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Quantification of nitrogen excretion rates for three lumbricid earthworms using ^{15}N

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Abstract Nitrogen excretion rates of ^{15}N-labeled earthworms and contributions of ^{15}N excretion products to organic (dissolved organic N) and inorganic (NH_4-N, NO_3-N) soil N pools were determined at 10 °C and 18 °C under laboratory conditions. Juvenile and adult *Lumbricus terrestris* L., pre-clitellate and adult *Aporrectodea tuberculata* (Eisen), and adult *Lumbricus rubellus* (Hoffmeister) were labeled with ^{15}N by providing earthworms with ^{15}N-labeled organic substrates for 5–6 weeks. The quantity of ^{15}N excreted in unlabeled soil was measured after 48 h, and daily N excretion rates were calculated. N excretion rates ranged from 274.4 to 744 μg N g^{-1} earthworm fresh weight day^{-1}, with a daily turnover of 0.3–0.9% of earthworm tissue N. The N excretion rates of juvenile *L. terrestris* were significantly lower than adult *L. terrestris*, and there was no difference in the N excretion rates of preclitellate and adult *A. tuberculata*. Extractable N pools, particularly NH_4-N, were greater in soils incubated with earthworms for 48 h than soils incubated without earthworms. Between 13 and 40% of excreted ^{15}N was found in the ^{15}N-mineral N (NH_4-N + NO_3-N) pool, and 13–23% was in the ^{15}N-DON pool. Other fates of excreted ^{15}N may have been incorporation in microbial biomass, chemical or physical protection in non-extractable N forms, or gaseous N losses. Earthworm excretion rates were combined with earthworm biomass measurements to estimate N flux from earthworm populations through excretion. Annual earthworm excretion was estimated at 41.5 kg N ha^{-1} in an inorganically-fertilized corn agroecosystem, and was equivalent to 22% of crop N uptake. Our results suggest that the earthworms could contribute significantly to N cycling in corn agroecosystems through excretion processes.

Key words Earthworm · Nitrogen excretion rate · ^{15}N · Agroecosystem · Stable isotopes

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Introduction

It is well established that earthworms can alter the forms and availability of N in terrestrial ecosystems through their modification of soil physical, chemical and biological properties (Lee 1985; Edwards and Bohlen 1996). Earthworms may influence microbially-mediated N transformations such as mineralization, nitrification and denitrification through their interactions with soil biota (Blair et al. 1995). In addition, earthworms contribute to the soil mineral N pool directly through the excretion of nitrogenous compounds in their mucus and urine. Earthworm mucus consists of mucoproteins which are secreted through gland cells in the epidermis to prevent desiccation, facilitate respiration, and provide lubrication for movement through the soil, and approximately one-half of daily N loss is from mucus excretion (Needham 1957). Earthworm urine is composed of ammonia, urea, and possibly uric acid and allantoin (Edwards and Bohlen 1996). While urea is excreted on the body surface through the nephridia, ammonia is thought to be excreted primarily through the gut with cast materials (Tillinghast 1967), which may explain why high concentrations of ammonia are often measured in fresh earthworm casts (Blair et al. 1995).

The excretion of nitrogenous compounds by earthworms has the potential to contribute significantly to nutrient cycling in terrestrial ecosystems. In agroecosystems, the quantity of N released from earthworm biomass through excretion and mortality has been estimated to range from 10 to 74 kg N ha^{-1} $year^{-1}$ (Anderson 1983; Christensen 1987; Parmelee and Crossley

1988; Curry et al. 1995; U. Böström, unpublished). In these studies, as much as 50% of the annual N flux through earthworms was from excretion of nitrogenous compounds. Satchell (1963) calculated that earthworms contributed over 30 kg N ha^{-1} $year^{-1}$ through N excretion alone in a forest ecosystem. While it appears that N excretion by earthworms can be substantial, most estimates are based on excretion rates for a few species under laboratory conditions.

Laboratory measurements of earthworm excretion rates have typically used the method of Needham (1957), who placed earthworms in flasks containing a small volume of water at 23 °C and analyzed the N content of the water after 24 h. Daily excretion (urine and mucus) of N by *Lumbricus terrestris* L. and *Allolobophora caliginosa* feeding on elm leaves were 268.8 μg N g^{-1} (fresh weight) day^{-1} and 87.5 μg N g^{-1} day^{-1}, respectively. Tillinghast (1967) measured excretion rates ranging from 60 to 160 μg N g^{-1} day^{-1} for *L. terrestris* with this method, while El-Duweini and Ghabbour (1971), who used paraffin oil instead of water, determined that 127 μg N g^{-1} day^{-1} was excreted in urine and mucus by *A. caliginosa* between 19 and 22 °C. Christensen (1987) found that fasting *A. caliginosa* incubated in small amounts of water at 6, 12 and 21 °C excreted 20–40 μg N g^{-1} day^{-1} in urine, and N excretion rates were greater with an increase in temperature ($Q_{10}=1.5$). The use of N excretion rates from fasting earthworms in a soil-free environment to estimate N flux through earthworm populations, however, is questionable.

A newer technique to estimate the N excretion rates of earthworms uses stable isotopes (Barois et al. 1987; Hameed et al. 1994; Curry et al. 1995). Typically, earthworms are provided with ^{15}N-labeled organic substrates for several weeks to incorporate ^{15}N into their tissues, and are then transferred to unlabeled soil where the rate of ^{15}N depletion from earthworm tissue through urine and mucus production is measured. Estimates of the turnover of ^{15}N from earthworm tissue range from 1 to 1.7% of earthworm N per day for *L. terrestris* (Hameed et al. 1994; Curry et al. 1995) and *Pontoscolex corethrurus* (Barois et al. 1987), and were influenced slightly by temperature ($Q_{10}=1.65$) (Hameed et al. 1994). The contribution of earthworms to N mineralization in a winter cereal agroecosystem through excretion was calculated from ^{15}N turnover data was between 29 and 36 kg N ha^{-1} $year^{-1}$ (Curry et al., 1995). However, this method may underestimate N excretion because ^{15}N elimination from earthworm tissue proceeds logarithmically (Barois et al. 1987), which suggests that the ^{15}N in earthworm tissue is present in both labile (rapidly eliminated) and recalcitrant (relatively stable) forms. N excretion rates may be more accurately quantified in relatively short-term (i.e. less than 1 week) experiments.

The objectives of this investigation were: (1) to quantify N excretion rates using ^{15}N for adults and juveniles of *L. terrestris*, *Lumbricus rubellus* and *Aporrectodea tuberculata*, (2) to determine the effect of soil temperature on excretion rates, and (3) to assess the contribution of excreted N to organic (dissolved organic N) and inorganic (NO_3-N, NH_4-N) N pools in soil.

Materials and methods

Soil and earthworm sampling

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, USA. The soil texture was silt loam (135 g sand kg^{-1}, 737 g silt kg^{-1}, 12.8 g clay kg^{-1}) with 23 g organic C kg^{-1}, 1.9 g N kg^{-1} and pH 6.3. Earthworms were collected from a site adjacent to the corn plots by hand-sorting and formalin extraction. Earthworms were separated into age classes on the basis of clitellum development and were categorized as juveniles, pre-clitellate adults (clitellum present but not fully developed) and clitellate adults (fully developed clitellum). Sexually mature specimens were identified to the species level using the key of Schwert (1990).

Experimental design

Earthworms were provided with ^{15}N-labeled organic substrates for 5–6 weeks to enrich their tissues with ^{15}N. Individuals of *L. terrestris* and *L. rubellus* were placed in containers with 150 g (dry weight basis) of soil and 0.5 g each of ^{15}N-labeled ryegrass leaves (11 atom% ^{15}N) and ^{15}N-labeled soybean leaves (12 atom% ^{15}N), while individuals of *A. tuberculata* were placed in containers with 75 g of soil and 0.25 g each of ^{15}N-labeled manure (4 atom% ^{15}N) and ^{15}N-labeled soybean leaves. Ryegrass and soybean leaves were enriched with ^{15}N by spraying about 500 g of finely-crushed leaves with 100 ml of solution containing 10 mg N l^{-1} from ^{15}N-labeled $(NH_4)_2SO_4$ (99 atom% ^{15}N) and allowing it to decompose at room temperature for 3 months. The ^{15}N-labeled manure was prepared using an in vitro rumen fermentation technique, and has been described by Bohlen et al. (1999). After 5–6 weeks, ^{15}N enrichment in earthworm tissues provided with ^{15}N-labeled organic substrates was between 0.4 and 2.5 atom% ^{15}N excess.

Earthworms were placed on wet filter paper for 24 h to clear the ^{15}N-labeled material from their guts, and fresh weights were determined. At least ten randomly selected individuals of each species and age category were sacrificed by freezing (−20 °C) and then freeze-dried, ground and analyzed to determine initial ^{15}N enrichment in earthworm tissue. The remaining earthworms were placed in 120 cm^3 specimen containers containing 20 g (dry weight basis) of unlabeled, sieved (<2 mm) soil and 0.25 g of unlabeled soybean leaves moistened to between 20 and 25% gravimetric soil water content [80 g of unlabeled, sieved (<2 mm) soil and 1 g of unlabeled soybean leaves for *L. terrestris* individuals]. The containers were incubated in the dark in environmentally controlled chambers set at 8–12 °C or 16–20 °C to simulate seasonal diurnal flux in soil temperature. At least seven replicates were incubated at each temperature for each earthworm species and age category analyzed. At least four containers containing unlabeled soil and soybean leaves without earthworms were also incubated at each temperature to serve as a control. Earthworms were removed from the soil after 48 h, killed immediately by freezing at −20 °C, then freeze-dried and ground for N analysis.

N excretion

Soil was oven-dried, ground and analyzed for total N and atom% ^{15}N excess (atom% ^{15}N in enriched samples – atom% ^{15}N in background samples) to quantify ^{15}N from earthworm excretion

products (casts, mucus, and urine). The soil from containers with unlabeled soil and soybean leaves that were incubated without earthworms for 48 h were used to establish background ^{15}N levels. N excretion was expressed as μg ^{15}N excreted g^{-1} earthworm (fresh weight) day^{-1}. Since only a small proportion of total earthworm N was labeled with ^{15}N, total N excreted (μg N g^{-1} earthworm day^{-1}) was calculated by dividing ^{15}N excreted g^{-1} earthworm day^{-1} by the initial atom% ^{15}N excess in earthworm tissue ($t=0$).

Tissue ^{15}N enrichment was determined by analyzing the freeze-dried, ground earthworm tissues for total N and atom% ^{15}N excess. Tissue ^{15}N enrichment was corrected for unlabeled material in the gut, since earthworms did not void their gut contents at the end of experiment, based on a linear regression between earthworm weight (live weight, gut not cleared) (EW) and the mass of gut contents (dry weight basis) (GW). For *L. terrestris*, the relationship between earthworm size and the mass of material in the gut was described by the equation:

$$GW = 0.1319 \times EW - 0.0337 (R^2 = 0.82, n = 10, p < 0.0001 \quad (1)$$

while for *L. rubellus*, the equation was:

$$GW = 0.0750 \times EW + 0.599 (R^2 = 0.86, n = 10, p < 0.0001 \quad (2)$$

and for *A. tuberculata*, the equation was:

$$GW = 0.2123 \times EW - 0.0654 (R^2 = 0.81, n = 10, p < 0.0001 \quad (3)$$

Soils were analyzed immediately after removal of the ^{15}N-labeled earthworms to determine the contribution of excretion products to organic (dissolved organic N) and inorganic (NO_3-N, NH_4-N) N pools. Mineral N (NO_3-N and NH_4-N) was determined in 0.5 M K_2SO_4 soil extracts (1:5 soil:extractant). NH_4-N and NO_3-N were measured using the phenate and cadmium reduction/diazotization methods with a Lachat AE flow-injection analyzer. 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).

The ^{15}N concentrations in the mineral N ($^{15}NH_4$-N + $^{15}NO_3$-N) and organic N (^{15}N-DON) pools were determined for six replicate soil extracts of each earthworm species, age class and soil temperature examined using a modification of the acid diffusion method described by Brooks et al. (1989). Disks 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 ml of 2.5 M $KHSO_4$. Ten ml of the soil extracts (containing 40–100 μg 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. ^{15}N-DON was determined by pipetting 20 ml of DON persulfate digest (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 days, after which the disks were removed and desiccated over concentrated H_2SO_4. N isotopic ratios (^{15}N:^{14}N) in soil extracts, bulk soil, and earthworm tissues were determined using a Carlo-Erba C and N analyzer coupled with a Europa Tracermass spectrophotometer (Michigan State University).

Statistical analysis

Data were log transformed to reduce variability and evaluated statistically by two-factor ANOVA in a general linear model (GLM) using SAS software (SAS Institute 1990). The effect of earthworm species, age class, soil temperature and the interaction of these variables on N excretion rates and extractable N concentrations were evaluated. Variables that significantly affected N excretion rates and extractable N concentrations were adjusted for multiple comparisons and analyzed statistically using a Tukey-Kramer test at the 95% confidence level. Values presented in the tables are untransformed means (± standard errors).

Results

Earthworm N excretion rates

Earthworms achieved ^{15}N enrichment of 0.4–2.5% ^{15}N above background levels after 5–6 weeks of processing ^{15}N-labeled organic substrates, and mean atom% ^{15}N excess values for each species and age class are presented in Table 1. However, there was considerable individual variation in tissue ^{15}N enrichment. For example, tissue ^{15}N enrichment of the adult *A. tuberculata* examined in the study ranged from 1.12 to 1.96 atom% ^{15}N excess, with a mean of 1.64 ± 0.07% ^{15}N (± standard error, $n=14$). Therefore, N excretion rates were determined on an individual earthworm basis by dividing the quantity of ^{15}N excreted g^{-1} earthworm day^{-1} by the initial atom% ^{15}N excess of earthworm tissue. Initial atom% ^{15}N excess in earthworm tissue was estimated for individuals by adding ^{15}N excreted during the 48 h incubation to ^{15}N remaining in earthworm tissues after 48 h.

The quantity of ^{15}N excreted in 48 h by earthworms ranged from 6.2 to 31.2 μg ^{15}N, and was lowest for adult *L. rubellus* (Table 1). The effects of temperature, earthworm species, and age class on the amount of ^{15}N

Table 1 Excretion of ^{15}N during 48 h incubation and N excretion rates for three lumbricid earthworm species. Mean values (±SE) followed by the same letter within a column are not statistically significantly different ($P<0.05$, Tukey-Kramer test)

Species	Age class	Temperature (°C)	Earthworm fresh weight (g)	Atom% ^{15}N excess (%)	^{15}N excreted in 48 h (μg ^{15}N)		N excretion rate (μg N g^{-1} fresh wt day^{-1})	
L. terrestris	Juvenile	10	1.98 ± 0.12	2.54 ± 0.15	31.2 ± 1.7	A	326.4 ± 24.6	BC
L. terrestris	Juvenile	18	2.03 ± 0.09	2.57 ± 0.11	27.7 ± 1.1	AB	278.4 ± 21.2	C
L. terrestris	Adult	10	4.98 ± 0.37	0.37 ± 0.09	17.1 ± 2.1	C	534.6 ± 62.8	A
L. terrestris	Adult	18	4.87 ± 0.25	0.36 ± 0.04	19.5 ± 2.9	BC	531.9 ± 67.0	A
A. tuberculata	Pre-clitellate	10	0.84 ± 0.04	1.55 ± 0.10	17.7 ± 2.2	C	624.5 ± 45.0	A
A. tuberculata	Pre-clitellate	18	0.85 ± 0.04	1.52 ± 0.08	14.9 ± 1.3	CD	543.1 ± 42.3	A
A. tuberculata	Adult	10	0.93 ± 0.05	1.78 ± 0.08	18.6 ± 3.3	BC	495.9 ± 75.1	AB
A. tuberculata	Adult	18	1.06 ± 0.04	1.56 ± 0.12	28.2 ± 3.9	AB	744.0 ± 62.3	A
L. rubellus	Adult	10	0.70 ± 0.10	0.82 ± 0.12	6.2 ± 0.7	D	543.1 ± 40.0	A
L. rubellus	Adult	18	0.55 ± 0.03	1.61 ± 0.10	9.4 ± 0.8	CD	613.6 ± 54.0	A

excreted were evaluated using a general linear model ($P<0.001$, $R^2=0.59$, C.V. = 32.3). Excreted ^{15}N was influenced by the species and age of earthworms, but not by soil temperature although the interaction between the main effects was significant ($P<0.02$). Juveniles of *L. terrestris* excreted significantly more ^{15}N than adult *L. terrestris* at 10 °C, although there was no difference in ^{15}N excretion at 18 °C (Table 1). For *A. tuberculata*, ^{15}N excretion was significantly greater for adult than for pre-clitellate individuals at 18 °C, although there was no difference in ^{15}N excretion at 10 °C (Table 1). The amount of ^{15}N excreted by earthworm species within the same age class was not affected significantly by soil temperature (Table 1).

N excretion rates, expressed as μg N excreted g^{-1} earthworm fresh weight day^{-1}, ranged from 278.4 to 744 μg N g^{-1} day^{-1} for *L. terrestris*, *A. tuberculata* and *L. rubellus* (Table 1). The effects of temperature, species and age class on the N excretion rate were evaluated using a general linear model ($P<0.001$, $R^2=0.50$, C.V. = 32.4), and N excretion rates were affected significantly by the age of earthworms ($P<0.001$) and the interaction between the main effects ($P<0.01$). N excretion rates were lower for juvenile *L. terrestris* than all other age classes of earthworm species examined (Table 1). N excretion rates of pre-clitellate and adult *A. tuberculata* did not differ significantly, and there was no difference in N excretion rates for adults of *L. terrestris*, *A. tuberculata* and *L. rubellus* (Table 1).

Influence of earthworms on soil N pools

Extractable soil N was measured after 48 h on soils incubated with and without earthworms, and the net change in soil N concentrations in the presence of earthworms was calculated. The amount of extractable N in soils incubated with earthworm species within an age class was not affected significantly by soil temperature, and data were pooled across temperatures for each age class of the three species studied.

Soils incubated with earthworms had higher NH_4-N and DON concentrations than soils without earthworms for all species examined, and NO_3-N concentrations were greater for all age classes of earthworm species except pre-clitellate *A. tuberculata* (Table 2). Increases in NH_4-N concentrations ranged from 4.2 to 27.3 μg N g^{-1}, and were significantly higher for adults of *A. tuberculata* and *L. rubellus* than juveniles and adults of *L. terrestris* and pre-clitellates of *A. tuberculata* (Table 2). The increase in NO_3-N concentration was greatest in soils with adult *L. terrestris*, and the increase in nitrate levels with earthworms were between 0 and 5 μg N g^{-1} (Table 2). DON concentrations did not differ significantly among the species of earthworms examined, and increases in DON of soils incubated with earthworms ranged from 1.2 to 5.8 μg N g^{-1} (Table 2).

Because changes in soil N pools in the presence of earthworms may result from processes other than the excretion of nitrogeneous compounds, we analyzed soil ^{15}N pools to determine the quantities and fate of earthworm ^{15}N-labeled excretion products. The total amount of ^{15}N excreted in 48 h ranged from 7.8 to 29.4 μg ^{15}N, and was likely influenced by initial earthworm tissue ^{15}N enrichment (Table 3). Hence, we could not evaluate statistically any differences in the quantity of ^{15}N excreted and transformed to ^{15}N-mineral N and ^{15}N-DON after 48 h, but we were able to calculate the contribution of ^{15}N excretion products to these pools. Between 13 and 40% of excreted ^{15}N was in the ^{15}N-

Table 2 Net change in extractable N pools of soils incubated with earthworms for 48 h. Mean values (±SE) followed by the same letter within a column are not statistically significantly different ($P<0.05$, Tukey-Kramer test)

Species	Age class	NH_4-N (μg N g^{-1})		NO_3-N (μg N g^{-1})		DON (μg N g^{-1})	
L. terrestris	Juvenile	4.2±0.7	B	0.2±0.1	B	1.2±0.3	A
L. terrestris	Adult	12.0±2.3	B	5.0±1.7	A	5.6±1.3	A
A. tuberculata	Pre-clitellate	8.1±1.1	B	0	B	4.2±0.9	A
A. tuberculata	Adult	27.3±2.2	A	0.8±0.2	B	5.8±3.1	A
L. rubellus	Adult	21.3±4.6	A	3.1±0.9	AB	2.8±0.9	A

Table 3 Quantities of earthworm ^{15}N-labeled excretion products (±SE) in extractable soil ^{15}N pools after 48 h

Species	Age class	Total ^{15}N excreted (μg)	^{15}N-mineral N (μg)	^{15}N-DON (μg)	Total ^{15}N in extractable ^{15}N pools (%)
L. terrestris	Juvenile	29.4±1.1	11.8±1.4	6.9±0.7	64
L. terrestris	Adult	18.3±1.8	4.9±0.8	2.4±0.4	40
A. tuberculata	Pre-clitellate	16.3±1.3	2.1±0.4	2.3±0.3	27
A. tuberculata	Adult	23.4±2.8	6.2±0.8	4.0±1.0	44
L. rubellus	Adult	7.8±0.6	2.5±0.4	1.3±0.2	49

mineral N (NH_4-N + NO_3-N) pool and 13–23% was in the ^{15}N-DON pool after 48 h. As much as 64% of the excreted ^{15}N was recovered in these extractable soil ^{15}N pools (Table 3). Approximately 36–73% of the ^{15}N excreted by earthworms was not recovered in the ^{15}N-mineral N and ^{15}N-DON pools, and may have been immobilized, chemically or physically stabilized in non-extractable soil N pools, or lost through denitrification or NH_3 volatilization.

Discussion

Few studies have assessed N excretion by earthworms, and, as far as we are aware, this is the first study to examine the fate of excreted N in soil. We were able to calculate N excretion rates in soil by labeling earthworms with ^{15}N, which Needham (1957) and others were not able to do previously. We were also able to determine the contribution of earthworm excretion products to organic and inorganic soil N pools.

We believe that short-term (e.g., less than 1 week) laboratory studies with ^{15}N-labeled earthworms provide more reliable estimates of N excretion rates than longer (e.g., several weeks) studies. The turnover of ^{15}N from earthworms during a 30-day study proceeded logarithmically (Barois et al. 1987), which suggests that ^{15}N in earthworm tissues is partitioned into metabolically active ^{15}N that is eliminated rapidly through excretion of ^{15}N in mucus and urine, and structural ^{15}N that turns over less rapidly. In addition, the results of longer ^{15}N turnover experiments may be compromised if earthworms have the opportunity to re-ingest eliminated ^{15}N.

N excretion rates in this study are among the highest reported and ranged from 278.4 to 744 μg N g^{-1} fresh weight day^{-1}. Needham (1957) reported a N excretion rate of 268.8 μg N g^{-1} day^{-1} for *L. terrestris*, which is similar to the 278.4–326.4 μg N g^{-1} day^{-1} excreted by juveniles of *L. terrestris* in this study. Our results were much higher than other excretion rates (60–160 μg N g^{-1} day^{-1}) that have been reported for *L. terrestris* (Tillinghast 1967; El-Duweini and Ghabbour 1971). If earthworm tissue contains 10% N on a dry weight basis (earthworm tissue contained 8.6–10.4% N in this study), and earthworm dry mass is 80% of earthworm fresh weight, then earthworm tissue N content is approximately 0.08 g N g^{-1} fresh weight. Daily N turnover from earthworm tissue, based on our excretion rates, ranged from 0.3 to 0.9% of earthworm tissue N. Our results are lower than the N turnover rates of 1–1.7% reported for *L. terrestris* and *P. corethrurus* (Barois et al. 1987; Hameed et al. 1994; Curry et al. 1995), and indicate that our N excretion rates would provide more conservative estimates of N flux via earthworm excretion.

Earthworm excretion rates using the method of Needham (1957) are measured on fasting earthworms in a soil-free environment. In this study, earthworms were provided with soybean leaves which had a C:N ratio of 12. It is well known that earthworm growth is influenced by the quality of the organic substrates provided, and earthworm growth is greater when substrates have a low C:N ratio than a high C:N ratio (Böström 1987; Shipitalo et al. 1988; Whalen and Parmelee 1999). We hypothesize that when earthworms are provided with N-rich organic substrates, N excretion will be greater than when earthworms are provided with N-poor or non-organic substrates, because earthworms reduce N excretion to conserve tissue N when N resources are limited. We did not explicitly test this hypothesis and suggest that future research on N excretion processes should investigate this further.

Soil temperatures of 10 and 18 °C did not affect N excretion rates of each age class of the three earthworm species examined. N excretion and N turnover has been found to increase slightly at higher temperatures (Christensen 1987; Hameed et al. 1994). However, soil temperatures of 10 or 18 °C did not significantly affect the growth rates of *L. terrestris* and *A. tuberculata* (Whalen and Parmelee 1999) or N excretion rates of *L. terrestris*, *A. tuberculata* and *L. rubellus* (this study). In field studies, we found that earthworm numbers and biomass were highest when soil temperatures ranged from 4 to 18 °C and declined dramatically when soil temperatures exceeded 22 °C (Whalen et al. 1998). These results suggest that soil temperatures ranging from 10 to 18 °C are optimal for metabolic processes of earthworms collected from our sites. Extrapolation of N excretion rates measured at temperatures exceeding 18 °C to earthworm populations in the field is questionable at best.

Soils incubated with earthworms tended to have higher concentrations of NH_4-N, NO_3-N and DON than soils incubated without earthworms for 48 h. Of the three soil N pools examined, the greatest increase in soils incubated with earthworms was in the NH_4-N pool. Changes in soil N pools may have been due to excretion of nitrogenous compounds by earthworms or earthworm-microbial interactions that stimulated N mineralization and nitrification processes. The fate of earthworm ^{15}N-labeled excretion products was quantified by measuring soil ^{15}N-mineral N and ^{15}N-DON pools, and between 27 and 64% of the ^{15}N excreted by earthworms was found in these pools after 48 h. Excreted ^{15}N not recovered in these pools may have been immobilized in microbial biomass, stabilized chemically or physically in non-extractable N pools, or liberated through gaseous losses. These results suggest that a substantial proportion of N excreted by earthworms may be readily available for microbial and plant uptake. Whalen et al. (1999) have found that much of the ^{15}N released from decomposing earthworm tissues cycled through microbial biomass within 4 days, and 70% of the ^{15}N from decomposing earthworms accumulated in plant shoot biomass after 16 days. Further work is required to determine how N excretion products are transformed within soil, microbial and plant N pools.

System-level estimates of the N flux through earthworm populations from excretion require accurate measurements of earthworm biomass and N excretion rates. In corn agroecosystems that have received longer term amendments of NH_4NO_3 fertilizer at a rate of 150 kg N ha^{-1} $year^{-1}$, mean monthly earthworm biomass from September 1994 to September 1995 was 5.28 g ash-free dry weight m^{-2} for *Lumbricus* species (primarily *L. terrestris*) and 2.86 g ash-free dry weight m^{-2} for *Aporrectodea* species (primarily *A. tuberculata*) (Whalen et al. 1998). Since most of the individuals collected were juveniles, we assumed that mean N excretion rates of 301 mg N g^{-1} fresh weight day^{-1} and 576 mg N g^{-1} fresh weight day^{-1} for *Lumbricus* species and *Aporrectodea* species, respectively, were representative of N excretion rates for earthworms under field conditions. Earthworm ash-free dry weight is 14% of earthworm fresh weight (Whalen and Parmelee 2000) and, if we assume earthworms are metabolically active for 180 days each year, then *Lumbricus* and *Aporrectodea* species may have excreted 2.04 g N m^{-2} and 2.11 g N m^{-2}, respectively, during 1994/95. These results are comparable with the 30 kg N ha^{-1} $year^{-1}$ from N excretion calculated by Satchell (1963) for a forest ecosystem and the 29–36 kg N ha^{-1} $year^{-1}$ estimated from ^{15}N turnover data by Curry et al. (1995). In corn agroecosystems, the N content of plant biomass, including the corn crop and weed biomass, was approximately 185 kg N ha^{-1} $year^{-1}$ in 1995 (Whalen and Parmelee 2000). The amount of N excreted by earthworms was equivalent to 22% of plant requirements. Further work will be required to determine earthworm excretion rates in the field and the contribution of nitrogenous excretion products to plant N nutrition. Our results suggest that the earthworms could make a significant contribution to N cycling in corn agroecosystems through excretion processes.

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References

Anderson NC (1983) Nitrogen turnover by earthworms in arable plots treated with farmyard manure and slurry. In: Satchell JE (ed) Earthworm ecology: from Darwin to vermiculture. Chapman and Hall, London, pp 139–150

Barois I, Verdier B, Kaiser P, Mariotti A, Rangel P, Lavelle P (1987) Influence of the tropical earthworm *Pontoscolex corethrurus* (Glossoscolecidae) on the fixation and mineralization of nitrogen. In: Bonvicini Pagliai AM, Omodeo P (eds) On earthworms. Mucchi, Modena, pp 151–158

Blair JM, Parmelee RW, Lavelle P (1995) Influences of earthworms on biogeochemistry. In: Hendrix PF (ed) Earthworm ecology and biogeography in North America. Lewis, Boca Raton, pp 127–158

Bohlen PJ, Parmelee RW, Allan MF, Ketterings QM (1999) Differential effects of earthworms on nitrogen cycling from various nitrogen-15-labeled substrates. Soil Sci Soc Am J 63:882–890

Böström U (1987) Growth of earthworms (*Allolobophora caliginosa*) in soil mixed with either barley, lucerne or meadow fescue at various stages of decomposition. Pedobiologa 30:311–321

Brooks PD, Stark JM, McInteer BB, Preston T (1989) Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Sci Soc Am J 53:1707–1711

Cabrera ML, Beare MH (1993) Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci Soc Am J 57:1007–1012

Christensen O (1987) The effect of earthworms on nitrogen cycling in arable soil. In: Proceedings of the 9th International Colloquium on Soil Zoology, Nauka, Moscow, pp 106–118

Curry JP, Byrne D, Boyle KE (1995) The earthworm population of a winter cereal field and its effects on soil and nitrogen turnover. Biol Fertil Soils 19:166–172

Edwards CA, Bohlen PJ (1996) Biology and ecology of earthworms, 3rd edn. Chapman and Hall, London

El-Duweini AK, Ghabbour SI (1971) Nitrogen contribution by live earthworms to the soil. In: d'Aguilar J (ed) IV Colloquium Pedobiologia. (Institut National des Recherches Agronomiques Publ 71–7) INRA, Paris, pp 495–501

Hameed B, Bouché MB, Cortez J (1994) Etudes *in situ* des transferts d'azote d'origine lombricienne (*Lumbricus terrestris* L.) vers les plantes. Soil Biol Biochem 26:495–501

Lee KE (1985) Earthworms: their ecology and relationship with soils and land use. Academic Press, Sydney

Needham AE (1957) Components of nitrogenous excreta in the earthworm *Lumbricus terrestris* L. and *Eisenia foetida* (Savigny). J Exp Biol 34:425–446

Parmelee RW, Crossley DA Jr (1988) Earthworm production and role in the nitrogen cycle of a no-tillage agroecosystem on the Georgia Piedmont. Pedobiologia 32:353–361

SAS Institute (1990) SAS procedures guide, version 6, 3rd edn. SAS Institute, Cary, N.C.

Satchell JE (1963) Nitrogen turnover by a woodland population of *Lumbricus terrestris*. In: Doeksen J, Van der Drift J (eds) Soil organisms. North Holland, Amsterdam, pp 60–66

Schwert DP (1990) Oligochaeta: Lumbricidae. In: Dindal DL (ed) Soil biology guide. Wiley, New York, pp 341–356

Shipitalo MJ, Protz R, Tomlin AD (1988) Effect of diet on the feeding and casting activity of *Lumbricus terrestris* and *L. rubellus* in laboratory culture. Soil Biol Biochem 20:233–237

Tillinghast EK (1967) Excretory pathways of ammonia and urea in the earthworm *Lumbricus terrestris* L. J Exp Zool 166:295–300

Whalen JK, Parmelee RW (1999) Growth of *Aporrectodea tuberculata* (Eisen) and *Lumbricus terrestris* L. under laboratory and field conditions. Pedobiologia 43:1–10

Whalen JK, Parmelee RW (2000) Earthworm secondary production and N flux in agroecosystems: a comparison of two approaches. Oecologia 123

Whalen JK, Parmelee RW, Edwards CA (1998) Population dynamics of earthworm communities in corn agroecosystems receiving organic or inorganic fertilizer amendments. Biol Fertil Soils 27:400–407

Whalen JK, Parmelee RW, McCartney DA, VanArsdale JL (1999) Movement of N from decomposing earthworm tissue to soil, microbial and plant N pools. Soil Biol Biochem 31:487–492