



Plant and microbial nitrogen use and turnover: Rapid conversion of nitrate to ammonium in soil with roots

M. Burger^{1,2} & L.E. Jackson¹

¹Department of Vegetable Crops, University of California, Davis CA 95616, U.S.A. ²Corresponding author*

Received 15 April 2003. Accepted in revised form 26 February 2004

Key words: isotope pool dilution, ¹⁵N, nitrogen cycling, rhizosphere ecology, tomato

Abstract

Immobilization of ammonium (NH₄⁺) by plants and microbes, a controlling factor of ecosystem nitrogen (N) retention, has usually been measured based on uptake of ¹⁵NH₄⁺ solutions injected into soil. To study the influence of roots on N dynamics without stimulating consumption of NH₄⁺, we estimated gross nitrification in the presence or absence of live roots in an agricultural soil. Tomato (*Lycopersicon esculentum* var. Peto76) plants were grown in microcosms containing root exclosures. When the plants were 7 weeks old, ¹⁵N enriched nitrate (NO₃⁻) was applied in the 0–150 mm soil layer. After 24 h, > 30 times more ¹⁵NH₄⁺ was found in the soil with roots than in the soil of the root exclosures. At least 18% of the NH₄⁺-N present at this time in the soil with roots had been converted from NO₃⁻. We estimated rates of conversion of NO₃⁻ to NH₄⁺, and rates of NH₄⁺ immobilization by plants and microbes, by simulating N-flow of ¹⁴⁺¹⁵N and ¹⁵N in three models representing mechanisms that may be underlying the experimental data: Dissimilatory NO₃⁻ reduction to NH₄⁺ (DNRA), plant N efflux, and microbial biomass nitrogen (MBN) turnover. Compared to NO₃⁻ uptake, plant NH₄⁺ uptake was modest. Ammonium immobilization by plants and microbes was equal to at least 35% of nitrification rates. The rapid recycling of NO₃⁻ to NH₄⁺ via plants and/or microbes contributes to ecosystem N retention and may enable plants growing in agricultural soils to capture more NH₄⁺ than generally assumed.

Introduction

Immobilization of soil NH₄⁺ by plants and microbes decreases the amount of N that is subject to nitrification and is therefore a controlling factor of N loss and environmental degradation. Nitrogen oxide gases, which contribute to atmospheric pollution, ozone depletion and N deposition, form during both nitrification and denitrification (Matson 1997), and NO₃⁻ is susceptible to leaching below the root zone and into groundwater. Knowledge of NH₄⁺ uptake rates by plants in situ and of mechanisms that increase availability of NH₄⁺ to plants may prove useful in light of evidence that NO₃⁻ assimilation by plants may be impaired under elevated CO₂ concentrations (Bloom et al., 2002). In many natural, N-limited ecosystems,

NH₄⁺ concentrations tend to be greater than NO₃⁻ concentrations, and both these pools turn over rapidly (Jackson et al., 1989; Stark and Hart, 1997). In these systems, NH₄⁺ may be available for plant uptake because it is relatively abundant. In contrast, in most well-drained agricultural soils in temperate zones, NO₃⁻ typically accumulates, regardless of the form of N inputs, and NO₃⁻ is thought to be the major form of N taken up by crops (Robertson, 1997). Crop NH₄⁺ uptake, on the other hand, has received little attention.

Because of rapid cycling of N between the microbial and the mineral fraction in soil (Bristow et al., 1987), N flow can only be followed over short time spans by using ¹⁵N. In soil without plants, remineralization of N immobilized by microbes has been shown to occur after 7 d (Bjarnason, 1988), and incubation times of 1–3 d have been considered short enough to

*FAX No: +1-651-649-5175.

E-mail: mburger@soils.umn.edu

avoid the problem of recycled N (Hart et al., 1994). However, rhizodeposition (Whipps, 1990; Merbach, et al. 1999), including root exudates of C and N compounds (Jaeger et al., 1999), and higher microbial and microfaunal populations affect N cycling in the rhizosphere (Griffiths, 1994; Bonkowski et al., 2000). Under the influence of live roots, N-turnover may be faster than in soil without plants (Clarholm, 1985; Jones et al., 1994).

Analytical solutions, initially developed by Kirkham and Bartholomew (1954), have been widely used to determine rates of gross ammonification and nitrification, and corresponding gross consumption, based on the ^{15}N isotope pool dilution principle. One of the assumptions of these analytical solutions is that no labeled N of the product pool re-enters the unlabeled source pool. A follow-up analytical solution by Kirkham and Bartholomew (1955) does not have this restriction, but this formula can only be used if the source pool that is being isotopically enriched is homogeneous and of a known size. Furthermore, differentially ^{15}N enriched organic N from a variety of N pools may be recycled. In contrast to analytical calculations, simulation modeling tracks changes in labeled and unlabeled organic and inorganic N pools, and N transformation rates are found by solving sets of simultaneous differential equations numerically (Mary et al., 1998). This approach is more flexible, as the flow of labeled N enriching an initially unlabeled N pool can easily be incorporated in a model.

In the few studies that have examined short-term N flow in the presence of plants, plant NH_4^+ and NO_3^- immobilization was estimated based on uptake of ^{15}N tracers injected into the soil (Jackson et al., 1989; Schimel et al., 1989; Zak et al., 1993; Norton and Firestone, 1996). In using such an approach, stimulation of consumption cannot be avoided when the concentration of the ambient inorganic N pool is low. Furthermore, to calculate NH_4^+ uptake based on ^{15}N tracers, corrections must be made to account for the $^{15}\text{NH}_4^+$ nitrified during the assay and taken up as $^{15}\text{NO}_3^-$ (Schimel et al., 1989). Estimates of plant N uptake as NH_4^+ mineralized from soil organic matter instead of $^{15}\text{NH}_4^+$ tracer solutions are rare. Immobilization of NH_4^+ by maize plants, grown in pots, was inferred from N mass balance together with the observation that the ^{15}N -labeled soil NO_3^- pool was not appreciably diluted (Haider et al., 1987).

The objective of the present study was to estimate NH_4^+ immobilization rates by plants and microbes in

Table 1. Soil characteristics and average yearly inputs of organic materials to the organic production soil used in the cylinders. The soil of the 0–150 mm layer was from an organic tomato/corn/legume cover crop rotation at LTRAS. The 150–450 mm layer was a sand/subsoil mixture. Values are means. $n = 3$

Soil layer	0–150 mm	150–450 mm
pH (H ₂ O 1:1)	6.5	7.8
CEC (meq 100 g ⁻¹ soil)	33.9	12.9
Sand (%)	19	80
Silt (%)	58	14
Clay (%)	23	6
Bulk density (Mg m ⁻³)	1.15	1.5
Organic C (g kg ⁻¹)	12.8	nd
Organic N (g kg ⁻¹)	1.4	nd
C:N ratio	9.3	nd
Dry matter inputs (Mg ha ⁻¹ yr ⁻¹)	22	0
N inputs (kg N ha ⁻¹ yr ⁻¹)	350	0

an agricultural soil with NO_3^- pools that are usually 5 to 20 times greater than NH_4^+ pools. In previous lab assays of this soil, gross ammonification and gross nitrification rates were similar (Burger and Jackson, 2003). However, it was unclear how the inclusion of plants would change the N dynamics. Immobilization of NH_4^+ by heterotrophic microbes was expected to occur at relatively low rates compared to nitrification, as previously found in this soil when no plants were present (Burger and Jackson, 2003). We hypothesized that roots could possibly take up a substantial part of freshly mineralized NH_4^+ before it is nitrified, and we expected that NH_4^+ uptake and assimilation by plants could be estimated from the difference in gross nitrification rates in the presence and absence of live roots. Simulation modeling was used to explore mechanisms potentially responsible for the conversion of ^{15}N labeled NO_3^- to NH_4^+ and to estimate the potential magnitude of N transformations under varied scenarios.

Materials and methods

Soil

The soil used for the experiment was from three 60 × 60 m plots of an organically-managed tomato/corn rotation at the University of California Davis Long Term Research on Agricultural Systems project (LTRAS; <http://ltrs.ucdavis.edu/>; 38°32'30" N, 121°52'30" W;

28 m elev.) (Table 1). The inputs into this soil are a winter legume cover crop, animal manure, and harvest residue. No pesticides or synthetic fertilizers are used. The system has been in place since 1993.

In the spring 2000, about two weeks after incorporation of the cover crop and manure, soil was collected in 200 mm deep \times 130 mm dia. cores from random locations in each of the three 60 \times 60 m plots. The soil types in these plots were Yolo silt loam, a fine-silty, mixed, nonacid, thermic Typic Xerorthent and Rincon silty clay loam, a fine montmorillonitic, thermic Typic Haploxeralf. The soil types were mixed, but the soil of each plot was kept separate. The soil, which was at a water potential (Ψ_s) of -1.5 MPa, was stored for two months at 20 °C.

Microcosms

Cylinders, 250 mm in diameter (dia.) and 450 mm deep, were fabricated from PVC pipe. The lower (150–450 mm) layer of the cylinders was packed to a bulk density of 1.5 Mg m^{-3} with a sand/subsoil mixture (Table 1). The 0–150 mm layer of the cylinders was packed to a bulk density of 1.15 Mg m^{-3} with the soil collected at LTRAS after passing it through a 10-mm sieve. The two soil layers were hydraulically separated by a layer of waxed paper towels that allowed unrestricted root growth (Gallardo et al., 1994). The wax layer was supported by galvanized steel mesh, fitted over two slightly arched intersecting steel rods. This arching shape was expected to facilitate drainage of water from the cusp towards the cylinder walls, where holes were drilled just above the base of the 0–150 mm layer. The lower layer could be watered separately *via* a perforated tube that allowed also for the exchange of air. The purpose of the stratified design was to construct an upper zone where roots would proliferate in field-derived soil and a lower zone that would provide a supply of water but not nutrients, and to prevent the movement of nutrients out of the upper layer.

Cylindrical root and mycorrhizal hyphae enclosures (140 mm deep \times 34 mm dia.) were constructed from nylon membrane material with a pore size of $0.45 \mu\text{m}$ (Sartolon, Sartorius AG, Germany). These root enclosure cylinders were placed in the 0–150 mm layer and packed to the same bulk density as the surrounding soil.

Three-prong time domain reflectometry (TDR) wave guides, protruding 110 mm into the cylinders, were installed horizontally at 80 and 300 mm depth.

Soil moisture was monitored *via* TDR by a Tektronix cable tester (Model 1502B) connected to a personal computer. The software used was WinTDR99 (Utah State University). Calibration curves were obtained (Dasberg and Hopmans, 1992) for the surface soil and for the sand/subsoil mixture used in the cylinders.

Plant material and growing conditions

The cylinders were placed in a growth chamber that was on a diurnal cycle of 15 h light, provided by a combination of sodium vapor and metal halide lamps ($350 \mu\text{mol m}^{-2} \text{ s}^{-1}$), at 25 °C, and 9 h dark at 20 °C. Relative humidity (RH) ranged from 70 to 90%. Tomatoes (*Lycopersicon esculentum*, var. Peto76) were direct-seeded. At sowing time, NO_3^- and NH_4^+ concentrations were approximately 60 and $2 \mu\text{g N g}^{-1}$ soil, respectively. Inorganic N concentrations in the 150–450 mm layer were $< 0.5 \mu\text{g NO}_3^- \text{-N} + \text{NH}_4^+ \text{-N g}^{-1}$ soil. During the 7-week growing period, Ψ_s was between -0.08 and -0.3 MPa in the upper layer, and about -0.03 MPa in the lower layer.

Pretreatment

When the plants were 7 weeks old, the 0–150 mm layer of soil was leached with two pore volumes of 0.01 M CaCl_2 to lower NO_3^- concentrations and also to reduce spatial variability of NO_3^- concentration that can make the ^{15}N isotope pool dilution method unfeasible. The effluent was allowed to flow out through the holes at the base of the 0–150 mm layer. It took, on average, $66 (\pm 3)$ h for the soil to reach a Ψ_s of -0.1 MPa. In this soil in the field, a Ψ_s of -0.1 MPa is typically reached three d after an irrigation of tomatoes. Thus, in our microcosms, we simulated moisture conditions not unlike an irrigation event.

^{15}N isotope pool dilution

Whenever the soil in one of the cylinders reached Ψ_s of -0.1 MPa, 267 1-mL injections of 25.7 mM $\text{K}^{15}\text{NO}_3^-$ at 23.8 atom % ^{15}N were made with a 19 gauge sideport (0.4 mm openings) needle attached to a repetitive syringe (Wheaton Instruments, Millville, NJ). The ^{15}N label was evenly distributed to a depth of 130 mm. To avoid contamination of the shoots with ^{15}N , the plants were bagged during application of the label. At the end of the injections (t_0), Ψ_s was -0.06 MPa.

Soil samples from three cylinders, one from each LTRAS plot, were taken at the end of the injection

period for determination of initial NO_3^- and NH_4^+ concentrations and their respective ^{15}N isotopic composition. Another 8 cylinders were harvested 24 h after the end of injections (t24). Subsamples of soil were used for moisture determination. Three 200 g-subsamples of the soil with roots and the remaining contents of the root enclosure (approx. 100 g soil) were immediately extracted with 2 M KCl at a liquid:soil ratio of 5:1. Almost all the soil of the root enclosures was extracted to ascertain that a reliable estimate of the inorganic N concentrations and their isotopic composition was obtained, which seemed preferable to diverting soil to sample microbial biomass (MB) N, especially since there was only one root enclosure, with a limited amount of soil, per microcosm. The samples were put on a reciprocating shaker for 1 h, centrifuged, and then, the supernatant was collected. In the soil with roots, MBN was determined by the 1-d chloroform-fumigation extraction method (Brookes et al., 1985) after removal of the roots. All soil extracts were frozen until further analysis.

At t24, the shoots were clipped, and approx. 25% of the bulk soil in the 0–150 mm layer was taken as subsample for root biomass. Soil and roots were briefly immersed in cold ($< 5^\circ\text{C}$) 0.5 M KCl to diminish further N uptake by the roots and remove ^{15}N labeled NO_3^- from the free space. The slurry was passed through screens (500 μm mesh) with additional water until the roots were free of soil. The roots were stored at 5°C in 5% propanol solution. After removal of organic matter debris, root length was measured on a Comair root scanner (Commonwealth Aircraft Corp. Ltd., Melbourne, Australia). The root volume was estimated by the displacement of water by the roots, which had been blotted dry beforehand, in a graduated cylinder.

Chemical analyses

The MB extracts and blanks were digested, using a modified Kjeldahl procedure to eliminate NO_3^- interference (Wyland et al., 1994). The 2 M KCl and the digested MBN extracts were analyzed for $\text{NO}_3^- + \text{NO}_2^-$ and NH_4^+ on a Lachat 8000 flow injection analyzer (Zellweger, Milwaukee, WI). To prepare the 2 M KCl extracts, digested MBN extracts, and blanks for ^{15}N analysis, diffusion techniques were used (Brooks et al., 1989; Stark and Hart, 1996). Separate diffusions were performed to trap NH_3 of the NH_4^+ and NH_3 evolved after reduction of NO_3^- to NH_4^+ in the soil extracts. These diffusions and the mass spectrometer

analyses were conducted at different times, and standards and blanks were included as quality controls. The Devarda's alloy used as reductant for the NO_3^- samples and blanks was weighed to be 0.4 ± 0.01 g for each sample. Acidified filter disks containing trapped NH_4^+ of 10 blanks were consolidated into a single tin capsule in order to obtain reliable estimates of mass and isotopic composition of blanks. Roots and shoots were dried at 65°C , chopped into small pieces, and ball-milled. Isotope ratios were determined by an Europa Integra mass spectrometer (PDZ Europa Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility.

Calculations and simulation modeling

To calculate ^{15}N recovered in the plant biomass, the mass of N in roots and shoots was multiplied by the respective ^{15}N enrichment (atom % ^{15}N excess/100). Recovery of ^{15}N in roots was estimated as ^{15}N measured in the roots of the 0–150 mm layer multiplied by 1.12 to account for the ^{15}N assimilated by roots in the 150–450 mm layer. This correction factor was obtained in a preliminary experiment, in which root ^{15}N assimilation in both layers was measured in a 24-h period by the same tomato cultivar under similar growing conditions. Evidence of within-plant N cycling *via* xylem and phloem is widespread (Marschner et al., 1997). In *Ricinus communis*, 17% of NO_3^- -N taken up was translocated *via* the phloem as amino acids prior to assimilation in the roots (Jeschke and Hartung, 2000). Microbial biomass ^{15}N ($\text{B}_{15\text{N}}$) was calculated as $\text{B}_{15\text{N}} = \text{F}^{15\text{N}}/0.54$, where $\text{F}^{15\text{N}} = \{\text{NH}_4^+ \text{-}^{15}\text{N} \text{ in the digested fumigated sample}\} - \{\text{NH}_4^+ \text{-}^{15}\text{N} \text{ in the digested non-fumigated sample}\}$ (Shen et al., 1984; Brookes et al., 1985).

For the soil in the root enclosures, gross nitrification and NO_3^- consumption rates were calculated according to Kirkham and Bartholomew (1954), and in addition, N process rates were estimated, using simulation modeling as described below. For the soil with roots, the following compartmental models of N-flow were explored: (a) Dissimilatory reduction of NO_3^- to NH_4^+ (DNRA), (b) plant N efflux (NLEAK), (c) MBN turnover (REMIN) (Figure 1). Each model was set up as double flows of $^{14+15}\text{N}$ and ^{15}N using STELLA software (HPS Inc., Hanover, NH). The program simultaneously solves a differential system of mass equations numerically, using the Runge-Kutta 4th order algorithm, based on rates of N-flow between compartments. The model outputs are the instantaneous pool sizes in the compartments. The initial

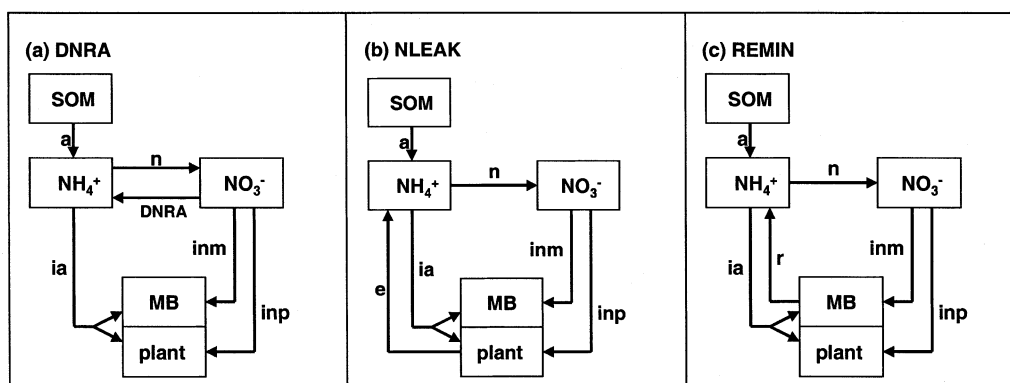


Figure 1. Compartmental models of N flow used for the simulations. The models are referred to as (a) dissimilatory nitrate reduction to ammonium (DNRA), (b) plant N efflux (NLEAK), (c) microbial biomass N turnover (REMIN). The rates of N flow are represented as a = ammonification, n = nitrification, inp = NO_3^- immobilization by plants, inm = NO_3^- immobilization by microbes, ia = NH_4^+ immobilization by plants and microbes, DNRA, e = plant N efflux, r = remineralization of MBN.

conditions of the $^{14+15}\text{N}$ compartments were the concentration of NO_3^- -N and NH_4^+ -N at t_0 . The starting values of the ^{15}N compartments were calculated as the concentration of NO_3^- -N and NH_4^+ -N multiplied by the respective ^{15}N enrichment (atom % $^{15}\text{N}/100$) at t_0 . For plant and microbial $^{14+15}\text{N}$ and ^{15}N compartments, the initial values were zero. All rates were assumed to be constant (zero-order). Losses of N due to NH_3 volatilization, denitrification, formation of stable OM, and fixation of newly formed NH_4^+ were assumed to be negligible. The organic N pool was considered a large reservoir of N compared to the initial inorganic N pools.

The input values for each scenario (roman numerals) of three models (DNRA, NLEAK, REMIN) are listed in Table 3. In all models, a gross ammonification rate equal to the minimum gross ammonification rate in the root exclusions (i, iii, iv, v) or an arbitrarily chosen, higher value (ii) was assumed. The NO_3^- immobilization rates were adjusted so that the mass of ^{15}N accumulated in the plant and microbial compartments, as indicated by the model at t_{24} , matched the measured ^{15}N recovery in the MBN and plant pools (corrected to represent total ^{15}N accumulated during the 24-h incubation). The ^{15}N plant and microbial compartments also included ^{15}N from $^{15}\text{NH}_4^+$ immobilization. Gross nitrification rates were set in order to match the model outputs of NO_3^- concentration and its ^{15}N isotopic enrichment with the experimentally measured values. Rates of conversion of NO_3^- to NH_4^+ and rates of NH_4^+ immobilization were adjusted in such a manner that the model output of $^{14+15}\text{NH}_4$ and its atom % ^{15}N corresponded to the values measured in

the experiment. Partitioning of NH_4^+ immobilization between plants and microbes was explored in various simulation runs. More details, pertaining to each model, follow below.

DNRA model

Dissimilatory NO_3^- reduction to NH_4^+ occurs under anoxic conditions and represents an alternative to denitrification as a result of anaerobic respiration by microorganisms. The ratio of C to electron acceptors is thought to control the partitioning between denitrification and DNRA, denitrification being favored when this ratio is low (Tiedje, 1988). The DNRA model has the following six rates of N flow: Ammonification, nitrification, plant NO_3^- immobilization, microbial NO_3^- immobilization, reduction of NO_3^- to NH_4^+ , and NH_4^+ immobilization by both plants and microbes. For simplicity, the plant and microbial NO_3^- immobilization were combined into one rate. For scenario (iii), a recently published constant rate of DNRA of $0.6 \mu\text{g N g}^{-1} \text{d}^{-1}$ was assumed (Silver et al., 2001). This rate was tested in a simulation because it was measured in an environment highly conducive to DNRA, and thus, could be regarded as upper limit of potential DNRA rates in our microcosms. We also simulated reduction of NO_3^- to NH_4^+ occurring only during the first two h of the incubation because DNRA is an anaerobic process that would have been more likely to take place at the beginning of the incubation, when WFPS was greater, than towards the end of the incubation.

Plant N efflux (NLEAK) model

Leakage of endogenously generated NH_4^+ by plants supplied with NO_3^- has been shown in barley, wheat,

and oat (Morgan and Jackson, 1988). Roots also release amino acids (Jaeger et al., 1999) that could be rapidly degraded to NH_4^+ by microorganisms (Mengel, 1996). Exudation of amino acids appears to take place as passive efflux due to the concentration gradient between the roots' cytoplasm and the soil solution (Jones et al., 1994). Leakage of N compounds *via* apoplast in developing roots, especially under root pressure in the absence of transpiration, is another potential pathway of root N exudation (Steudle and Peterson, 1998). The NLEAK model has six rates: ammonification, nitrification, plant NO_3^- immobilization, microbial NO_3^- immobilization, efflux of NH_4^+ or reduced N compounds, and NH_4^+ immobilization by both plants and microbes. We modeled N efflux at two levels of ^{15}N enrichment of the leaked NH_4^+ . The first one was the instantaneous atom % ^{15}N of the NO_3^- pool (i, ii, v). The maximum atom % of the leaked NH_4^+ had to be the highest atom % of the NO_3^- pool. The lower level of ^{15}N enrichment of the leaked N was 6.4 atom % ^{15}N (iv). This isotopic composition was found to be the lowest possible ^{15}N enrichment of the leaked N to produce the measured atom % ^{15}N of the NH_4^+ pool. We also simulated a process where ^{15}N efflux took place only in the last two h of the incubation period (v). In this scenario, the efflux of reduced N, as well as NH_4^+ consumption, were assumed to occur during the first 22 h at the same rate as during the last two h, the difference being that the ^{15}N label started to appear in the NH_4^+ pool only after 22 h. This scenario portrays delayed release of $^{15}\text{NH}_4^+$ by roots after plant $^{15}\text{NO}_3^-$ uptake and assimilation.

MBN turnover (REMIN) model

Many studies have shown that C loss from roots in the form of exudates, sloughed off cells and decomposing roots lead to larger microbial biomass in the rhizosphere compared to the bulk soil (Newman, 1985; Jensen and Sorensen, 1994). Microbial N turnover and production of NH_4^+ may be taking place when bacteria are grazed by microfauna that excrete excess NH_4^+ (Clarholm, 1985; Griffiths and Robinson, 1992). Another mechanism of remineralization of microbial N may be starvation and death of microbes due to lack of substrate, and mineralization of this organic matter by subsequent microbial populations. Exudates vary in abundance and composition along roots (Maloney et al., 1997; Jaeger et al., 1999), and these changes in substrate availability may lead to MB turnover cycles. The REMIN model has six rates: Am-

monification, nitrification, plant NO_3^- immobilization, microbial NO_3^- immobilization, remineralization, and NH_4^+ immobilization by plants and microbes. In this model, microbial biomass that has taken up labeled $^{15}\text{NO}_3^-$ is mineralized. The N mineralized from MBN was assumed to have either the ^{15}N enrichment of NO_3^- (i, ii, v) or 8.75 atom % ^{15}N (iv). If the latter ^{15}N enrichment of MBN was assumed, N of microbial origin would have been mineralized at about the same rate as MB would have been immobilizing NO_3^- .

Furthermore, we simulated a delay in the appearance of $^{15}\text{NH}_4^+$ after $^{15}\text{NO}_3^-$ has been taken up by MB (v). Analogous to the plant N efflux model, remineralization of MBN and consumption of NH_4^+ were assumed to occur during the first 22 h at the same rate as during the last 2 h.

Results

Experimental conditions

There was no difference in water content between the soil with roots and the soil in the root enclosures either at t0 or at t24 (Table 2). During the actual experiment, after application of the ^{15}N label, Ψ_s in the 0–150 mm layer remained in a relatively narrow range of -0.08 (t0) to -0.1 MPa (t24). All estimates of soil water content via TDR immediately before harvest were within 2.5% (mean = -0.3%) of the value determined after drying of soil samples at 105°C . The leaching pre-treatment lowered NO_3^- concentration to about $2 \mu\text{g N g}^{-1}$ soil within both the root enclosures and the soil with roots. Among the three cylinders harvested at t0, NO_3^- concentrations and ^{15}N enrichment were not different between the soil with roots and the soil in the root enclosures. The roots in the 0–150 mm layer were comprised mostly of very fine roots (average dia. 0.1 mm). Dense root growth was observed on the outside surface of the root enclosures.

N processes in root enclosures

In the root enclosures, the mean gross nitrification and NO_3^- consumption rates according to the Kirkham and Bartholomew model (1954) were $2.75 (\pm 0.40)$ and $9.88 (\pm 0.50) \mu\text{g NO}_3^- \text{-N g}^{-1} \text{ soil d}^{-1}$, respectively. Ammonium concentrations did not change significantly during the incubation ($P > 0.05$; *t*-test) (Table 2). Modeling the flow of ^{14}N and $^{14+15}\text{N}$ with STELLA yielded almost identical gross nitrification

Table 2. Plant biomass, soil moisture, concentrations and isotopic composition of inorganic N, and microbial biomass N. The t0 sampling took place after injection of the $^{15}\text{NO}_3^-$, and t24 was 24 h later. Values are means (SE), $n = 3$ (t0) or 8 (t24)

	Soil with roots		Root exclosures	
	t0	t24	t0	t24
Shoots (g dwt. plant ⁻¹)		17.1 (0.9)		
Atom % $^{15}\text{N}_{\text{shoots}}$		3.0 (0.1)		
Roots (g dwt. plant ⁻¹)		4.3 (0.3)		
Atom % $^{15}\text{N}_{\text{roots}}$		2.3 (.2)		
Roots allocation (root g dwt./total g dwt.)		0.20 (0.01)		
Root length density (km m ⁻³ soil)		91 (8)		
Soil moisture (%)	26.9 (0.5)	24.1 (0.3)	27.1 (0.5)	23.7 (0.4)
WFPS (%)	55	49	55	49
NO_3^- ($\mu\text{g N g}^{-1}$ soil)	14.9 (0.6)	3.0 (0.2)	14.9 (0.8)	7.8 (0.8)
Atom % $^{15}\text{NO}_3^-$	20.3 (0.4)	15.7 (0.3)	19.7 (0.6)	15.4 (0.5)
NH_4^+ ($\mu\text{g N g}^{-1}$ soil)	0.28 (0.04)	0.40 (0.04)	0.22 (0.03)	0.16 (0.03)
Atom % $^{15}\text{NH}_4^+$	0.37	4.03 (0.65)	0.37	0.65 (0.03)
MBN ($\mu\text{g N g}^{-1}$ soil)	nd	48.5 (1.3)	nd	nd
Atom % $^{15}\text{N}_{\text{MBN}}$		0.81 (0.06)		

rates ($2.82 \mu\text{g N g}^{-1} \text{d}^{-1}$) although a conversion rate of NO_3^- to NH_4^+ of $0.04 \mu\text{g N g}^{-1} \text{d}^{-1}$ to account for the ^{15}N enrichment of the NH_4^+ pool (0.3 atom % excess ^{15}N) was included (Table 3). The gross ammonification rate of $2.75 \mu\text{g NH}_4^+ \text{N g}^{-1} \text{d}^{-1}$ that fit the experimental data can be considered as the minimum rate since besides nitrification, no NH_4^+ consuming processes, e.g. microbial immobilization of NH_4^+ , were included in this model.

N processes in soil with roots

Recovery of the ^{15}N label ranged from 93.2–109.0% (mean = 98.8%). Roots and shoots, where 76% of the applied ^{15}N was recovered, were the greatest sink for inorganic N (Table 2). Net microbial assimilation accounted for 7% of the ^{15}N label, and 16% of the ^{15}N label was found in the inorganic N pool, mostly as NO_3^- .

At harvest, NH_4^+ pools in the soil with roots were substantially enriched even though only NO_3^- had been labeled with ^{15}N (Table 2). In the soil with roots, the mean atom % excess of soil NH_4^+ was 3.7% while the mean atom % excess of soil NO_3^- was between 19.9% (t0) and 15.3% (t24). Therefore, at least 18%

of the NH_4^+ present at harvest had been converted from ^{15}N labeled NO_3^- .

The conversion of ^{15}N labeled NO_3^- to NH_4^+ violated one of the assumptions of the Kirkham and Bartholomew model (1954). The model assumes that labeled N immobilized over the experimental period is not remineralized. The mean gross nitrification rate in the soil with roots according to this model was $1.97 \mu\text{g N g}^{-1} \text{d}^{-1}$, or $0.78 \mu\text{g N g}^{-1} \text{d}^{-1}$ lower than in the root exclosures (significant at $P < 0.05$; t -test). However, this gross nitrification rate, calculated via the Kirkham and Bartholomew equations (1954), is probably an underestimate. Newly formed $^{15}\text{NH}_4^+$ was likely nitrified since NH_4^+ concentrations in the soil with roots barely increased during the 24-h incubation, and this $^{15}\text{NH}_4^+$, once nitrified, would not have lowered the ^{15}N enrichment of the NO_3^- pool, as nitrified NH_4^+ with an isotopic composition of natural abundance would. Gross consumption rates, and net microbial and plant NO_3^- immobilization values probably also are erroneous since ^{15}N could have been taken up as $^{15}\text{NH}_4^+$.

Table 3. Relationships of rates under varied scenarios (i–v) in the three models. Model inputs were the starting concentrations and ^{15}N enrichment of NH_4^+ and NO_3^- pools, as well as ammonification rates, atom % of N source of $^{15}\text{NH}_4^+$, and duration of $^{15}\text{NH}_4^+$ production. Nitrification, NO_3^- immobilization, NO_3^- conversion and NH_4^+ immobilization rates were found by matching model outputs with the measured ending concentrations and atom % ^{15}N of the NH_4^+ and NO_3^- pool, and ^{15}N recovery in plant and microbial biomass. In the DNRA (iii) model, the NO_3^- conversion rate was an assumed value, and the ammonification rate was found by the above procedure. More details on the models and scenarios are given in the text.

Model	Scenario	Model inputs				Modeled rates			
		Ammonification	Source of	atom % of	Duration of	Nitrification	NO_3^- immobilization	NO_3^- conversion ^a	Immobilization of
		$\mu\text{g N g}^{-1} \text{d}^{-1}$	$^{15}\text{NH}_4^+$	N source of	$^{15}\text{NH}_4^+$	$\mu\text{g N g}^{-1} \text{d}^{-1}$	$\mu\text{g N g}^{-1} \text{d}^{-1}$	$\mu\text{g N g}^{-1} \text{d}^{-1}$	NH_4^+
		production h		Plants		Microbial biomass		$\mu\text{g N g}^{-1} \text{d}^{-1}$	
Root exclosure		2.75	NO_3^-	NO_3^-	0-24	2.82	9.88	0.04	–
DNRA	(i)	2.75	NO_3^-	NO_3^-	0-24	2.48	13.58	0.81	0.96
	(ii)	3.50	NO_3^-	NO_3^-	0-24	2.55	13.44	0.98	1.80
	(iii)	2.16	NO_3^-	NO_3^-	0-24	2.42	13.73	0.60	0.25
NLEAK	(i)	2.75	plant N	NO_3^-	0-24	2.46	13.22	0.79	0.95
	(ii)	3.50	plant N	NO_3^-	0-24	2.46	13.22	1.0	1.92
	(iv)	2.75	plant N	6.40	0-24	2.47	13.22	4.38	4.54
	(v)	2.75	plant N	NO_3^-	22-24	1.97	12.60	1.68	2.35
	(i)	2.75	MBN	NO_3^-	0-24	2.54	12.36	0.84	0.94
REMIN	(ii)	3.50	MBN	NO_3^-	0-24	2.54	12.36	1.08	1.92
	(iv)	2.75	MBN	8.75	0-24	2.64	12.36	2.16	2.23
	(v)	2.75	MBN	NO_3^-	22-24	1.97	12.36	1.56	2.21

^arefers to either DNRA, plant NH_4^+ or amino acid leakage, and remineralization of MBN, depending on the model used.

^brefers to immobilization of NH_4^+ by plants and microbes.

Simulation modeling

We hypothesized that each of the mechanisms explored in the three models (DNRA, NLEAK, REMIN) could explain the conversion of NO_3^- to NH_4^+ . For clarity, the mechanisms were treated as mutually exclusive although more than one of these processes may have been taking place simultaneously. The relationships between rates of gross ammonification, gross nitrification, NO_3^- immobilization, conversion of NO_3^- to NH_4^+ , and NH_4^+ immobilization were explored, using the conditions and assumptions listed in the materials and methods section. In each scenario, once the parameters were adjusted, all model outputs (concentrations of NH_4^+ and NO_3^- and the respective isotopic composition, mass of ^{15}N accumulated in plants and MB) were within $< \text{SE}$ of the measured variables.

If conversion of $^{15}\text{NO}_3^-$ to $^{15}\text{NH}_4^+$ was assumed to take place during the entire 24 h of the incubation (i–iv; Table 3), the gross nitrification rates obtained *via* simulation modeling were higher than indicated by the calculations according to Kirkham and Bartholomew (1954) because simulation modeling included nitrification of ^{15}N -labeled NH_4^+ .

The lowest rates of NO_3^- conversion to NH_4^+ , and of NH_4^+ immobilization by plants and microbes, were found if (1) the ^{15}N enrichment of the NO_3^- pool was used as atom % ^{15}N of the N source being converted to NH_4^+ , and (2) the ammonification rate was set equal to the minimum gross ammonification rate in the root exclusions (i). This was the case in all the models, with the exclusion of scenario iii (DNRA). Higher rates of NH_4^+ immobilization were obtained if a gross ammonification rate larger than the minimum gross ammonification rate in the root exclusions was assumed (ii).

Greater NO_3^- conversion rates and greater NH_4^+ immobilization rates were also found if the atom % ^{15}N of the source of $^{15}\text{NH}_4^+$ was lower than the atom % ^{15}N of the NO_3^- pool (iv), a possibility that existed only in the NLEAK and the REMIN models. In the NLEAK and the REMIN models, it could be shown that the ^{15}N enrichment of the NH_4^+ pool would also have occurred if a small amount of ^{15}N entered the NH_4^+ pool at the end of the incubation period (v). If $^{15}\text{NH}_4^+$ started to appear only after 22 h, as in the example shown, a gross nitrification rate of $1.97 \mu\text{g N g}^{-1} \text{d}^{-1}$ fit the experimental data. However, the rates of conversion of NO_3^- to NH_4^+ would be lar-

ger than rates found under the assumption that $^{15}\text{NO}_3^-$ was converted to $^{15}\text{NH}_4^+$ at a constant rate during the entire 24 h.

If a DNRA rate of $0.6 \mu\text{g N g}^{-1} \text{d}^{-1}$ was assumed (Silver et al., 2001), a lower rate of NH_4^+ immobilization than in all other scenarios was found, and a gross ammonification rate lower than the minimum gross ammonification rate in the root exclusions was necessitated by the experimental data (iii). Reduction of NO_3^- to NH_4^+ only at the beginning of the incubation period would have made a very high rate of DNRA necessary. For example, if conversion of NO_3^- to NH_4^+ was assumed to occur only during the first two h of the incubation, a DNRA rate $> 5 \mu\text{g N g}^{-1} \text{d}^{-1}$ was indicated (modeling results not shown).

Modeling did not provide any indications how NH_4^+ immobilization was partitioned between plants and MB because the mass of $^{15}\text{NH}_4^+$ flowing into these two pools was quite low compared to the mass of $^{15}\text{NO}_3^-$ taken up by plants and microbes. However, the value of total NH_4^+ immobilization was virtually unaffected by variations in partitioning.

Discussion

The influence of roots on N dynamics

The presence of live roots had a strong influence on soil N transformations. Within only 24 h, some of the ^{15}N -labeled NO_3^- appeared in the NH_4^+ pool. Any of the proposed mechanisms that could have caused this N conversion would be the result of plant-soil interactions. Roots could have contributed to decreased soil O_2 content as a result of respiration (DNRA model). Roots may have leaked reduced N compounds after plant NO_3^- uptake and assimilation (NLEAK model). Or, roots may also have provided C substrate for enhanced microbial activity and possibly a third trophic level that may have led to accelerated N cycling (REMIN model). Root length density was higher than reported for tomatoes in the field (Jackson and Bloom, 1990), where roots explore a much greater volume of soil. Higher root length densities may also have been due to the continuously moist growing conditions in the microcosms.

Nitrate uptake rates by the tomato plants were high, which is not surprising given the high Ψ_s and the high concentration of NO_3^- in the soil solution (3.9 to 0.9 mM). At about $40 \mu\text{g NO}_3^- \text{N g}^{-1}$ dry weight root d^{-1} , uptake rates were well below the V_{max} of

72 $\mu\text{g NO}_3^- \text{-N g}^{-1}$ dry weight root d^{-1} measured in solution culture at an external NO_3^- concentration of 0.125 mM (Smart and Bloom, 1988). The NO_3^- concentration at t0 (15 $\mu\text{g NO}_3^- \text{-N g}^{-1}$ soil) is a typical value at mid-season in California tomato cropping systems (Cavero et al., 1996).

Roots probably influenced N dynamics in the root exclosures too. The high NO_3^- consumption rates measured in the root exclosures suggest that roots must have taken up NO_3^- from within the root exclosures. It is likely that NO_3^- moved by mass flow to roots that were congregated on the outside surface of the root exclosures. The finding that, at t24, soil water content inside and outside of the root exclosures did not differ indicates that there was good contact between soil and membrane, and water movement was not impeded between the two compartments. Compared to the soil with roots, the mass of NO_3^- converted to NH_4^+ in the root exclosures was much lower. Although DNRA and MBN turnover appear to be the most likely mechanisms responsible for the conversion of NO_3^- to NH_4^+ in the root exclosures, plant N efflux could have played a role too because of the high root density on the outside surface of the root exclosures.

Simulation modeling

Simulation modeling was used to explore the potential magnitude of the conversion from NO_3^- to NH_4^+ , and the likelihood of different mechanisms potentially responsible for it. Another goal was to estimate NH_4^+ immobilization by plants and microbes under various scenarios.

DNRA

In an upland humid tropical forest soil that is conducive to DNRA due to its high C content (7–14%) and small NO_3^- pools ($<1 \mu\text{g NO}_3^- \text{-N g}^{-1}$), the average rate of DNRA (Silver et al., 2001) was lower than indicated by simulation modeling in our soils. Assuming this rate in our agricultural soil did not fit the experimental data well. Furthermore, at an average WFPS of 56 to 48%, the soil in the microcosms could not be considered conducive to denitrification or DNRA (Paul and Clark, 1996) although anaerobic microsites due to root respiration or microbial respiration stimulated by root-produced carbon could have existed in our soil. In any case, denitrification would have been more likely to occur than DNRA since NO_3^- as electron acceptor was not in short supply. Moreover, an anaerobic process such as DNRA would

have been more likely to take place at the beginning of the incubation since moisture levels declined because of evapotranspiration. However, simulation modeling showed that NH_4^+ at natural abundance entering the NH_4^+ pool in the later phase of the incubation would have necessitated an unrealistically high rate of DNRA in the early phase of the incubation. We conclude that in our case, DNRA was probably not the principal mechanism underlying the conversion of NO_3^- to NH_4^+ .

Plant N efflux (NLEAK)

Ammonium and amino acid efflux have been measured in solution and sterile sand cultures. Feng et al. (1998), using 7-d old maize seedlings grown with NH_4^+ as the sole N source, reported sustained release of NH_4^+ from endogenous sources, mainly soluble organic N, over 3 d. Under the assumption that NH_4^+ efflux is proportional to plant biomass, those NH_4^+ efflux rates would be about 1.0 $\mu\text{g NH}_4^+ \text{-N g}^{-1}$ soil d^{-1} . Similar rates of NH_4^+ efflux, corresponding to about 0.9 $\mu\text{g NH}_4^+ \text{-N g}^{-1}$ soil d^{-1} , were observed for 20-d old barley plants, as derived from net NH_4^+ efflux rates per g root fresh weight, and using a root fresh weight:dry weight ratio of 10 (Morgan and Jackson, 1989). However, net NH_4^+ efflux lasted only 1 to 2 h after transferring plants from a NO_3^- into a NH_4^+ containing solution, and in the absence of exogenous NH_4^+ , no NH_4^+ efflux was found (Morgan and Jackson, 1989). To our knowledge, NH_4^+ efflux in a situation where both NO_3^- and NH_4^+ are available for plant uptake, as in our microcosms, has not been investigated in solution culture. In the sterile root zone of 25-d-old sand-grown forage rape (*Brassica napus* L.), 100 $\mu\text{mol g}^{-1}$ root dry weight amino acids were collected after 3.5 h (Shepherd and Davies, 1994), which would correspond to a rate of about 2.6 $\mu\text{g amino-N g}^{-1}$ soil d^{-1} in our microcosms. Jones (1993) on the other hand, observed efficient recapture of most of the amino acids released by 12-d-old maize seedlings in solution culture. All the above values of NH_4^+ or amino-N efflux are comparable to our lowest estimates of NH_4^+ efflux according to simulation modeling albeit the measurement periods were often shorter.

According to simulation modeling, if the atom % of the leaked N was lower than the atom % of the NO_3^- pool, the rate of $^{15}\text{NH}_4^+$ efflux or $^{15}\text{NH}_4^+$ formation would increase. We do not know what the ^{15}N isotopic composition of the potential endogenous source N in the tomato plants would have been. Moreover,

the ^{15}N enrichment of this pool could have been subject to change during the 24-h incubation. Therefore, estimating the range of potential efflux rates is not possible.

MBN turnover

In addition to bacterial substrate, microfaunal activity resulting in mineralization of MBN requires soil moisture (Clarholm, 1989; Bouwman and Zwart, 1994; Cowling, 1994). The high Ψ_s during and preceding the actual ^{15}N pool dilution experiment would have been conducive in supporting active grazers. Estimates of protozoan N excretion rates vary by several orders of magnitude among studies (Zwart et al., 1994). Our estimate of $0.94 \mu\text{g N g}^{-1} \text{d}^{-1}$ would be on the low end of these published values. We do not know if MBN turnover due to starvation of microbes and mineralization of this organic matter by other microbes could have taken place within the short time frame of this experiment.

According to simulation modeling, at least $0.84 \mu\text{g NO}_3^- \text{N g}^{-1} \text{d}^{-1}$ was converted to NH_4^+ via remineralization of MBN, and plants and microbes immobilized at least $0.94 \mu\text{g N g}^{-1} \text{d}^{-1}$ as NH_4^+ . Both these values would probably be underestimates. First, some time had to elapse before ^{15}N labelled NO_3^- was taken up by microbes and remineralized. Modeling showed that both conversion rates and NH_4^+ immobilization rates would be higher if $^{15}\text{NH}_4^+$ appeared only towards the end of the incubation period. We expressed these conversion rates as daily rates under the assumption that conversion of $^{14}\text{NO}_3^-$ to $^{14}\text{NH}_4^+$ also took place earlier during the incubation period. Second, ammonification rates may have been greater in the soil with roots compared to the soil in the root enclosures, and this would have led to an increase in NH_4^+ immobilization by plants and microbes. Several studies have shown increased mineralization of SOM in the presence of plants (Clarholm, 1985; Haider et al., 1987; Kuikman et al., 1990; Bottner et al., 1991). Third, on average, the ^{15}N enrichment of the remineralized MBN probably would have been lower than the atom % ^{15}N of the NO_3^- pool.

The ^{15}N enrichment of MBN as a whole was only 0.81 atom % ^{15}N , and remineralization of MBN at 0.81 atom % ^{15}N would have led to a drastic, unrealistic reduction of the MBN pool. Remineralization of MBN with an isotopic composition of at least 8.75 atom % ^{15}N would have had to take place where microbes were immobilizing $^{15}\text{NO}_3^-$, i.e. where new microbial growth was occurring, probably in the

rhizosphere. If $^{15}\text{NO}_3^-$ immobilization and remineralization of MBN were preferentially occurring in the rhizosphere, accordingly, the rates of these N transformations had to be higher in the rhizosphere than our estimated NO_3^- conversion rates based on average inorganic N concentrations and ^{15}N enrichment in the soil with roots. Because the ^{15}N enrichment of MBN as a whole would not result in realistic MBN turnover rates, this mechanism as explanation for the conversion of NO_3^- to NH_4^+ raises questions that cannot be answered without estimates of N transformation rates in the rhizosphere proper.

Significance of proposed mechanisms

Other workers have observed conversion of NO_3^- to NH_4^+ in ^{15}N isotope pool dilution experiments, but considered it negligible because the mass of NH_4^+ derived from NO_3^- at the time of extraction was small compared to the source (NO_3^-) pool (Davidson et al., 1991; Norton and Firestone, 1996). We explored the potential magnitude, origin, and fate of recycled NO_3^- because in this soil, the mean residence time of NH_4^+ (initial NH_4^+ concentration/ NH_4^+ consumption rate) was quite short (< 2 h). Therefore, even a small amount of ^{15}N found at a given moment in the NH_4^+ pool can be an indication of substantial N flow from NO_3^- to NH_4^+ .

Plant NH_4^+ uptake was modest compared to plant NO_3^- immobilization, regardless of the mechanism and actual rate of NH_4^+ immobilization. Nevertheless, NH_4^+ uptake by roots is probably greater than generally assumed in agricultural soil because of the very rapid N cycling between organic and inorganic fractions that we demonstrated. According to the REMIN model, rapid remineralization would be a mechanism that would increase NH_4^+ availability, potentially for plant uptake. This rapid turnover of MBN could give plants an opportunity to capture NH_4^+ . Moreover, plants retain N longer than microbes (Hodge et al., 2000). Overall, this mechanism would likely contribute to ecosystem N retention.

Plants, which under these conditions immobilized approx. 10-fold more NO_3^- than microbes, were the most important NO_3^- immobilizers. In addition, NH_4^+ was immobilized by plants and microbes after recycling of NO_3^- at a rate equal to at least 35% of the gross nitrification rate, whereas net microbial NO_3^- immobilization rates were also equal to at least 35% of the gross nitrification rate. According to the NLEAK

model, the immobilized NH_4^+ would be of the same magnitude as N previously lost by roots. The loss of N in the form of amino acids or NH_4^+ by the tomato plants would be about 6.5% of the N gained via NO_3^- uptake. Reduction of NO_3^- to NH_4^+ consumes about 10 ATP, and for the conversion of NH_4^+ to glutamate, about 2 ATP are required (Bloom et al., 1992). Simulation modeling showed that NH_4^+ uptake by roots is a possible fate of the leaked N. Under such a scenario, the loss of N and the loss in energy by the plant would be relatively small, and the effect of root N leakage on ecosystem N retention would probably be neutral.

This study highlighted the importance of microbial processes and microbial-plant interactions to plant mineral nutrition in the vicinity of roots, most likely the rhizosphere, where future research efforts should be directed in order to gain a better understanding of the modifications of the belowground environment by plants.

Acknowledgements

We thank Donald Herman, James Richards, Kate Scow, and two anonymous reviewers for their critical comments on earlier versions of this manuscript, David Harris for the mass spectrometer analyses, and Sharon Schnabel and Matthew Quok for their help with the experiments. This work was supported by the U.S. Department of Agriculture National Research Initiative Soils and Soil Biology grant 0000991 to LEJ.

References

- Bjarnason S 1988 Calculation of gross nitrogen immobilization and mineralization in soil. *J. Soil Sci.* 39, 393–406.
- Bloom A J, Smart D R, Nguyen D T and Searles P S 2002 Nitrogen assimilation and growth of wheat under elevated carbon dioxide. *Proc. Natl. Acad. Sci. U.S.A.* 99, 1730–1735.
- Bloom A J, Sukrapanna S S and Warner R L 1992 Root respiration associated with ammonium and nitrate absorption and assimilation by barley. *Plant Physiol.* 99, 1294–1301.
- Bonkowski M, Cheng W, Griffiths B S, Alpehi J and Scheu S 2000 Microbial-faunal interactions in the rhizosphere and effects on plant growth. *Eur. J. Soil Biol.* 36, 135–147.
- Bottner P, Cortez J and Sallih Z 1991 Effect of living roots on carbon and nitrogen of the soil microbial biomass. *In* *Plant Root Growth*, Ed. D Atkinson. pp. 201–210. Blackwell, Oxford.
- Bouwman L A and Zwart K B 1994 The ecology of bacterivorous protozoans and nematodes in arable soil. *Agric. Ecosyst. Environ.* 51, 145–160.
- Bristow A W, Ryden J C and Whitehead D C 1987 The fate at several time intervals of ^{15}N -labelled ammonium nitrate applied to an established grass sward. *J. Soil Sci.* 38, 245–254.
- Brookes P C, Landman A, Pruden G and Jenkinson D S 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.
- Brooks P D, Stark J M, McInTeer B B and Preston T 1989 Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. *Soil Sci. Soc. Am. J.* 53, 1707–1711.
- Burger M and Jackson L E 2003 Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. *Soil Biol. Biochem.* 35, 29–37.
- Cavero J, Plant R E, Shennan C and Friedman D B 1996 The effect of nitrogen source and crop rotation on the growth and yield of processing tomatoes. *Nutr. Cycl. Agroecosyst.* 47, 271–282.
- Clarholm M 1989 Effects of plant-bacterial-amoebal interactions on plant uptake of nitrogen under field conditions. *Biol. Fertil. Soils* 8, 373–378.
- Clarholm M 1985 Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. *Soil Biol. Biochem.* 17, 181–188.
- Cowling A J 1994 Protozoan distribution and adaptation. *In* *Soil Protozoa*, Ed. J F Darbyshire. pp. 5–42. CAB International, Wallingford, UK.
- Dasberg S and Hopmans J W 1992 Time Domain Reflectometry calibration for uniformly and nonuniformly wetted sandy and clayey loam soils. *Soil Sci. Soc. Am. J.* 56, 1341–1345.
- Davidson E A, Hart S C, Shanks C A and Firestone M K 1991 Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. *J. Soil Sci.* 42, 335–349.
- Feng J, Volk R J and Jackson W A 1998 Source and magnitude of ammonium generation in maize roots. *Plant Physiol.* 118, 835–841.
- Gallardo M, Turner N C and Ludwig C 1994 Water relations, gas exchange and abscisic acid content of *Lupinus cosentinii* leaves in response to drying different proportions of the root system. *J. Exp. Bot.* 45, 909–918.
- Griffiths B and Robinson D 1992 Root-induced nitrogen mineralisation: A nitrogen balance model. *Plant Soil* 139, 253–264.
- Griffiths B S 1994 Microbial-feeding nematodes and protozoa in soil: Their effects on microbial activity and nitrogen mineralization in decomposition hotspots and the rhizosphere. *Plant Soil* 164, 25–33.
- Haider K, Mosier A and Heinemeyer O 1987 The effect of growing plants on denitrification at high soil nitrate concentrations. *Soil Sci. Soc. Am. J.* 51, 97–102.
- Hart S C, Stark J M, Davidson E A and Firestone M K 1994 Nitrogen mineralization, immobilization, and nitrification. *In* *Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties*. Eds. R W Weaver, S Angle, P Bottomley, D Bezdicek, S Smith, A Tabatabai and A Wollum. pp. 985–1018. SSSA, Madison, WI.
- Hodge A, Robinson D and Fitter A 2000 Are microorganisms more effective than plants at competing for nitrogen? *Trends Plant Sci.* 5, 304–308.
- Jackson L E and Bloom A J 1990 Root distribution in relation to soil nitrogen availability in field-grown tomatoes. *Plant Soil* 128, 115–126.
- Jackson L E, Schimel J P and Firestone M K 1989 Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. *Soil Biol. Biochem.* 21, 409–415.
- Jaeger C H, Lindow S E, Miller W, Clark E and Firestone M K 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.
- Jensen L S and Sorensen J 1994 Microscale fumigation-extraction and substrate-induced respiration methods for measuring microbial biomass in barley rhizosphere. *Plant Soil* 162, 151–161.
- Jeschke W D and Hartung W 2000 Root-shoot interactions in mineral nutrition. *Plant Soil* 226, 57–69.
- Jones D L and Darrah P R 1993 Influx and efflux of amino acids from *Zea mays* L. roots and their implications for N nutrition and the rhizosphere. *Plant Soil* 155–156, 87–90.
- Jones D L, Edwards A C, Donachie K and Darrah P R 1994 Role of proteinaceous amino acids released in root exudates in nutrient acquisition from the rhizosphere. *Plant Soil* 158, 183–192.
- Kirkham D and Bartholomew W V 1954 Equations for following nutrient transformations in soil, utilizing tracer data. *Soil Sci. Soc. Am. Proc.* 18, 33–34.
- Kirkham D and Bartholomew W V 1955 Equations for following nutrient transformations in soil, utilizing tracer data: II. *Soil Sci. Soc. Am. Proc.* 19, 189–192.
- Kuikman P J, Jansen A G, Van Veen J A and Zehnder A J B 1990 Protozoan predation and the turnover of soil organic carbon and nitrogen in the presence of plants. *Biol. Fertil. Soils* 10, 22–28.
- Maloney P E, Van Bruggen A H C and Hu S 1997 Bacterial community structure in relation to the carbon environments in lettuce and tomato rhizospheres and in bulk soil. *Microb. Ecol.* 34, 109–117.
- Marschner H, Kirkby E A and Engels C 1997 Importance of cycling and recycling of mineral nutrients within plants for growth and development. *Bot. Acta* 110, 265–273.
- Mary B, Recous S and Robin D 1998 A model for calculating nitrogen fluxes in soil using ^{15}N tracing. *Soil Biol. Biochem.* 30, 1963–1979.
- Matson P 1997 NO_x emission from soils and its consequences for the atmosphere and biosphere: Critical gaps and research directions for the future. *Nutr. Cycl. Agroecosyst.* 48, 1–6.
- Mengel K 1996 Turnover of organic nitrogen in soils and its availability to crops. *Plant Soil* 181, 83–93.
- Merbach W, Mirus E, Knof G, Remus R, Ruppel S, Russow R, Gransee A and Schulze J 1999 Release of carbon and nitrogen compounds by plant roots and their possible ecological importance. *J. Plant Nutr. Soil Sci.* 162, 373–383.
- Morgan M A and Jackson W A 1989 Reciprocal ammonium transport into and out of plant roots: Modifications by plant nitrogen status and elevated root ammonium concentration. *J. Exp. Bot.* 20, 207–214.
- Morgan M A and Jackson W A 1988 Suppression of ammonium uptake by nitrogen supply and its relief during nitrogen limitation. *Physiol. Plant.* 73, 38–45.
- Newman E I 1985 The rhizosphere: carbon sources and microbial populations. *In Ecological Interactions in Soil. Plants, Microbes, Animals.* Ed. A H Fitter. pp. 107–122. Blackwell Scientific Publications, Oxford.
- Norton J M and Firestone M K 1996 N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. *Soil Biol. Biochem.* 28, 351–362.
- Paul E A and Clark F E 1996 *Soil Microbiology and Biochemistry.* Academic Press, San Diego. xiii, 340 pp.
- Robertson P G 1997 Nitrogen use efficiency in row-crop agriculture: Crop nitrogen use and soil nitrogen loss. *In Ecology in Agriculture.* Ed. L E Jackson. pp. 347–365. Academic Press, San Diego.
- Schimel J P, Jackson L E and Firestone M K 1989 Spatial and temporal effects on plant microbial competition for inorganic nitrogen in a California annual grassland. *Soil Biol. Biochem.* 21, 1059–1066.
- Shen S M, Pruden G and Jenkinson D S 1984 Carbon and nitrogen turnover in adjacent grassland and cropland ecosystems. *Biogeochem.* 16, 437–444.
- Shepherd T and Davies H V 1994 Effect of exogenous amino acids, glucose and citric acid on the patterns of short-term accumulation and loss of amino acids in the root-zone of sand-cultured forage Rape (*Brassica Napus* L.). *Plant Soil* 158, 111–118.
- Silver W L, Herman D J and Firestone M K 2001 Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. *Ecology* 82, 2410–2416.
- Smart D R and Bloom A J 1988 Kinetics of ammonium and nitrate uptake among wild and cultivated tomatoes. *Oecologia* 76, 336–340.
- Stark J M and Hart S C 1996 Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. *Soil Sci. Soc. Am. J.* 60, 1846–1855.
- Stark J M and Hart S C 1997 High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* 385, 61–64.
- Stedtle E and Peterson C A 1998 How does water get through roots? *J. Exp. Bot.* 49, 775–788.
- Tiedje J M 1988 Ecology of denitrification and dissimilatory nitrate reduction to ammonium. *In Biology of Anaerobic Microorganisms.* Ed. A J B Zehnder. pp. 179–244. John Wiley, New York.
- Whipps J M 1990 Carbon economy. *In The Rhizosphere,* Ed. J M Lynch. pp. 59–98. Wiley, Chichester.
- Wyland L J, Jackson L E and Brooks P D 1994 Eliminating nitrate interference during Kjeldahl digestion of soil extractions for microbial biomass determination. *Soil Sci. Soc. Am. J.* 58, 357–360.
- Zak D R, Pregitzer K S, Curtis P S, Terri J A, Fogel R and Randlett D L 1993 Elevated atmospheric carbon dioxide and feedback between carbon and nitrogen cycles. *Plant Soil* 151, 105–117.
- Zwart K B, Kuikman P J and van Veen J A 1994 Rhizosphere protozoa: Their significance in nutrient dynamics. *In Soil Protozoa.* Ed. J F Darbyshire. pp. 93–122. CAB International, Wallingford, UK.

Section editor: S. Recous