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Applied Soil Ecology 13 (1999) 199–208

Applied Soil Ecology

www.elsevier.com/locate/apsoil

Quantification of nitrogen assimilation efficiencies and their use to estimate organic matter consumption by the earthworms *Aporrectodea tuberculata* (Eisen) and *Lumbricus terrestris* L.

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Received 22 September 1998; received in revised form 12 May 1999; accepted 14 May 1999

Abstract

Earthworms affect nitrogen cycling directly through the consumption and assimilation of organic N and the turnover of N through excretion and mortality. Although earthworms can process large quantities of organic matter, organic-matter consumption by earthworms based on their nitrogen requirements has not been quantified. Organic-matter consumption and the efficiency of nitrogen assimilation by earthworms were determined using ^{15}N-labelled litter-soil mixtures. Consumption rates were influenced by the type of mixture provided and ranged from 8.5 to 13.2 mg organic matter g^{-1} earthworm day^{-1} for *Aporrectodea tuberculata* (Eisen) and from 1.4 to 2.7 mg g^{-1} day^{-1} for *Lumbricus terrestris* L. Consumption rates of ^{15}N-labelled soyabean–soil mixtures (^{15}N-SOY) were higher than ^{15}N-labelled ryegrass–soil mixtures (^{15}N-RYE). The addition of glucose to ^{15}N-labelled soyabean–soil mixtures (^{15}N-SOY+G) to stimulate microbial activity did not influence the consumption rate by *A. tuberculata* or *L. terrestris* compared to the ^{15}N-SOY mixture. However, the addition of glucose to ^{15}N-labelled ryegrass–soil mixtures (^{15}N-RYE+G) significantly increased the consumption rate of *A. tuberculata* by 36% compared to the ^{15}N-RYE mixture. The efficiency of nitrogen assimilation from the ^{15}N-labelled mixtures ranged from 10.0% to 25.8% for *A. tuberculata* and from 25.4% to 30.1% for *L. terrestris*. *A. tuberculata* had lower efficiency of nitrogen assimilation from the ^{15}N-RYE mixture than from all other mixtures. However, there was no difference in the efficiency of nitrogen assimilation from ^{15}N-labelled mixtures by *L. terrestris*. The efficiencies of nitrogen assimilation and estimates of the nitrogen flux from *A. tuberculata* and *L. terrestris* populations in manure-amended and inorganically fertilized corn agroecosystems were used to estimate system-level consumption of organic matter by earthworms. Based on hypothetical diets, we estimated that earthworms consumed 11.8 to 17.1 Mg organic matter ha^{-1} year^{-1}, which was equivalent to 19–24% of the total organic matter in crop residues and the top 15 cm of soil each year.

Keywords: Nitrogen assimilation efficiency; Organic matter; Consumption rate; Earthworms; Corn agroecosystem

1. Introduction

It has been estimated that earthworms can process 2–15 Mg of organic matter ha^{-1} year^{-1} (Satchell, 1967; Shipitalo et al., 1988; Lavelle et al., 1989;

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0929-1393/99/$ – see front matter
PII: S0929-1393(99)00033-5

Hendriksen, 1991) and 4–10% of the organic matter in the top 15 cm of soil and surface residues in one year (James, 1991). The processes by which nitrogen is assimilated from organic materials and used in earthworm metabolism are of interest because there is evidence that the nitrogen in earthworm tissues is rapidly turned over through the excretion of urine and mucus. In a classic study, Needham (1957) estimated that the daily nitrogen excretion rates of *Lumbricus terrestris* and *Allolobophora caliginosa* fed elm leaves were 268.8 and 87.5 μg N g^{-1} live worm day^{-1}, respectively. In studies using ^{15}N, nitrogen excretion rates for *L. terrestris* have been found to range from 278.3 to 326.7 μg N g^{-1} live worm day^{-1} (R.W. Parmelee, personal communication), while excretion of mucus and urine accounted for the turnover of 1–1.7% of earthworm tissue N per day for *L. terrestris* and *Pontoscolex corethrurus* (Barois et al., 1987; Hameed et al., 1994; Curry et al., 1995). It is clear that nitrogen losses from earthworm tissues are substantial, and it has been estimated that the flux of nitrogen from earthworm populations in agroecosystems through excretion and mortality ranges from 10 to 74 kg N ha^{-1} $year^{-1}$ (Andersen, 1983; Christensen, 1987; Böström, 1988; Parmelee and Crossley, 1988; Curry et al., 1995).

Since earthworms do not appear to conserve nitrogen in their tissues, they may satisfy their nitrogen requirements either by selectively consuming organic matter with a high nitrogen content or by having a high efficiency of nitrogen assimilation from litter or soil. The efficiency of nitrogen assimilation by earthworms is the proportion of nitrogen consumed that is assimilated into earthworm tissue. Earthworm weight gain under laboratory and field conditions is greater when they feed on organic materials with a high nitrogen content than materials with a low nitrogen content (Böström, 1987; Shipitalo et al., 1988; Heine and Larink, 1993). Furthermore, microcosm and field studies have shown that earthworms preferentially remove litter and soil organic matter fractions with lower C : N ratios (Bohlen et al., 1997; Ketterings et al., 1997). Since the colonization of organic materials by microorganisms affects their nitrogen content and the degree of decomposition, it is of interest to examine how the microbial activity in litter–soil mixtures affects their consumption by earthworms.

While we are not aware of any studies that have measured directly the efficiency of nitrogen assimilation by earthworms, we calculated that *L. terrestris* provided with ^{15}N-labelled ryegrass litter had an efficiency of nitrogen assimilation of 27% using data from Binet and Trehen (1992). Using the REAL model, which simulates the role of earthworms in nitrogen dynamics, Bouché et al. (1997) estimated that the efficiencies of nitrogen assimilation by *Nicodrilus longus* and *L. terrestris* were 30%. The paucity of experimental studies on the efficiency of nitrogen assimilation by earthworms represents a considerable gap in our understanding of basic earthworm biology and ecology. This study presents a new methodology that directly measures the efficiency of nitrogen assimilation by earthworms using ^{15}N-labelled litter–soil mixtures. Traditionally, the assimilation of nutrients by animals has been calculated by subtracting the quantity of nutrients defecated and excreted from the quantity that was ingested. The advantage of using ^{15}N-labelled materials is that it allows us to quantify nitrogen assimilated into earthworm tissues directly and provides a more accurate calculation of assimilation efficiency.

Measurement of the efficiency of nitrogen assimilation not only increases our understanding of earthworm physiology, but also allows us to determine the quantity of organic nitrogen that earthworms must consume to satisfy their nitrogen requirements. We define the nitrogen requirement of an earthworm population as the quantity of nitrogen that must be assimilated into their tissues to compensate for nitrogen lost through excretion and mortality. Since earthworms assimilate only a portion of the nitrogen ingested, it is then possible to estimate, based on their nitrogen requirements and efficiencies of nitrogen assimilation, how much organic matter they consume. We will provide sample calculations to show how the quantity of organic matter processed annually by the *L. terrestris* and *Aporrectodea tuberculata* populations in a corn agroecosystem can be estimated. We believe this approach may provide more reliable estimates of system-level organic matter processing by earthworms than are available currently.

The purposes of this study were to:

(i) develop a method to measure the efficiency of nitrogen assimilation by *L. terrestris* and

A. tuberculata, the dominant earthworm populations in our corn agroecosystems;
(ii) determine how environmental conditions (temperature) and litter quality (nitrogen content and microbial activity) influence the consumption and assimilation of nitrogen by earthworms; and
(iii) estimate the annual organic matter consumption by *L. terrestris* and *A. tuberculata* in a corn agroecosystem based on the nitrogen requirements of these populations and their efficiency of nitrogen assimilation from organic substrates.

2. Materials and methods

2.1. Preparation of ^{15}N-labelled litter–soil mixtures

Soil used in this study was obtained from the A horizon (0–15 cm) of a fine, mixed, mesic Fragiudaulf soil of the Canfield series adjacent to established corn plots in Wooster, OH. The soil texture was silt loam (13.5% sand, 73.7% silt, 12.8% clay) with a pH of 6.3 and an organic-matter content of 3.7%. The total carbon and nitrogen contents of the soil were 23 g C kg^{-1} and 1.9 g N kg^{-1}. More information on this soil is provided in Bohlen and Edwards (1995). The earthworms used in this study, juveniles of *A. tuberculata* and *L. terrestris* species, were collected from this field site by handsorting and extraction with 0.5% formalin. To obtain ^{15}N-labelled leaves, soyabean (*Glycine max* L.) and ryegrass (*Lolium perenne* L.) plants were grown to maturity in low organic-matter (<1%) soil. Approximately one-half of the nitrogen applied to the soyabean plants was unlabelled $(NH_4)_2SO_4$ (0.367% atom ^{15}N) and the remainder was ^{15}N-labelled $(NH_4)_2SO_4$ (99% atom ^{15}N). The ryegrass plants were grown in a raised outdoor bed after another crop to scavenge ^{15}N remaining in the soil. Nearly one-quarter of the total plant-available N was ^{15}N-labelled $(NH_4)_2SO_4$ (99% atom ^{15}N) and the remainder was unlabelled NH_4NO_3 (D. McCartney, personal communication).

^{15}N-labelled litter–soil mixtures, hereafter referred to as ^{15}N-SOY and ^{15}N-RYE, were prepared by mixing 100 g (dry weight) of unlabelled, sieved (<2 mm) soil with either 10 g (dry weight) of finely ground (<1 mm) ^{15}N-labelled soyabean leaves (44.5% C, 1.6% total N, 42% atom ^{15}N) or 10 g of finely ground ^{15}N-labelled ryegrass leaves (38.6% C, 1.2% total N, 22% atom ^{15}N). Mixtures were moistened to 25% (w/w) soil water content, corresponding with 71% of soil field capacity, and incubated at room temperature for 72 h. Twenty-four hours prior to offering the mixtures to the earthworms, one-half of each was placed in a separate container and 0.1 ml of a 10% glucose solution g^{-1} soil was added to stimulate microbial activity. We will refer to the ^{15}N-labelled mixtures with glucose as ^{15}N-SOY + G and ^{15}N-RYE + G.

The ^{15}N-labelled mixtures were weighed, ground and analyzed for total N and atom% ^{15}N. Nitrogen isotopic ratios ($^{15}N/^{14}N$) were determined as described in Section 2.2. Microbial activity was determined using the dehydrogenase assay of Casida (1977) modified by Subler et al. (1997). One gram of each mixture was reacted at 40°C for 6 h in test tubes containing 1 ml of 2,3,5-triphenyltetrazolium chloride (TTC) in 0.5 M TRIS buffer (pH 7.6). The enzyme-cleaved product, triphenyl formazan (TPF), was determined in methanol extracts (10 ml) using a Lachat AE flow-injection autoanalyzer at 480 nm. The dehydrogenase activity of the ^{15}N-SOY and ^{15}N-SOY+G mixtures were 31.1 and 91.6 μg TPF g^{-1} soil h^{-1}, while the activity of the ^{15}N-RYE and ^{15}N-RYE + G mixtures were 13.9 and 47.3 μg TPF g^{-1} soil h^{-1}, respectively.

2.2. Consumption and assimilation of ^{15}N-labelled mixtures

In a preliminary study, it was found that earthworms would burrow into substrates when they were given direct access to them. This problem was overcome by inserting two 1000 μl pipette tips end-to-end so that the earthworms crawled into an empty pipette tip and consumed substrate from the other pipette tip (Fig. 1). This design worked well for both *L. terrestris* and *A. tuberculata*, although it was necessary to vary the size of the opening to the pipette containing the substrate, depending on the species, to prevent the earthworms from burrowing into it. Based on the preliminary study, two pipettes containing ^{15}N-labelled mixture were provided in each container so that consumption and assimilation of nitrogen were not limited by substrate availability. The pipette tips were installed at the base of a 300-cm^3 container, and between 0.7 and 1.1 g (dry weight basis) of ^{15}N-labelled mixture was placed into pre-weighed 1000 μl pipette tips.

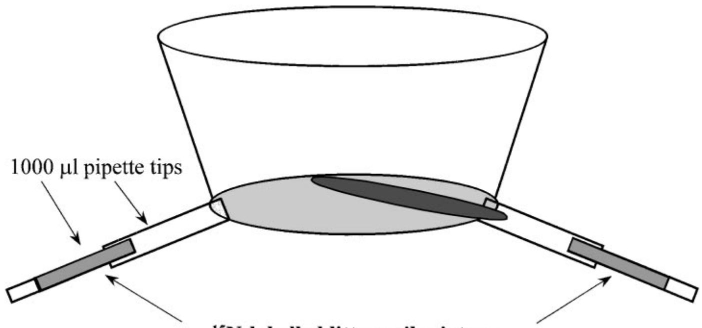

Fig. 1. Feeding system used to measure consumption and assimilation of nitrogen by earthworms.

One juvenile of *L. terrestris* (1.5–3 g fresh weight) or *A. tuberculata* (0.3–0.5 g fresh weight) was placed in each container with one ml of water to prevent desiccation. Consumption and assimilation of nitrogen from ^{15}N-labelled mixtures was measured on juvenile earthworms because they are actively growing and represent the greatest proportion of the earthworm population under field conditions (Whalen et al., 1998). To reduce variability in nitrogen consumption and assimilation, we did not allow the earthworms to void their gut contents prior to the study. For each species, 24 replicate containers were prepared for each of the four ^{15}N-labelled mixtures. The influence of temperature on consumption and assimilation of nitrogen by earthworms was determined by randomly placing 12 replicate containers with each substrate in controlled environment chambers set at either 8–12°C (average daily temperature = 10°C) or 16–20°C (average daily temperature = 18°C) to simulate daily diurnal flux. These temperatures were chosen to simulate mean seasonal temperature fluxes in field soil during the spring and autumn when earthworms are most active.

After 48 h, the earthworms were removed from the containers, anaesthetized by spraying with 95% ethanol, and dissected. Earthworm tissue and guts were collected on pre-weighed Whatman 42 filter paper, and blood and coelomic fluid that may have contained ^{15}N were washed from the dissecting pan into a plastic scintillation vial. Earthworm casts were collected from the containers on pre-weighed Whatman 42 filter paper, and the containers were rinsed into a plastic scintillation vial to collect any remaining ^{15}N. The pipette containing unconsumed ^{15}N-labelled mixture and the filter papers were dried in a forced-air oven at 60°C for 48 h. Earthworm tissue, guts and casts were weighed, ground and analyzed for total N and atom% ^{15}N. Nitrogen isotopic ratios (^{15}N/^{14}N) were determined using a Carlo–Erba C and N analyzer coupled with a Europa Tracermass spectrophotometer (Michigan State University). Percentage atom ^{15}N excess was calculated by subtracting background levels of atom% ^{15}N for each variable measured. Due to methodological problems, we were unable to determine the nitrogen isotopic ratios of earthworm blood and coelomic fluid.

2.3. Calculation of N assimilation efficiency

Traditionally, the quantity of nutrients which are assimilated into animal tissues has been calculated from equations of animal energetics (Brafield and Llewellyn, 1982):

$$A = I - F - E \tag{1}$$

where A is the quantity of nutrients assimilated into tissue, I the quantity of nutrients ingested, F the quantity of nutrients defecated, and E the quantity of nutrients excreted during some period of time. Using ^{15}N, we were able to directly quantify nitrogen assimilated into earthworm tissues (A) rather than calculate nitrogen assimilation by difference. We are aware that discrimination in biochemical pathways between ^{15}N and ^{14}N can change the ^{15}N abundance in nitrogen-containing compounds of plant and animal tissues by 0.001–0.002 atom% ^{15}N of natural abundance levels (Hobson et al., 1996; Robinson et al., 1998). Since the variance in our

^{15}N measurements of ^{15}N-labelled mixtures and earthworm tissues was about 0.005 atom% ^{15}N of natural abundance levels, discrimination or fractionation of ^{15}N in the nitrogen pools of these materials could not be detected.

The efficiency of N assimilation (AE) is the proportion of nitrogen ingested that becomes incorporated into earthworm tissue:

$$\mathrm{AE} = (A/I) \times 100\% \tag{2}$$

where A was the quantity (μg) of ^{15}N excess in earthworm tissue and I the quantity (μg) of ^{15}N excess consumed. The recovery of ^{15}N was calculated by subtracting the quantity of ^{15}N that was assimilated, defecated, or present in the earthworm gut from the quantity of ^{15}N ingested. We assumed that ^{15}N losses through excretion of mucus and metabolic byproducts (e.g. urine) were negligible during this short (48 h) experiment.

2.4. *Estimation of organic matter consumption by A. tuberculata and L. terrestris*

Organic-matter consumption by earthworms was calculated from information on their nitrogen requirements and their efficiency of nitrogen assimilation from ^{15}N-labelled mixtures. Annual N flux from earthworm populations, mortality and excretion was calculated by multiplying the percentage N content of earthworms by earthworm secondary production and adding N lost through excretion of mucus and metabolic byproducts (Parmelee and Crossley, 1988). Earthworm secondary production, which is the accumulation of biomass through growth and reproduction, was calculated using the instantaneous growth rate method (Benke, 1984) which combines field biomass estimates (g ash-free dry weight m^{-2}) and field growth rates (g weight gain g^{-1} earthworm day^{-1}).

The secondary production of *A. tuberculata* and *L. terrestris* populations in corn agroecosystems amended with 150 kg N ha^{-1} $year^{-1}$ (applied as either straw-packed cow manure or NH_4NO_3 fertilizer) was calculated with the instantaneous growth rate method using earthworm biomass measurements from Whalen et al. (1998) and species-specific growth rates (Whalen and Parmelee, 1999). Nitrogen excretion rates for these species (R.W. Parmelee, personal communication) were added, and the direct flux of nitrogen through *A. tuberculata* and *L. terrestris* populations during 1994–1995 was estimated to be 38.1 and 45.2 kg N ha^{-1} $year^{-1}$, respectively, in manure-amended agroecosystems. In inorganically-fertilized agroecosystems, nitrogen flux was 21.1 and 26.1 kg N ha^{-1} $year^{-1}$ for *A. tuberculata* and *L. terrestris* populations, respectively (Whalen, 1998).

The quantity of organic nitrogen consumed by *A. tuberculata* and *L. terrestris* populations to account for the nitrogen lost from their tissues through mortality and excretion was calculated from:

$$I_N = (\mathrm{N\ flux})/\mathrm{AE} \tag{3}$$

where I_N is organic N ingested, N flux the total nitrogen released from earthworm tissues through mortality and excretion, and AE the efficiency of nitrogen assimilation by earthworms from ^{15}N-labelled mixtures. Total organic-matter consumption by earthworm populations was:

$$I_{OM} = I_N \times (\mathrm{RES}_{C:N}/\mathrm{RES}_{\%C}) \tag{4}$$

where I_{OM} is the organic matter consumed, $RES_{C:N}$ the C : N ratio of an organic resource, and $RES_{\%C}$ is the percentage carbon content of an organic resource. The ratio $RES_{C:N}/RES_{\%C}$ is equivalent to the percentage nitrogen content of the organic resource.

2.5. *Statistical analysis*

Data were log transformed to equalize variance and analyzed using ANOVA in a general linear model (GLM) with SAS software (SAS Institute, 1990). The effects of temperature and the nitrogen content and microbial activity of ^{15}N-labelled mixtures on the consumption rates and efficiencies of nitrogen assimilation were determined using two-factor ANOVAs. Variables that significantly affected consumption rates and efficiencies of nitrogen assimilation were adjusted for multiple comparisons and analyzed using a *t*-test (LSD) at the 95% confidence level.

3. Results

Recovery of the ^{15}N tracer was determined for individual earthworms. Although consumption and assimilation were somewhat variable, $83 \pm 6\%$ of the consumed ^{15}N was recovered in earthworm tissues, guts and casts after 48 h. Since we were unable to assess ^{15}N in earthworm blood and coelomic fluid due to methodological problems, we believe our estimates of the efficiencies of nitrogen assimilation by juvenile *A. tuberculata* and *L. terrestris* are conservative. The mean total N and ^{15}N concentrations in earthworms provided with the ^{15}N-SOY mixture after 48 h were 11.7% N and 0.66% atom ^{15}N for *A. tuberculata*, and 10.2% N with 0.53% atom ^{15}N for *L. terrestris*. Nitrogen concentrations in earthworms provided with the ^{15}N-RYE mixture were 12.3% N and 0.44 atom% ^{15}N for *A. tuberculata* and 13.8% N and 0.39 atom% ^{15}N for *L. terrestris*.

The C : N ratio of the soyabean leaves (C : N = 27.8) used in the ^{15}N-SOY and ^{15}N-SOY + G mixtures was lower than the C : N ratio of the ryegrass leaves (C : N = 32.2) in the ^{15}N-RYE and ^{15}N-RYE + G mixtures due to the slightly higher nitrogen content of soyabean leaves than ryegrass leaves. The addition of glucose to the ^{15}N-SOY + G and ^{15}N-RYE + G mixtures stimulated microbial activity, and the dehydrogenase activity of the ^{15}N-SOY + G and ^{15}N-RYE + G mixtures was approximately three times greater than the dehydrogenase activity of the ^{15}N-SOY and ^{15}N-RYE mixtures.

Neither the rate at which ^{15}N-labelled mixtures were consumed by *A. tuberculata* and *L. terrestris*, nor the efficiency of nitrogen assimilation of these species was significantly affected by temperature between 10°C and 18°C. Therefore, we pooled results across temperatures for each ^{15}N-labelled mixture provided to *A. tuberculata* and *L. terrestris*.

3.1. *Consumption rates for A. tuberculata and L. terrestris*

Consumption of ^{15}N-labelled mixtures by *A. tuberculata* and *L. terrestris* is presented as mg organic matter consumed g^{-1} earthworm (on a fresh weight basis) day^{-1}. The rate at which juvenile *A. tuberculata* consumed substrates ranged from 8.5 to 13.2 mg g^{-1} day^{-1} and was significantly (p <0.05, LSD) greater than the rate of consumption by *L. terrestris*, which ranged from 1.4 to 2.7 mg g^{-1} day^{-1} (Table 1).

For *A. tuberculata*, the consumption rates of ^{15}N-SOY and ^{15}N-SOY + G mixtures were not significantly different. However, the consumption rate of the ^{15}N-RYE + G mixture was significantly higher (p <0.05, LSD) than the consumption rate of the ^{15}N-RYE mixture (Table 1). For *L. terrestris*, the consumption rates of the four different mixtures did not differ significantly (Table 1).

3.2. *Efficiencies of nitrogen assimilation by A. tuberculata and L. terrestris*

The quantities of ^{15}N consumed (μg consumed ^{15}N) and ^{15}N assimilated into earthworm tissue (μg tissue ^{15}N) after two days were determined, and efficiencies of nitrogen assimilation were calculated on an individual earthworm basis. Mean consumed ^{15}N and tissue ^{15}N values for *A. tuberculata* and *L. terrestris* are given in Table 1. The efficiencies of nitrogen assimilation ranged from 10.0% to 25.8% for

Table 1
Effect of resource quality on consumption rate (mg organic matter g^{-1} earthworm wet weight day^{-1}), excess quantity of ^{15}N consumed and assimilated per worm into earthworm tissues during a 48-h experiment, and N assimilation efficiencies of *A. tuberculata* and *L. terrestris*

Species	Mixture	Consumption rate [a,b] (mg g^{-1} day^{-1})	Consumed [a] ^{15}N (μg ^{15}N)	Tissue ^{15}N[a] (μg ^{15}N)	Efficiency of N assimilation [a,b] (%)
A. tuberculata	^{15}N-SOY	9.8 ± 1.7 A	61.2 ± 12.0	14.6 ± 2.7	25.8 ± 3.7 AB
A. tuberculata	^{15}N-SOY + G	8.9 ± 1.7AB	60.6 ± 10.7	9.8 ± 2.1	18.0 ± 5.3 B
A. tuberculata	^{15}N-RYE	8.5 ± 1.8 B	18.0 ± 3.0	1.9 ± 0.5	10.0 ± 3.0 C
A. tuberculata	^{15}N-RYE + G	13.2 ± 2.1 A	27.3 ± 3.8	5.2 ± 0.6	17.6 ± 3.0 B
L. terrestris	^{15}N-SOY	2.6 ± 0.3 C	73.2 ± 8.0	16.3 ± 1.7	25.9 ± 3.1 AB
L. terrestris	^{15}N-SOY + G	2.3 ± 0.4 C	56.6 ± 7.2	12.5 ± 1.1	25.4 ± 2.5 AB
L. terrestris	^{15}N-RYE	1.4 ± 0.2 C	16.7 ± 2.0	5.1 ± 0.6	28.5 ± 0.9 A
L. terrestris	^{15}N-RYE + G	2.7 ± 0.5 C	30.1 ± 5.4	10.1 ± 1.8	30.1 ± 1.5 A

[a] Means presented are untransformed values ± standard errors.
[b] Means within a column followed by the same letter are not statistically significantly different (p <0.05, LSD).

A. tuberculata and 25.4% to 30.1% for *L. terrestris* (Table 1). The efficiency of nitrogen assimilation by *A. tuberculata* was highest for the ^{15}N-SOY mixture, and the efficiency of nitrogen assimilation from the ^{15}N-RYE mixture was significantly lower ($p < 0.05$, LSD) than all other mixtures offered to *A. tuberculata* (Table 1). The efficiencies of nitrogen assimilation by *L. terrestris* were not affected significantly by the type of mixture consumed (Table 1).

3.3. Estimation of annual organic-matter consumption by A. tuberculata and L. terrestris

In the ecological classification of lumbricids (Bouché, 1977), *A. tuberculata* is an endogeic species since it inhabits the top 10–15 cm of soil and is thought to feed primarily on particulate organic matter, while *L. terrestris* is an anecic species which forms permanent vertical burrows in the soil and feeds primarily on surface litter. There is little information concerning the diets of earthworms and what proportion of their energy and mineral nutritional requirements are derived from living microbial biomass and organic matter (dead microbial biomass, plant residues, etc.) (Edwards and Bohlen, 1996). Therefore, we calculated annual organic-matter consumption by *A. tuberculata* and *L. terrestris* in corn agroecosystems with hypothetical diets of particulate organic matter (POM) or surface litter (LIT), respectively. The C : N ratio of POM was 20, and the C : N ratio of LIT was 30 in manure-amended agroecosystems and 40 in inorganically fertilized agroecosystems (Bohlen et al., 1997; Ketterings et al., 1997). We assumed a C content of 45% for POM and LIT in both the agroecosystems (Paul and Clark, 1996). The C : N ratio of POM was similar to the ^{15}N-SOY mixture, and the quantity of POM consumed by *A. tuberculata* was calculated using efficiencies of nitrogen assimilation by this species from the ^{15}N-SOY mixture. For *L. terrestris*, the consumption of the hypothetical LIT diet was also calculated using efficiencies of nitrogen assimilation from the ^{15}N-RYE mixture.

Organic-matter consumption by *A. tuberculata* and *L. terrestris* populations was estimated at 6.6–10.5 Mg ha^{-1} year^{-1} and 3.6–8.1 Mg ha^{-1} year^{-1} in manure-amended and inorganically fertilized agroecosystems, respectively (Table 2). The soil organic-matter content of the corn agroecosystems is 3.7%

Table 2
Estimated organic-matter consumption (kg organic matter ha^{-1} year^{-1}) by *A. tuberculata* and *L. terrestris* populations with hypothetical diets in corn agroecosystems receiving manure or inorganic fertilizer amendments

Earthworm species	Corn agroecosystem	
	Manure	Inorganic fertilizer
A. tuberculata [a]	6563	3634
L. terrestris [b]	10 573	8140
Total	17 136	11 774

[a] Hypothetical diet of *A. tuberculata*: particulate organic matter (POM).
[b] Hypothetical diet for *L. terrestris*: surface litter (LIT).

with a bulk density of 1.0 g cm^{-3} (Bohlen and Edwards, 1995), which is equivalent to 55.5 Mg organic matter ha^{-1} to a depth of 15 cm. In manure-amended agroecosystems, 10 Mg organic matter ha^{-1} is added each year in straw-packed cow manure. An additional 6.5 Mg organic matter ha^{-1} accumulates each year from the litter and residues from crop production and weed biomass. Thus, *A. tuberculata* and *L. terrestris* populations consumed 6–9% and 13–15%, respectively, of the organic matter in residues and soil to a 15 cm depth in corn agroecosystems.

4. Discussion

4.1. Consumption rates and nitrogen assimilation efficiencies

Litter type or microbial activity did not greatly affect the consumption rate of ^{15}N-labelled mixtures by earthworms, although consumption rates were greater for *A. tuberculata* than *L. terrestris*. While we are not aware of any studies that have reported consumption rates for *A. tuberculata*, organic-matter consumption by *Allolobophora caliginosa*, another endogeic species, ranged from 40 to 80 mg g^{-1} day^{-1} (Barley, 1959; Piearce, 1972; Böström, 1986; Böström, 1987). Organic-matter consumption by the smaller endogeic species *Allolobophora rosea* was considerably higher and ranged from 96 to 280 mg g^{-1} day^{-1}, depending on the age of the earthworm (Bolton and Phillipson, 1976). Cortez and

Hameed (1988) reported that the consumption rates for *L. terrestris* provided with ryegrass litter were as high as 84 mg g^{-1} day^{-1}, although most estimates of organic-matter consumption by *L. terrestris* are from 6 to 27 mg g^{-1} day^{-1} (Needham, 1957; Böström, 1988; Shipitalo et al., 1988; Daniel, 1991; Binet and Trehen, 1992). The rates at which *L. terrestris* consumed different ^{15}N-labelled mixtures were lower than values reported in the literature.

The efficiencies of nitrogen assimilation by *A. tuberculata* ranged from 10.0% to 25.8%, and were affected by litter type and microbial activity. However, the efficiency of nitrogen assimilation by *L. terrestris* did not differ by litter type or microbial activity, and *L. terrestris* tended to be more efficient at assimilating nitrogen than *A. tuberculata* when both species were provided with the same type of litter–soil mixture. It seems likely that *A. tuberculata* and *L. terrestris* have different physiological mechanisms for deriving nitrogen from different types of mixtures. Although our calculation of the efficiencies of nitrogen assimilation by earthworms may be conservative because we did not include ^{15}N from blood and coelomic fluid in our measurement of ^{15}N assimilation, our results are similar to the 27–30% values calculated for *L. terrestris* using data from Binet and Trehen (1992) and Bouché et al. (1997). With the exception of the ^{15}N-RYE mixture, the efficiencies of nitrogen assimilation by both, *A. tuberculata* and *L. terrestris* tended to be higher than the efficiencies of carbon assimilation that have been reported for earthworms, which range from 2% to 15% (Bolton and Phillipson, 1976; Dash and Patra, 1977; Huchinson and King, 1979; Martin et al., 1992; Rozen, 1994). These results suggest that either the nitrogen requirements of *A. tuberculata* and *L. terrestris* are much higher than their energy requirements or that much of the carbon ingested by earthworms is not easily assimilated (e.g. carbon compounds may be physically protected in organomineral complexes or chemically resistant to digestion and assimilation by earthworms).

4.2. Annual organic-matter consumption by *A. tuberculata* and *L. terrestris*

We have demonstrated that the efficiency of nitrogen assimilation by earthworms may be combined with estimates on nitrogen flux through earthworm populations to yield information on agroecosystem-level consumption of organic matter by earthworms. Based on our assumptions, earthworms may consume as much as 19% of the organic matter in the top 15 cm of inorganically fertilized corn agroecoystems, and 24% of total organic matter in manure-amended corn agroecosystems, which is approximately equivalent to annual net primary production. It seems likely that the greater consumption of organic matter in manure-amended, as compared with inorganically fertilized agroecosystems was due to greater earthworm biomass and secondary production in the manure-amended agroecosystems (Whalen et al., 1998; Whalen, 1998).

Our estimates of organic-matter processing by earthworm populations are greater than those of James (1991), namely that earthworms consumed 10% of the total soil organic matter in the top 15 cm of a tallgrass prairie. If earthworms derive some portion of their diet from soil microbial biomass, or if the efficiency of nitrogen assimilation by earthworms is higher than what we have found, then total organic-matter consumption by the earthworm populations in our corn agroecosystems would be lower than the estimates we have presented.

Although our estimates of organic-matter consumption by earthworms are based on hypothetical diets and efficiencies of nitrogen assimilation from laboratory studies, we present our methodology as an alternative approach for determining the direct role of earthworms in organic matter cycling. Since the nitrogen requirements of earthworms seem to be greater than their requirements for carbon, our approach may provide a more accurate estimate of organic-matter processing by earthworms. Our calculations would be improved by measuring the efficiency of nitrogen assimilation by earthworms for particulate organic matter and corn litter. In addition, it would be useful to determine what proportion of the earthworm diet is derived from these and other organic materials in the field. Our estimates indicate that the quantity of organic matter processed by earthworms in corn agroecosystems was between 11.8 and 17.1 Mg ha^{-1} $year^{-1}$. These values are within, or slightly exceeding, the range of 2–15 Mg ha^{-1} $year^{-1}$ of organic-matter consumption by earthworms that have been reported in the literature and indicate that, based on their nitrogen requirements, earthworms have an important role in the ecosystem-level processing of organic matter.

Acknowledgements

Thanks are extended to Jessie VanArsdale for technical assistance. Keith Paustian, Jim Curry and two anonymous reviewers provided valuable comments on the manuscript. This work was supported by a grant from the US Department of Agriculture.

References

Andersen, N.C., 1983. Nitrogen turnover by earthworms in arable plots treated with farmyard manure and slurry. In: Satchell, J.E. (Ed.), Earthworm Ecology: From Darwin to Vermiculture. Chapman and Hall, London. pp. 139–150.

Barley, K.P., 1959. The influence of earthworms on soil fertility. II. Consumption of soil and organic matter by the earthworm *Allolobophora caliginosa*. Aus. J. Agr. Res. 10, 179–185.

Barois, I., Verdiers, 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: Bonviscini Pagliai, A.M., Omodeo, P. (Eds.), On Earthworms. Mucchi, Modena. pp. 151–158.

Benke, A.C., 1984. Secondary production of aquatic insects. In: Resh, V.H., Rosenberg, D.M. (Eds.), The Ecology of Aquatic Insects. Praeger, New York. pp. 289–322.

Binet, F., Trehen, P., 1992. Experimental microcosm study of the role of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) on nitrogen dynamics in cultivated soils. Soil Biol. Biochem. 24, 1501–1506.

Bohlen, P.J., Edwards, C.A., 1995. Earthworm effects on nitrogen dynamics and soil respiration in microcosms receiving organic and inorganic nutrients. Soil Biol. Biochem. 27, 341–348.

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

Bolton, P.J., Phillipson, J., 1976. Burrowing, feeding, egestion and energy budgets of *Allolobophora rosea* (Savigny) (Lumbricidae). Oecologia 23, 225–245.

Böström, U., 1986. Growth of earthworms (*Allolobophora caliginosa*) fed on shoots and roots of barley, meadow fescue, and lucerne: studies in relation to particle size, protein, crude fiber and toxicity. Pedobiologia 29, 1–12.

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

Böström, U., 1988. Ecology of earthworms in arable land. Population dynamics and activity in four cropping systems. Ph.D. thesis, Rep. 34, Swedish University of Agricultural Sciences, Uppsala.

Bouché, M.B., 1977. Stratégies lombiciennes. In: Lohm, U., Persson, T. (Eds.), Soil Organisms as Components of Ecosystems. Ecol. Bull. (Stockholm) 25, 122–132.

Bouché, M.B., Al-Addan, F., Cortez, J., Hameed, R., Heidet, J.-C., Ferriere, G., Mazaud, D., Samih, M., 1997. The earthworm role in the nitrogen cycle: a falsifiable assessment. Soil Biol. Biochem. 29, 375–380.

Brafield, A.E., Llewellyn, M.J., 1982. Animal energetics. Blackie and Son, Glasgow. pp. 168.

Casida Jr., L.E., 1977. Microbial metabolic activity as measured by dehydrogenase determinations. Appl. Environ. Microbiol. 34, 630–636.

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

Cortez, J., Hameed, R., 1988. Effets de la maturation des litières de ray-gras (*Lolium perenne* L.) dans le sol sur leur consommation et leur assimilation par *Lumbricus terrestris* L.. Rev. Ecol. Biol. Sol. 25, 397–412.

Curry, J.P., Byrne, D., Boyle, K.E., 1995. The earthworm population of a winter cereal field and its effects on soil and nitrogen turnover. Biol. Fertil. Soils 19, 166–172.

Dash, M.C., Patra, U.C., 1977. Density, biomass and energy budget of a tropical earthworm population from a grassland site in Orissa. India. Rev. Ecol. Biol. Sol. 14, 461–471.

Daniel, O., 1991. Leaf-litter consumption and assimilation by juveniles of *Lumbricus terrestris* L. (Oligochaeta, Lumbricidae) under different environmental conditions. Biol. Fertil. Soils 12, 202–208.

Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworms, third edn. Chapman and Hall, London. pp. 426.

Hameed, R., Cortez, J., Bouché, M.B., 1994. Biostimulation de la croissance de *Lolium perenne* L. par l'azote excrete par *Lumbricus terrestris* L.—mesure au laboratoire de ce debit. Soil Biol. Biochem. 26, 483–493.

Heine, O., Larink, O., 1993. Food and cast analysis as a parameter of turn-over of materials by earthworms (*Lumbricus terrestris* L.). Pedobiologia 37, 245–256.

Hendriksen, N.B., 1991. Gut load and food-retention time in the earthworm *Lumbricus festivus* and *L. castaneus*: a field study. Biol. Fertil. Soils 11, 170–173.

Hobson, K.A., Schell, D.M., Renouf, D., 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can. J. Fish. Aquatic. Sci. 53, 528–533.

Huchinson, K.J., King, K.L., 1979. Consumers. In: Coupland, R. (Ed.), Grassland Systems of the World: Analysis of Grasslands and their Uses. Cambridge University Press, Cambridge. pp. 259–265.

James, S.W., 1991. Soil, nitrogen, phosphorus, and organic matter processing by earthworms in tallgrass prairie. Ecology 72, 2101–2109.

Ketterings, Q.M., Blair, J.M., Marinissen, J.C.Y., 1997. The effects of earthworms on soil aggregate stability and carbon and nitrogen storage in a legume cover crop agroecosystem. Soil Biol. Biochem. 29, 401–409.

Lavelle, P., Schaefer, R., Zaidi, Z., 1989. Soil ingestion and growth in *Millsomia anomala*, a tropical earthworm, as influenced by the quality of the organic matter ingested. Pedobiologia 33, 379–388.

Martin, A., Mariotti, A., Balesdent, J., Lavelle, P., 1992. Soil organic matter assimilation by a geophagous tropical earthworm based on $\delta^{13}C$ measurements. Ecology 73, 118–128.

Needham, A.E., 1957. Components of nitrogenous excreta in the earthworm *L. terrestris* and *E. feotida*. J. Exp. Biol. 34, 425–446.

Parmelee, R.W., Crossley Jr., D.A., 1988. Earthworm production and role in the nitrogen cycle of a no-tillage agroecosystem on the Georgia Piedmont. Pedobiologia 32, 353–361.

Paul, E.A., Clark, F.E., 1996. Soil Microbiology and Biochemistry, second edn. Academic Press, San Diego. pp. 340.

Piearce, T.G., 1972. The calcium relations of selected Lumbricidae. J. Anim. Ecol. 41, 167–188.

Robinson, D., Handley, L.L., Scrimgeour, C.M., 1998. A theory for $^{15}N/^{14}N$ fractionation in nitrate-grown vascular plants. Planta 205, 397–406.

Rozen, A., 1994. The annual cycle in populations of earthworms (Lumbricidae, Oligochaeta) in three types of oak-hornbeam of the Niepolomicka Forest. III. Energy flow through earthworm populations. Pedobiologia 38, 28–35.

SAS Institute Inc., 1990. SAS procedures guide, Version 6, third edn. SAS Institute, Cary, NC. pp. 705.

Satchell, J.E., 1967. Lumbricidae. In: Burges, A., Raw, F. (Eds.), Soil Biology. Academic Press, New York. pp. 259–322.

Shipitalo, M.J., Protz, R., Tomlin, A.D., 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.

Subler, S., Baranski, C.M., Edwards, C.A., 1997. Earthworm additions increased short-term nitrogen availability and leaching in two grain-crop agroecosystems. Soil Biol. Biochem. 29, 413–421.

Whalen, J.K., 1998. Effects of earthworms on nitrogen flux and transformations in agroecosystems. Ph.D. thesis, Ohio State University, Columbus, Ohio, USA.

Whalen, J.K., Parmelee, R.W., Edwards, C.A., 1998. Population dynamics of earthworm communities in corn agroecosystems receiving inorganic and organic fertilizer amendments. Biol. Fertil. Soils 27, 470–475.

Whalen, J.K., Parmelee, R.W., 1999. Growth of *Aporrectodea tuberculata* (Eisen) and *Lumbricus terrestris* L. under laboratory and field conditions. Pedobiologia 43, 1–10.