

# The Effect of Invasive Earthworm *Lumbricus terrestris* on the Distribution of Nitrogen in Soil Profile

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## Abstract

The purpose of this study was to determine if *Lumbricus terrestris*, an invasive earthworm in Northern Michigan, is redistributing nitrogen from the organic soil layer to the deeper, mineral soil layer. *L. terrestris* burrow 2 meters vertically into the ground and emerge to feed on freshly fallen leaf litter. The study included collecting of *L. terrestris* in 16 0.5 m square plots by method of electro-shock. Soil cores from a depth of 0-5 and 30-40 cm as well as leaf litter were taken from each plot to determine nitrogen content and nitrogen isotope ratios. Data analysis resulted in no significance between plots with earthworms and without earthworms in both nitrogen, N, isotope ratios and N content. Plots with *L. terrestris* showed no difference between the organic and mineral soil layer. This result suggests that *L. terrestris* are homogenizing soil layers. However, smaller than ideal sample sizes limit interpretive capacity of the results. Further research needs to be completed to confirm these perceived trends. The analysis of nitrogen isotope ratios suggest that there is another source of  $^{15}\text{N}$  other than leaf litter and *L. terrestris* that is contributing to soil composition and therefore the contribution of each was not conclusively determined.

## Introduction

Invasion of an exotic species into an ecosystem is one of the leading threats to biologically diverse ecosystems throughout the world. Exotic species are initially introduced as a solution for food, farming, aesthetic purposes, or even accidentally. In undisturbed areas, exotic species may become invasive if they are superior competitors or have no natural enemies. Invaders can alter ecosystems by changing the hydrology, nutrient cycling, and other processes that control the natural order of the system. These dramatic changes are difficult for ecosystems to recover from and organisms suffer (Wittenberg & Cock 2001).

Exotic earthworms have been colonizing temperate forests since the last glacial maximum when any native earthworms went locally extinct because top soil was removed (Bohlen et al. 2004). Earthworms have been spreading northward in North America from the southern part of the United States since they were brought from Europe in ship ballasts and imported vegetation (Edwards & Bohlen 1996).

Earthworms manipulate the soil of natural forests and can help to facilitate the invasion of non-native plant species. The forest of northern Michigan has developed without the presence of earthworms for about the last 10,000 years and during that time period did not experience

manipulation of the soils by earthworm activity. Earthworms burrow into soil and create channels and pores that increase flow of water that causes leaching of nutrients away from the roots of plants (Parkin & Berry 1999). They can also cause a decrease in the availability of nutrients in upper soil horizons, decrease populations of beneficial microorganisms, and damage thin root systems. These effects can be beneficial for adapted plants because of aeration of soil, but are more often detrimental to non-adaptive native plants in forests, particularly those with shallow root systems that are not accustomed to a nutrient regime altered by earthworms (Hale et al. 2008).

Earthworms ingest leaf litter, or the organic soil layer, and then redistribute the nutrients to the A horizon or lower. Redistribution occurs because individuals digest litter and burrow into the soil where material is excreted or the earthworm dies. Their presence is associated with a reduction in the amount of nitrogen on the forest floor and the redistribution of organic matter in the soil profile (Bohlen et al. 2004; Mackay 1999). Loss of nitrogen is due to leaching and denitrification, the breakdown of organic nitrogen into atmospheric nitrogen by earthworms (Bohlen et al. 2004). Reduction of available nitrogen is detrimental to native plants with shallow roots because nitrogen is necessary for plant growth. Therefore, plants that are not capable of reaching nitrogen at lower depths will be more nitrogen stressed because of this change in nitrogen abundance. Forests invaded by earthworms have shown an increase in plant mortality and thus a decrease in density of plants (Hale et al. 2008).

*Lumbricus terrestris*, commonly known in Michigan as the nightcrawler, is a large, multi-segmented, hermaphroditic annelid that typically ranges from eight to fifteen centimeters in length. *Lumbricus terrestris* is an anecic species, meaning that it lives in deep vertical burrows of 2 meters and generally only emerges to feed on surface litter. Because of this characteristic burrowing, anecic species such as *L. terrestris* are associated with the mixing of soil horizons in areas that they invade (Postma-Blaauw et al. 2006). When feeding, *L. terrestris* prefers fresh litter rather than accumulated organic matter in the upper soil horizon (Hale et al. 2005).

There are two broad categories of mechanisms through which *L. terrestris* can alter the soil (Devliegher & Verstraete 1997). The first, known as nutrient enrichment processes, are due to the incorporation and mixing of organic matter into the soil. The second, known as gut associative processes, are due to the transport of material through the gut of the earthworm.

These processes affect soil chemistry and composition in characteristic ways that can be used to help assess *L. terrestris*' effect on soil conditions (Devliegher & Verstraete 1997). Nutrient enrichment processes simply move nutrients such as nitrogen and calcium in an unaltered form and redistribute them to lower soil horizons. Gut associative processes can also redistribute nutrients but do so in a way that alters their isotopic signature or elemental concentrations in a measurable way. Nutrients contained within the body of the worm bear this altered chemical signature and their decomposition can also contribute to nutrient redistribution (Devliegher & Verstraete 1997).

There is a great deal of research assessing *L. terrestris*' effect on soil in a variety of ecological settings. It has been shown that *L. terrestris* can incorporate leaf litter into the mineral soil layers of forests and reduce the C:N ratios as well as the carbon storage of the upper mineral soil layers (Bohlen et al. 2004). In agricultural soils, *L. terrestris* can increase nitrogen fluxes by increasing the amount of leachate through enhancing soil permeability. These findings may also bode importance for forest soils, particularly those in riparian zones where there is a lot hydrologic through flow (Costello & Lamberti 2008). Furthermore, burrowing activity by *L. terrestris* can allow O<sub>2</sub> to reach greater depth and possibly change the dominant nitrogen transformation that takes place. Specifically, previously anoxic soils where denitrification dominates could potentially support nitrogen fixation or ammonification under aerobic conditions (Costello & Lamberti 2008).

<sup>15</sup>N isotopes are molecules of nitrogen with 15 instead of the normal 14 neutrons. The <sup>15</sup>N molecule occurs naturally in all environments and biological processes, such as protein synthesis, preferentially takes in a ratio of <sup>15</sup>N to <sup>14</sup>N known as the δ<sup>15</sup>N. Each organism has a unique δ<sup>15</sup>N; therefore there is a potential to determine the possible source of nitrogen in the soil (Peterson & Fry 1987). Since heavier isotopes generally bioaccumulate in the food chain, an earthworm has a higher δ<sup>15</sup>N than leaf litter (Heller 2005). If the isotope signature of the soil is more similar to *L. terrestris* than the leaf litter then *L. terrestris* has more of an influence on soil nitrogen composition.

The purpose of this experiment is to determine if *L. terrestris* affects the distribution of nitrogen in different soil horizons and if the soil is receiving more nitrogen from either the leaf litter or the body of *L. terrestris*. If the nitrogen levels in the deep soil are greater where

earthworms are present than where they are not present, then *L. terrestris* could be redistributing the nitrogen in from higher to lower soil layers. If there is no difference in the nitrogen at the top layer compared to the bottom layer of soil where earthworms are present and where they are absent, then *L. terrestris* has had no effect on the vertical nutrient distribution in previously undisturbed soil.

## Materials and Methods

### *Plot Requirements*

To test nitrogen levels and the  $\delta^{15}\text{N}$ , samples of soils, leaf litter, and worm bodies were taken at 16 independent plots. The study area was a mid-successional temperate forest on sandy Rubicon series soils (USDA 1991) off of the D7 transect in the FASET project plots at the University of Michigan Biological Station. This site was chosen because it was judged to be most likely to contain the target species, *L. terrestris*. Plots were 0.5 meters on each side and chosen in generally low-lying areas to minimize micro-topographical moisture gradients. Each plot was also chosen to be at least two meters from any trees greater than four cm DBH to avoid areas of high root density, and at least 10 meters apart to achieve a gradient in samples.

### *Leaf litter and Moisture*

Plot boundaries were temporarily defined with a PVC frame and 10 cm<sup>2</sup> of leaf litter at the center of the frame was collected for analysis. Soil moisture was measured with a Hydrosense that was inserted at the center of each plot. The soil samples were taken at 0-5 cm and 30-40 cm with a pipe-corer on the northern side of each plot outside the boundary to determine nitrogen isotope ratios and content near the surface in the organic horizon and in the deeper mineral soil.

### *Earthworm Sampling*

Worm density was determined using an electro-shocking method. The apparatus included a 120 volt generator attached to eight 60 cm steel rods, 4 rods on opposite sides of the plot, approximately 12 cm apart, inserted into the soil to a depth of 50 cm. Two plots were electrified simultaneously for 20 minutes and surfacing worms that appeared to be *L. terrestris*

were collected. Each plot was then electro-shocked alone for five minutes at a higher voltage to cause any remaining worms to surface.

Sampling was conducted on four different days. On May 23, 2009 4 plots were sampled for soils, worms, and leaf litter. The second day, May 27, 2009 was rainy; the last 12 plots were identified, and sampled for soils. The third day, May 30, 2009 was intermittently rainy; the remaining plots were electro-shocked for worms. We returned to the plots on June 6, 2009 when it was dry to take another soil moisture measurement that would provide more consistent measurements among plots.

### *Preparing Samples*

In the lab, collected worms were refrigerated with some leaf litter and allowed to void their guts. *L. terrestris* individuals were identified, counted, weighed, lyophilized and then ground in a ball grinder into a fine powder to prepare them for analysis. Soil samples were hand mixed to preliminarily homogenize them and hand sifted to remove visible rocks and fine roots before a subsample of between 0.4 and 0.8 milligrams was taken. The samples were then frozen at -80°C for two hours, dehydrated in the lyophilizer overnight, and ground in a ball grinder for four minutes. Each leaf litter sample was oven dried at 60°C over night then crumbled by hand to preliminarily homogenize the sample. A subsample was then ground for analysis by mass spectrometry. Micrograms of each sample were then analyzed using a mass spectrometer for nitrogen content and  $\delta^{15}\text{N}$ .

### *Data analysis*

The effect of earthworms on nitrogen distribution within the soil was determined by comparing between plots with and without worms, the absolute amount of nitrogen,  $\delta^{15}\text{N}$ , and the ratio of N and  $\delta^{15}\text{N}$  between the 0-5 cm and 30-40 cm soil layers. Since all the data other than that of total nitrogen content between plots with and without worms were normal, means were compared using a t-test. Total nitrogen content was compared using Wilcoxon signed ranks test.

The  $\delta^{15}\text{N}$  of the leaf litter, soil, and worms were all adjusted according to a standard sample. The standard, air, has a  $\delta^{15}\text{N}$  ratio set at zero. In formula 1, the nitrogen isotope ratio is determined for each sample. By using formula 2, it is possible to find the amount of nitrogen

from *L. terrestris* that is contributing to soil composition. The term a  $\delta^{15}\text{N}$  refers to the ratio of  $^{15}\text{N}$  to  $^{14}\text{N}$  compared to the standard.

$$\text{Formula 1: } \delta^{15}\text{N} = [(N_{\text{sample}}/N_{\text{standard}})-1]*100 \text{ (Moran et al. 2001)}$$

$$\text{Formula 2: } \%N_{\text{worms}} = [(\delta^{15}\text{N}_{\text{soil}} - \delta^{15}\text{N}_{\text{leaf litter}}) / (\delta^{15}\text{N}_{\text{worms}} - \delta^{15}\text{N}_{\text{leaf litter}})]$$

(Heller 2005)

## Results

### *Nitrogen content between 0-5 cm and 30-40 cm*

Total nitrogen between the two soil layers was significantly higher in 0-5cm than in 30-40 cm for plots for all plots (paired  $t=5.203$ ,  $df=15$ ,  $p=0.001$ ). However, when nitrogen content was assessed between 0-5 cm and 30-40 cm separately for plots where *L. terrestris* were present and where *L. terrestris* were absent, only plots without *L. terrestris* showed a significantly higher N content in the 0-5 cm soil layer than in the 30-40 cm soil layer (*L. terrestris* present: Wilcoxon signed ranks test=-1.461,  $df=3$ ,  $p=0.144$ ; *L. terrestris* absent: Wilcoxon signed ranks test=-2.981,  $df=11$ ,  $p=0.003$ ).

Table 1: Total Nitrogen Content

Depth (cm)	Mean	t	df	P-value
0-5	0.375	5.203	15	0.001
30-40	0.0606			

Table 2: Nitrogen Content of Plots With and Without *L. terrestris*

Worms	Depth (cm)	Mean	Z	df	P-Value
Absent	0-5	0.4225	-2.981	11	0.003
	30-40	0.0475			
Present	0-5	0.2325	-1.461	3	0.144
	30-40	0.1			

Figure 1: Total Nitrogen in Top and Bottom Layers

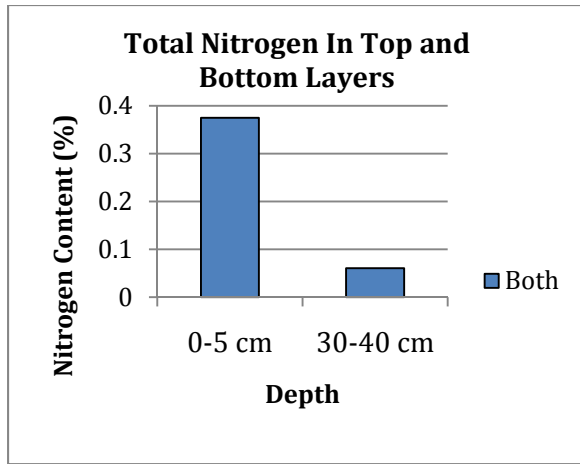
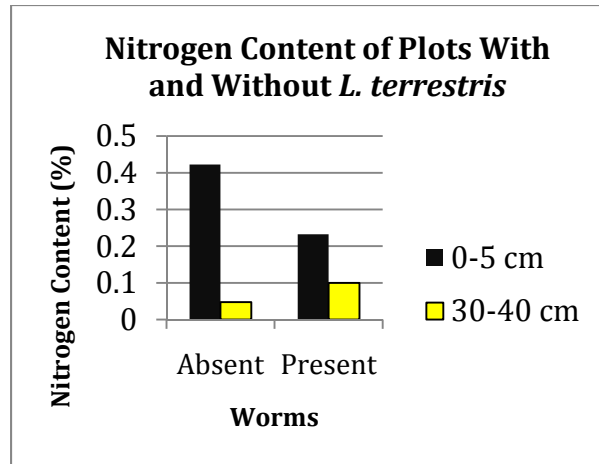


Figure 2: Nitrogen Content of Plots With and Without *L. terrestris*



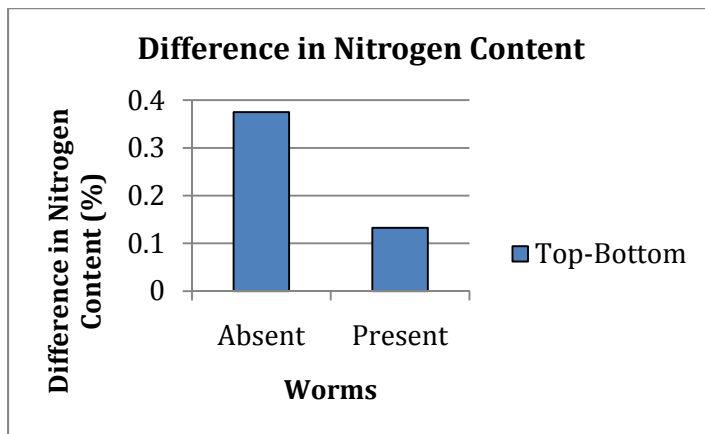
*Difference in nitrogen content in plots with and without L. terrestris*

There was not a significant difference in the mean differences of nitrogen content between 0-5 cm and 30-40 cm for plots with and without worms (Independent samples  $t=1.879$ ,  $df=14$ ,  $p=0.081$ ).

Table 3: Difference in Nitrogen Content Between Plots With and Without *L. terrestris*

Worms	Mean Difference	t	df	P-Value
Absent	0.375	1.879	14	0.081
Present	0.1325			

Figure 3: Difference in Nitrogen Content Between 0-5cm and 30-40cm



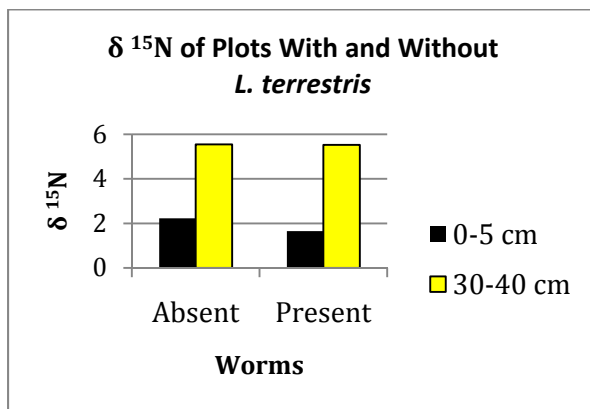
$\delta^{15}\text{N}$  between 0-5 cm and 30-40 cm for plots with and without *L. terrestris*

There is a significantly higher  $\delta^{15}\text{N}$  in 30-40 cm soil layer than in the 0-5 cm soil layer for plots where *L. terrestris* was present and where *L. terrestris* was absent (*L. terrestris* absent: paired  $t=-7.965$ ,  $df=11$ ,  $p=0.001$ ; *L. terrestris* present: paired  $t=-7.566$ ,  $df=3$ ,  $p=0.005$ ).

Table 4:  $\delta^{15}\text{N}$  of Plots With and Without Worms *L. terrestris*

Worms	Depth (cm)	Mean	t	df	P-value
Absent	0-5	2.225	-7.965	11	0.001
	30-40	5.55			
Present	0-5	1.65	-7.566	3	0.005
	30-40	5.525			

Figure 4:  $\delta^{15}\text{N}$  of Plots With and Without *L. terrestris*



Mean difference in  $\delta^{15}\text{N}$  for plots with and without *L. terrestris*

There is not a significant difference in the mean differences of  $\delta^{15}\text{N}$  between 0-5cm and 30-40 cm in plots with and without *L. terrestris* (Independent samples  $t=0.697$ ,  $df=14$ ,  $p=0.497$ ).



Table 5: Mean Difference of  $\delta^{15}\text{N}$  Between Plots With and Without *L. terrestris*

Worms	Mean Difference	t	df	P-value
Absent	-3.325	0.697	14	0.497
Present	-3.875			

## Discussion

*The presence of L. terrestris may affect the distribution of nitrogen in the upper and lower soil layers*

Where *L. terrestris* was not present, there was a consistently higher amount of nitrogen in shallow soils than in deeper layers. The plots where *L. terrestris* was found to not show a difference between the nitrogen content of the surface organic layers and the deeper mineral soils. This is evidence for the alternative hypothesis that *L. terrestris* is altering nitrogen distribution in the soil profile. In particular this result suggests that the presence of the deep burrowing earthworm is homogenizing the gradient of nitrogen between soil horizons. Data for absolute differences in nitrogen content were not significant ( $p=.081$ ) at the 95% confidence level, but the trend could be further explored with a larger sample size. Plots with worms showed nominally lower differences in nitrogen content indicating that worm activity may be homogenizing soil nitrogen levels. This evidence suggests that the worms are actively or passively increasing the amount of nitrogen in deeper soils. They do this by bringing leaf litter into their burrows, and excreting them as nitrogen rich casts, or passively by increasing the porosity of soils and allowing more nitrogen to leach to deeper soils. The evidence only confirms a relationship, and the trend may be due to other possible confounding variables. *L. terrestris* density may be associated with overall worm density. If this is the case it may be the total quantity of worms redistributing nitrogen, and not solely related *L. terrestris* activity.

*There is no difference in  $\delta^{15}\text{N}$  in the soil layers of plots with and without *L. terrestris*.*

There was no statistically significant association between  $\delta^{15}\text{N}$  in areas with and without *L. terrestris*. There was an observable difference between soils, worm, and leaf litter isotope

ratios, the highest  $\delta^{15}\text{N}$  being found in soils at 30-40cm depth. This may be attributable to natural decompositional or trophic processes and not to worm activity.  $\delta^{15}\text{N}$  increases at each trophic level; the levels of  $\delta^{15}\text{N}$  found in soils were higher than found in either worms or leaves, indicating the effect of a confounding or noisy variable increasing  $\delta^{15}\text{N}$  in deep soils. The effect of a species of earthworm we did not study may contribute to leaching in upper soil horizons. *Lumbricus rubellus* is a shallow burrowing earthworm that mixes the organic and shallow mineral soils. Its presence increases porosity of shallow soils and may increase leaching of minerals and nitrogen (Bohlen et al. 2004). *Lumbricus rubellus* was more prevalent than *L. terrestris* in our plots and may have had a larger effect on the soils, including the nitrogen isotope signature. Our samples were not extensively sieved, and included some root mass in the sample that was analyzed with the mass spectrometer. This could have affected our results, but leaf and root nitrogen isotope ratios do not vary enough to have produced the results observed (Dijkstra et al 2003). Unanalyzed factors including nematodes and other soil biota could be contributing the extra observed  $^{15}\text{N}$  to soil isotope ratios.

#### *Suggestions for Further Research*

Further research into the effects of earthworms on the distribution of nitrogen in the soil profile would be necessary to more definitively answer these questions, and many things about the experimental design would be revised before continuing with the work. The *L. terrestris* that were found in the plots were all juveniles. The adults typically make the deep burrows and the juveniles remain nearer to the surface, and so were more susceptible to our sampling technique. The sampling equipment used may not have been as effective to the depth in the soil where the target species lives, limiting the sampling ability. In the future, the effects of a different worm species *L. rubellus* could be investigated with these same methods possibly with better success in worm sampling.

#### *Ecological Implications*

A proposed consequence of worm activity is that by enriching deep soils with nitrogen they change soil conditions to favor invasive species that are adapted to an altered nutrient profile. A study in a Wisconsin forest observed higher densities of invasive European buckthorn in areas invaded by earthworms (Hendrix 2006). Worm colonization also cause native

understory plants to suffer increased mortality following introduction (Hale et al. 2008).

Earthworm activity is generally considered to be beneficial in agricultural settings where changes in nitrogen levels and soil aeration must otherwise be accomplished manually. In forest soils no such benefit has been observed. While earthworm induced increases in nitrogen levels leads to an increase in biomass of agricultural species, no change is observed in the biomass of forest species. Furthermore, the physical changes that earthworms make to soil structure can increase leaching and lead to a decrease in nitrogen over time, as observed in soils colonized by worms for several years (Hale 2008). While it is unlikely that earthworm invasion will result in a complete loss of the forests they occupy, their presence is already changing basic elements of the forest structure from the ground up. Most essentially, earthworms can completely strip the forest floor of leaf litter by the end of the summer, making seeds more vulnerable to seed predators, and generally causing an increase in temperature right at the soil level, which can effect seedling survival (Bohlen 2004). This relationship may have consequences for future forest ecology and conservation tactics. Most forest preservation initiatives do not include restoration of soil health in their management of the forest areas which may limit their effectiveness in the long term. Soil health is an essential part of forest ecosystem integrity and such a consideration is crucial for the maintenance and health of forest systems in the future.

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