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Movement of N from decomposing earthworm tissue to soil, microbial and plant N pools

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Abstract

A microcosm experiment was made to determine the fate of nitrogen released from ^{15}N-labelled decomposing earthworms (*Lumbricus terrestris*) in soil in the presence or absence of ryegrass seedlings (*Lolium perenne*). Earthworm tissue (2.0% ^{15}N atom enriched) was added to each microcosm. Nitrogen movement from earthworm tissue to soil N [mineral N (NH_4-N + NO_3-N), dissolved organic N (DON) and organic N], microbial biomass N and plant shoot N pools was determined by destructive sampling at 1, 2, 4, 8 and 16 d. Earthworm tissues decomposed rapidly, and no tissue was visible after 4 d. Initially in pots without plants, most of the N from earthworm tissue was found in the organic N pool, however, as much as 55% of the N from decomposing earthworm tissue was incorporated into microbial biomass after 2 d. Much less of the N from earthworm tissue was transformed into DON and mineral N forms after 2 d. The DON and mineral N pools contained 13–18% and 4–7% of the N from earthworm tissue, respectively, from d 2 to 16. By the end of the experiment, N from earthworm tissue in the microbial biomass N pool declined to 29% while the amount of N from earthworm tissue in the organic N pool increased to 49%. The increase in the organic N may have resulted from the production of new organic compounds such as microbial by-products. In pots with plants, N from earthworm tissue was rapidly incorporated into microbial biomass, and by d 2, the microbial biomass N pool contained 40% of the N from earthworm tissue. Mineral N, DON and microbial biomass N concentrations were lower in pots with ryegrass seedlings compared to pots without plants, and after d 2 declined to almost undetectable amounts because of rapid plant uptake. Between 42–52% of the N from earthworm tissue was found in the organic N pool from d 1 to 8, and then declined to 19% by d 16. After 16 d, over 70% of the N added as earthworm tissue was incorporated into plant shoot biomass. Our results demonstrate that the movement of N from dead earthworm tissue into microbial biomass was extremely rapid, and in pots without plants, much of this N was transformed into organic N forms, while in pots with ryegrass, most of the N from earthworm tissue accumulated in ryegrass shoots.

1. Introduction

The decomposition of organic residues and mineralization of nitrogen are largely mediated by microorganisms; however, there is growing evidence that earthworms can significantly affect N mineralization rates in forest, grassland and agricultural ecosystems (Haimi and Huhta, 1990; James, 1991; Blair et al., 1995). Earthworms influence N mineralization indirectly, through the alteration of soil physical properties (water holding capacity, structure and aeration), fragmentation of organic material, and interactions with other soil biota (e.g. grazing on microorganisms), and directly through the release of N from metabolic processes and mortality (Lee, 1985). It is difficult to quantify the indirect effects of earthworms on N mineralization. However, estimates of the direct flux of N through earthworm biomass in agroecosystems have been made, ranging from 10–74 kg N ha^{-1} y^{-1} (Anderson, 1983; Christensen, 1987; U. Böström unpubl. PhD thesis, Swedish University of Agricultural Sciences, Uppsala, 1988; Parmelee and Crossley, 1988; Curry et al., 1995).

Research on the direct effects of earthworms on nitrogen availability has focused primarily on the N dynamics of earthworm excretion casts (Martin and Marinissen, 1993; Parkin and Berry, 1994). Little is

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known about the transformations of N released from earthworm tissue through mortality. Earthworm tissue contains about 10% N on a dry weight basis, and is rapidly decomposed at death. Christensen (1988) found that 50% of the N in dead earthworm tissue was mineralized in 7 d, while Satchell (1967) estimated that 70% of earthworm tissue N was mineralized in 10–20 d. The release of mineral N through mortality may be significant since earthworm biomass can turn over up to three times a year (Parmelee and Crossley, 1988). Christensen (1987) estimated that 90% of the annual contribution of N by earthworms to agroecosystems was derived from the decomposition of earthworm tissue. Edwards and Bohlen (1996) reported that estimates of the availability of N to plants from excretion and mortality ranged from 6–38% of crop N requirements. Both the quantity and quality of N released through earthworm mortality is of interest, since it may be readily available for microbial and plant uptake, or loss through leaching, volatilization and denitrification.

Our purpose was to determine the fate of N released from decomposing earthworm tissue in soil in the absence or presence of plants. We used an ^{15}N tracer to follow the transformations of N released from earthworm tissue to organic (^{15}N-microbial biomass N, ^{15}N-dissolved organic N) and inorganic ($^{15}NH_4$-$N + {}^{15}NO_3$-N) nitrogen pools in soil. We also examined the availability of this N to ryegrass seedlings.

2. Materials and methods

2.1. Soil

Soil used in this experiment was obtained from the A horizon (0–15 cm) of a fine, mixed, mesic Fragiudaulf soil of the Canfield series from established corn plots in Wooster, Ohio, U.S.A. The soil texture was silt loam (13.5% sand, 73.7% silt, 12.8% clay) with pH 6.3 and contained 2.3% C and 0.19% N. Further information on the properties of the soil used in this experiment has been reported (Bohlen and Edwards, 1995).

2.2. Experimental design

Lumbricus terrestris L. juveniles were placed in containers with 150 g of soil and 0.5 g each of crushed ^{15}N-labelled ryegrass leaves (11 at% ^{15}N enriched) and ^{15}N-labelled soybean leaves (12 at% ^{15}N enriched) to incorporate the ^{15}N tracer into their tissues. After 6 weeks, the earthworms had reached an average of 2.0 at% ^{15}N enrichment. Earthworms were placed on wet filter paper for 24 h to void their guts and then were killed by freezing. Approximately 1.0 ± 0.01 g (fresh weight; dry weight was 17% of fresh weight) of earthworm tissue was added below the soil surface to pots (pot volume = 120 ml). Small cores of soil were removed at a 4 cm depth at three randomly selected locations in each pot, about one-third of the earthworm tissue was placed in each hole, and the soil was replaced. Each pot contained either a) 80 g (oven-dry basis) of air-dried (48 h) sieved (< 2 mm) soil or b) 80 g (oven-dry basis) of air-dried (48 h) sieved (< 2 mm) soil plus six *Lolium perenne* L. seedlings (planted 3 weeks earlier). The pots had no drains in order to prevent N loss through leaching. All pots (with or without plants) were maintained in a greenhouse (mean daily temperature ranged from 20–27°C) to facilitate plant growth during the 3 weeks prior to the beginning of the study, and maintained at 62–76% of field capacity. After the addition of earthworm tissue, the pots were returned to the greenhouse for the duration of the study. Movement of earthworm tissue N into other N pools was determined by destructively sampling five replicates of the two treatments at 1, 2, 4, 8 and 16 d after the beginning of the experiment.

2.3. Nitrogen analysis

Fresh soil was sieved (< 2 mm) and 20 g of soil was immediately analyzed to determine mineral N and microbial biomass N concentrations. The remaining soil was oven-dried at 60°C for 48 h, and ground in preparation for total N and ^{15}N analysis. Mineral N (NH_4-$N + NO_3$-N) was determined in 0.5 M K_2SO_4 soil extracts (1:5 soil:extractant). NH_4-N was determined colorimetrically using the modified indophenol blue technique (Sims et al., 1995) and measured on a Bio-Tek EL211sx automated microplate reader. NO_3-N was measured using the cadmium reduction–diazotization method with a Lachat AE flow-injection autoanalyser. Dissolved organic N (DON) was calculated as the difference between the NO_3-N concentration in an alkaline persulfate digestion of the soil extract and the mineral N concentration of the initial soil extract (Cabrera and Beare, 1993). Microbial biomass N (MBN) was determined using the chloroform fumigation-direct extraction method followed by persulfate digestion and calculated as (Brookes et al., 1985; Joergensen and Mueller, 1996)

[(total extractable N after fumigation
− total extractable N before fumigation)/0.54]

The ^{15}N concentration in the mineral N ($^{15}NH_4$-$N + {}^{15}NO_3$-N) pool was determined using a modification of the acid diffusion method described by Brooks et al. (1989). Disks (0.5 cm dia) 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 $KHSO_4$. 10 ml of the soil extracts (containing 40–100 µg mineral N) were pipetted into acid-washed specimen cups, and 0.4 g of Devarda's alloy, 0.2 g of MgO and two filter disks sealed in Teflon tape were added quickly to each cup before the cup was sealed. ^{15}N-DON and ^{15}N-MBN were determined by pipetting 20 ml of DON and MBN persulfate digests (containing 40–100 µg N) into acid-washed specimen cups, and adding 0.2 g of Devarda's alloy, 1 ml of 5 M NaOH and two filter disks sealed in Teflon tape. The cups were swirled vigorously once or twice daily for 7 d, after which the disks were removed and desiccated over concentrated H_2SO_4. Ryegrass shoots were harvested, oven-dried, ground and analyzed for total N and at% ^{15}N excess. Samples of the earthworm tissue added to each pot were also oven-dried, ground and analyzed for total N and at% ^{15}N excess. Nitrogen isotopic ratios (^{15}N/^{14}N) in soil extracts and bulk soil, ryegrass shoots, and earthworm tissue were determined using a Carlo-Erba C and N analyzer coupled with a Europa Tracermass spectrophotometer (Michigan State University).

2.4. Statistical analysis

Data were log transformed to equalize variance and evaluated statistically by two-factor ANOVA in a general linear model (GLM) using SAS software (SAS Institute Inc., 1990). Since the interaction between treatment (pots without plants, pots with plants) and sampling date was statistically significant ($p < 0.0001$), means for each treatment at each date were adjusted for multiple comparisons and analyzed statistically using a Tukey–Kramer test at the 95% confidence level.

3. Results

Recovery of the ^{15}N tracer added as earthworm tissue was determined on a per pot basis, and while recovery was somewhat variable, 97 ± 6% of the added ^{15}N was recovered; hence, we presumed that losses due to denitrification and volatilization were negligible. The mean total N content of *L. terrestris* juveniles was 7.9% N on a dry mass basis, with an average enrichment of 2.0 at% ^{15}N. The average quantity of ^{15}N added to each pot was 270 µg ^{15}N. Earthworm tissue decomposed rapidly, and could not be visibly detected after 4 d.

The mean changes in the ^{15}N concentration (µg ^{15}N) of soil, microbial biomass, and plant pools at five sampling dates during earthworm decomposition were determined (Figs. 1 and 2). The percentage of ^{15}N in soil, microbial biomass, and plant N pools derived from ^{15}N-labelled earthworm tissue was used as a measure of the proportion of total N that was transformed from earthworm tissue during the experiment

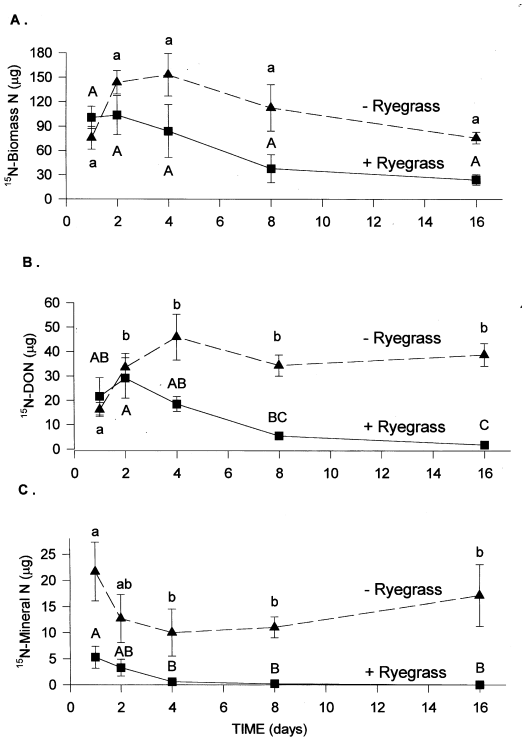

Fig. 1. Changes in (A) ^{15}N-microbial biomass N, (B) ^{15}N-DON, and (C) ^{15}N-mineral N concentrations (µg total ^{15}N in each soil pool) during earthworm decomposition (mean ± standard error) in pots with ryegrass (■) and pots without ryegrass (▲). Means with the same letter are not significantly different at $p < 0.05$ (Tukey–Kramer test), where capital letters denote differences between sampling dates for pots with plants, and small letters denote differences between sampling dates for pots without plants.

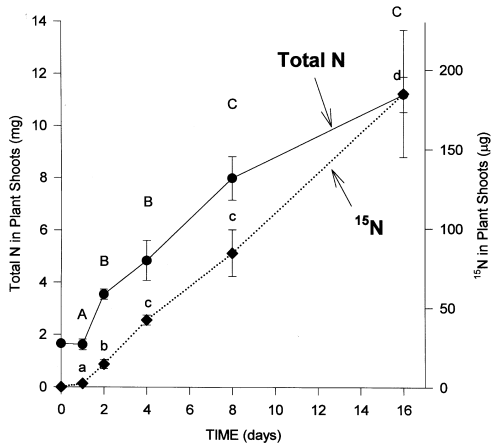

Fig. 2. Changes in total N (●) and ^{15}N (◆) concentrations in ryegrass shoot biomass during earthworm decomposition (mean ± standard error). Means with the same letter are not significantly different at $p < 0.05$ (Tukey–Kramer test) where capital letters denote differences between sampling dates for total N concentrations (mg total N) and small letters denote differences between sampling dates for ^{15}N concentrations (µg total ^{15}N) in ryegrass shoots.

Table 1
Proportion of ^{15}N from decomposing earthworm tissue present in microbial biomass ^{15}N and soil ^{15}N pools, and plant ^{15}N during earthworm decomposition expressed as a percentage of added ^{15}N

	Pots without plants				Pots with plants				
Sampling day	^{15}N-MBN (%)	^{15}N-DON (%)	^{15}N-mineral N (%)	^{15}N-organic N (%)	^{15}N-MBN (%)	^{15}N-DON (%)	^{15}N-mineral N (%)	^{15}N-organic N (%)	^{15}N-plant N (%)
1	29	6	8	57	38	8	2	51	1
2	55	13	5	27	40	11	1	42	6
4	58	18	4	20	32	7	< 1	45	16
8	43	13	4	40	14	2	< 1	52	32
16	29	15	7	49	9	1	< 1	19	71

(Table 1). In a preliminary experiment, the chloroform fumigation-direct extraction method for determination of microbial biomass N also extracted N from lysed cells of earthworm biomass. When 0.5 g of earthworm tissue was mixed with 12 g of sterilized sand, 3% of the ^{15}N was extractable with 0.5 M K_2SO_4, while an additional 14% was released by chloroform fumigation and K_2SO_4 extraction. During the first 4 d of the experiment, therefore, our ^{15}N-microbial biomass N pool likely also contained a small amount of N from earthworm biomass. ^{15}N-organic N was not directly measured, but calculated as the difference between the ^{15}N measured in bulk soil minus ^{15}N recovered in the extractable soil N pools (^{15}N-mineral N, ^{15}N-DON and ^{15}N-MBN) and plant shoots. The ^{15}N-organic N pool also includes plant roots, which were not separated from bulk soil.

3.1. N transformations in pots without plants

Of the added ^{15}N 29% was incorporated into microbial biomass N (MBN) after 1 d, and by d 4 over half of the added ^{15}N was present in the ^{15}N-MBN pool (Table 1). Although the ^{15}N-MBN concentration declined during the last two sampling dates, this change was not statistically significant (Fig. 1(A)). The amount of added ^{15}N in the ^{15}N-MBN pool declined to 29% by d 16 (Table 1).

The dissolved organic nitrogen (DON) pool increased significantly ($P < 0.05$) between d 1 and 2 from about 16.2 to 33.5 μg of ^{15}N-DON (Fig. 1(B)). The quantity of ^{15}N-DON did not change significantly after d 2, and 13–18% of the added ^{15}N remained in the ^{15}N-DON pool (Table 1). There was an increase in the ^{15}N-mineral N pool after 1 d of decomposition, however the ^{15}N-mineral N concentration declined significantly ($P < 0.05$) by d 4 (Fig. 1(C)). The ^{15}N-mineral N pool contained 4–8% of the ^{15}N released from earthworm tissue during decomposition (Table 1).

The greatest proportion of added ^{15}N after 1 d was found in the ^{15}N-organic N pool (Table 1). After 4 d of decomposition, the concentration of ^{15}N-organic N declined to 20% of the added ^{15}N. However, the quantity of ^{15}N in this pool increased to 40–49% of the added ^{15}N during the last two sampling dates (Table 1).

3.2. N transformations in pots with plants

The greatest proportion of extractable soil N (38%) was in the ^{15}N-MBN pool after 1 d of earthworm decomposition (Table 1). The percentage of ^{15}N in MBN then declined to 9% by d 16 (Table 1). However, the concentration of ^{15}N-MBN did not change significantly during earthworm decomposition (Fig. 1(A)). Although the concentration of ^{15}N-MBN tended to be smaller in pots with ryegrass than in pots without plants, the difference was found to be significant only at $p < 0.10$ (Tukey–Kramer test) (Fig. 1(A)).

The DON concentration in pots with ryegrass was greatest on d 2 and then decreased to very low concentrations by the end of the experiment (Fig. 1(B)). Of the added ^{15}N 11% was transformed to ^{15}N-DON by d 2, decreasing to 1–2% by d 8 and 16 (Table 1). The ^{15}N-DON concentration in pots with ryegrass seedlings was significantly lower than in pots without plants after d 4 of the experiment (Fig. 1(B)).

The ^{15}N-mineral N pool constituted a relatively small proportion (2%) of the added ^{15}N after 1 d of decomposition and declined to very low amounts (< 1%) after d 4 (Table 1, Fig. 1(C)). Concentrations of ^{15}N-mineral N were significantly lower in pots with plants compared to pots without plants (Fig. 1(C)).

Most of the added ^{15}N was initially in the ^{15}N-organic soil N pool, which contained 51% of the added ^{15}N after 1 d (Table 1). The quantity of ^{15}N-organic N remained relatively constant during the first 8 d of earthworm decomposition, however, between d 8 and 16, the proportion of added ^{15}N declined from 52% to 19% in the ^{15}N-organic N pool (Table 1).

The ryegrass seedlings had signs of N stress (yellowish leaves) before the addition of dead earthworm tissue. The percentage N in shoot tissue increased from 1% N to about 3% N by d 16 and the average shoot

mass increased from 0.1 g (dry weight) to nearly 0.4 g (dw). The plants were visibly greener by the end of the experiment The total N and ^{15}N concentrations in ryegrass shoots increased significantly by d 2 (Fig. 2). The proportion of added ^{15}N that accumulated in the ^{15}N-plant N pool was substantial, and approximately doubled each sampling date from d 4 to 16 (Table 1). Of the added ^{15}N 71% was incorporated into ryegrass shoots by the end of the experiment.

4. Discussion

Few studies have examined the fate of N released from earthworm tissue through mortality, and, as far as we are aware, this is the first study which examines the availability of this N to plants. By labelling the earthworm tissue with an ^{15}N tracer, we were able to quantify the transformations of N from earthworm tissue to soil N, microbial biomass N and plant shoot N pools during earthworm decomposition.

Earthworm tissue decomposed quickly, and N from earthworm tissue was rapidly incorporated into microbial biomass in pots without plants during the first 4 d of the experiment. We hypothesize that the decline in the ^{15}N-MBN pool and the increase in non-extractable ^{15}N-organic N pool after d 4 resulted from the release of organic N compounds in microbial by-products. These compounds may be non-extractable due to chemical or physical stabilization (Voroney et al., 1991). The ^{15}N-organic N pool may have contained undecomposed earthworm tissue, including chaetae and cuticle, as well as newly-formed non-extractable ^{15}N-organic N. In pots with ryegrass seedlings, the decline of microbial biomass N after the d 2 of the experiment could not be accounted for by an increase in the concentration of the organic N pool, which included non-extractable soil N and ryegrass root N compounds, since the proportion of N added in this pool was relatively unchanged during the first 8 d of the experiment. However, the concentration of ^{15}N-organic N declined by more than 60% between d 8 and 16, while the ^{15}N-plant N concentration in ryegrass shoots increased by more than 50%, which suggests that ^{15}N from the ^{15}N-organic N pool was incorporated into ryegrass shoots during this time.

Studies of N mineralization from dead earthworm tissue conducted by Christensen (1987, 1988); Satchell (1967) found that 70% of earthworm tissue N was converted to NO_3-N after 10–20 d. Interestingly, less than 10% of the ^{15}N added was found in the ^{15}N-mineral N in pots without plants after 16 d. The proportion of ^{15}N added that was transformed to ^{15}N-mineral N and ^{15}N-DON was much lower than that in the ^{15}N-MBN and ^{15}N-organic N pools throughout the experiment. Neither the ^{15}N-mineral N nor the ^{15}N-DON pools changed significantly after d 2, which suggests that either these pools were relatively stable during earthworm decomposition or that mineral N and DON were recycled through microbial biomass.

Our results clearly demonstrate that N from decomposing earthworm tissue is readily available for plant uptake and growth. In pots with ryegrass, over 70% of the ^{15}N added was incorporated into plant shoots after 16 d. Since we only measured ^{15}N that was taken up into plant shoot tissue and not in roots, it is likely that the uptake of N from earthworm tissue by plants was greater than 70% during the experiment. In pots with ryegrass, ^{15}N-mineral N and ^{15}N-DON pools were likely available sources of N for plants and were rapidly depleted by uptake. There is growing evidence that the roots of cultivated plants can successfully compete for soil organic N with microbial communities, and that plants may derive a significant proportion of their N requirement from soil organic N in some ecosystems (Jones and Darrah, 1993; Watson and Miller, 1996).

Earthworms have often been credited with increasing plant growth and yield through a variety of mechanisms, including altering soil physical characteristics, accelerating nutrient cycling, and interacting with microorganisms to promote the release of enzymes and possibly phytohoromones (Edwards and Bohlen, 1996). The effects of earthworms on plant growth are generally examined experimentally in soil microcosms. However, there is often a decrease in earthworm biomass during microcosm experiments due to weight loss and mortality (Cortez et al., 1989; Haimi and Boucelham, 1991; Scheu, 1993; Bohlen and Edwards, 1995). Since dead earthworm tissue provides a readily-available source of N for plant growth, caution should be exercised in separating the effect of earthworm activity on plant growth from increases in plant-available nutrients due to earthworm mortality.

In the field, earthworm biomass may turn over between one to three times per year (U. Böström, 1988; Parmelee and Crossley, 1988). Depending on the size of the earthworm population, a substantial amount of N could be released from earthworm biomass each year. Christensen (1988) estimated that 90% of the direct flux of N through earthworms in some Danish agroecosystems was due to mineralization of dead earthworm tissue. A significant proportion of mineralized earthworm tissue may be available for plant uptake.

We have biomass measurements of earthworm populations in corn agroecosystems which have received longer-term amendments of either straw-packed cow manure or NH_4NO_3 fertilizer at a rate of 150 kg N ha^{-1} y^{-1} (J. K. Whalen et al., unpublished). During 1994–1996, the mean monthly earthworm biomass in the manure and inorganic fertilizer plots was

9.93 and 6.70 g ash-free dry weight m^{-2}, respectively. The production-to-biomass (P-to-B) ratios of earthworm populations in our corn agroecosystems range from 2–4, indicating that earthworm biomass turns over between two and four times each year (J. K. Whalen and R. W. Parmelee, unpublished). Since earthworm tissue contains 7.9% N and earthworm biomass in our corn agroecosystems turns over, on average, 3 times each year, then mortality and decomposition of earthworms contributes 23.5 kg N ha^{-1} y^{-1} in the manure-amended agroecosystem and 15.9 kg N ha^{-1} y^{-1} in the inorganic fertilizer-treated agroecosystem. Total N uptake by the corn crop in these agroecosystems is about 90 kg N ha^{-1} y^{-1} (Stinner et al., 1997). If we assume that all of the N from earthworm tissue becomes available for uptake by plants, then the turnover of earthworm biomass in the manure-amended and inorganic fertilizer-treated agroecosystems can contribute 26% and 18%, respectively, of the N required by the corn crop. Our results indicate that, through their mortality, earthworms have a significant direct effect on nitrogen cycling in our corn agroecosystems.

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