

## Effects of *Lumbricus terrestris* L. on nitrogen dynamics beyond the burrow

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### Abstract

The degree to which earthworms can affect the availability of plant nutrients depends on their distribution following formation in burrow soil. We conducted a mesocosm-scale laboratory experiment to test the hypothesis that anecic earthworms (those that build semi-permanent vertical burrows) can affect C and N dynamics beyond the confines of the soil immediately surrounding their burrows. Nitrate and ammonium concentrations and the rate of C mineralization were determined in burrow (defined as soil within 5 mm from the macropore wall, regardless of its origin) and bulk soil of treatments amended with *Lumbricus terrestris* (WORM) and in treatments containing artificial burrows (ARTF) and artificial burrows containing corn leaves (LEAF) periodically over the course of 16 weeks. Comparisons were made to soil in unamended treatments (CTRL) under two different moisture regimes, WET and DRY, during the course of the experiment. Nitrate concentration was significantly higher in WORM and LEAF bulk soil than in CTRL soil, but only under WET conditions. Differences in nitrate concentrations appeared after incubation for 5 weeks and persisted for 11 weeks. Ammonium concentration and C mineralization in bulk soil were not significantly different from CTRL soil for any of the treatments regardless of moisture regime, although values for both variables were significantly higher in burrow than in bulk soil in WORM and LEAF treatments. Anecic earthworms can enhance nitrate concentrations in soil beyond the confines of the burrow, a process that appears to be facilitated with increased soil moisture.

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### 1. Introduction

Management of the detrital foodweb is an integral part of sustainable agricultural production systems (Hendrix et al., 1986; Beare, 1997). Within this foodweb, anecic earthworms (those that build semi-permanent vertical burrows) act to change the physical

structure, gas and water fluxes, nutrient dynamics, and microbial ecology of soil (Edwards, 2004). These effects are generally presumed to be confined to soil that has come directly into contact with the earthworm, either through casting or burrowing activities. However, the effects of anecic earthworms need not be limited to cast and drilosphere soil. A number of studies have shown that the effects of other biological “hot spots” are observed beyond the soil immediately in contact with the resource driving enhanced activity. For example, Frey et al. (2000) have shown that fungal N translocation is a significant mechanism for soil N inputs and can account for the observed net N immobilized by surface residues decomposing in the field. Fungi are also important in

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the translocation of nutrients across the soil/litter interface in forest soils (Hart and Firestone, 1991). Gaillard et al. (1999) showed that microbial activity is enhanced and nitrogen derived from straw is found in soil beyond that immediately in contact with the plant residues.

Whether anecic earthworms exert effects on soil beyond the confines of burrows and casts has implications for understanding the importance of earthworms in agroecosystems. Enrichment of soil with nutrients and carbon beyond casts and burrows extends the potential for anecic earthworms to enhance availability of plant nutrients and to alter the structure and function of the microbial community. Alternatively, depletion of nutrients and carbon in the bulk soil would suggest that anecic earthworms could have a negative effect on the fertility and microbial activity of bulk soil. Furthermore, the timing of such effects would be critical to assessment of the role of anecic earthworms in controlling soil fertility and plant nutrition.

The present study is part of a larger experiment in which we examined the faunal and microbial ecology and biogeochemistry of C and N transformations in earthworm burrow soil (Amador et al., 2003, 2005; Savin et al., 2004). We observed that C and N mineralization were enhanced in the drilosphere of *Lumbricus terrestris* to a greater extent than in empty macropores and in macropores containing corn leaves (Amador et al., 2003). Furthermore, N dynamics were dominated by the accumulation of nitrate in earthworm burrow soil and in the soil surrounding macropores containing corn leaves (Amador et al., 2003). In addition, the effects of *L. terrestris* on N speciation in burrow soil were dependent on soil moisture content, with nitrate becoming the dominant inorganic N species at high soil moisture contents (Amador et al., 2005). In a separate experiment we also observed that transfer of  $^{15}\text{N}$  from corn litter to corn plants in mesocosms amended with *L. terrestris* took place in the absence of root interception of burrows (Amador and Görres, 2005). These results led us to examine the possibility that anecic earthworms may affect microbial processes in bulk soil.

In the current study we examine the hypothesis that anecic earthworms can affect C and N dynamics beyond the confines of the earthworm drilosphere. Specifically, we assessed potential effects of *L. terrestris* on the concentration of inorganic N and on C mineralization rates in the bulk soil adjacent to burrows. Because the interaction between burrow and bulk soil may be mediated by the presence of water, two different moisture regimes were employed to investigate whether

water facilitated transport of N across the burrow–bulk soil boundary. Additional treatments (artificial burrows and artificial burrows containing leaf litter) were included to determine whether such effects were strictly associated with the activities of anecic earthworms.

## 2. Materials and methods

### 2.1. Soil

Soil employed in the experiment was an Enfield silt loam (coarse-silty over sandy or sandy-skeletal, mixed, active, mesic Typic Dystrudepts) with an organic matter content of 3.6%. Details of soil collection, storage, and processing are described in Amador et al. (2005).

### 2.2. Plant litter and earthworms

Surface litter consisted of partially decomposed corn leaves from a no-till, organic corn field cut into ~2 cm long pieces (Amador et al., 2005). The litter had a mean C content of the litter was 38%, with an N content of 1.8% and a C/N ratio of 21.1 (Amador et al., 2005). Earthworms (*L. terrestris*) were purchased from a commercial outlet in North Kingstown, RI, 1 day before the beginning of the experiment and stored in bark chips in the dark at 4 °C.

### 2.3. Mesocosms

An experimental unit consisted of a 10.16 cm i.d. and 0.5 m long white polyvinyl chloride (PVC) cores filled with soil to a depth of 40 cm and packed to an average bulk density of 1.3 g/cm<sup>3</sup>. Nine grams (dry wt.) of corn litter (corresponding to 3.42 g C and 0.16 g N) was placed on the surface of the soil of all mesocosms. Litter was not replenished during the course of the experiment in order to mimic field conditions. Water (1.25 cm) was added to all mesocosms at the beginning of the experiment. Four treatments were employed:

- (I) no earthworms present (CTRL);
- (II) artificial burrows containing no plant residues (ARTF);
- (III) artificial burrows containing plant residues (LEAF);
- (IV) earthworms present (WORM).

Two moisture regimes were established for each treatment. Mesocosms in the WET regime received water on weeks 1, 3, 7, and 10 at a rate of 2 cm/mesocosm; mesocosms in the DRY regime received

0.5 cm/mesocosm. The watering schedule was the same for both regimes. WET and DRY mesocosms received a total of 9.25 and 3.25 cm of water/mesocosm, respectively (including the 1.25 cm added initially to all mesocosms). This corresponds to approximately one-third and one-ninth the average precipitation from April to June in Kingston, RI (NOAA, 1975) for the WET and DRY regimes, respectively.

A total of 24 mesocosms per treatment were constructed for the WET moisture regime, and 12 mesocosms per treatment for the DRY regime, with three replicates analyzed per treatment and moisture regime at each sampling time. All mesocosms were incubated in the laboratory at 18–20 °C.

Treatments are described in detail in Amador et al. (2005). Briefly, mesocosms in the CTRL treatment had no burrows. The ARTF and LEAF treatments had two artificial, 9 mm diameter burrows/mesocosm. Burrows in the ARTF treatment remained empty, whereas burrows in the LEAF treatment contained half a corn leaf (1.50 g dry wt.; corresponding to 0.49 g C and 0.023 g N per LEAF mesocosm). The WORM treatment consisted of adding three adult individuals of *L. terrestris* per mesocosm, representing an initial population density of 370 worms/m<sup>2</sup>.

#### 2.4. Sampling

Mesocosms were sampled destructively after 0, 1, 3, 5, 7, 10, 13, and 16 weeks of incubation for the WET moisture regime, and after 0, 3, 7, and 13 weeks for the DRY regime. Litter and earthworms were removed from the cores prior to soil sampling. Soil within 5 mm of the macropore wall (regardless of its origin) was defined as burrow soil. This definition of burrow soil is based on our own previous observations of the distance over which morphological differences are found in the soil surrounding burrows of *L. terrestris*. Mesocosms were sectioned into 10 cm lengths and burrow soil excavated with a spatula. Burrow and bulk soil were separated and soil from different depths pooled and stored at 4 °C in plastic bags.

#### 2.5. Soil analyses

Details of the analyses for C mineralization, inorganic N, and moisture content can be found in Amador et al. (2005). Briefly, C mineralization was determined by measuring CO<sub>2</sub> evolved from soil samples incubated in sealed serum vials using gas chromatography. The concentration of NO<sub>3</sub> and NH<sub>4</sub> was measured by automated colorimetric analysis of

soil extracts. Moisture content of soil and plant materials was determined gravimetrically.

#### 2.6. Statistical analyses

Statistical analyses were performed using a one-way analysis of variance. Significant differences between means were determined using a multiple comparisons versus control group procedure (Dunnnett's method).

### 3. Results

The initial soil moisture content was 0.11 g/g for all treatments. Subsequently the difference between WET and DRY regimes remained constant at 0.07 g/g for the duration of the experiment (Amador et al., 2005), with no significant differences observed between burrow and bulk soil in any of the treatments.

All of the mesocosms in the WORM treatment had three separate burrows, most of which extended to a depth of 35 cm (Amador et al., 2003). Death of earthworms was observed in mesocosms under the WET regime prior to sampling on week 13, resulting in a population density of 290 individuals/m<sup>2</sup> for the remainder of the experiment (Amador et al., 2003). It is safe to assume that earthworms did not die more than 1 week before sampling, since earthworm tissue in close contact with soil decomposes completely within 2–3 days after death (Whalen et al., 1999).

For the WET regime, nitrate concentration was significantly higher in bulk than in control soil in both WORM and LEAF treatments (Fig. 1). Although statistically significant differences between burrow and control soil were observed on every sampling date in LEAF and after week 3 in WORM treatments, differences between bulk and control soil were statistically significant only from week 5 on. In contrast, in the ARTF treatment differences between bulk and control soil were observed only on week 13. On weeks 1, 13, and 16 burrow soil nitrate values in ARTF were also significantly higher than in control soil (Fig. 1).

Under the DRY regime, no statistically significant differences between bulk and control soil in nitrate concentration were observed for WORM, LEAF, or ARTF treatments (Fig. 2) even on sampling dates when the same treatments under the WET regime had developed significant differences between bulk and control soil. Nitrate values were significantly higher in burrow than in control soil only for the WORM treatment (Fig. 2).

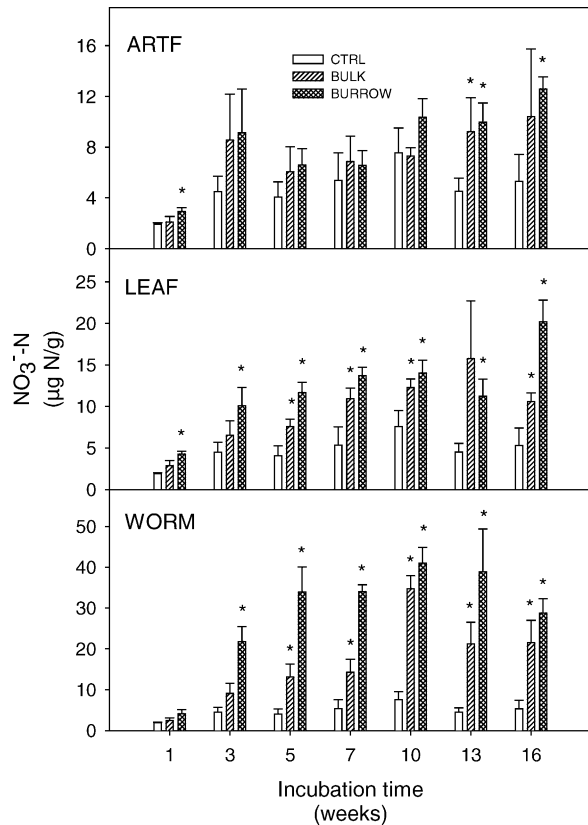


Fig. 1. Nitrate-N levels in CTRL soil and in bulk and burrow soil of ARTF, LEAF, and WORM treatments under WET conditions. Statistically significant differences ( $P < 0.05$ ) from CTRL values are indicated with an asterisk. Bars represent one standard deviation ( $n = 3$ ).

No statistically significant differences in ammonium content were observed between bulk and control soil in either WET or DRY conditions for any of the treatments, with values ranging from 1 to  $20 \mu\text{g NH}_4\text{-N/g}$ . Similarly, C mineralization in bulk soil was not significantly different from control soil for any treatment regardless of moisture regime, with values ranging from 1 to  $5 \mu\text{g CO}_2\text{-C/g/day}$ . By contrast, C mineralization was significantly higher in burrow than in CTRL soil for the WORM and LEAF treatments on most sampling dates for both moisture regimes (Amador et al., 2003). Levels of  $\text{NH}_4$  in burrow were significantly higher than in CTRL soil for the WORM and LEAF treatments under WET conditions only after incubation for 1 week. Under DRY conditions burrow soil had significantly higher levels of  $\text{NH}_4$  than CTRL only for the WORM treatment after incubation for 3 and 7 weeks. Statistical analyses of additional carbon and nitrogen variables (microbial biomass C, DOC, soil C,  $\text{NO}_3 + \text{NH}_4$ , soil N) did not reveal

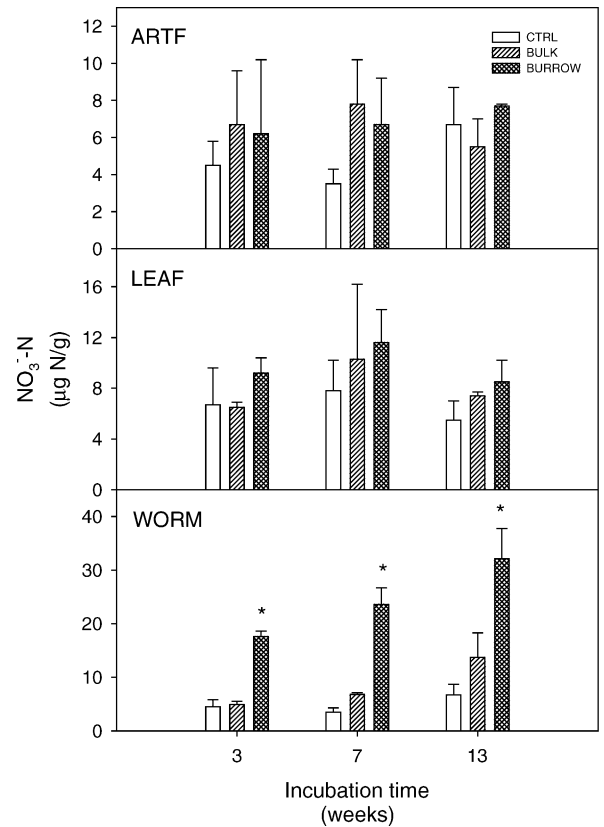


Fig. 2. Nitrate-N levels in CTRL soil and in bulk and burrow soil of ARTF, LEAF, and WORM treatments under DRY conditions. Statistically significant differences ( $P < 0.05$ ) from CTRL values are indicated with an asterisk. Bars represent one standard deviation ( $n = 3$ ).

significant differences between bulk and control soil for any of the treatments or moisture regimes evaluated.

#### 4. Discussion

Our results allow us to accept the hypothesis that anecic earthworms can affect nutrient dynamics beyond the confines of their burrows only for nitrate, since no other variables exhibited either enrichment or depletion in bulk soil relative to control soil regardless of treatment or moisture regime. Furthermore, the effects were apparent only at higher moisture levels. Our data also show that the effects are not unique to earthworms, inasmuch as similar enrichment of bulk soil, as well as the timing of enrichment, were observed in LEAF and ARTF treatments under the WET regime. Together, these results suggest a mechanism for the effects of anecic earthworms on nitrate concentration beyond the burrow soil:

1. As observed in a previous study, nitrate accumulation in burrow soil is enhanced considerably under the WET moisture regime (Amador et al., 2005). Movement of nitrate can then occur by diffusion from the burrow, following a concentration gradient, or by convection with water movement from burrow to bulk soil. Ammonium, a cation, was not enriched in the bulk soil, although it occurred in greater concentrations in the burrow soil. Nitrate, an anion, is more mobile than ammonium, and is more likely to diffuse or move with the water flow when sufficient water is available. Indeed, enrichment of bulk soil with nitrate only occurred in the WET treatment, not the DRY treatment. When water content is too low, pore spaces are not connected sufficiently to support diffusion. Bulk water flow is also unlikely because the capillary forces between bulk and burrow are either at equilibrium or support water flow, driven by evaporation, towards the macropore. Gaillard et al. (1999) observed movement of  $^{15}\text{NO}_3$  originating from  $^{15}\text{N}$ -labeled straw in contact with soil, albeit over a relatively short distance (4 mm).
2. The enrichment of bulk soil with nitrate does not require the presence of either an earthworm or a leaf residue. The generation of nitrate and its accumulation within a burrow, regardless of its origins or the presence of resources, is sufficient to cause enrichment of bulk soil with nitrate for the incubation period investigated here. However, the rate and magnitude of enrichment of bulk soil with nitrate do appear to depend on the presence of plant residues and/or earthworms, with plant residues and earthworms serving as sources of nitrogen for nitrate formation. This increases the concentration of nitrate in burrows, creating a steeper nitrate gradient between burrow and bulk soil than observed in ARTF treatments that hastens the diffusion of nitrate into adjacent bulk soil and enriches bulk soil with nitrate. Tiunov and Scheu (1999) observed a nitrogen concentration gradient in soil surrounding N-enriched *L. terrestris* burrows over distances in the order of cm.

Translocation by fungi may also be involved in the movement of N from burrow to bulk soil. In a companion study, Savin et al. (2004) found that active fungal biomass in burrow soil increased consistently with incubation time in the WORM treatment, with the lowest values observed in the CTRL treatment.

We calculated the total amount of nitrate-N in bulk soil above CTRL values in ARTF, LEAF, and WORM treatments under the WET regime on the sampling dates

for which this difference was statistically significant and highest (ARTF = 13 weeks; LEAF = 7 weeks; WORM = 10 weeks; Fig. 1) assuming a bulk density of  $1.3 \text{ g/cm}^3$ . The amount of nitrate was calculated using the equation:

$$M_{\text{NO}_3} = [\text{NO}_3] \times V \times \rho_{\text{B}} \quad (1)$$

where  $M_{\text{NO}_3}$  is the mass of  $\text{NO}_3\text{-N}$  (mg N/mesocosm),  $[\text{NO}_3]$  the concentration of nitrate-N in soil (mg N/g soil),  $V$  the volume of soil in the mesocosm ( $\text{cm}^3$ ), and  $\rho_{\text{B}}$  is the bulk density of the soil ( $\text{g/cm}^3$ ). The observed enrichment in nitrate concentration above CTRL treatment corresponded to 93, 19, and 11 mg  $\text{NO}_3\text{-N}$ /mesocosm for the WORM, LEAF, and ARTF treatments, respectively. Assuming the background level of  $\text{NO}_3$  in WORM and LEAF treatments was equal to that in ARTF treatment, we calculated that 82 and 8 mg  $\text{NO}_3\text{-N}$ /mesocosm would have to be produced from external inputs in WORM and LEAF treatments, respectively. This is considerably less than the inputs of N calculated based on disappearance of litter from surface and burrow soil (Amador et al., 2003), indicating that N in litter inputs is sufficient to account for the extra nitrate-N found in the bulk soil of WORM and LEAF treatments. Other N sources, including earthworm exudates, likely contributed to enrichment of bulk soil with nitrate. However, our experiment was not designed to elucidate the sources of N enrichment.

These results have implications for the fate of nitrogen produced from the consumption of crop residues in anecic earthworm burrows. Denitrification and leaching are considered potentially important fates for nitrate produced in burrows (e.g. Edwards et al., 1992; Parkin and Berry, 1999; Amador and Görres, 2005). Our data indicate that movement of nitrate from the drilosphere into bulk soil also needs to be considered as a potentially important fate for nitrate produced in anecic earthworm burrows. The enrichment of bulk soil with nitrate also removes the spatial constraints imposed by the need for burrow interception by roots in order for plants to take advantage of the inorganic N released through the actions of *L. terrestris*. This interpretation is supported by recent findings indicating that the transfer of  $^{15}\text{N}$  from corn litter to corn plants in mesocosms amended with *L. terrestris* can take place in the absence of burrow interception (Amador and Görres, 2005).

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