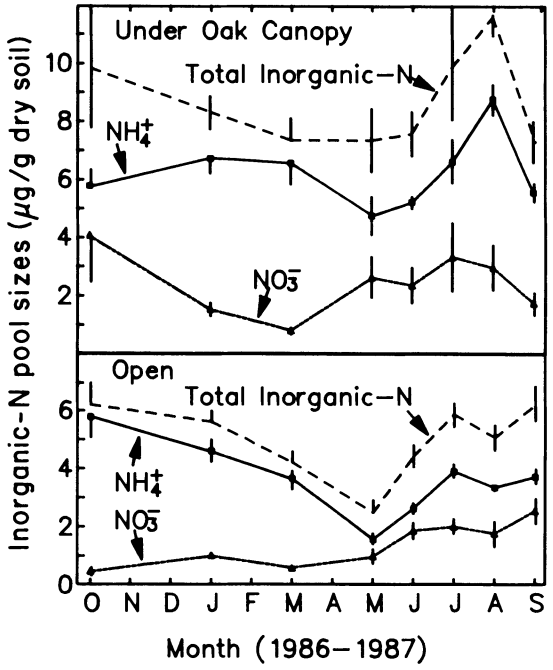

FIG. 2. Ammonium and nitrate pool means (± SE) for open-grass plots and plots under oak canopy. Means of NH_4^+ and NO_3^- are summed to plot total inorganic-N. The effect of month and the interaction of month and vegetation type are significant at $P = .01$ for NH_4^+, NO_3^-, and total inorganic-N. The vegetation type effect (open vs. canopy) is not significant for NO_3^-, is significant at $P = .01$ for NH_4^+, and is significant at $P = .05$ for total inorganic-N.

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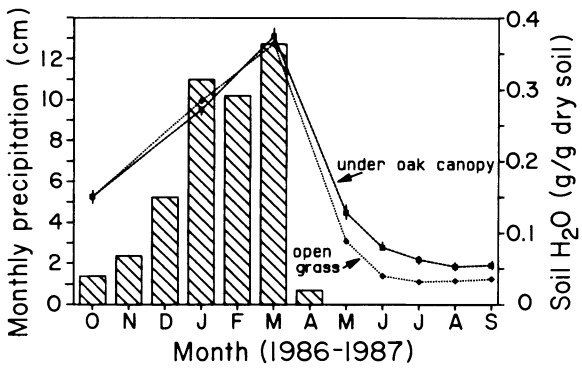

FIG. 3. Monthly precipitation (histogram bars) and gravimetric soil moisture means ± SE (points with vertical lines). The effects of month, vegetation type, and their interaction are significant at $P = .01$ for soil moisture.

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 NH_4^+-N never varied by more than a factor of 3 (Fig. 2), 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_{max} might indicate a recent increase in resource availability, but is unlikely to be sustained. If NH_4^+ availability is the primary factor limiting nitrification, the existing population would respond quickly to an increase in NH_4^+ by increasing its gross rate. Using the energy from this enhanced activity, the population could grow, causing V_{max} to increase and the ratio of gross nitrification rate to V_{max} to decrease. Similarly, if a recent decline in resource availability caused gross rates to drop very low relative to V_{max}, then maintenance energy requirements of the existing population might not be met. The population size and V_{max} would decline, and the ratio of gross rate to V_{max} would increase. In short, changes in resource availability could cause temporary changes in the ratio of gross rate to V_{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_{max} ratios in two soil habitats suggest that the ratio may be independent of ecosystem characteristics. Perhaps homeostasis of gross rate-to-V_{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 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}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.

Season	Gross mineralization	Gross nitrification	Microbial immobilization		Plant uptake	
			NH_4^+	NO_3^-	NH_4^+	NO_3^-
	Rates of N transformation [$\mu g \cdot (g \text{ dry soil})^{-1} \cdot d^{-1}$]					
Open grass						
Winter	5.01 (0.38)	0.59 (0.13)	6.60 (0.35)	0.81 (0.11)	0.58 (0.06)	0.29 (0.04)
Spring	4.90 (0.77)	0.81 (0.11)	7.29 (0.50)	1.76 (0.06)	0.79 (0.17)	0.60 (0.18)
Under canopy						
Winter	6.96 (1.28)	1.38 (0.29)	8.12 (1.05)	1.49 (0.27)	no data	
Spring	9.14 (2.47)	3.47 (0.52)	11.67 (2.98)	3.69 (0.51)	no data	
	Significant effects					
Open vs. canopy	*	**	NS	*		
Winter vs. spring	NS	**	NS	**		
Interaction	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 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.

Month	Gross nit. / Gross min.	Gross nit. / Nit. pot.	Net nit. / Gross nit.	NO_3^- imm. / Gross nit.
		Open grass		
Jan	0.12 (0.02)	0.25 (0.07)	0.56 (0.17)	1.67 (0.37)
Mar	0.18 (0.04)	0.35 (0.10)	0.27 (0.02)	2.32 (0.34)
		Under-oak canopy		
Jan	0.22 (0.04)	0.21 (0.03)	0.37 (0.10)	2.07 (1.24)
Mar	0.46 (0.21)	0.48 (0.21)	0.17 (0.04)	1.09 (0.07)
		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.

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 survived 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 potential fell well below −9 MPa indicates that mechanisms exist for tolerating severe desiccation. Attributing population decline entirely to desiccation stress may be an oversimplification of process-level dynamics. 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:

$$J = \frac{(c_o - c_b)D_o k\theta^3}{s}, \qquad (1)$$

where J is the rate of NH_4^+ diffusion; c_o 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_o is the diffusion coefficient for NH_4^+-N in H_2O (2×10^{-9} m^2/s^1); k is a constant (2.8); θ is the volumetric soil water content; and s is the diameter of a bacterial cell (0.5×10^{-6} m). For simplicity, bulk soil solution concentration of NH_4^+ was assumed to equal total KCl-extractable NH_4^+ divided by the gravimetric 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 providing 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 θ 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 indicate that movement of N through the NO_3^- pool was

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

Month	Volumetric moisture (cm^3/cm^3)	$[NH_4^+$-N] ($\mu g/g$)	$[NH_4^+$-N] (mg/L)	NH_4^+-N diffusion rate ($mg \cdot m^{-2} \cdot s^{-1}$)
		Open grass		
Oct	0.207	5.77	38.7	3.84
Jan	0.396	4.59	16.1	11.17
Mar	0.507	3.65	10.0	14.56
May	0.124	1.55	17.4	0.37
Jun	0.056	2.60	65.0	0.12
Jul	0.044	3.89	121.6	0.12
Sep	0.050	3.71	103.1	0.14
		Under-oak canopy		
Oct	0.182	5.76	38.1	2.57
Jan	0.329	6.71	24.6	9.80
Mar	0.452	6.55	17.5	18.05
May	0.155	4.73	36.7	1.54
Jun	0.096	5.20	65.0	0.65
Jul	0.076	6.59	104.6	0.51
Sep	0.064	5.54	104.5	0.30

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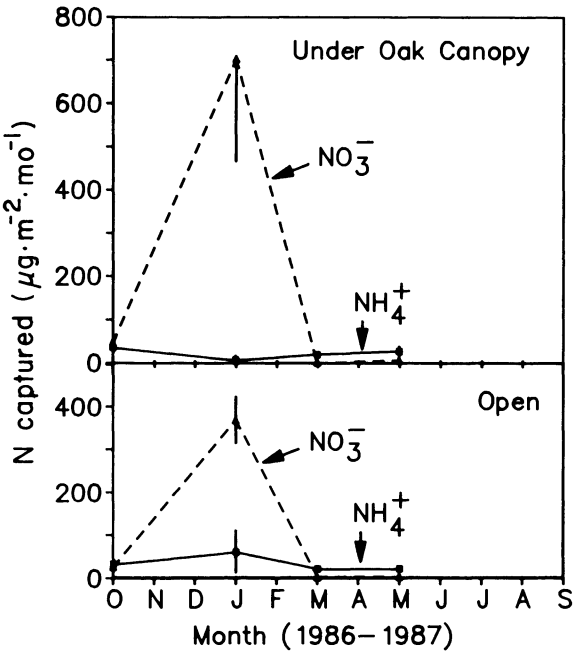

FIG. 4. Nitrogen content of NH_4^+ and NO_3^- captured on resin bags in open-grass plots and plots under oak canopy (means ± SE). No effects are significant for NH_4^+. For NO_3^-, the effect of month is significant at $P = .01$, the effect of vegetation type (open vs. canopy) is not significant, and the interaction is significant at $P = .05$.

also very rapid, with a turnover time between 0.3 and 1.6 d. Small NO_3^- pool sizes have been interpreted as an indication that NO_3^- was unimportant in the internal N cycle of a grassland ecosystem (Woodmansee et al. 1981). At the California annual grassland site of the present study and the shortgrass steppe of Colorado (Schimel and Parton 1986), high rates of both NO_3^- production and consumption result in an important role for NO_3^- in N cycling within the system, despite small NO_3^- pool sizes.

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 at 2 μg/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 confounded 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

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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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