



Role of the anecic earthworm *Lumbricus terrestris* L. in the distribution of plant residue nitrogen in a corn (*Zea mays*)–soil system

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Abstract

While the benefits of earthworms to crop production are widely acknowledged, the mechanisms involved are poorly understood. We examined the effects of an anecic earthworm (*Lumbricus terrestris*) on the distribution of plant residue N in a corn (*Zea mays*)/soil system. Soil (mixed Ap and B horizons) mesocosms (10 cm diameter, 39 cm deep) were amended with ¹⁵N-labeled corn litter, inoculated with one earthworm per mesocosm (WORM) or none (CTRL), and pre-incubated for 1, 2 or 3 weeks. Earthworms and remaining plant residues were removed and sweet corn grown in the mesocosms in a greenhouse for 3 weeks. Litter, earthworms, shoots, roots and bulk and burrow soil were analyzed for total N and ¹⁵N. Plant and earthworm biomass were also determined. Earthworms had no significant effect on the N content of shoots, roots or bulk soil. Recovery of ¹⁵N ranged from 92.6 to 101.9% in CTRL and 60.2 to 83.2% in the WORM treatment. The ¹⁵N content of bulk soil in the WORM treatment was significantly higher than in CTRL and increased with pre-incubation time. Excess at.% ¹⁵N of burrow soil was 10–100 times higher than in bulk soil. Incorporation of ¹⁵N by shoots and roots was significantly higher in the WORM treatment and increased significantly with pre-incubation time only in the WORM treatment. In WORM mesocosms pre-incubated for 3 weeks, the distribution of added ¹⁵N was 9.8% in litter, 6.5% in plant, 31.5% in soil, 12.0% in earthworms and 39.8% presumably lost as gas; in CTRL mesocosms, the values were 75.7% in litter, 3.2% in plant, 13.7% in soil and 7.4% in presumed gas losses. The activities of *L. terrestris* altered the distribution of plant residue N significantly, increasing the transfer of N to plants and soil and enhancing losses of N in the gas phase as pre-incubation time increased.

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1. Introduction

It is commonly accepted that the activities of earthworms benefit plant growth and productivity, particularly in pastures (Stockdill, 1982) and agricultural

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production systems (Edwards and Batey, 1992; Hopp and Slater, 1949). In a recent analysis of the literature, Scheu (2004) reported that shoot biomass of plants was found to increase significantly in 79% of published studies when earthworms were present. Mounting evidence of the beneficial role of earthworms in crop production has led to the suggestion that the health and activity of earthworms be considered when assessing and implementing agronomic practices (Lavelle et al., 1989; Linden et al., 1994). While positive effects have been demonstrated, the direct and indirect effects of earthworms on soil fertility and plant production are not well understood. Elucidation of the mechanisms involved must receive further attention if earthworms are to be used successfully in agroecosystem management.

The beneficial effects of earthworms on the productivity of ecosystems and on soil and water conservation have been attributed to a number of physical, ecological and biogeochemical factors. The positive physical effects of earthworms include improved soil structure (Shipitalo and Protz, 1988), increased macroporosity (Binet et al., 1997) with concomitant effects on aeration (Dziejowski et al., 1997) and water dynamics (Francis and Fraser, 1998), as well as provision of paths for root exploration (Cortez and Bouché, 1992). Earthworms also alter the ecology of the soil to suppress plant pathogens and/or promote the growth of microflora and fauna that may be beneficial to crops (Clapperton et al., 2001). Physical and ecological mechanisms affect plant growth indirectly by improving the soil conditions, allowing the plant to grow better. By contrast, the biogeochemical mechanisms affect plant growth and ecosystem productivity directly because they control the availability of plant nutrients, especially nitrogen (e.g. Devliegher and Verstraete, 1996; Görres et al., 1997; Subler et al., 1998; Amador et al., 2003).

Because of the relatively high cost of fertilizer N and concerns with the fate of this often-limiting nutrient, much effort has been focused on assessing the extent to which the dynamics of nitrogen transformations in soil are altered by the activities of earthworms. Despite the relatively large number of studies that have evaluated the transformations of nitrogen in earthworm-altered soil, a number of questions on the role of earthworms in crop nutrition remain largely unanswered.

Our research objective was to investigate the distribution of litter-N in an earthworm–plant soil system in the presence and absence of earthworms. Specifically, we employed ^{15}N -labeled corn litter as a tracer in mesocosms planted to corn (*Zea mays*) to elucidate whether crop plants benefit from litter-N more when the soil is amended with earthworms than in their absence. In agriculture, and particularly in low-input systems, appropriate timing of N availability and plant needs is indispensable for greater productivity. We, thus, also investigated the effect of timing and duration of litter-N release relative to planting of corn seedlings on the N nutrition and productivity of corn plants. To this end, prior to planting corn, ^{15}N -litter was added to mesocosms and incubated for 1, 2 and 3 weeks to determine how time, which presumably controls the release of inorganic N from litter, affected N transfers within the earthworm–plant–soil system.

2. Materials and methods

2.1. Experimental design

The experiment consisted of two treatments: mesocosms amended with *Lumbricus terrestris* (WORM) and mesocosms without earthworms (CTRL). The experiment was conducted in two stages. The first stage involved a pre-incubation period of 1, 2 or 3 weeks during which we expected N transformations to proceed (Fig. 1). Three replicates were employed for each combination of treatment and pre-incubation time. In addition, a single replicate of each combination containing only unlabeled litter was employed. Variations in pre-incubation time allowed us to assess the timing of N release by earthworms relative to plant growth. During the second stage of the experiment, corn plants were grown for 3 weeks after removal of remaining litter and earthworms. This experimental design reflects the limitations farmers face with respect to planting crops in relation to earthworm activities, since only the time of planting is subject to their control.

Mesocosms consisted of white polyvinyl chloride (PVC) pipes (10 cm diameter, 50 cm height) filled with soil to a depth of 39 cm and a bulk density of 1.15 g cm^{-3} . All mesocosms received 2.20 g (dry

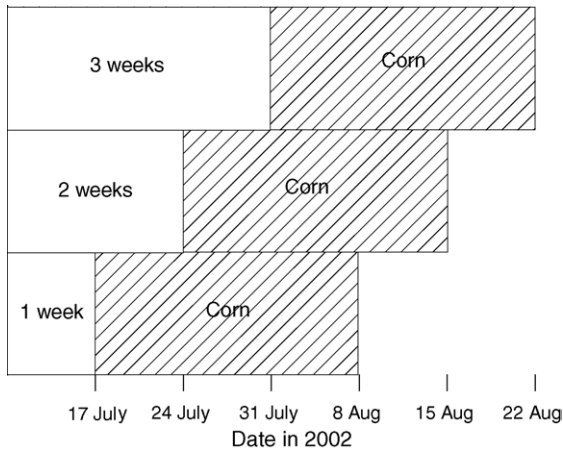


Fig. 1. Time course of experimental treatments. Mesocosms were amended with ^{15}N -labeled corn litter and pre-incubated with (WORM) or without (CTRL) *L. terrestris* for 1, 2 or 3 weeks (open bars). After pre-incubation, the litter and earthworms were removed, the mesocosms planted with a corn seedling, and the plants grown in a greenhouse for 3 weeks (hatched bars).

weight) of aged corn litter (rate equivalent of 2.6 Mg ha^{-1}). This corresponded to 30.14 mg N and $4.45 \text{ mg } ^{15}\text{N}$ per mesocosm. A fiberglass mesh, secured in place with duct tape, was used to cover the top and bottom of the cores. Mesocosms were filled with a 1:1 (w/w) mixture of soil from the Ap and B horizons of an Enfield silt loam (coarse-silty over sandy or sandy-skeletal, mixed, active, mesic Typic Dystrudepts) with an organic matter content of 36 g kg^{-1} and a pH of 5.0 (Amador et al., 2003). The initial N and ^{15}N content of the soil was 1.7 g kg^{-1} and $0.3756 \text{ at.}\%$, respectively.

The experiment was started on 17 July 2002 with the addition of plant residues to all mesocosms and of earthworms to the WORM mesocosms (one per mesocosm; equivalent to $127 \text{ individuals m}^{-2}$). ^{15}N -labeled corn litter was produced by growing corn in a greenhouse using $(^{15}\text{NH}_4)_2\text{SO}_4$. Unlabeled litter was produced under identical conditions except that unlabeled ammonium sulfate was used. In both cases, the leaves were harvested after 50 d, dried for 30 d at room temperature, cut into 2 cm long pieces, and inoculated with a small amount of soil. The inoculated leaf litter was aged at room temperature ($20\text{--}22^\circ\text{C}$) for 30 d and stored frozen until used. Total N and ^{15}N content were measured prior to use. ^{15}N -labeled litter had a nitrogen content of 13.7 g kg^{-1} and a ^{15}N

content of $15.123 \text{ at.}\%$. Unlabeled litter had a nitrogen content of 13.5 g kg^{-1} and a ^{15}N content of $0.369 \text{ at.}\%$. Earthworms had an N content of 61.2 g kg^{-1} and a ^{15}N content of $0.366 \text{ at.}\%$

Mesocosms were placed in a controlled temperature chamber and incubated for 1, 2 or 3 weeks. Soil temperature and soil water potential were monitored at a depth of 10 cm. Soil temperature ranged from 15.0 to 16.5°C and water potential was maintained between -20 and -40 kPa by periodic watering.

2.2. Greenhouse incubation

Earthworms and litter were removed from the soil prior to planting, and processed and analyzed as described below. Mesocosms were planted with one corn seedling that was sprouted previously on moist paper towels for 5–6 d. Mesocosms were sampled destructively after incubation for 3 weeks in a greenhouse.

2.3. Sampling and processing

2.3.1. Litter

All visible litter was removed from the surface of pre-incubated mesocosms by hand prior to planting. Only litter that was clearly incorporated into middens was left in place. Litter from each mesocosm was placed in a separate sealable plastic bag and stored frozen.

2.3.2. Earthworms

To remove earthworms from pre-incubated mesocosms, the top and bottom mesh was removed, the core placed on top of a 40 cm long metal cylinder (with a diameter slightly smaller than the internal diameter of the core), and the PVC cylinder slid down to expose the soil in 13 cm sections. Earthworms were removed by hand, and the length and diameter of burrows and the presence of plant detritus in burrows determined. After earthworm removal, the soil core was slid back into the PVC cylinder and the mesh on the bottom replaced.

Recovered earthworms were placed individually in a glass Petri dish filled with water for approximately 15 min, and dried by placing on paper towels. After determining the fresh weight (f.w.) of the earthworm, it was placed in a sealable plastic bag and killed by

freezing. Frozen worms were thawed and dried to a constant weight in a vacuum desiccator, and dried further by freeze-drying. Dried earthworms were ground (40-mesh) using a Wiley mill. The ground earthworm tissue was stored in a desiccator at room temperature.

2.3.3. Shoot biomass

Shoot height was determined just prior to sampling. After sampling the fresh weight of shoots was determined, the shoots were dried to a constant weight at 65 °C, and the dry weight determined. Dried shoots were subsequently cut into 2–3 cm pieces and ground (40-mesh) using a Wiley mill. The ground shoots were stored in a desiccator at room temperature.

Weeds were dug out of the soil with a metal spatula and the small amount of soil associated with the roots removed carefully with a stream of water as described for roots (below). They were subsequently dried to a constant weight at 65 °C and the dry weight determined. Because of their small biomass, dried weeds from each pre-incubation time were combined before grinding (40-mesh) using a Wiley mill. Ground weeds were stored in a desiccator at room temperature.

2.3.4. Soil and roots

To sample soil and roots the bottom mesh was removed from each mesocosm, the mesocosm placed on top of a 40 cm long metal cylinder with a diameter slightly smaller than the internal diameter of the core, and the PVC cylinder slid down to expose the soil in three consecutive, 13 cm segments. The above-ground portion of the plant was cut and processed as described above. In cores that received earthworms, burrow interception by roots was noted and the burrow soil (within 4 mm of burrow wall) was sampled through the length of the burrow within each 13 cm segment. The bulk soil from each segment was placed in a large, sealable plastic bag, and stored at 4 °C. Roots were removed from the soil sample within 24 h of collection. Soil was stored in the dark at 4 °C after root removal and dried at 105 °C prior to analysis.

To remove roots, soil from each section of a mesocosm was placed on a plastic tray, and the soil broken up gently (to minimize root breakage) by hand to reveal roots. Roots were removed by hand, with soil turned over repeatedly until no more roots were visible. The roots were shaken to remove all the soil particles,

placed on a 0.5 mm mesh metal colander, and washed with three 1000 ml volumes of water using a hose fitted with a showerhead. Soil particles were removed from the sieve between washings. The washed roots were dried to a constant weight at 65 °C and their weight recorded. After root dry weight was determined, the roots from all three sections of a particular core were pooled, ground (40-mesh) in a Wiley mill and stored in a desiccator at room temperature.

2.4. Nitrogen analyses

The concentration of ammonium and nitrate in soil samples was determined by extraction of 1 g soil with 10 ml of 2N KCl, followed by filtration and measurement of NH_4^+ and NO_3^- by colorimetric analyses using an automated nutrient analyzer (Alpkem Flow Solution IV, OI Analytical, College Station, TX, USA).

The total N and ^{15}N content of litter, shoots, roots, soil and earthworms were determined at the Stable Isotope Facility, University of California, Davis, CA, USA. Analysis was performed using an integrated stable isotope analyzer (model Integra, PDZ Europa Ltd., Cheshire, England).

2.5. Statistical analyses

Data were analyzed using a two-way analysis of variance, with α values considered significant at the $P \leq 0.05$ level unless stated otherwise. The two factors were earthworms and pre-incubation time, with the results checked for interaction. Changes in litter mass with pre-incubation time were also analyzed using linear regression. Statistical analyses were conducted using version 2.03 of SigmaStat (SPSS Inc., Chicago, IL, USA). Differences among means were evaluated using pairwise multiple comparison procedures (Tukey's test).

3. Results

3.1. Earthworms and litter

3.1.1. Earthworm biomass and moisture content

Live earthworms were recovered and burrows were found in all WORM mesocosms. Burrows had a

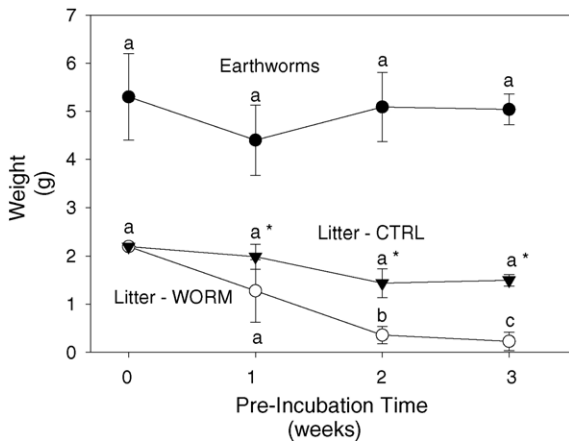


Fig. 2. Earthworm fresh weight and amount of litter remaining in mesocosms without (CTRL) and with (WORM) *L. terrestris* initially and after pre-incubation for 1, 2 and 3 weeks. Values are means ($n = 3$). Bars represent one standard deviation. Values followed by the same letter for a particular variable were not significantly different. Litter values followed by an asterisk (*) were significantly different within a pre-incubation time.

diameter of about 1 cm and depth of 20–25 cm, and were between 15 and 30° off the vertical. Corn litter was present in all burrows prior to planting. Root interception of burrows was observed in 11 of the 12 mesocosms sampled.

There were no significant differences between initial earthworm fresh weight and fresh weight after pre-incubation for 1, 2 or 3 weeks (Fig. 2), nor were there differences in dry weight (data not shown). The moisture content of earthworms after incubation was $78.0 \pm 3.0\%$, with no significant differences observed

among pre-incubation times. This value is within the normal range reported by Edwards and Bohlen (1996). Average earthworm fresh weight loss after pre-incubation for 3 weeks was 9.4%.

3.1.2. Earthworm total N and ¹⁵N content

Pre-incubation time had no statistically significant effect on the N content of earthworms, which ranged from 54.3 to 61.2 g kg⁻¹ (Table 1). The ¹⁵N content of earthworms after pre-incubation for 2 weeks (395 μg ¹⁵N kg⁻¹) was almost double that after 1 week (229 μg ¹⁵N kg⁻¹), but was not significantly different between 2 and 3 weeks (Table 1), suggesting that earthworms had reached equilibrium with respect to ¹⁵N dynamics. Pre-incubation for 2 and 3 weeks resulted in earthworm tissue with significantly higher excess at.% ¹⁵N than after 1 week (Table 1).

3.1.3. Litter total N and ¹⁵N content

The amount of litter remaining above ground was significantly lower in WORM than in CTRL treatments, with statistically significant differences between treatments observed after pre-incubation for 2 and 3 weeks (Fig. 2). A two-way analysis of variance showed that the mass of litter declined significantly with pre-incubation time only in the WORM treatment, with 90% of the litter disappearing in mesocosms after pre-incubation for 3 weeks (Fig. 2). However, linear regression analysis of the data showed a significant declining trend in litter mass with pre-incubation time for both treatments ($P < 0.05$).

The N content of litter ranged from 11.6 to 16.3 mg kg⁻¹ and was not affected significantly by the

Table 1
Nitrogen content, ¹⁵N content and at.% excess ¹⁵N of earthworms and litter in mesocosms incubated with (WORM) and without (CTRL) *L. terrestris* for 1, 2 or 3 weeks ($n = 3$)

Pool	Property	Pre-incubation time (weeks)					
		1		2		3	
		WORM	CTRL	WORM	CTRL	WORM	CTRL
Earthworms	N content (g kg ⁻¹)	61.2a	NA	54.9a	NA	54.3a	NA
	¹⁵ N content (μg kg ⁻¹)	229a	NA	395b	NA	397b	NA
	Excess ¹⁵ N (at.%)	0.405a	NA	0.912b	NA	0.720b	NA
Litter	N content (g kg ⁻¹)	14.2a	12.9a	15.0a	16.3a	13.8a	11.6a
	¹⁵ N content (mg kg ⁻¹)	2002a	1821a	2115a	2358b	1895a	1576c
	Excess ¹⁵ N (at.%)	14.061a	14.127a	14.075a	14.484a	13.713b	13.597b

Values followed by the same letter within a row for a particular treatment were not significantly different. Bold indicates significant differences between treatments for a particular pre-incubation time. NA: not applicable.

presence of earthworms or by pre-incubation time (Table 1). Pre-incubation time had no significant effect on the ^{15}N content of litter in the WORM treatment, which ranged from 1895 to 2115 $\text{mg } ^{15}\text{N kg}^{-1}$ (Table 1). In contrast, there were significant differences in the ^{15}N content of litter among pre-incubation times in the CTRL treatment, with the highest values observed at 2 weeks and the lowest values after 3 weeks. Litter in WORM mesocosms had a significantly higher ^{15}N content than CTRL mesocosms only after pre-incubation for 3 weeks. Litter in mesocosms pre-incubated for 3 weeks had a lower excess at $\% ^{15}\text{N}$ than after 1 or 2 weeks, and no differences were observed between treatments for a particular pre-incubation time (Table 1).

3.2. Shoots and roots

3.2.1. Biomass

Pre-incubation time had no significant effect on the height, fresh or dry weight of corn shoots in the CTRL treatment (Fig. 3). In contrast, shoot height, fresh and dry weight were significantly higher in the WORM treatment after pre-incubation for 2 weeks than for 1 or 3 weeks. These properties were significantly higher in WORM than in the CTRL treatment only in mesocosms pre-incubated for 2 weeks. In addition, there was a statistically significant interaction between earthworm treatment and pre-incubation time.

Neither pre-incubation time nor the presence of earthworms had a significant effect on root dry weight except for mesocosms pre-incubated for 2 weeks, where root weight was significantly higher in the WORM treatment (Fig. 3). Root weight was significantly greater in the WORM treatment at 0–13 and 13–26 cm, and only in cores pre-incubated for 2 weeks (data not shown).

The mean dry weight of weeds (whole plant) was 0.03, 0.08 and 0.06 g for pre-incubation times of 1, 2 and 3 weeks, respectively, and no significant effects of treatment or pre-incubation time were found. Weeds accounted for 2.3–3.9% of the total dry plant matter (roots + shoots + weeds) sampled.

3.2.2. ^{15}N and total N content

Neither pre-incubation time nor the presence of earthworms had a significant effect on the N content of corn shoots, which ranged from 40.2 to 45.7 g kg^{-1}

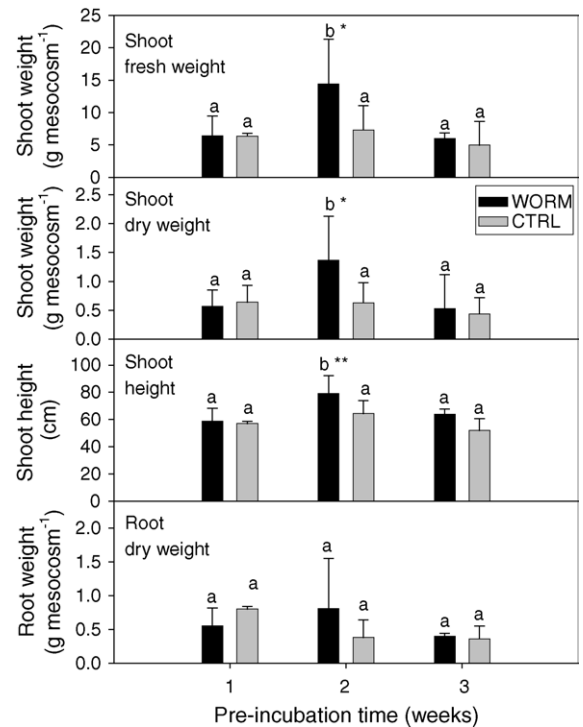


Fig. 3. Corn (*Z. mays*) shoot fresh and dry weight, shoot height and root dry weight after growing for 3 weeks in soil that was pre-incubated with (WORM) or without (CTRL) *L. terrestris* for 1, 2 or 3 weeks. Values are means ($n = 3$). Bars represent one standard deviation. Values followed by the same letter for a particular treatment were not significantly different. Values followed by an asterisk (*) were significantly different within a pre-incubation time.

(Table 2). The N content of roots increased with pre-incubation time in both treatments with significant differences observed among all pre-incubation times (Table 2); however, there were no significant differences between treatments regardless of pre-incubation time.

The N content of weeds ranged from 30.0 to 46.4 g N kg^{-1} . The low total biomass of weeds required pooling from different replicates prior to analyses, precluding statistical analyses of the data.

The ^{15}N content of corn shoots in the CTRL treatment ranged from 242 to 317 $\mu\text{g } ^{15}\text{N kg}^{-1}$ and it was not affected significantly by pre-incubation time (Table 2). In contrast, the ^{15}N content of shoots in the WORM treatment increased significantly with pre-incubation time, with 307 and 483 $\mu\text{g } ^{15}\text{N kg}^{-1}$ in mesocosms pre-incubated for 1 and 3 weeks,

Table 2

Nitrogen content, ^{15}N content and at.% excess ^{15}N of shoots and roots of corn (*Z. mays*), and of weeds grown for 3 weeks in soil pre-incubated with (WORM) and without (CTRL) *L. terrestris* for 1, 2 and 3 weeks ($n = 3$ except for weeds, where $n = 1$)

Pool	Property	Pre-incubation time (weeks)					
		1		2		3	
		WORM	CTRL	WORM	CTRL	WORM	CTRL
Shoots	N content (g kg^{-1})	44.1a	41.3a	44.1a	45.7a	40.2a	44.3a
	^{15}N content ($\mu\text{g kg}^{-1}$)	307a	242a	342b	271a	483c	317a
	Excess ^{15}N (at.%)	0.685a	0.583a	0.761b	0.608a	1.215c	0.719a
Roots	N content (g kg^{-1})	13.7a	13.4a	15.9b	15.9b	19.4c	18.7c
	^{15}N content ($\mu\text{g kg}^{-1}$)	62a	43a	121b	57a	219c	60a
	Excess ^{15}N (at.%)	0.463a	0.320a	0.744b	0.351a	1.160c	0.317a
Weeds	N content (g kg^{-1})	37.4	NA	30.0	46.4	30.9	32.4
	^{15}N content ($\mu\text{g kg}^{-1}$)	104	NA	61	69	227	65
	Excess ^{15}N (at.%)	1.587	NA	1.575	1.148	2.996	1.395

Values followed by the same letter within a row for a particular treatment were not significantly different. Bold indicates significant differences between treatments for a particular pre-incubation time. NA: not applicable.

respectively. In addition, corn shoots in the WORM treatment had a significantly higher ^{15}N content than those in the CTRL treatment in mesocosms pre-incubated for 2 and 3 weeks (Table 2). The ^{15}N content of weeds ranged from 61 to 227 $\mu\text{g }^{15}\text{N kg}^{-1}$, lower than that for corn shoots in treatments pre-incubated for the same amount of time.

Pre-incubation time had no significant effect on the ^{15}N content of corn roots in the CTRL treatment, with values ranging from 43 to 60 $\mu\text{g }^{15}\text{N kg}^{-1}$ (Table 2). In the WORM treatment, the ^{15}N content of roots nearly doubled with every week that the mesocosms were pre-incubated with values ranging from 62 to 219 $\mu\text{g }^{15}\text{N kg}^{-1}$ after 1 and 3 weeks, respectively.

Enrichment of corn shoots with ^{15}N in the CTRL treatment ranged from 0.583 to 0.719 at.% and was not significantly affected by pre-incubation time (Table 2). Pre-incubation in the WORM treatment increased ^{15}N enrichment of corn shoots significantly, with values of 0.685 and 1.215 at.% after pre-incubation for 1 and 3 weeks, respectively. The level of ^{15}N enrichment of corn shoots was significantly higher in the WORM than in the CTRL treatment in mesocosms pre-incubated for 2 and 3 weeks.

No significant effect of pre-incubation time was observed on ^{15}N enrichment of corn roots in the CTRL treatment, which ranged from 0.317 to 0.351 at.% (Table 2). In the WORM treatment, ^{15}N enrichment of corn roots was enhanced significantly by pre-incubation time, ranging from 0.463 to 1.160 at.% after pre-

incubation for 1 and 3 weeks, respectively. Roots in the WORM treatment had significantly higher excess ^{15}N than CTRL in mesocosms pre-incubated for 2 and 3 weeks.

3.3. Soil

Levels of inorganic N, nitrate-N and ammonium-N did not differ significantly among bulk soil from WORM and CTRL and burrow soil from WORM treatment (data not shown). Nitrate-N constituted the bulk (80–95%) of the soil inorganic nitrogen pool for bulk and burrow soil regardless of treatment and pre-incubation time. Inorganic N concentration at 13–26 and 26–39 cm was roughly twice that found at 0–13 cm in all cases (data not shown).

The total N content of bulk soil ranged from 1.6 to 1.8 g N kg^{-1} soil, with no significant differences observed in response to pre-incubation time or the presence of earthworms (Table 3). Burrow soil N was within the range of values observed for bulk soil. Depth had no apparent effect on bulk or burrow soil total N content.

The ^{15}N content of bulk soil in the top 13 cm of the CTRL treatment declined with pre-incubation time from 492 to 308 $\mu\text{g }^{15}\text{N kg}^{-1}$ for 1 and 3 weeks, respectively, although the differences were not significant (Table 3). In contrast, the ^{15}N content of bulk CTRL soil at 13–26 and 26–39 cm increased with incubation time, although again the differences were

Table 3

Nitrogen content, ^{15}N content, and at.% excess ^{15}N of bulk and burrow soil at different depths in mesocosms after pre-incubation with (WORM) and without (CTRL) *L. terrestris* for 1, 2 or 3 weeks and subsequently planted with corn (*Z. mays*) for 3 weeks ($n = 3$)

Pool	Depth (cm)	Property	Pre-incubation time (weeks):					
			1		2		3	
			WORM	CTRL	WORM	CTRL	WORM	CTRL
Bulk soil	0–13	N content (g kg^{-1})	1.7a	1.7a	1.7a	1.7a	1.8a	1.7a
		^{15}N content ($\mu\text{g kg}^{-1}$)	530a	492a	952b	440a	746b	308a
		Excess ^{15}N (at.%)	0.026a	0.023a	0.050b	0.020a	0.036a	0.013a
	13–26	N content (g kg^{-1})	1.6a	1.7a	1.7a	1.7a	1.7a	1.6a
		^{15}N content ($\mu\text{g kg}^{-1}$)	120a	80a	167a	254b	262b	150a
		Excess ^{15}N (at.%)	0.003a	0.001a	0.011a	0.005a	0.011a	0.005a
	26–39	N content (g kg^{-1})	1.7a	1.7a	1.7a	1.7a	1.7a	1.7a
		^{15}N content ($\mu\text{g kg}^{-1}$)	43a	55a	102b	63a	97b	85a
		Excess ^{15}N (at.%)	0.001a	0.001a	0.001a	0.001a	0.001a	0.001a
Burrow soil	0–13	N content (g kg^{-1})	1.7a	NA	1.7a	NA	1.8a	NA
		^{15}N content ($\mu\text{g kg}^{-1}$)	5221a	NA	4478b	NA	3684b	NA
		Excess ^{15}N (at.%)	0.294a	NA	0.267a	NA	0.203a	NA
	13–26	N content (g kg^{-1})	1.8a	NA	1.7a	NA	1.8a	NA
		^{15}N content ($\mu\text{g kg}^{-1}$)	954a	NA	1150a	NA	797a	NA
		Excess ^{15}N (at.%)	0.048a	NA	0.072a	NA	0.039a	NA

Values followed by the same letter within a row for a particular treatment were not significantly different. Bold indicates significant differences between treatments for a particular pre-incubation time. NA: not applicable.

not significant. The ^{15}N content of bulk soil from the CTRL treatment decreased with depth, with the most marked differences observed after pre-incubation for 1 week. Depth differences became less apparent with longer pre-incubation time.

Bulk soil in the WORM treatment had a significantly higher ^{15}N content than CTRL soil in mesocosms pre-incubated for 2 and 3 weeks at 0–13 cm, 3 weeks at 13–26 cm and for 2 weeks at 26–39 cm (Table 3). The ^{15}N content of bulk soil in the WORM treatment generally increased with pre-incubation time at all depths (Table 3). The highest ^{15}N content was observed in the top 13 cm of soil, with values decreasing with depth for all pre-incubation times.

Burrow soil in the WORM treatment had a ^{15}N content that was 5–10 times higher than in the bulk soil at 0–13 cm and three–seven times higher than in the bulk soil at 13–26 cm (Table 3). The ^{15}N content of burrow soil at 0–13 cm was highest in mesocosms pre-incubated for 1 week ($5221 \mu\text{g } ^{15}\text{N kg}^{-1}$), declining significantly for mesocosms pre-incubated for 2 ($4478 \mu\text{g } ^{15}\text{N kg}^{-1}$) and 3 ($3684 \mu\text{g } ^{15}\text{N kg}^{-1}$) weeks. Burrow soil at 0–13 cm had a ^{15}N content that was four–five times higher than burrow soil at 13–26 cm for all pre-incubation times.

Enrichment with ^{15}N was not affected significantly by pre-incubation time in the CTRL treatment bulk soil at any depth (Table 3). In WORM soil only, pre-incubation for 2 weeks resulted in significantly higher enrichment, and only at 0–13 cm. WORM soil had a significantly higher level of excess ^{15}N than CTRL soil only at 0–13 cm in mesocosms pre-incubated for 2 and 3 weeks. The level of ^{15}N enrichment decreased drastically with soil depth in all treatments. In burrow soil, excess at.% ^{15}N was one and two orders of magnitude higher at 0–13 and 13–26 cm, respectively, than in bulk soil. Pre-incubation time had no significant effect on excess at.% ^{15}N of burrow soil at any depth.

3.4. ^{15}N recovery

We examined the effects of *L. terrestris* on the distribution of ^{15}N in WORM and CTRL mesocosms as a function of pre-incubation time. The distribution of ^{15}N differed considerably among treatments, and these differences became more pronounced with increased pre-incubation time (Table 4). In the CTRL treatment recovery of ^{15}N ranged from 93 to 102%, with litter accounting for the majority of ^{15}N (76–82%), followed by soil (14–18%), plant (including

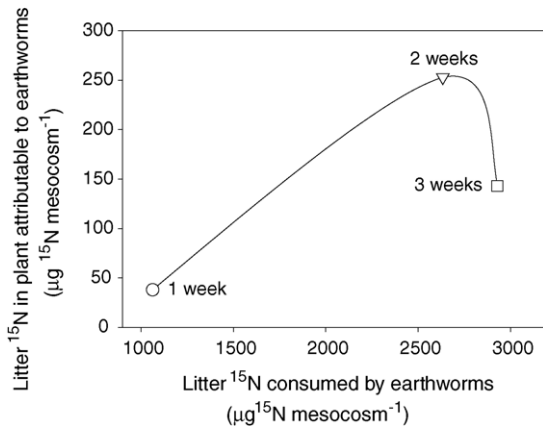


Fig. 4. Relationship between litter ¹⁵N consumed by *L. terrestris* and litter-derived ¹⁵N in corn (*Z. mays*) plants that was attributable to the presence of *L. terrestris*. Labels next to symbols indicate pre-incubation times.

between 25 and 30% (Whalen and Parmelee, 1999), indicating that most of the ingested litter N has the potential to be excreted and become available to plants. In the present study, the relationship between amount of litter N consumed by earthworms and the amount of litter N found in corn plants exhibited a maximum when mesocosms were pre-incubated for 2 weeks, with approximately 10% of the litter N consumed by earthworms subsequently found in the plant biomass (Fig. 4). These results suggest that there may be a relatively short time for plants to take advantage of litter N released through the activities of anecic earthworms before competing processes become more important.

The vertical distribution of N originating in the litter was altered by the activities of earthworms. This was indicated by higher levels of ¹⁵N enrichment found in at greater depths in burrow soil. Deeper distribution of litter-derived N in the soil was also caused by *M. anomala* (Gilot-Villenave et al., 1996). We did not observe significant differences in inorganic N or total N content between burrow and surrounding bulk soil, or soil from mesocosms without worms. This is likely the result of uptake by corn plants, which are active sinks for this nutrient. Accumulation of nitrate in earthworm burrow soil observed in previous studies is, thus, attributable to the absence of a plant sink, as suggested earlier (Amador et al., 2003). We did observe significant ¹⁵N enrichment of the bulk soil

in the WORM treatment as well as in burrow soil N, indicating that litter-derived N made up a more significant portion of the soil N pool when earthworms were present.

The significant enhancement in shoot and root biomass in mesocosms pre-incubated with earthworms for 2 weeks appears to support the hypothesis that the presence of earthworms increases crop biomass. However, the fact that the enhancement was limited to the 2-weeks pre-incubation period is difficult to explain. The response was unique to the WORM treatment, since no significant differences in shoot fresh or dry biomass or height, or root dry biomass were observed in response to pre-incubation time in the CTRL treatment. These results rule out differences in environmental conditions (e.g. solar radiation and temperature) among sampling dates as the reason for the effect. The water potential in all the mesocosms ranged from -20 to -50 kPa for the duration of the experiment, suggesting that water availability was not involved. Similarly, there were no significant differences in weed biomass between treatments or pre-incubation times, such that competition for N by weeds is an unlikely explanation. The difference was not associated with enhanced N content of shoots or roots, since there were no significant differences in these properties, ruling out additional N release. Weeds accounted for a very small (2.3–3.9%) fraction of the plant biomass in the mesocosms, and their biomass was not significantly affected by treatment or pre-incubation time. Thus, weed growth does not appear to account for differences in corn plant biomass. Enhanced growth may be the result of limiting factors that were not evaluated in the present study. Increased plant growth associated with the presence of earthworms is a well-established phenomenon (Scheu, 2004), although positive effects are not observed consistently. The tropical endogeic earthworm *M. anomala* increased production of maize stalks and grains by 12 and 18%, respectively (Gilot-Villenave et al., 1996). Similarly, Whalen et al. (2001) observed that *Aporrectodea tuberculata*, an endogeic earthworm, increased shoot and root production in annual ryegrass (*Lolium multiflorum* L.) and Hameed et al. (1994) found that *L. terrestris* increased the biomass and total N content of perennial ryegrass (*Lolium perenne* L.). In contrast, Stinner et al. (1997) found greater early season maize biomass in treat-

ments in which earthworms were reduced. Devliegher and Verstraete (1996) observed that the dry matter yield of maize and its N content were lower in soil worked by *L. terrestris*.

Litter N transfer to corn plants also occurred to a limited extent in the absence of earthworms. The fraction of litter N in CTRL mesocosms incorporated into either shoots or roots did not change significantly with pre-incubation time. Transfer of litter N to soil in the absence of earthworms may occur subsequent to microbial mineralization of litter N via leaching. Nitrogen can also be transferred by fungi capable of bridging the gap between litter and soil (Frey et al., 2000, 2003). These mechanisms are most effective near the soil surface, especially given that water was added at the surface and only in quantities to keep the soil at field capacity. The differences in ^{15}N content of litter observed in the CTRL treatment with pre-incubation time likely reflect changes in the relative rates of net C and N losses. No differences were observed in the WORM treatment. These results suggest that the presence of *L. terrestris* affects the processes involved in the loss of these elements from litter at the soil surface.

The results of our experiment show that enrichment of the soil N pool with ^{15}N was limited to the top 13 cm in the absence of earthworms, while in the presence of earthworms the bulk soil ^{15}N pool was enriched deeper in the mesocosms, possibly as a result of movement from burrow soil. These data indicate that the feeding activities of anecic earthworms may either remove, circumvent, or otherwise change the limitations imposed on litter N transfer to corn plants.

We observed that a longer pre-incubation period translated into lower ^{15}N concentration in burrow soil. For the 3-week pre-incubation period, the concentration of ^{15}N in burrow soil in the top 13 cm was about two-thirds that for the 1-week pre-incubation period. The reduction of burrow soil ^{15}N concentration observed with increasing pre-incubation time was likely the result of plant uptake, particularly for mesocosms pre-incubated for 1 and 2 weeks.

In general, losses of ^{15}N from the WORM mesocosms not accounted for by any of the pools that we measured were assumed to result from gaseous losses of N. The amount of ^{15}N lost from the mesocosms was a function of time, proceeding at a rate of $600 \mu\text{g } ^{15}\text{N mesocosm}^{-1} \text{ week}^{-1}$. This

suggests a continuous process with time, such as denitrification, when conditions are kept constant. Other explanations are less likely. We did not irrigate the mesocosms sufficiently to incur leaching losses, and analysis of ^{15}N accounted for both inorganic and organic forms of nitrogen. Brown and Doube (2004) have suggested that earthworm casts and burrows may be important microsites for denitrification because they possess larger populations of denitrifiers, higher levels of soluble C and NO_3 , and higher water contents than the surrounding soil. Knight et al. (1992) found enhanced rates of denitrification in earthworm casts than in a surrounding pasture soil.

Fragoso et al. (1997) have argued that the synchrony of earthworm activities with crop N demands and synlocalization within the sphere of root growth are essential to the enhancement of primary production by earthworms. Our results suggest that the benefits of earthworms to the N nutrition of corn may be confined to the time shortly after litter translocation and N mineralization take place. At longer time scales, plants may compete with microbial processes that result in net removal of N from the system, such as denitrification, which may also be affected by earthworms. Similarly, leaching losses of NO_3 can be enhanced by the activities of anecic earthworms (Edwards et al., 1992; Li and Ghodrati, 1995).

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