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Root Influence on Nitrogen Mineralization and Nitrification in *Avena barbata* Rhizosphere Soil

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ABSTRACT

Micro–^{15}N pool dilution was used to quantify rates of gross N mineralization, consumption, and nitrification in bulk soil and in soil within 2 mm of root sections of *Avena barbata* (slender wild oats), an annual grass common to California oak woodland-savannas. Rates of gross N mineralization in rhizosphere soil (9.2 mg N $kg^{-1}d^{-1}$) were about ten times higher than in bulk soil (1.0 mg N $kg^{-1}d^{-1}$). Total bacterial numbers in soil adjacent to roots were slightly higher than in bulk soil; protozoa biomass was not measurably different. Changes in bacterial numbers or standing stocks of bacterial N could not account for rates of N mineralization. Nitrification potential values were similar in bulk and rhizosphere soil, yet gross rates of nitrification were highly dependent on location along the root. Gross nitrification rates in soil near the root tip were the same as those in bulk soil, while rapid uptake of NH_4 by older sections of root (8–16 cm from the tip), appeared to limit nitrification rates. Only small differences in microbial community structure between bulk and rhizosphere soil were detected by terminal restriction fragment length polymorphism (TRFLP) analysis. While the small increases in bacterial numbers and changes in community composition may in-part explain the increased rates of N mineralization, other microbial-root interactions are likely involved in accelerating the flux of N from organic sources to the plant-available NH_4 pool. The high rates of N mineralization observed in soil immediately adjacent to roots should facilitate plant access to N. Most of the stocks and fluxes determined in these studies exhibited distinct spatial patterns along the plant root that may have significantly impacted N-availability to the plant.

In temperate terrestrial ecosystems, N is the nutrient most limiting to plant productivity (Vitousek and Howarth, 1991). Most temperate plants cannot directly access the large pool of N tied up in complex macromolecular soil organic matter, relying primarily on pools of inorganic N in soil solution (Jones et al., 2005). Microorganisms however, are less commonly N limited than plants because they have the enzymatic capacity to access and utilize macromolecular soil organic N (Paul and Clark, 1996). Heterotrophic soil microbes are thought to be generally C limited (Paul and Clark, 1996). In mutualistic models of root–microbe interaction, roots supply soil microbes C while the microbes access soil organic N and make this N available to roots (Harte and Kinzig, 1993; Kinzig and Harte, 1998).

The interactions of plant roots and microbes in the rhizosphere have been studied extensively because of their broad-ranging importance in nutrient availability, pathology, and soil C dynamics (Pinton et al., 2000). Plant roots exude large amounts (Minchin and Pate, 1973; Norton et al., 1990) and complex arrays of organic compounds into the nearby soil (Juma and McGill, 1986; Paul and Clark, 1996; Kennedy, 1998). Primarily in response to elevated C availability, bacterial, fungal, and protozoal numbers are generally higher in rhizosphere soil than in bulk soil (Kennedy, 1998). Some types of microbial activity have also been found to be higher in rhizosphere than bulk soil (Hojberg and Sorensen, 1993; Sorensen, 1997; Naseby and Lynch, 1997; Yang and Crowley, 2000).

The quality and quantity of root exudates vary temporally and spatially along the root (Klein et al., 1990; Newman, 1985; Jaeger et al., 1999; Bringhurst et al., 2001). We have mapped sugar and amino acid exudate patterns in soil adjacent to *Avena barbata* roots using engineered bacterial reporter gene systems (two different strains of *Erwinia herbicola* 299r) and found that sucrose/fructose availability was highest near the root tip and declined with distance from the tip (Jaeger et al., 1999).

Increased numbers of microorganisms in rhizosphere soil can represent a potentially labile stock of organic N near plant roots. There are several ways that N contained in microbial biomass can become available to plants. If the supply of labile C is high near young roots and declines substantially in older root sections, then C-limited heterotrophs in a mature rhizosphere would mineralize NH_4 during catabolism of N-rich cell components (Myrold, 1998). Such a spatial pattern of C-availability along roots (high C availability near root tips and low C availability near mature roots) could in itself result in N mineralization. Alternatively, root-C enhancement of microbial numbers and activity may attract bacterivores, which on consumption of low C/N microbial biomass, release N as NH_4 into the rhizosphere. Protozoa and other soil fauna excrete an estimated 30% of consumed bacterial N into the rhizosphere (Griffiths et al., 1992), where it is available for plant uptake (Elliott et al., 1984; Clarholm, 1985). Infection of rhizosphere bacteria by bacteriophage would also result in cell lysis and biomass N mineralization. Finally, rhizosphere soil is a zone of water potential fluctuation as a result of evapotranspiration during the day followed by re-equilibration with surrounding soil water during the night (Papendick and Campbell, 1975). Such relatively rapid fluctuations in soil water potential could also result in N mineralization from the rhizosphere microbial biomass as N-rich cellular materials are released during cell water potential equilibration with the surrounding

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Abbreviations: AM, arbuscular mycorrhizal; CFDE, chloroform fumigation-direct extraction; PCR, polymerase chain reaction; TRFLP, terminal restriction fragment length polymorphism.

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soil solution (Bottner, 1985; Kieft et al., 1987; Halverson et al., 2000).

The current study was designed to quantify rates of gross N mineralization in rhizosphere soil and relate these rates to spatial patterns of root-microbial interaction. The experimental design allowed testing of the first two mechanisms discussed in the preceding paragraph: N mineralization resulting from C-starvation in older root zones and protozoal grazing of bacterial cells. We grew *Avena barbata* in microcosms designed for calibrated temporal and spatial access to roots and rhizosphere soil (here within 2 mm of root). Using this simplified system we have: (i) quantified gross N mineralization and nitrification rates along the root and in bulk soil using a micro–^{15}N pool dilution technique; (ii) mapped the density of native bacteria in soil around the root using direct microscopic counts; (iii) characterized the total bacterial community through DNA-based techniques; and (iv) mapped the biomass of protozoal grazers around the root using a standard MPN technique. These data are analyzed to determine if rates of gross N transformation are directly impacted by the presence of roots and if so, how.

MATERIALS AND METHODS

Plants and Soils

Studies were conducted using the annual grass *Avena barbata* in microcosms incubated in growth chambers; seed was collected at the Jasper Ridge Biological Preserve, Palo Alto, CA. The microcosms (Fig. 1), modified from the designs of James et al. (1985) and Norton et al. (1990), comprise a large main compartment and a 5-mm deep thin compartment, with a Plexiglas divider separating the two compartments. Seeds were planted in the main compartment, which was filled with a mixture of 1:1 soil and 50 to 70 mesh quartz sand. Assays were performed only on soil from the thin compartments of the microcosms, which were filled with a 1:1 mix of sand and Yolo silt loam (fine-silty mixed, superactive, nonacid, thermic, typic Mollic Xerofluvent) from the University of California, Davis, CA. The Yolo soil/sand mix was used in the thin compartments to standardize these studies with preceding work on spatial pattern of C flow (Jaeger et al., 1999). An *Avena barbata* seedling (5–8 d old) was planted in the main compartment of each microcosm, then placed in growth chambers with a 25°C day, a 15°C night, and a 12-h photoperiod. Plants received tap water three times a week and a dilute solution of a balanced plant nutrient solution with micronutrients (J.R. Peter's Inc., Allentown, PA) once a week. The microcosms were wrapped in aluminum foil to prevent light from reaching the roots.

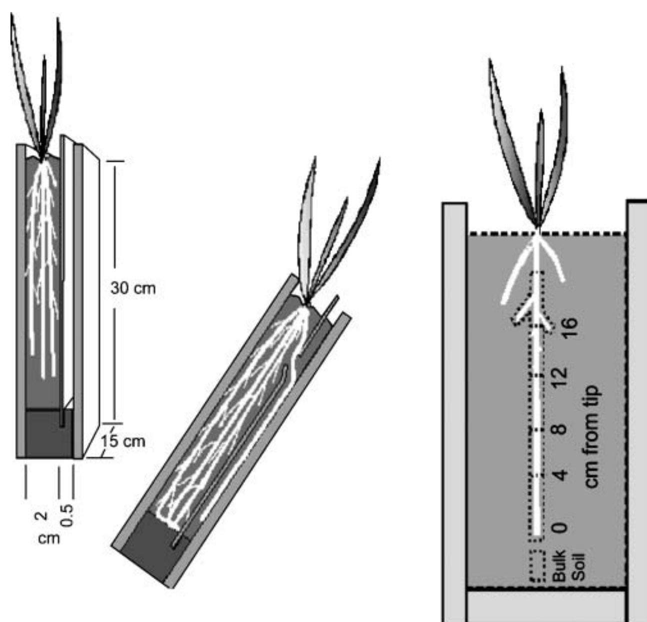

Fig. 1. Diagram of plant growth microcosms. About 8 wk after *Avena barbata* germination, the solid divider separating the two compartments of the microcosm was replaced by a divider with a 5-mm high horizontal slot approximately 3 cm below the soil line. The pattern of root and soil harvest is shown on the right.

Experimental Approach

When the plants reached peak vegetative size and were producing large numbers of rapidly growing primary roots from their stem bases (usually 8 wk after germination), the solid divider separating the two compartments of the microcosm was replaced by a divider with a 5-mm high horizontal slot approximately 3 cm below the soil line (Fig. 1). The microcosms were placed back in the growth chambers and held in racks at a 45° angle. Under the influence of gravity, new roots initiated from stem bases grew along the divider, entered the thin compartment of the microcosm through the slot in the divider, and grew through the soil in the thin compartment, along the face of the microcosm. The progress of the roots was monitored daily with colored wax pencils on the face of the microcosms to determine root growth rate.

Mapping Native Bacteria and Protozoa

Roots were allowed to grow through the thin compartment for 9 to 13 d, until new roots were within 2 to 3 cm of the bottom of the thin compartment. After removal of the faceplate, two roots and the 2-mm radius of rhizosphere soil surrounding each root were excised. Each root was divided into four sections: 0 to 4, 4 to 8, 8 to 12, and 12 to 16 cm from the root tip. For each section, the soil from the two roots was removed by gentle agitation in a preweighed tube containing 0.2 *M* Trizma buffer. This process was replicated for a total of 10 microcosms (n = 10). Bulk soil control samples were taken from each microcosm at a distance >1.5 cm from any root. The samples were stored at 5°C or frozen at −80°C depending on analysis to be performed. A large (~16 g) soil sample was composited from multiple different areas in the microcosm, weighed, dried at 105°C to calculate the gravimetric soil water content for each microcosm. Soil samples were diluted to yield a 10-fold dilution series to 1:10^{-5} and used for assays of bacterial and protozoal numbers. Bacterial numbers were determined using the two-part stain BacLight Bacterial Viability Kit (Molecular Probes Inc., Eugene OR), and counted using epiflourescence microscopy to determine live and dead bacterial numbers. Serial dilutions of soil were made in phosphate buffered saline (0.14 *M* NaCl + 9 m*M* PO_4), sonicated, stained, and viewed within 48 h. At least two slides were prepared per sample, one for each dilution, and ten fields of view were counted per slide. Total bacterial counts are a sum of live plus dead counts. Protozoal populations were determined by a most probable number method adapted from that of Ingham (1994). Protozoal biomass was then calculated based on the average biomass of the three major groups of protozoa (ciliates, amoebae, and flagellates) as described by Ingham (1994).

Community Analysis

DNA was extracted from five frozen rhizosphere soil samples, five frozen bulk soil samples and two control soil samples. The control samples consisted of DNA extracted from the soil before it was added to the growth chamber. DNA was extracted from all soil samples using the BIO 101 soil DNA extraction kit (Qbiogene, Carlsbad, CA) according the protocol provided by the manufacturer.

TRFLP patterns of the total bacterial community were constructed using the following PCR reaction: 10 ng of soil DNA, 0.2 μM of primers 6FAM- 27F (5′ 6-FAM- AGAGTTTGATCCTGGCTCAG 3′) and 1492R (5′ TACGGYTACCTTGTTACGACTT 3′), 2.5 m*M* MgCl2, 1 × Taq buffer and 5 units of Taq polymerase enzyme combined in a total volume of 50 μL. The PCR reaction was initiated with a hot start for 3 min at 92°C followed by 30 cycles of 30 s of 92°C, 30 s of 53°C, and 60 s of 72°C. The PCR reaction was cleaned with a QIAGEN PCR cleanup kit (Qiagen Inc., Valencia CA), digested with 10 units of MspI and analyzed on a ABI 3700 automatic DNA sequencing system (Applied Biosystems, Foster City CA).

Gross Nitrogen-Mineralization Rates

Nitrogen mineralization rates were determined using ^{15}N pool dilution experiments. After new roots had colonized the thin compartments of the microcosms, the faceplates were removed from five microcosms at a time, and 60 mL of ^{15}N label-containing solution was sprayed onto the soil surface of the five microcosms. The label solution was 400 mg N L^{-1} as $(NH_4)_2SO_4$ at 29.7 atom% ^{15}N, and was applied at a rate of 14 mg N kg^{-1} soil. This application rate was necessary to minimize the effect of the variability of background soil NH_4, and the resulting concentration was comparable with a fertilization event. After labeling all microcosms, the faceplates were replaced and the microcosms returned to the growth chamber.

Since homogeneity of label application is critical in pool dilution experiments (Davidson et al., 1991), we tested the uniformity of label application within and among microcosms. We applied NH_4 to the surfaces of five unplanted microcosms, and collected a total of five 3-g soil samples from each microcosm. We found no differences among microcosms (data not shown), although the top third of the microcosms were lower ($P < 0.05$) than the middle and bottom thirds (24 vs. 29 mg NH_4–N kg^{-1}). With respect to penetration of label applied to a soil surface, Murphy et al. (2003) suggest the soil depth be restricted to <2 cm; the 5-mm depth of the experimental compartment and the 2-mm radius of rhizosphere soil we harvest is well within that limit.

It has been reported that nonuniform exploitation of indigenous and applied N pools occurs in short-term pool dilutions (Watson et al., 2000), and it has therefore been suggested that at least 24 h should elapse after label application before initial sampling (Murphy et al., 2003). We performed pool dilution trials up to 31 h long, but found that beyond 7 h, standard deviations exceeding 5 atom% ^{15}N were typical in rhizosphere soil. This rendered rate calculations uninterpretable. For this reason, and to avoid overestimation of gross rates due to nonlinear changes in pool sizes imparted by root assimilation, we used incubation times not exceeding 3 h. At 2.3 h, for example, sufficient label remained in the soil (Table 1) as to avoid problems of label exhaustion and excessively high variability.

We performed pool dilution experiments to determine gross N mineralization rates on two separate occasions; the results presented here are the combined results for the two experiments. We collected bulk and rhizosphere soil from the microcosms after 15 min and 2 to 3 h of incubation. The initial 15 min allowed for abiotic reactions involving the added label (Davidson et al., 1991). We excised roots with the surrounding 2 mm of soil, and cut the roots into four sections: 0 to 4, 4 to 8, 8 to 12, and 12 to 16 cm from the root tip. We harvested rhizosphere soil by gently agitating the root sections in 30 mL of 2 *M* KCl, combining soil from four different roots (generally harvested from two or three microcosms) into each extraction cup. This yielded approximately 3 g of soil for each 4-cm section, and gave four or five replicate samples for each 4-cm section. We also collected bulk soil from each microcosm, combining microcosms as with the rhizosphere soil, for a total of five extractions of 3 g soil in 30 mL of 2 *M* KCl chilled to 5°C. Ammonium concentration was quantified using flow-injection analysis (Lachat QC8000 flow injection analyzer, Lachat Instruments, Milwaukee, WI) and isotopic composition using isotope ratio mass spectrometry (Tracermass isotope ratio mass spectrometer, PDZ Europa Ltd. Crewe, U.K.). We calculated gross rates of NH_4 mineralization and consumption using standard isotope dilution equations (Hart et al., 1994).

Table 1. Distribution of NH_4 in microcosm soil before and after label addition.

Soil	Soil NH_4		
	Before label	2.3 h after label addition	
	mg N kg^{-1}	mg N kg^{-1}	atom% ^{15}N
Bulk	0.16 ± 0.03	15.8 ± 3.1	23.6 ± 0.4
0–4 cm rhizosphere	0.33 ± 0.12	13.5 ± 4.4	21.9 ± 0.2
4–8 cm rhizosphere	0.25 ± 0.03	14.6 ± 4.6	22.5 ± 1.1
8–12 cm rhizosphere	0.38 ± 0.10	10.3 ± 1.5	21.7 ± 0.1
12–16 cm rhizosphere	0.47 ± 0.16	7.3 ± 1.7	20.6 ± 0.8

Values are means ± standard errors from Experiment 1 only. The bulk soil NH_4 pool is significantly smaller ($P < 0.05$) than the rhizosphere NH_4 pool before label addition.

Gross Nitrification Rates

In a separate experiment, gross rates of nitrification were determined using methods analogous to gross mineralization. However, $K^{15}NO_3$ was substituted for $^{15}NH_4$, the incubation period was 6 h, and root sections were 8 cm long, rather than 4 cm. Rates of gross nitrification were calculated using standard isotope dilution equations (Hart et al., 1994).

Nitrification Potential

Soil nitrification potentials were assayed according to the method developed by Schmidt and Belser (1982) and modified by Hart et al. (1994). Roots were divided into 4-cm sections, and soil from two or three roots was composited for each replicate. Soil was washed from roots into buffered solution containing NH_4 in phosphate buffer (Hart et al., 1994). Aliquots were taken from shaken slurry incubation over a 24-h period and analyzed for NO_3 content using flow-injection analysis (Lachat QC8000 flow injection analyzer, Lachat Instruments, Milwaukee, WI). A regression of nitrate concentration against time was used to estimate potential nitrification rates.

Biomass Nitrogen

We estimated bulk soil microbial biomass N by chloroform fumigation-direct extraction (CFDE; Brookes et al., 1985) as well as from direct bacterial counts. For the CFDE method, we collected approximately 5 g oven-dry equivalent of bulk soil from each microcosm at the 3-h harvest, as well as from the

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planted-unlabeled microcosms, using half of each sample as the nonfumigated control.

We also estimated biomass N in individual root sections and bulk soil from direct cell counts of total (live + dead) bacteria. We assumed rod-shaped cells of 0.5-μm diameter and 1.5-μm length, a bacterial cell density of 1.1 mg mm^{-3}, a solids content of 0.4, and a carbon content of 0.45 g g^{-1} (Paul and Clark, 1996). Then we multiplied biomass C per cell by the number of cells per gram of soil to estimate biomass C on a unit soil weight basis. Finally, we estimated biomass N assuming a C/N ratio of 6:1.

Statistical Analysis

For background NH_4 pools, we tested differences between bulk and rhizosphere soil by pooling rhizosphere sections, and performing a Student's *t* test. We tested for differences in bacterial cell numbers and protozoa biomass using randomized block designs; rhizosphere sections and bulk soil were blocked by microcosm. We analyzed gross mineralization results by first pooling all four rhizosphere sections, but not bulk soil; we then tested differences between rhizosphere and bulk soil using a factorial design, with experiment (1 or 2) and soil source (rhizosphere or bulk) as main effects. Bacterial numbers, protozoa biomass, mineralization rates, and NH_4 pools were log-normally distributed, and so were $\log_{10}$–transformed before analysis. Statistics were performed using Statistix 7 software (Analytical Software, Tallahassee, FL). Principal component analysis of TRFLP patterns was performed using JUMP software (SAS Institute Inc. Cary, NC) TRFLP fragments were binned if they were within one base-pair in size of each other. Only fragments with intensities higher than 0.5% of the total fluorescence were used in the analysis.

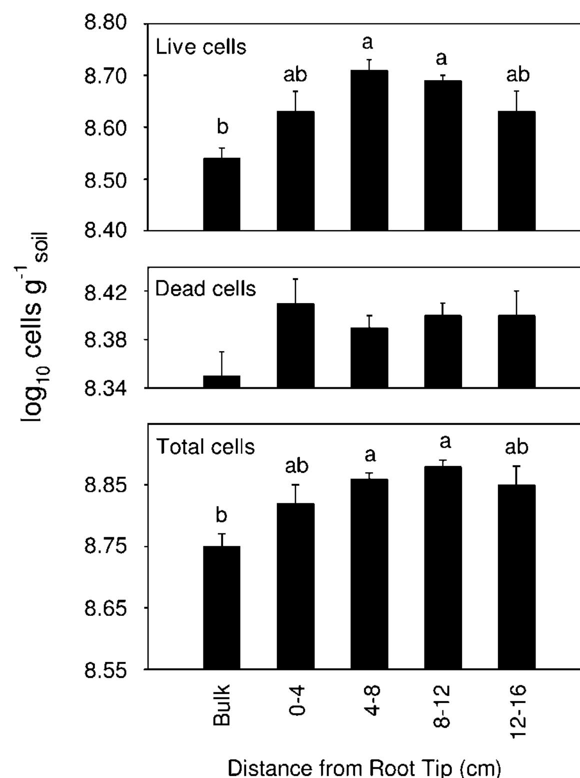

Fig. 2. Live, dead, and total bacterial numbers in rhizosphere and bulk soils. Total cells = live cells + dead cells. Cell numbers were $\log_{10}$ transformed before analysis. Error bars indicate standard error of the means.

RESULTS

Bacterial Numbers and Protozoal Biomass

Live, dead, and total (live + dead) bacterial numbers in rhizosphere soil adjacent to root zones are shown in Fig. 2. Numbers of live bacteria were higher in rhizosphere soil sections than in the bulk soil, reaching maximum numbers in the 4- to 8-cm zone. The numbers of dead bacteria are substantially smaller than live and exhibit smaller differences among the bulk and rhizosphere soil samples. There were no significant differences among counts of dead bacterial cells and hence no evidence for increased numbers of dead or starved cells in the older root sections. Thus the pattern for total bacterial numbers is similar to that of the pattern for live bacterial numbers. Protozoal biomass ranged from 0.17 mg g^{-1} in bulk soil to a maximum of 0.22 mg g^{-1} in the 12- to 16-cm section (Table 2). There were no significant differences between protozoal biomass in the bulk and rhizosphere soils.

Rates of Gross N Mineralization and N Flux through Microbial Biomass

The average rates of N mineralization in rhizosphere soil were higher than in bulk soil ($p < 0.0001$; Table 2). Average N mineralization for soil adjacent to the four root sections (0–16 cm) is 9.2 mg N $kg^{-1}d^{-1}$ compared with 1.0 mg N $kg^{-1}d^{-1}$ for bulk soil.

Calculated values for bacterial biomass N in rhizosphere soil are shown in Table 2. The bacterial biomass N value for bulk soil (3.0 mg N kg^{-1}) is about 25% of the value for microbial biomass N found by chloroform fumigation direct extraction [12.8 (± 1.0) mg N kg^{-1}]. If the standing stock of microbial biomass N decreases over time (that is, along the root), then that change in biomass N should result in net N mineralization. The calculated values for bacterial biomass N shown in Table 2 can thus be used to estimate net rates of N mineralization that could result from the changes in the standing stock of microbial biomass N. The greatest decrease in bacterial biomass N (0.3 mg N kg^{-1}) occurs between the 8- to 12-cm and 12- to 16-cm root sections. Under these experimental conditions, *A. barbata* roots were growing at an average of about 2 cm d^{-1} (data not shown). This translates to a net rate of N mineralization of 0.3 mg N kg^{-1}/2 d = 0.15 mg N kg^{-1} d^{-1}.

Alternatively, the flux of N through the microbial biomass can be calculated as the biomass N turnover time, by dividing the standing stock of biomass N by the gross rate of N mineralization. This approach assumes that most of the N mineralized comes through bacterial biomass N but does not necessarily originate from the microbial biomass. The calculated values for N-turnover are shown in Table 2. The turnover time for N in the microbial biomass in bulk soil is 3 d. The turnover time for microbial biomass N in rhizosphere soil is much shorter and very rapid, ranging from 0.3 to 0.6 d. To the extent that direct microscopic counts underestimate bacterial numbers and CFDE underestimates total microbial biomass, the actual

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Table 2. Gross N mineralization, protozoal biomass, total bacterial numbers and calculated rates of bacterial biomass N turnover.

Soil	Gross N mineralization	Protozoal biomass	Bacteria	Bacterial biomass-C	Bacterial biomass-N	Bacterial N-turnover
cm	mg N kg^{-1} d^{-1}	mg g^{-1}	Cells × 10^8† g^{-1}	mg kg^{-1}	mg kg^{-1}	d
Bulk	1.0	0.17	5.7	18.3	3.0	3.0
0–4	9.1	0.19	6.7	21.8	3.6	0.4
4–8	8.1	0.18	7.4	23.8	4.0	0.5
8–12	6.8	0.18	7.6	24.6	4.1	0.6
12–16	12.8	0.22	7.2	23.2	3.9	0.3

† Multiply the reported numbers by 10^8 to obtain the actual numbers.

turnover rates of the biomass in these soils would be slower than those calculated here.

Bacterial Community Analysis

The TRFLP patterns indicate there were few differences between the bacterial communities of the bulk soil and rhizosphere samples. Principle component analysis of the TRFLP patterns (Fig. 3) showed no discernable differences in the first principle component (44% variability). However, six terminal fragments (out of the total 109 TRFLP peaks) constituted significantly different portions of the total TRFLP pool in rhizosphere vs. bulk soil as shown in Fig. 4. Five new fragments appeared or increased significantly in rhizosphere soil while one fragment (71 bp) declined in rhizosphere soils.

Potential and Gross Nitrification

Spatial patterns of potential nitrification are shown in Fig. 5. Nitrification potentials in the young root sections are higher than those in the bulk soil, while soil adjacent to the oldest root section (16–20 cm) has potentials substantially lower than soil adjacent to the root tip.

The spatial patterns of gross nitrification (Fig. 5) are quite different than those of potential nitrification. Rates of nitrification in soil adjacent to the 0- to 8-cm root tip are similar to those of the bulk soil. However, gross rates of nitrification are much lower near the 8- to 16-cm root zone, in fact, statistically indistinguishable from zero. Thus the potential for nitrification in soil near the 8- to 16-cm root zone is comparable with that of the bulk soil, but the actual rate of gross nitrification occurring in this soil is effectively zero.

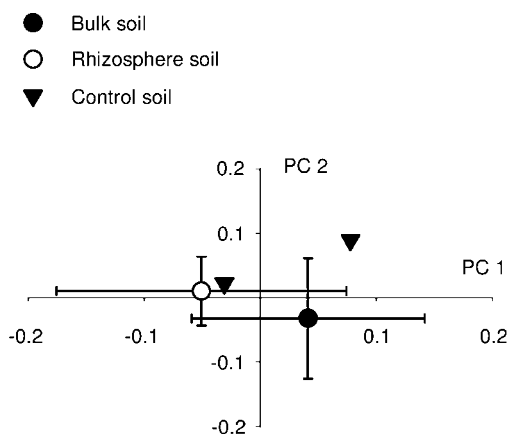

Fig. 3. Principal component analysis plot of terminal restriction fragment length polymorphism (TRFLP) patterns generated from rhizosphere soil, bulk soil, and control soil. Error bars represent two standards of deviation. Principal component 1 explained 44% of the variation in the data set while component 2 represented 20% of the variation.

Spatial Pattern of NH_4 in Soil

The distribution of NH_4 in the microcosm soil before the addition of labeled-N substrate is shown in Table 1. Before the spray application of ^{15}N-label, average ammonium concentrations in the rhizosphere zones were slightly higher than in the bulk soil ($p < 0.05$). All ammonium concentrations were low (<1 mg N kg^{-1}soil) before spraying on the labeled NH_4 solution. During the first 30 min following label application, there was a substantial decline in the NH_4 pool surrounding the 8- to 12- and 12- to 16-cm root sections; this did not occur in the 0- to 4- and 4- to 8-cm sections (data not shown). From 30 min to 2.3 h, the NH_4 pool surrounding the 8- to 12- and 12- to 16-cm sections appeared to recover somewhat, while NH_4 surrounding the 0- to 4- and 4- to 8-cm sections remained relatively unchanged (Table 1). These changes in pool size reflect simultaneous mineralization and NH_4 consumption. While we have determined the gross rate of mineralization during the 0.5- to 2.3-h interval, we cannot parse out gross consumption into microbial and plant assimilation. It would be interesting to determine if short-term dynamics of root assimilation changed in response to the NH_4 application.

Patterns of Gross Nitrogen Consumption

The ^{15}N pool dilution method for determining N mineralization also yields values for total N-consumption from the soil (Fig. 5). Nitrogen-consumption mea-

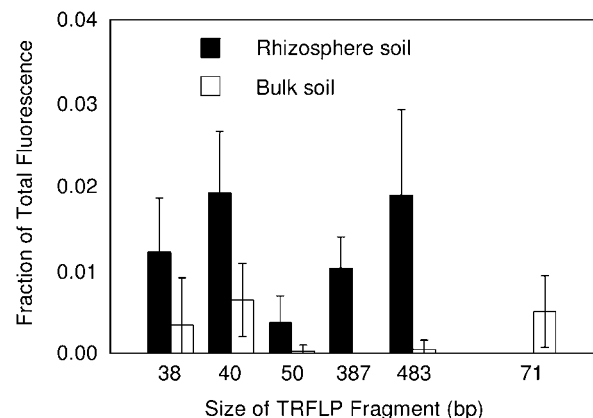

Fig. 4. Terminal restriction fragment length polymorphism (TRFLP) fragments present in significantly ($p < 0.05$) different amounts between rhizosphere and bulk soils. Error bars represent two standards of deviation.

sured by this method includes all possible fates of NH_4 including nitrification, microbial assimilation, and plant uptake. In the area near the root tip (0–8 cm), the rates of nitrification (about 11 mg N kg^{-1} d^{-1}) were similar in magnitude to the gross N consumption shown in Fig. 5. Although the $^{15}NH_4$ label addition may stimulate root assimilation of NH_4, gross nitrification rates were measured in a separate experiment using $^{15}NO_3$, and so were unaffected by the NH_4 addition. In the root zones 8 to 16 cm from the root tip, the high rates of gross N consumption (60 to 90 mg N $kg^{-1}d^{-1}$) exceeded even nitrification potential rates, and hence could not have resulted from nitrifier consumption of NH_4. These high rates of consumption most likely reflected root uptake of NH_4. It appears that rapid NH_4 uptake from the root zones 8 to 16 cm from the root tip severely depressed the rates of gross nitrification by depleting soil solution NH_4.

DISCUSSION

In this study, we report gross rates of N mineralization in rhizosphere soil that are about 10 times higher than those in bulk soil. While protozoal biomass did not correlate with gross rates of N mineralization, involvement of grazing by other bacterial predators, particularly nematodes and/or bacteriophage could also have been responsible for bacterial biomass turnover. Rapid growth of new bacterial biomass in high-C soil near young roots, followed by starvation of large numbers of bacteria in C-limited soil near older root sections, also did not appear to be an important mechanism for N mineralization in this system. Frequent watering of the planted microcosms excluded water potential fluctuation of a magnitude common to California annual grasslands, so this mechanism of N mineralization should not have been significant in this study.

Increased rates of gross N mineralization demonstrated increased flux of N into the NH_4 pool. Decreases in the standing stock of bacterial biomass N were too small to account for the increased rates, so bacterial biomass was not the ultimate source of the N being mineralized. Mobilization of organic N must have increased to enable the increased rates of N mineralization.

The direct microscopic counts used in this study only estimated bacteria; we have no data on fungal numbers or biomass. In these mesocosms however, the fact that the roots under study ranged from 2 to 12 d old and that the soil and sand had been recently mixed and added to the experimental compartments, likely minimized the importance of fungi. Annual grasses, including *Avena barbata*, form arbuscular mycorrhizal (AM) associations (Hawkes et al., 2006). It has been suggested that AM hyphae may accelerate rates of decomposition of N-containing organic materials by stimulating soil bacteria (Hodge et al., 2001); thus the impacts of AM associations on rhizosphere N mineralization are potentially quite important.

We found only small differences in bacterial community composition by TRFLP analysis. By its nature however, TRFLP analysis is not a particularly sensitive method for assessing subtle differences in microbial communities. The results of TRFLP analysis did show that several bacterial populations were enriched in the rhizosphere. Whether the changes in bacterial community found between rhizosphere and bulk soil can explain differences in N-dynamics in the rhizosphere is as yet unknown.

Rates of N mineralization are significantly enhanced in rhizosphere soil; yet potential rates of N uptake by roots are greater than the rates of N supplied by N mineralization in rhizosphere soils. While the total N-demand of the roots would likely be supplied by advection/diffusion processes in combination with mycorrhizal N

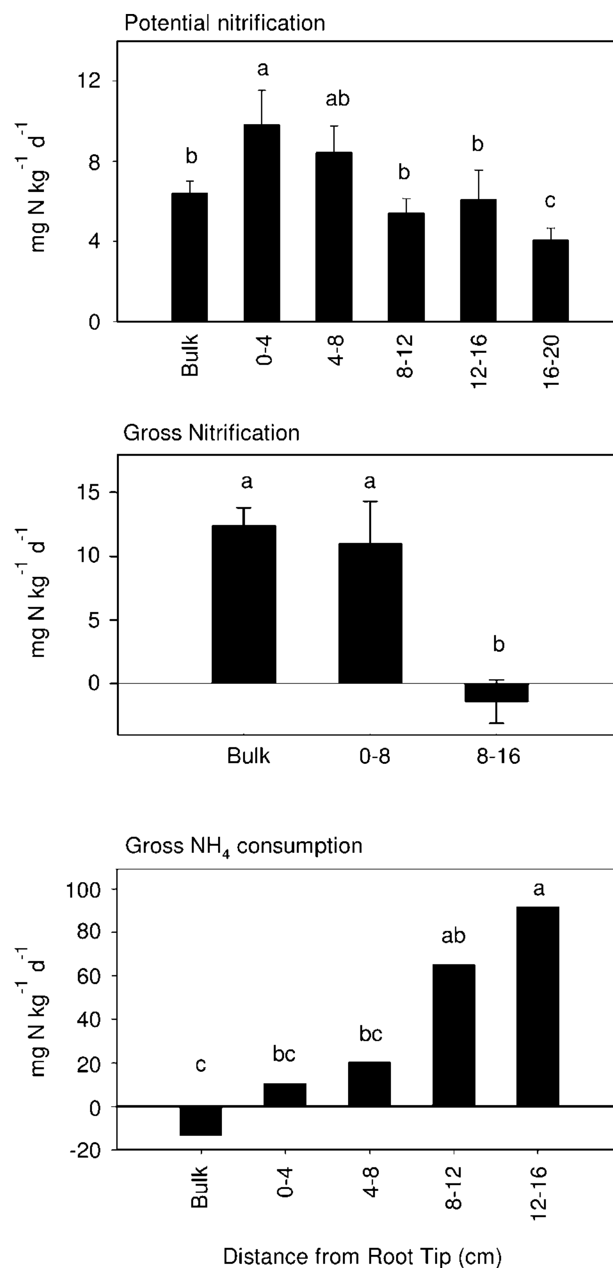

Fig. 5. Rates of potential nitrification, gross nitrification, and NH_4 consumption in rhizosphere soil along *Avena barbata* roots. Error bars indicate standard error of the means. Means sharing a common letter within the same graph are not significantly different ($P < 0.05$).

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supply to the plants, rapid uptake of N by roots likely depletes NH_4 in soil near roots actively involved in N acquisition. Gross rates of nitrification were effectively zero in soil adjacent to root zones most rapidly consuming NH_4. While nitrification potential rates indicated a functional nitrifying community all along the root, the measured potential rates varied by more than two-fold over the period of root growth. If nitrification potential is an accurate index of nitrifier number, then nitrifier numbers (as well as gross nitrification) were depressed relatively rapidly by root competition for NH_4. Previous work by Norton and Firestone (1996) investigating the N dynamics associated with Ponderosa pine roots, also demonstrated significant interaction between root uptake and rhizosphere nitrification; however in that case, the nitrifiers held their own against root competition.

Avena barbata is a common species in California annual grasslands. In the shallow rooting zone of California annual grasslands (0–15 cm), root density is commonly very high (900 g m^{-2}, Jackson et al., 1988). A relatively large proportion of soil would then be expected to be functioning as rhizosphere soil (within 2 mm of a root) as plant biomass approaches peak standing crop.

In current models of ecosystem N dynamics, plant roots function primarily as passive assimilators of mineral (and amino acid) N diffusing/advecting into the root zone (Schimel and Bennett, 2004). Nitrogen mineralization has been viewed as a steady state microbial process in which C-limited soil microbes mineralize organic matter and release excess inorganic N into the soil (Paul and Clark, 1996). Both plants and microbes are seen as passive recipients of products from each other (as in MIT models), rather than as strong interactors. Ecosystem models based on this view commonly determine plant N availability and N cycling rates simply on the basis of C/N ratio of decomposing organic material and soil temperature and moisture. The underlying assumption is that mechanisms of N mineralization and uptake are highly complex and not really important at the ecosystem level. There is mounting evidence however, that this description is inadequate. Numerous studies have found that plants assimilate more N during a growing season than is available from net N mineralization (Chapin et al., 1988; Nadelhoffer et al., 1991; Shaver and Chapin, 1991; Fisk et al., 1998).

Plants and free-living soil microorganisms appear to interact more strongly in the cycling of N than has generally been recognized. If root influences on N mineralization are significant, then net N mineralization incubations conducted in the absence of live roots underestimate the actual N cycling rates in terrestrial ecosystems. Measurements of net N mineralization in the absence of active plant roots are the basis of most estimates of N cycling in terrestrial ecosystems. Gross rates of N mineralization are not only substantially higher than net rates (Booth et al., 2005), but show the direct effects of living-root processes. Given the interaction of plant roots and soil microorganisms that we have demonstrated in rhizosphere N mineralization, it is not surprising that environmental perturbations that affect plant C balance and root exudation can have substantial effect on ecosystem N cycling (Hu et al., 2001).

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REFERENCES

Booth, M.S., J.M. Stark, and E. Rastetter. 2005. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. Ecol. Monogr. 75:139–157.

Bottner, P. 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with 14C- and 15N-labelled plant material. Soil Biol. Biochem. 17:329–337.

Bringhurst, R.M., Z.G. Cardon, and D.J. Gage. 2001. Galactosides in the rhizosphere: Utilization by *Sinorhizobium meliloti* and development of a biosensor. Proc. Natl. Acad. Sci. USA 98:4540–4545.

Brookes, P.C., A. Landman, G. Pruden, and D.S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17:837–842.

Chapin, F.S., III, N. Fetcher, K. Kielland, K.R. Everett, and A.E. Linkins. 1988. Productivity and nutrient cycling of Alaskan tundra: Enhancement by flowing soil water. Ecology 69:693–702.

Clarholm, M. 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biol. Biochem. 17: 181–187.

Davidson, E.A., S.C. Hart, C.A. Shanks, and M.K. Firestone. 1991. Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. J. Soil Sci. 42:335–349.

Elliott, E.T., D.C. Colemen, R.E. Ingham, and J.A. Troymow. 1984. Carbon and energy flow through microflora and microfauna in the soil subsystem of terrestrial ecosystems. *In* M.J. Klug, and C.A. Reddy (ed.) Current perspective in microbial ecology. Am. Soc Microbiol., Washington, DC.

Fisk, M.C., S.K. Schmidt, and T.R. Seastedt. 1998. Topographic patterns of above- and belowground production and nitrogen cycling in alpine tundra. Ecology 79:2253–2266.

Griffiths, B.S., R. Welschen, J.J.C.M. van Arendonk, and H. Lambers. 1992. The effect of nitrate-nitrogen supply on bacteria and bacterial-feeding fauna in the rhizosphere of different grass species. Oecologia 91:253–259.

Halverson, L.J., T.M. Jones, and M.K. Firestone. 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Sci. Soc. Am. J. 64:1630–1637.

Hart, S.C., J.M. Stark, E.A. Davidson, and Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification. p. 269–290. *In* R.W. Weaver et al. (ed.) Methods of soil analysis. Part 2. SSSA, Madison WI.

Harte, J., and A.P. Kinzig. 1993. Mutualism and competition between plants and decomposers: Implications for nutrient allocation in ecosystems. Am. Nat. 141:829–846.

Hawkes, C.H., J. Belnap, C. D'Antonio, and M.K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of exotic grasses. Plant Soil 281:367–379.

Hodge, A., C.D. Campbell, and A.H. Fitter. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297–299.

Hojberg, O., and J. Sorensen. 1993. Microgradients of microbial oxygen consumption in a barley rhizosphere model system. Appl. Environ. Microbiol. 59:431–437.

Hu, S., F.S. Chapin, III, M.K. Firestone, C.B. Fields, and N.R. Chiariello. 2001. Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. Nature 409:188–191.

Ingham, E.R. 1994. Protozoa. p. 491–515. *In* R.W. Weaver et al. (ed.) Methods of soil analysis. Part 2. SSSA Book Ser. No. 5. SSSA. Madison WI.

Jackson, L.E., R.B. Strauss, M.K. Firestone, and J.W. Bartolome. 1988. Plant and soil nitrogen dynamics in California grassland. Plant Soil 110:9–17.

Jaeger, C.H., III, S.E. Lindow, W. Miller, E. Clark, and M.K. Firestone. 1999. Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. Appl. Environ. Microbiol. 65:2685–2690.

James, B.R., R.J. Bartlett, and J.F. Aamadon. 1985. A root observation and sampling chamber (rhizotron) for pot studies. Plant Soil 85: 291–293.

Jones, D.L., J.R. Healey, V.B. Willett, J.F. Farrar, and A. Hodge. 2005. Dissolved organic nitrogen uptake by plants—An important N uptake pathway? Soil Biol. Biochem. 37:413–423.

Juma, N.G., and W.B. McGill. 1986. Decomposition and nutrient cycling in agro-ecosystems. p. 74–137. *In* M.F. Mitchell and J.P. Nakas (ed.) Microfloral and faunal interactions in natural and agro-ecosystems. Martinus-Nijhoff/DR W. Junk Publishers, Boston, MA.

Kennedy, A.C. 1998. The rhizosphere and spermosphere. p. 389–407. *In* J.J. Fuhrmann, et al. (ed.) Principles and applications of soil microbiology. DM Sylvia, Prentice Hall Inc, Upper Saddle River, NJ.

Kieft, T.L., E. Soroker, and M.K. Firestone. 1987. Microbial biomass response to a rapid increase in water potential when a dry soil is wetted. Soil Biol. Biochem. 19:119–126.

Kinzig, A.P., and J. Harte. 1998. Selection of micro-organisms in a spatially explicit environment and implications for plant access to nitrogen. J. Ecol. 86:841–853.

Klein, D.A., J.L. Salzwedel, and F.B. Dazzo. 1990. Microbial colonization of plant roots. p. 189–225. *In* J.P. Nakas, C. Hagedorn (ed.) Biotechnology of plant-microbe interactions. McGraw-Hill Publishing Co., New York.

Minchin, F.R., and J. Pate. 1973. The carbon balance of a legume and the functional economy of its root nodules. J. Exp. Bot. 24:259–271.

Murphy, D.V., S. Recous, E.A. Stockdale, I.R.P. Fillery, L.S. Jensen, D.J. Hatch, and K.W.T. Goulding. 2003. Gross nitrogen fluxes in soil: Theory, measurement, and application of ^{15}N pool dilution techniques. Adv. Agron. 79:69–118.

Myrold, D.D. 1998. Transformations of nitrogen. p. 259–294. *In* D.M. Sylvia et al. (ed.) Principles and applications of soil microbiology. Prentice Hall, Upper Saddle River, NJ.

Nadelhoffer, K.J., A.E. Giblin, G.R. Shaver, and J.A. Laundre. 1991. Effects of temperature and substrate quality on element mineralization in six arctic soils. Ecology 72:242–253.

Naseby, D.C., and J.M. Lynch. 1997. Rhizosphere soil enzymes as indicators of perturbations caused by enzyme substrate addition and inoculation of a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. Soil Biol. Biochem. 29:1353–1362.

Newman, E. 1985. The rhizosphere: Carbon sources and microbial populations. p. 107–121. *In* A. Fitter et al. (ed.) Ecological interactions in soil. Blackwell Scientific Publications, Oxford.

Norton, J.M., and M.K. Firestone. 1996. N dynamics in the rhizosphere of *Pinus Ponderosa* seedlings. Soil Biol. Biochem. 28:351–362.

Norton, J.M., J.L. Smith, and M.K. Firestone. 1990. Carbon flow in the rhizosphere of ponderosa pine seedlings. Soil Biol. Biochem. 22: 449–455.

Papendick, R.I., and G.S. Campbell. 1975. Water potential in the rhizosphere and plant methods of measurement and experimental control. p. 39–49. G.W. Bruehl (ed.) Biology and control of soil-borne pathogens. The American Phytopathological Society, St. Paul, MN.

Paul, E.A., and F.E. Clark. 1996. Soil microbiology and biochemistry. Academic Press, San Diego.

Pinton, R., Z. Varanini, and P. Nannipieri. 2000. The Rhizosphere. Marcel Dekker, New York.

Schimel, J.P., and J. Bennett. 2004. Nitrogen mineralization: Challenges of a changing paradigm. Ecology 85:591–602.

Schmidt, E.L., and L.W. Belser. 1982. Nitrifying bacteria. p. 1027–1042. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. No. 9 ASA, Madison, WI.

Shaver, G.R., and F.S. Chapin, III. 1991. Production: Biomass relationships and element cycling in contrasting arctic vegetation types. Ecol. Monogr. 61:1–31.

Sorensen, J. 1997. The rhizosphere as a habitat for soil microorganisms. p. 21–45. *In* J.D. Van Elsas et al. (ed.) Modern soil microbiology. Marcel Dekker, Inc New York.

Vitousek, P.M., and R.W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemisty 13:78–116.

Watson, C.J., G. Travers, D.J. Kilpatrick, A.S. Laidlae, and E. O'Riordan. 2000. Oversestimation of gross N transformation rates in grassland soils due to non-uniform exploitation of applied and native pools. Soil Biol. Biochem. 32:2019–2030.

Yang, C.H., and D.E. Crowley. 2000. Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Appl. Environ. Microbiol. 66:345–351.

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