PERGAMON

Soil Biology & Biochemistry 33 (2001) 1937–1945

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Short-term nitrogen transformations in bulk and root-associated soils under ryegrass

J.K. Whalen[a,*], P.J. Bottomley[a,b], D.D. Myrold[a]

[a]*Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331, USA*
[b]*Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA*

Received 18 August 2000; received in revised form 26 February 2001; accepted 27 April 2001

Abstract

The balance between gross rates of N mineralization and N consumption in soils is influenced both by the presence or absence of plants, and by physical soil disturbance. Studies are needed which evaluate the impact of disturbances caused either by plant harvest (grazing or clipping) or by the activities of soil fauna on soil N transformations. Using ^{15}N pool dilution techniques, we compared gross N transformations in bulk and root-associated soils under ryegrass grown in greenhouse conditions. Over a period of 98 d, ryegrass plants were clipped periodically, or left unclipped in the presence or absence of earthworms. After 98 d of ryegrass growth, $^{15}NH_4$–N (33 atom% ^{15}N) or $^{15}NO_3$–N (10 atom% ^{15}N) solutions were injected throughout the soil. Virtually all of the ^{15}N added was recovered in ryegrass and soil after 48 h of incubation, but up to 30% of the $^{15}NH_4$–N and 44% of the $^{15}NO_3$–N were unaccounted for after 30 d. Gross N mineralization and NH_4–N consumption rates were higher in the root-associated than in bulk soils of all treatments. The rate of gross N mineralization was highest in the pots with clipped plants, followed by the unclipped plus earthworm and the unclipped minus earthworm treatments. Our results indicate that rates of gross N transformations differ between bulk and ryegrass root-associated soils, and that clipping of plants and the presence of earthworms exert marked effects on short-term N transformations.

Keywords: Gross N mineralization rate; Ryegrass; Root-associated soil; Bulk soil; SOM fractions

1. Introduction

Soil N transformations are affected by the quality of carbon substrates, and plant roots exert considerable influence on soil microbial communities through the depletion of nutrients and water in the rhizosphere, and the secretion of protons, enzymes, and carbon compounds from root surfaces. Relatively few studies have investigated gross N transformations in the rhizosphere. Norton and Firestone (1996) found gross rates of mineralization and microbial assimilation of NH_4–N were 50% higher in soil adjacent to pine seedling roots than in soil a few millimeters away. Reydellet et al. (1997) found the available N pool was 50% larger, and gross N mineralization rates were 35% higher, in the rhizosphere of an alfisol under ryegrass than in soil without plants, and attributed their results to stimulation of microbial activity from rhizodeposition.

The quantity and quality of carbon secreted by plant roots is affected by many factors, including plant species, physiological state and age of roots, and environmental conditions (Hale and Moore, 1979; Grayston et al., 1996). The removal of above-ground biomass by clipping or grazing is known to alter the proportion of photosynthate allocated to root and stem tissues and long-term, above-ground biomass removal can reduce root biomass and change root morphology and architecture (McNaughton et al., 1983; Waller and Jones 1989). Root exudation, root respiration, and soil respiration are often greater in pots with plants that have been clipped or grazed than in pots with unclipped or ungrazed plants (Holland et al., 1996; Mawdsley and Bardgett, 1997; Bardgett et al., 1998). We are not aware of any studies that have attempted to assess how gross N transformations in soil are affected by above-ground biomass removal.

In addition, it is well established that both the activity and size of soil microbial communities are affected by predation from amoebae, flagellates, nematodes, and mites, and that the numbers of micro- and meso-fauna are greater in rhizosphere than in bulk soils (Anderson, 1988). Grazing of below-ground microbial communities by soil micro- and meso-fauna increases decomposition and N mineralization (Ingham et al., 1985; Beare et al., 1992). Soil macrofauna,

* Corresponding author: Department of Natural Resource Sciences, Macdonal Campus, 21,111 Lakeshore Road, Ste. Anne de Bellevue, PA, H9X 3V9, Canada. Tel.: +1-514-398-7943; fax: +1-514-398-7990.
E-mail address: whalenj@nrs.mcgill.ca (J.K. Whalen).

0038-0717/01/$ - see front matter
PII: S0038-0717(01)00121-3

such as earthworms, alter soil physical structure through litter comminution and burrow construction, and have multiple effects on microbial activity and nutrient cycling (Blair et al., 1995). Microbial biomass is lower, but microbial activity is higher in soils with elevated earthworm populations (Bohlen et al., 1997). Earthworms likely affect gross N transformations due to their modification of the soil environment, grazing on soil micro-organisms, and their own turnover. We are not aware of any studies that have evaluated the impact of earthworms on gross rates of N cycling.

The objectives of this study were to: (1) compare gross N transformations in bulk and root-associated soils under ryegrass, (2) determine the effect of periodic removal of above-ground biomass on gross N transformations in bulk and root-associated soils, and (3) determine whether the presence of earthworms affects gross N transformations in bulk and root-associated soils.

2. Materials and methods

2.1. Soil

In March 1998, soil was collected from the A horizon (0–15 cm) of replicate plots containing fescue grass from the North Willamette Research and Extension Center, Aurora, OR, USA. Fescue grass was planted on these plots in 1989 and has been minimally maintained without animal grazing. From 1978 to 1988, the site was under conventional winter wheat production. The soil is a Willamette silt loam (Pachic Ultic Argixeroll) containing 18 g organic C kg^{-1} and 1.1 g N kg^{-1} with pH 5.6. Other properties of the soil have been discussed elsewhere (Burket and Dick, 1998; Whalen et al., 2000). Soil samples were composited, air-dried, and coarsely-sieved (<12.5-mm mesh). Air-dried soil (1885 g, oven-dry weight) was weighed into individual 4-l pots. The pots were undrained to prevent nutrient losses through leaching. Soils were moistened to 75% of field capacity and planted with annual ryegrass (*Lolium multiflorum* L.). Fourteen days after emergence, seedlings were thinned to leave approximately 50 individuals within the inner 100 mm of each pot. Pots were watered regularly to between 75 and 85% of field capacity. Nitrogen fertilizer (100 mg N kg^{-1}) was added 35 d after emergence due to the appearance of N deficiency symptoms in the foliage (yellowing leaves).

2.2. Experimental design

To accommodate the two N forms (NH_4–N and NO_3–N) required for the isotope dilution study, three sampling times [2, 48 and 720 h (i.e. 30 d)], and five replicates of each treatment, 30 pots were prepared for each of three major experimental treatments. The major experimental treatments were assigned to the pots at random 37 d after seedling emergence, and pots were distributed completely at random in the greenhouse. Gross N transformations were measured in bulk and ryegrass root-associated soils of pots in which above-ground biomass was not harvested, and to which earthworms were not added. The effect of removing above-ground biomass on gross N transformations in bulk and root-associated soils was determined on another 30 pots. Approximately one-half of the above-ground biomass was harvested periodically (37, 52, 75 and 97 d after seedling emergence). In this treatment, a total of 1.32 ± 0.07 g above-ground biomass (dry weight) per pot was removed from ryegrass plants through clipping. Gross N transformations in bulk and root-associated soils were also measured in 30 replicate pots to which earthworms were added. Five to seven individuals of *Aporrectodea tuberculata* (Eisen) with a total mass of 2 g (fresh weight, gut cleared for 24 h) were added 37 d after seedling emergence to each pot. *A. tuberculata* is an endogeic earthworm that inhabits the top 10–15 cm of soil and is thought to consume primarily soil organic matter (Edwards and Bohlen, 1996). Most of the earthworms were dead by the time the experiment ended (98–128 d after seedling emergence). Surviving earthworms were weighed, euthanized with 5% ethanol, oven-dried (60°C for 48 h), ground and analyzed for total N and atom% ^{15}N.

2.3. ^{15}N labelling

Gross rates of N mineralization and nitrification were determined using ^{15}N isotope dilution methodology (Hart et al., 1994). Soils were labelled with either 50 ml of a $^{15}NH_4$–N (about 33 atom% ^{15}N) or a $^{15}NO_3$–N (about 10 atom% ^{15}N) solution that contained 60 μg N ml^{-1}. Because the mean (±standard error) concentrations of inorganic N in pots prior to injection of ^{15}N were 2.1 ± 0.2 mg NH_4–N kg^{-1} and 0.21 ± 0.03 mg NO_3–N kg^{-1}, addition of ^{15}N-labelled solutions increased the mean NH_4–N concentration almost 2-fold, and the mean NO_3–N concentration 8-fold. The ^{15}N-labelled solution was injected into soil 1–2 ml at a time using a 60-mm long side-bore needle. Numerous injections were made throughout each pot to ensure even distribution of the ^{15}N label. Five replicate pots were destructively sampled 2 h, 48 h and 30 d, respectively, after the ^{15}N label was added. The form of N added to each pot and time of destructive sampling were assigned randomly to each experimental treatment.

2.4. Nitrogen analysis

Above-ground plant biomass (shoots and seed heads) was clipped at the soil surface, oven-dried (60°C for 48 h), ground and analyzed for total N and atom% ^{15}N. Visual examination indicated that most of the roots were in the innermost 100 mm of each pot, whereas soil outside this region had few roots. Soil adhering to roots in the middle 100 mm of each pot was defined as root-associated soil and the remainder was defined as bulk soil. We chose not to recover rhizosphere soil senso stricto because of the time it would have taken to obtain the small amounts of soil

closely adhering to the roots of our many treatments and the possibility of changes in labelling patterns during the process. About 40% of the total soil mass was root-associated, and most root-associated soil was gently removed from the roots by hand. Subsamples of bulk and root-associated soils were oven-dried (105°C for 48 h), ground and analyzed for total N and atom% ^{15}N. Roots were oven-dried for 3 d at 60°C, rinsed on a 0.5-mm mesh sieve with distilled water to remove remaining soil, and then oven-dried (60°C for 48 h), ground and analyzed for total N and atom% ^{15}N.

Soil organic matter from bulk soils was separated by density into light fraction (LF) and heavy fraction (HF) following the procedure of Whalen et al. (2000) modified from Strickland and Sollins (1987). About 100 g of sieved (<2-mm mesh) soil was mixed with 100 ml of a 1.6 g cm^{-3} sodium polytungstate (SPT) solution for 30 s at $4000 \times g$ and then dispersed for 2 min with a probe-type sonic disrupter at 2000 J (Model 350, Branson Sonic Power Company, Danbury, CT, USA). Suspended LF material was collected from the surface of the SPT solution, and the LF and HF materials were rinsed several times with deionized water to remove the SPT. Light and heavy fractions were oven-dried (105°C for 48 h), ground and analyzed for total N and atom% ^{15}N.

Fresh soil was sieved (<2 mm) and extracted immediately to determine mineral N concentrations. About 20 g of soil was extracted with 0.5 M K_2SO_4 (1:5 soil:extractant) for mineral N (NH_4–N and NO_3–N) determination. NH_4–N was determined colorimetrically using the modified indophenol blue technique (Sims et al., 1995) and measured at 650 nm using Titertek Multiscan MCC/340 automated microplate reader (Huntsville, AL, USA). NO_3–N was measured using the cadmium reduction–diazotization method with a Technicon II flow-injection autoanalyzer (Technicon Industrial Systems, Tarrytown, NY).

The ^{15}N concentration in the mineral N ($^{15}NH_4$–N and $^{15}NO_3$–N) pools was determined using a modification of the acid diffusion method described by Brooks et al. (1989). Disks (5 mm diameter) were cut from glass fiber filter circles (Whatman GF/D), placed in a muffle furnace at 500°C for 2 h, and then acidified with 15 μl of 2.5 M $KHSO_4$. Extracts from root-associated and bulk soils contained between 3 and 19 μg N ml^{-1} as NH_4–N and <2 μg N ml^{-1} as NO_3–N. We pipetted 2.5 ml of soil extract and 10 ml of 0.5 M K_2SO_4 solution into an acid-washed 120-ml specimen cup. At least 10 ml of liquid is required in our modified diffusion procedure so that the MgO and Devarda's alloy are dissolved and react properly with the soil extracts. Our first diffusion trial showed that inorganic N pools, particularly the NO_3–N pool, were only slightly labelled with ^{15}N, and when we had to repeat our diffusions, we were limited by the total amount of extract remaining. Because the diffusion procedure requires between 40 and 100 μg N in solution for good recovery of the ^{15}N tracer, the soil extracts were spiked with 1 ml of 100 mg N l^{-1} solution (from NH_4–N or NO_3–N) containing 0.3663 atom% ^{15}N (natural abundance) and 1 ml of 100 mg N l^{-1} solution containing 5.1 atom% ^{15}N (from $^{15}NH_4$–N) or 5.0 atom% ^{15}N (from $^{15}NO_3$–N). Although spiking solutions decreased the precision and accuracy of results, the alternative was to obtain no data at all. The ^{15}N recovery of diffused samples was compared to spiked NH_4–N and NO_3–N standards that were diffused at the same time with the same procedures as the soil extracts, and the variation among replicate standards ranged from 0.0036 atom% ^{15}N to 0.0115 atom% ^{15}N. The isotopic abundance of NH_4–N was determined by adding 0.2 g of MgO and two filter disks sealed in Teflon tape; that of NO_3–N was determined by adding 0.4 g of Devarda's alloy, 0.2 g of MgO and two filter disks sealed in Teflon tape to each cup. The cups were swirled vigorously once or twice daily for 7 d, after which the disks were removed, desiccated over concentrated H_2SO_4, and analyzed for total N and atom% ^{15}N. Nitrogen isotopic ratios ($^{15}N/^{14}N$) in soil extracts, plant shoots and roots, earthworms, bulk and root-associated soils, and light and heavy fractions were determined using a Roboprep combustion unit coupled with a Tracermass isotope ratio mass spectrometer (Europa Scientific, Crewe, UK).

2.5. Nitrogen calculations

The atom% ^{15}N in the NH_4–N pool was calculated from the equation:

$$At^{15}N_{sample} \times (AN_{extract} + AN_{spike}) = (AN_{extract} \times At^{15}N_{extractNH4}) + (AN_{spike} \times At^{15}N_{spike}) \quad (1)$$

where $At^{15}N_{sample}$ is the atom% ^{15}N measured from the diffusion disks, $AN_{extract}$ is the μg of NH_4–N in soil extracts, and AN_{spike} is the μg of NH_4–N added in the $^{15}NH_4$–N spike. The $At^{15}N_{spike}$ is the atom% ^{15}N added in the spike, and the unknown in the equation is $At^{15}N_{extractNH4}$, the atom% ^{15}N in the extract NH_4–N pool. The atom% ^{15}N excess in the NH_4–N pool was calculated by subtracting 0.3663 (natural abundance). We attempted to calculate the atom% ^{15}N excess in the NO_3–N pool in a similar manner but this proved impossible because of the low amount of soil NO_3–N relative to that added in the spike.

Gross N mineralization and NH_4–N consumption were calculated from changes in the $^{15}NH_4$–N and $^{14+15}NH_4$–N pools in $^{15}NH_4$–N amended soils between 2 and 48 h after ^{15}N addition. We used the $m = i$ (mineralization equals immobilization) equation of Kirkham and Bartholomew (1954) because there was no significant change in the NH_4–N pool during the incubation.

2.6. Statistical analysis

Data were evaluated statistically by ANOVA in a general linear model (GLM) using SAS software (SAS Institute, 1990). Pots were arranged in a completely random design,

Table 1
Mean shoot and root weights and total N concentrations after 98–128 d of ryegrass growth [means in a column within a sampling date followed by the same letter are not significantly different ($P < 0.05$, LSD)]

Treatment[a]	Shoot weight (g dry weight)	Shoot N (g N kg^{-1})	Root weight (g dry weight)	Root N (N kg^{-1})
After 98 and 100 d of growth (pooled data)				
Clipped	3.36 a	7.7 b	3.20 a	4.5 a
Unclip. + EW	5.47 b	6.2 a	4.90 b	4.6 a
Unclip. − EW	3.63 a	6.0 a	3.76 a	4.9 a
After 128 d of growth				
Clipped	4.40 a	7.3 c	3.11 a	4.0 a
Unclip. + EW	6.40 b	5.2 a	4.95 b	4.8 a
Unclip. − EW	4.06 a	6.1 b	3.21 a	4.6 a

[a] Treatments were clipped (about one-half of above-ground biomass was removed each month during ryegrass growth, no earthworms in pots), unclipped plus earthworm (plants were unclipped, five to seven individuals of *A. tuberculata* with a mass of about 2 g were added to pots 1 month after seeding), and unclipped minus earthworm (plants were unclipped, there were no earthworms in pots). Shoot yields for the clipped treatment include biomass removed prior to harvest. The unclipped plus earthworm treatment = Unclip. + EW; the unclipped minus earthworm treatment = Unclip. − EW

and plant biomass and nutrient concentrations at harvest were evaluated using a one-way ANOVA. Plant yields did not differ significantly ($P > 0.05$) in pots that received injections of $^{15}NH_4$–N or $^{15}NO_3$–N at any sampling dates, so the data were pooled among the ^{15}N sources for analysis. Plant yields did not differ significantly ($P > 0.05$) in samples harvested between 98 and 100 d of growth, and data for these dates were pooled for statistical analysis. The biomass and nutrient concentration in plants harvested between 98 and 100 d or after 128 d of growth were compared for clipped, unclipped plus earthworm and unclipped minus earthworm treatments with an LSD test at the 95% confidence level. Treatment means (clipped, unclipped plus earthworm and unclipped minus earthworm) for the ^{15}N concentration in soil and plant pools at each sampling time (2 h, 48 h, 30 d) were evaluated with a two-way ANOVA and compared with an LSD test at the 95% confidence level.

3. Results

3.1. Ryegrass growth and nutrient content

The masses and N concentrations in shoots and roots harvested after 98–100, or 128 d of growth were evaluated statistically. Shoot and root yields were significantly ($P < 0.05$, LSD) higher for the unclipped plus earthworm treatment than the clipped or unclipped minus earthworm treatments after 98–100 d of growth (Table 1). Shoot yield for the clipped treatment in Table 1 includes biomass removed through periodic clipping prior to harvest. Clipped plants contained a significantly ($P < 0.05$, LSD) greater concentration of N in their shoots than did plants from either the unclipped plus earthworm or unclipped minus earthworm treatments (Table 1). Root N concentrations did not differ among the treatments (Table 1). A significantly ($P < 0.05$, LSD) greater amount of N was assimilated by plants in the unclipped plus earthworm treatment which was due primarily to the greater root growth in this treatment (Table 1). Similar trends in biomass yield and N concentration for roots and shoots from these treatments were observed after 128 d of growth (Table 1). About 20% of the earthworms added to pots were recovered at harvest, and they had an average N concentration of 111.1 ± 5.3 mg N g^{-1} (oven-dry weight) with 0.005 atom% ^{15}N excess.

3.2. Recovery of ^{15}N added to bulk and root-associated soils

Between 96 and 111% of the ^{15}N from $^{15}NH_4$–N, and between 94 and 107% of the ^{15}N from $^{15}NO_3$–N were recovered in soil and plant pools 2 h and 48 h after injection (Tables 2 and 3). In contrast, nearly 30% of the $^{15}NH_4$–N and 32–44% of the $^{15}NO_3$–N added to the pots was not recovered after 30 d (Tables 2 and 3). Because the pots were not drained, it is likely that the ^{15}N missing from these soils was lost through denitrification.

The quantity of ^{15}N in plant roots and shoots increased significantly ($P < 0.05$, LSD) in most treatments between 2 and 48 h after injection of $^{15}NH_4$–N and $^{15}NO_3$–N (Tables 2 and 3). Between 6 and 13% of the $^{15}NH_4$–N added was found in plant shoots and roots within 2 h of injection (Table 2). The amount of $^{15}NH_4$–N in plant shoots and roots ranged from 22 to 27%, and 21 to 28% of the total applied within 48 h and 30 d of injection, respectively (Table 2). The proportion of $^{15}NO_3$–N assimilated in plant shoots and roots increased from 5 to 19% after 2 h to between 29 and 40% after 48 h of incubation (Table 3). The proportion of ^{15}N in plant shoots and roots in the $^{15}NO_3$–N treated pots declined to between 18 and 20% after 30 d of incubation (Table 3).

There was considerable variation in the ^{15}N content of bulk soils, and no clear trends emerged to explain ^{15}N fluctuations in bulk soils at time intervals following $^{15}NH_4$–N and $^{15}NO_3$–N injections (Tables 2 and 3). The quantity of ^{15}N in

Table 2
Percentage of ^{15}N from $^{15}NH_4$–N in plant shoots, plant roots, bulk and root-associated soils after 2 h, 48 h and 30 d [means in a column followed by the same letter are not significantly different ($P < 0.05$, LSD); all treatments received 1111 μg ^{15}N at time = 2 h, 1099 μg ^{15}N at time = 48 h, and 1114 μg ^{15}N at time = 720 h; bulk soil was 60% and root-associated soil was 40% of total soil mass]

Treatment[a]	Time (h)	Plant shoots (%)	Plant roots (%)	Bulk soil (%)	Root-associated soil (%)	^{15}N recovery[b] (%)
Clipped	2	2 a	5 a	36 b	55 b	98 ± 8
Unclip. + EW	2	2 a	11 b	29 ab	59 b	101 ± 6
Unclip. − EW	2	0.4 a	6 a	37 b	53 b	96 ± 10
Clipped	48	13 bc	9 b	29 ab	57 b	108 ± 11
Unclip. + EW	48	16 cd	11 b	20 a	64 b	111 ± 8
Unclip. − EW	48	11 b	11 b	20 a	65 b	107 ± 10
Clipped	720	19 d	5 a	27 ab	22 a	73 ± 2
Unclip. + EW	720	19 d	9 b	23 a	21 a	72 ± 2
Unclip. − EW	720	16 cd	5 a	24 ab	25 a	71 ± 3

[a] See footnote under Table 1 for explanation of experimental treatments.
[b] ^{15}N recovery data are mean values ± standard errors; $n = 5$.

Table 3
Percentage of ^{15}N from $^{15}NO_3$–N in plant shoots, plant roots, bulk and root-associated soils after 2 h, 48 h and 30 d [means in a column followed by the same letter are not significantly different ($P < 0.05$, LSD); all treatments received 320 μg ^{15}N at time = 2 h, 312 μg ^{15}N at time = 48 h, and 404 μg ^{15}N at time = 720 h; bulk soil was 60% and root-associated soil was 40% of total soil mass]

Treatment[a]	Time (h)	Plant shoots (%)	Plant roots (%)	Bulk soil (%)	Root-associated soil (%)	^{15}N recovery[b] (%)
Clipped	2	16 bc	4 a	29 a	46 c	95 ± 10
Unclip. + EW	2	1 a	4 a	48 b	47 c	100 ± 5
Unclip. − EW	2	1 a	4 a	44 b	46 c	94 ± 7
Clipped	48	29 d	11 bc	27 a	38 c	107 ± 8
Unclip. + EW	48	20 bcd	9 b	35 ab	33 bc	97 ± 10
Unclip. − EW	48	25 cd	14 c	23 a	42 c	105 ± 10
Clipped	720	16 bc	4 a	21 a	15 a	56 ± 8
Unclip. + EW	720	14 bc	6 a	25 a	18 a	62 ± 5
Unclip. − EW	720	13 b	5 a	29 a	21 ab	68 ± 4

[a] See footnote under Table 1 for explanation of experimental treatments.
[b] ^{15}N recovery data are mean values ± standard errors; n = 5.

root-associated soils from all treatments declined significantly ($P < 0.05$, LSD) between 2 h and 30 d after $^{15}NH_4$–N and $^{15}NO_3$–N injections, possibly due to microbial assimilation and turnover via denitrification (Tables 2 and 3).

The quantities of ^{15}N (μg ^{15}N g^{-1} soil) recovered in bulk and root-associated soil pools of each treatment 2 h after $^{15}NH_4$–N and $^{15}NO_3$–N injection were evaluated with a two-way ANOVA [Fig. 1(A) and (B)]. Significantly ($P < 0.05$) more ^{15}N was found in root-associated than bulk soils of all treatments, which may have been due to poor distribution of the label or rapid transport of the label from bulk to root-associated soils shortly after ^{15}N injection.

3.3. Recovery of ^{15}N in LF and HF pools

Subsamples of bulk soil from each treatment were fractionated by density separation to collect LF and HF of soil organic matter. About 97.8 ± 0.1% of the bulk soil was the HF of soil organic matter. The C/N ratio of LF ranged from 30 to 68, whereas the C/N ratio of HF ranged from 13 to 18. Following injection with either $^{15}NH_4$–N or $^{15}NO_3$–N, the atom% ^{15}N excess in LF was higher in most treatments after 48 h and 30 d than after 2 h (Tables 4 and 5). There were fewer differences in the atom% ^{15}N excess in HF collected from the three treatments. Generally less ^{15}N was recovered in the HF from pots incubated with $^{15}NO_3$–N than with $^{15}NH_4$–N (Tables 4 and 5). No ^{15}N was detected in the HF of the unclipped minus earthworm treatment injected with $^{15}NO_3$–N solution (Table 5).

3.4. Gross ^{15}N transformations

There was substantial dilution of label in the NH_4–N pool of all but the bulk soil of the unclipped treatments, indicating rapid production and consumption of NH_4–N (Table 6). Changes in NH_4–N pool sizes were usually small, and not statistically significant (*t*-test, $P > 0.1$), suggesting that production and consumption of NH_4–N were balanced. In all cases, gross rates were higher in root-associated compared to bulk soil. In root-associated soil, rates were 56% higher in the presence of earthworms and 94% higher when plants were clipped (Table 6). Nitrate concentrations were low (1 mg N kg^{-1} soil or less) in all treatments, but consistently decreased between 2 and 48 h, suggesting that

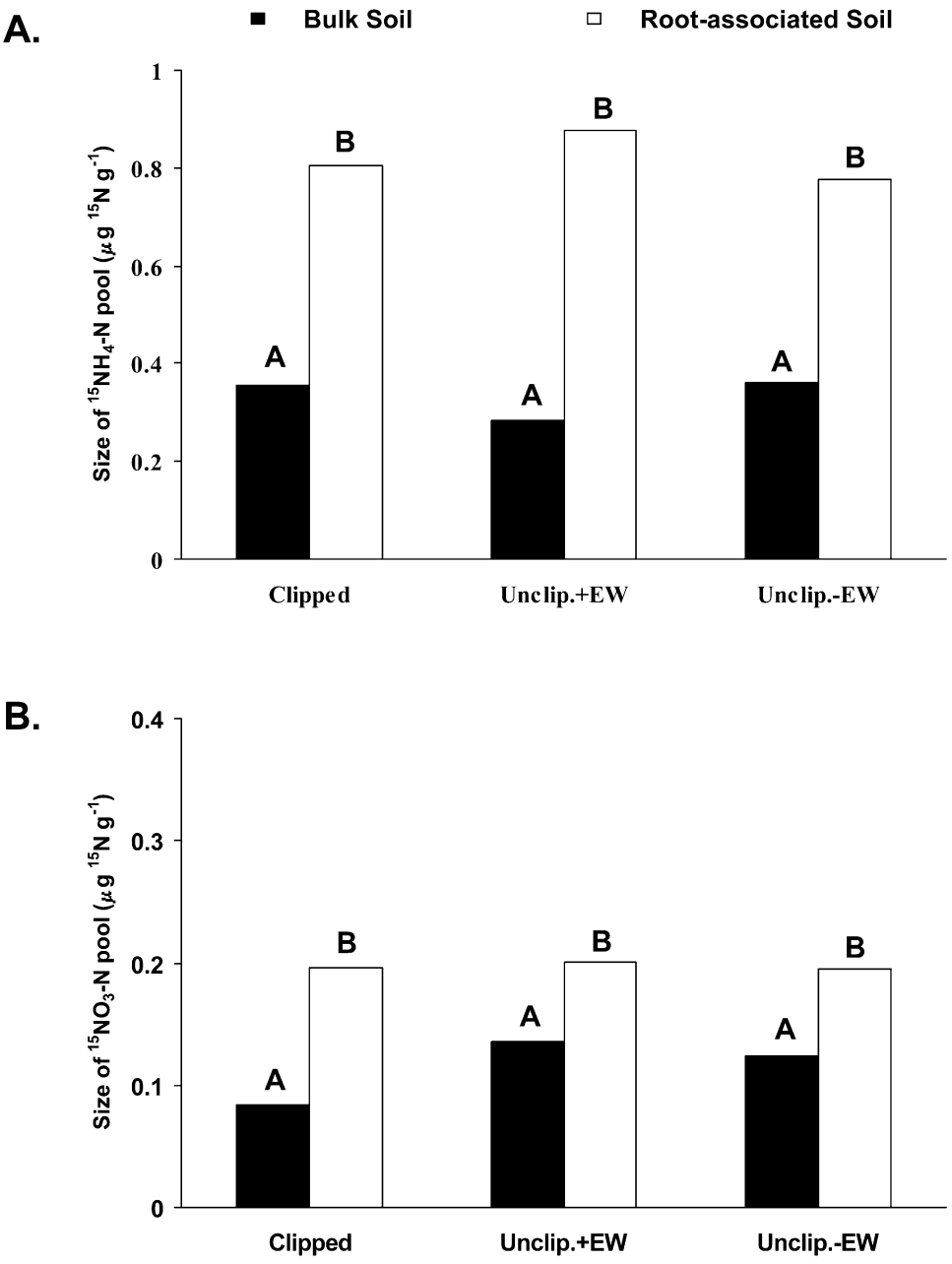

Fig. 1. Quantities of ^{15}N recovered in bulk and root-associated soil pools of ryegrass under different treatments 2 h after (A) $^{15}NH_4$–N injection and (B) $^{15}NO_3$–N injection. See footnote under Table 1 for explanation of experimental treatments. Treatment means followed by the same letter are not significantly different ($P < 0.05$, LSD).

NO_3–N consumption was greater than nitrification (data not shown).

4. Discussion

4.1. Short-term N transformations in bulk and root-associated soils

The distribution of ^{15}N in soil pools changed significantly in the 46 h following ^{15}N injection, and up to 27% of the $^{15}NH_4$–N and 41% of the $^{15}NO_3$–N was assimilated into plant roots and shoots. Between 48 h and 30 d after $^{15}NH_4$–N injection, there was a net increase in the ^{15}N content of ryegrass shoots and a net decrease in the ^{15}N of ryegrass roots, suggesting translocation of ^{15}N from roots to shoots. Between 48 h and 30 d after $^{15}NO_3$–N injection, there was no net change in the ^{15}N content of ryegrass shoots, but the ^{15}N content of ryegrass roots was generally lower. These results suggest there was an N form interaction with ryegrass that caused losses of NO_3–N from the plants treated with nitrate. It has been suggested that nitrate could

Table 4
Mean N and ^{15}N content of LF and HF in soil organic matter 2 h, 48 h and 30 d after soil was injected with $^{15}NH_4$–N [means in a column followed by the same letter are not significantly different ($P < 0.05$, LSD)]

Treatment[a]	Time (h)	Total N in LF (g N kg^{-1})	Atom% ^{15}N excess in LF	Total N in HF (g N kg^{-1})	Atom% ^{15}N excess in HF
Clipped	2	2.8 a	0.011 a	1.1 ab	0.011 a
Unclip. + EW	2	2.2 a	0.021 ab	1.1 ab	0.019 ab
Unclip. − EW	2	2.7 a	0.013 a	1.1 ab	0.001 a
Clipped	48	2.1 a	0.035 b	1.1 ab	0.009 a
Unclip. + EW	48	1.9 a	0.049 c	1.2 b	0.038 b
Unclip. − EW	48	2.4 a	0.058 bc	1.1 ab	0.007 a
Clipped	720	1.8 a	0.041 bc	1.0 a	0.015 a
Unclip. + EW	720	2.2 a	0.033 bc	1.1 ab	0.011 a
Unclip. − EW	720	2.2 a	0.039 bc	1.1 ab	0.003 a

[a] See footnote under Table 1 for explanation of experimental treatments.

Table 5
Mean N and ^{15}N content of LF and HF in soil organic matter 2 h, 48 h and 30 d after soil was injected with $^{15}NO_3$–N [means in a column followed by the same letter are not significantly different ($P < 0.05$, LSD)]

Treatment[a]	Time (h)	Total N in LF (g N kg^{-1})	Atom% ^{15}N excess in LF	Total N in HF (g N kg^{-1})	Atom% ^{15}N excess in HF
Clipped	2	2.4 a	0.007 a	1.0 a	0.003 a
Unclip. + EW	2	2.7 a	0.011 a	1.3 b	0.013 c
Unclip. − EW	2	2.4 a	0.003 a	1.1 a	0 a
Clipped	48	2.0 a	0.023 b	1.0 a	0.005 b
Unclip. + EW	48	2.0 a	0.025 b	1.1 a	0.003 a
Unclip. − EW	48	2.1 a	0.029 b	1.1 a	0 a
Clipped	720	2.8 a	0.021 b	1.0 a	0.007 b
Unclip. + EW	720	2.1 a	0.011 a	1.1 a	0.005 b
Unclip. − EW	720	2.5 a	0.011 a	1.1 a	0 a

[a] See footnote under Table 1 for explanation of experimental treatments.

be lost from plants through exudation of NO_3–N and organic N compounds from roots to soils (Bailey, 1976). We did not measure exudates of ryegrass roots, and this factor warrants future investigation.

Nitrogen transformations were not coupled tightly with plant N requirements under our experimental conditions, and as much as 30% of the $^{15}NH_4$–N and 44% of the $^{15}NO_3$–N injected were lost after 30 d. The average rate of loss between 48 h and 30 d after ^{15}N addition was 6 ng ^{15}N g^{-1} soil d^{-1} in pots injected with $^{15}NH_4$–N and 3 ng ^{15}N g^{-1} soil d^{-1} in pots injected with $^{15}NO_3$–N. We did not monitor denitrification, but greater denitrification has been found in planted than in bulk soil, due to a combination of carbon supply (Smith and Tiedje, 1979; Bodelier et al., 1997; Mahmood et al., 1997) and oxygen depletion in the root zone (Klemedtsson et al., 1987; Prade and Trolldenier, 1988; Mahmood et al., 1997). Further work should be conducted to determine if spatial distribution of denitrifiers in soil-plant systems, coupled with temporal variation in oxygen concentrations, could lead to competition for nitrate between denitrifiers and plants.

We also examined changes in soil organic matter pools of bulk soil to determine if ^{15}N was stabilized in the light or heavy fraction of SOM. The ^{15}N content increased in the LF between 2 and 48 h after injection of $^{15}NH_4$–N and $^{15}NO_3$–N, indicating that the LF was a sink for mineral N. The ^{15}N incorporated in the LF pool appeared to be dynamic, as there were substantial declines in the ^{15}N content of the unclipped plus earthworm and unclipped minus earthworm treatments between 48 h and 30 d after injection of $^{15}NH_4$–N and $^{15}NO_3$–N. Similar declines were not observed in the clipped treatment where the ^{15}N content of LF remained constant or increased slightly between 48 h and 30 d after injection of $^{15}NH_4$–N and $^{15}NO_3$–N. The HF also was rapidly labeled with ^{15}N in both treatments, but its labeling tended to change less over time than the LF fraction, presumably because most of the HF is relatively recalcitrant. The types of ^{15}N-containing compounds in LF and the mechanisms responsible for ^{15}N stabilization in LF are unknown, and are worthy of further study.

4.2. Short-term N transformations in agricultural soils with clipping and earthworm treatments

Neither shoot nor root yields differed in the clipped and unclipped minus earthworm treatments at the two harvest dates. Interestingly, the clipped treatment had more total N in shoots than the other two treatments, although the total N content of roots did not differ. These results suggest that clipping of ryegrass stimulated N uptake and translocation to shoots, depending on the form of N available for plant uptake. Ryegrass shoot and root biomass was greater in the

Table 6
Concentration and atom% ^{15}N excess of NH_4–N, and gross rates of N mineralization (M) and NH_4–N consumption (C_A) in bulk and root-associated soils under ryegrass

Treatment[a]	Soil	Time (h)	NH_4–N (mg N kg^{-1})	Atom% ^{15}N excess (%)	M or C_A (mg N kg^{-1} d^{-1})
Clipped	Bulk	2	6.6 ± 0.9	3.65 ± 1.88	3.04
		48	6.8 ± 1.5	1.53 ± 0.67	
Clipped	Root-associated	2	8.5 ± 1.3	5.94 ± 2.31	6.09
		48	10.3 ± 1.7	1.71 ± 0.67	
Unclipped	Bulk	2	5.4 ± 1.3	0.33 ± 0.33	0
+ Earthworm		48	3.5 ± 0.7	0.53 ± 0.53	
Unclipped	Root-associated	2	7.7 ± 2.2	1.93 ± 0.91	4.90
+ Earthworm		48	9.0 ± 2.9	0.63 ± 0.34	
Unclipped	Bulk	2	8.3 ± 1.8	0.25 ± 0.25	0
− Earthworm		48	7.9 ± 0.6	0.31 ± 0.28	
Unclipped	Root-associated	2	8.6 ± 1.2	2.13 ± 1.04	3.14
− Earthworm		48	6.1 ± 1.5	0.94 ± 0.58	

[a] See footnote under Table 1 for explanation of experimental treatments. Extractable NH_4–N and atom% ^{15}N excess data are mean values ± standard errors; $n = 5$. Mineralization and consumption rates were 0 when there was no dilution of ^{15}N in the NH_4–N pool between 2 and 48 h after the label was added.

unclipped plus earthworm than the unclipped minus earthworm treatment. It has been speculated that earthworms can increase plant growth by grazing on micro-organisms and stimulating N mineralization, by altering soil physical characteristics that enhance root growth (e.g. through burrowing), or by secreting plant growth hormones (Blair et al., 1995; Edwards and Bohlen, 1996).

Whalen et al. (1999) found that the N in dead earthworm tissues was rapidly cycled through microbial biomass and assimilated by ryegrass, and up to 70% of the N from earthworm tissues was incorporated in ryegrass shoots after 16 d. We added earthworms with a mass of 2 g (fresh weight, equivalent to about 0.4 g oven-dry weight) to each pot in the earthworm treatment. The endogeic *A. tuberculata* aestivates and eventually dies when the soil environment is inhospitable, so it is unlikely that the decline in earthworm numbers during this study was from earthworms escaping the pots. Earthworms contained about 110 mg N g^{-1} and only about 20% of them survived to harvest, so the estimated contribution from dead earthworm tissues was 35.2 mg N to pots in the earthworm treatment. Plants harvested after 98 and 100 d of growth in the unclipped plus earthworm treatment contained, on average, 33.9 mg N in shoots and 22.5 mg N in roots, whereas plants from the unclipped minus earthworm treatment contained 21.8 mg N in shoots and 18.4 mg N in roots, a difference of 16.2 mg N per plant. It seems possible that decomposed earthworms could have provided extra N for plant uptake in the unclipped plus earthworm treatment. Further work will be required to separate the effects of earthworms on N transformations due to their burrowing and grazing activities from N fluxes resulting from the turnover of earthworm tissues.

4.3. Gross N mineralization in bulk and root-associated soils with clipping and earthworm treatments

Gross NH_4–N consumption rates were similar to gross N mineralization rates, and these patterns differed when plants were clipped or grown in the presence of earthworms than when they were unclipped and grown in soil without earthworms. These rates were of the same magnitude as plant N uptake rates (1.5–5.8 mg N kg^{-1} d^{-1}) calculated from the increase in ^{15}N and weighted average atom% ^{15}N values of the NH_4–N pools between 2 and 48 h (Tables 2 and 6).

Gross N mineralization rates were higher in the root-associated than bulk soils under ryegrass, which is consistent with results reported by Norton and Firestone (1996) and Reydellet et al. (1997). The highest gross N mineralization rates were measured in root-associated soils of the clipped treatment, followed by the unclipped plus earthworm and unclipped minus earthworm treatments. Although both clipping of plant biomass and the presence of earthworms appeared to stimulate N mineralization, the mechanisms governing this process are unknown. It seems possible that clipping would alter photosynthate allocation, and the response to clipping may be to enhance root activity and/or turnover that could stimulate microbial activity and N cycling processes. Other researchers have found greater root exudation, root respiration and soil respiration in clipped than control plants (Holland et al., 1996; Mawdsley and Bardgett, 1997; Bardgett et al., 1998). Greater N mineralization in the unclipped treatment with earthworms than without earthworms may have resulted from direct earthworm–microbial interactions (e.g. grazing on micro-organisms), indirect earthworm–microbial interactions (e.g. alteration of soil physical and chemical properties), and/or N flux from earthworm tissues. Further research will be required to elucidate the mechanisms responsible for increasing gross N transformations under plants that are clipped or grown in the presence of earthworms.

Acknowledgements

Thanks are extended to Nan Ritchie for mass spectrometer analysis, and Kris Lippert, Brenda Patterson, Kirk

Myrold, Janna Carefoot and Sherry Foran for assistance with sample preparation. Two anonymous reviewers provided helpful suggestions on this manuscript. This work was supported by a grant from the US Department of Agriculture National Research Initiative Competitive Grants Program to DDM and PJB. Oregon Agricultural Experiment Station technical paper 11,799.

References

Anderson, J.M., 1988. Spatiotemporal effects of invertebrates on soil processes. Biology & Fertility of Soils 6, 216–227.

Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biology & Biochemistry 30, 1867–1878.

Bailey, L.D., 1976. Effects of temperature and root on denitrification in a soil. Canadian Journal of Soil Science 56, 79–87.

Beare, M.H., Parmelee, R.W., Hendrix, P.F., Cheng, W., Coleman, D.C., Crossley Jr, D.A., 1992. Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. Ecological Monographs 62, 569–591.

Blair, J.M., Parmelee, R.W., Lavelle, P., 1995. Influences of earthworm on biogeochemistry. In: Hendrix, P.F. (Ed.). Earthworm Ecology and Biogeography in North America. Lewis Publishers, Chelsea, pp. 127–158.

Bodelier, P.L.E., Wijlhuizen, A.G., Blom, C.W.P.M., Laanbroek, H.J., 1997. Effects of photoperiod on growth of and denitrification by *Pseudomonas chlororaphis* in the root zone of *Glyceria maxima*, studied in a gnotobiotic microcosm. Plant & Soil 190, 91–103.

Bohlen, P.J., Parmelee, R.W., McCartney, D.A., Edwards, C.A., 1997. Earthworms effects on carbon and nitrogen dynamics of surface litter in corn agroecosystems. Ecological Applications 7, 1341–1349.

Brooks, P.D., Stark, J.M., McInteer, B.B., Preston, T., 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Science Society of America Journal 53, 1707–1711.

Burket, J.Z., Dick, R.P., 1998. Microbial and soil parameters in relation to N mineralization in soils of diverse genesis under differing management systems. Biology & Fertility of Soils 27, 430–438.

Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworms. 3rd ed. Chapman and Hall, London.

Grayston, S.J., Vaughan, D., Jones, D., 1996. Rhizosphere carbon flows in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5, 29–56.

Hale, M.G., Moore, L.D., 1979. Factors affecting root exudation—II: 1970–1978. Advances in Agronomy 31, 93–124.

Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, M.K., 1994. Nitrogen mineralization, immobilization and nitrification. In: Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A. (Eds.). Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. Soil Science Society of America, pp. 985–1018.

Holland, J.N., Cheng, W., Crossley Jr, D.A., 1996. Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. Oecologia 107, 87–94.

Ingham, R.E., Trofymow, J.A., Ingham, E.R., Coleman, D.C., 1985. Interactions of bacteria, fungi and their nematode grazers: effects on nutrient cycling and plant growth. Ecological Monographs 55, 119–140.

Kirkham, D., Bartholomew, M.V., 1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Proceedings 18, 33–34.

Klemedtsson, L., Svensson, B.H., Rosswall, T., 1987. Dinitrogen and nitrous oxide produced by denitrification and nitrification in soil with and without barley plants. Plant & Soil 99, 303–319.

Mahmood, T., Ali, R., Malik, K.A., Shamsi, S.R.A., 1997. Denitrification with and without maize plants (*Zea mays* L.) under irrigated field conditions. Biology & Fertility of Soils 24, 323–328.

Mawdsley, J.L., Bardgett, R.D., 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the microbial population of an upland grassland soil. Biology & Fertility of Soils 24, 52–58.

McNaughton, S.J., Wallace, L.L., Coughenour, M.B., 1983. Plant adaptation in an ecosystem context: effects of defoliation, nitrogen and water on growth of an African C4 sedge. Ecology 64, 307–318.

Norton, J.M., Firestone, M.K., 1996. N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. Soil Biology & Biochemistry 28, 351–362.

Prade, K., Trolldenier, G., 1988. Effect of wheat roots on denitrification at varying soil air-filled porosity and organic carbon content. Biology & Fertility of Soils 7, 1–6.

Reydellet, I., Laurent, F., Oliver, R., Siband, P., Ganry, F., 1997. Quantification par méthode isotopique de l'effet de la rhisophère sur la minéralisation de l'azote (cas d'un sol ferrugineux tropical). Agronomie 320, 843–847.

SAS Institute, 1990. SAS Procedures Guide, Version 6. 3rd ed. SAS Institute, Cary.

Sims, G.K., Ellsworth, T.R., Mulvaney, R.L., 1995. Microscale determination of inorganic nitrogen in water and soil extracts. Communications in Soil Science & Plant Analysis 26, 303–316.

Smith, M.S., Tiedje, J.M., 1979. The effect of roots on soil denitrification. Soil Science Society of America Journal 43, 951–955.

Strickland, T.C., Sollins, P., 1987. Improved method for separating light- and heavy-fraction organic matter from soil. Soil Science Society of America Journal 51, 1390–1393.

Waller, D.A., Jones, C.G., 1989. Measuring herbivory. Ecological Entomology 14, 479–481.

Whalen, J.K., Parmelee, R.W., McCartney, D.A., VanArsdale, J.L., 1999. Movement of N from decomposing earthworm tissue to soil, microbial and plant N pools. Soil Biology & Biochemistry 31, 487–492.

Whalen, J.K., Bottomley, P.J., Myrold, D.D., 2000. Carbon and nitrogen mineralization from light- and heavy-fraction additions to soil. Soil Biology & Biochemistry 32, 1345–1352.