



# Earthworms increase soil microbial biomass carrying capacity and nitrogen retention in northern hardwood forests



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## ARTICLE INFO

### Article history:

Received 24 November 2014

Received in revised form

19 March 2015

Accepted 24 March 2015

Available online 21 April 2015

### Keywords:

Carbon

Earthworms

Isotopes

Microbial biomass

Nitrification

Nitrogen

## ABSTRACT

Earthworms have been shown to produce contrasting effects on soil carbon (C) and nitrogen (N) pools and dynamics. We measured soil C and N pools and processes and traced the flow of <sup>13</sup>C and <sup>15</sup>N from sugar maple (*Acer saccharum* Marsh.) litter into soil microbial biomass and respirable C and mineralizable and inorganic N pools in mature northern hardwood forest plots with variable earthworm communities. Previous studies have shown that plots dominated by either *Lumbricus rubellus* or *Lumbricus terrestris* have markedly lower total soil C than uncolonized plots. Here we show that total soil N pools in earthworm colonized plots were reduced much less than C, but significantly so in plots dominated by contain *L. rubellus*. Pools of microbial biomass C and N were higher in earthworm-colonized (especially those dominated by *L. rubellus*) plots and more <sup>13</sup>C and <sup>15</sup>N were recovered in microbial biomass and less was recovered in mineralizable and inorganic N pools in these plots. These plots also had lower rates of potential net N mineralization and nitrification than uncolonized reference plots. These results suggest that earthworm stimulation of microbial biomass and activity underlie depletion of soil C and retention and maintenance of soil N pools, at least in northern hardwood forests. Earthworms increase the carrying capacity of soil for microbial biomass and facilitate the flow of N from litter into stable soil organic matter. However, declines in soil C and C:N ratio may increase the potential for hydrologic and gaseous losses in earthworm-colonized sites under changing environmental conditions.

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## 1. Introduction

Soils contain the largest pools of carbon (C) and nitrogen (N) in most terrestrial ecosystems (Schlesinger and Bernhardt, 2013). The factors that control the size and dynamics of these pools have a profound influence on atmospheric chemistry, water quality and ecosystem fertility (Cotrufo et al., 2013). For C, the depletion of soil pools can increase the carbon dioxide (CO<sub>2</sub>) content of the atmosphere, contributing to global warming (Lal, 2005). For N, there are multiple concerns: production of the greenhouse gas N<sub>2</sub>O, leaching of NO<sub>3</sub><sup>-</sup> (a drinking water pollutant and cause of eutrophication) and loss of soil fertility (N commonly limits terrestrial productivity; Davidson et al., 2012). There is great interest in the ability of

ecosystems to retain N inputs and to prevent their movement to the atmosphere or to receiving waters (Galloway et al., 2003).

Soil microorganisms and fauna transform primary production into soil organic matter pools and regulate the long-term depletion or accumulation of these pools. The activities of soil biota are strongly driven by environmental factors such as temperature, moisture and pH and soil community composition has been shown to be a regulator of soil C and N dynamics (Fierer et al., 2009; Wallenstein and Hall, 2012). Changes in soil fauna can be particularly important and challenging to study. Fauna can exert a strong influence on C and N dynamics but their distribution and dynamics are complex and regulated by more complex ecological interactions than microbes (Coleman et al., 2004; Burke et al., 2011; Blouin et al., 2013; Crumsey et al., 2013).

Recent studies have characterized changes in forest soils of North America, where invasion by earthworm species from Europe and Asia has caused marked changes in soil C and N pools and

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dynamics (Bohlen et al., 2004c; Hale et al., 2005; Hendrix, 2006; Szlavetz et al., 2011). Earthworm effects on C and N dynamics have been found to vary greatly in different studies (Bartlett et al., 2010; Crumsey et al., 2014). There is particular variation in reported effects of earthworms on N cycling and retention. While several studies have shown marked stimulation of N cycling and loss by earthworms (Gorres et al., 1997; Amador et al., 2006; Eisenhauer et al., 2007; Costello and Lamberti, 2008, 2009; Hale et al., 2008; Greiner et al., 2012), others have found that earthworms stimulate nitrogen retention and/or reduce nitrogen availability or loss (Groffman et al., 2004; Bohlen et al., 2004b; Hale et al., 2005; Wironen and Moore, 2006). Understanding this variation is important to evaluating forest productivity (Vitousek and Howarth, 1991), concerns about N saturation (Aber et al., 1989), and the ability of forests to function as significant sinks for atmospheric N deposition (Pardo et al., 2011).

Much of the uncertainty surrounding earthworm effects on soil C and N dynamics centers on their effects on soil microbial biomass. Microbial biomass is the critical interface between new sources of organic matter such as leaf litter and more stable pools of soil organic matter (Cotrufo et al., 2013). While some studies have suggested that earthworms decrease microbial biomass, others have shown increases (McLean et al., 2006). These evaluations have been complicated by differential enrichment of earthworm casts and bulk soil and by earthworm alteration of the depth and density of soil horizons. They may also be complicated by differences in earthworm species composition, as earthworms with different feeding strategies could affect microbial biomass and N retention in different ways. For example, epigeic and endogeic earthworms are expected to mix organic matter relatively uniformly throughout surface soils, whereas burrowing activities by anecic earthworms can introduce substantial heterogeneity by concentrating organic matter in and around burrows (Stromberger et al., 2012; Andriuzzi et al., 2013). Longer gut transit times within anecic earthworms such as *Lumbricus terrestris* could also alter the chemistry of processed organic material compared to organic matter processed by other species (Dempsey et al., 2013). There is a strong need for coupling data on microbial biomass and activity with estimates of soil mass for soils with different earthworm communities to evaluate the integrated, ecosystem-scale effects of earthworm invasion on soil C and N pools and processes.

We have been studying effects of European earthworms on forest soil C and N dynamics at a site in New York, USA since 1997. Comparison of earthworm-invaded and uncolonized reference sites found marked depletion of soil C, but not soil N pools (Bohlen et al., 2004b). Earthworm invasion increased microbial biomass C and N pools which appeared to stimulate immobilization and retention of soil N (Li et al., 2002; Groffman et al., 2004) while at the same time facilitating decomposition and loss of soil C (Dempsey et al., 2013). More recently, we initiated studies using  $^{13}\text{C}$  and  $^{15}\text{N}$ -labeled sugar maple (*Acer saccharum* Marsh.) litter to analyze soil C and N dynamics in more detail (Fahey et al., 2013b). These studies confirmed that earthworms reduce soil C and C:N ratio in these sites by eliminating organic surface soil horizons and by accelerating the decomposition of litter (Fahey et al., 2013a, 2013b). The role of microbial biomass and activity in N cycling on these sites remains uncertain. After two years, recovery of  $^{15}\text{N}$  in bulk soil was much higher than recovery of  $^{13}\text{C}$ , suggesting that significant amounts of N were retained in the soil profile presumably as a result of mechanisms mediated by microbial activity. These uncertainties are the focus of the study reported here.

In this study, we examined microbial biomass and activity, including  $^{13}\text{C}$  and  $^{15}\text{N}$  dynamics, across three years in the plots studied by Fahey et al. (2013a; 2013b). This study builds on our previous analyses of interactions between earthworms and

microbial biomass (Li et al., 2002; Groffman et al., 2004) by evaluating differences in total pools of microbial biomass C and N as well as the flow of  $^{13}\text{C}$  and  $^{15}\text{N}$  from litter into and through these pools over a three year period. We hypothesized that earthworm mixing of soil and litter increases the carrying capacity of soil for microbial biomass, which in turn facilitates immobilization and retention of total and litter N. This hypothesis is based on the concept that mixing improves habitat quality and provides physical protection from predation for microbes. We also hypothesized that earthworm communities dominated by the relatively large anecic earthworm, *L. terrestris* would have greater effects on microbial processing and retention of litter N than those dominated by the epigeic species, *Lumbricus rubellus*. We tested these hypotheses by comparing the size and  $^{13}\text{C}$  and  $^{15}\text{N}$  content of microbial biomass C and N, respirable C and inorganic and mineralizable N pools in uncolonized reference plots and in earthworm invaded plots dominated by either *L. rubellus* or *L. terrestris* treated with  $^{13}\text{C}$  and  $^{15}\text{N}$ -labeled sugar maple litter over a three year period.

## 2. Methods

### 2.1. Research site

The research site was located in Central New York State (42°15' N, 76°40' W), USA at the Arnot Teaching and Research Forest operated by Cornell University (Fain et al., 1994). Climate at this location is temperate continental with mean temperature of 4.8 °C in January and 22.8 °C in July, with evenly distributed mean annual precipitation of 90 cm. Soils are acidic (pH 4.5–5.0), stony (22% by volume) Dystrichrepts derived from glacial till overlying shale with approximately 25% clay content in the top 10 cm of the mineral soil. In the absence of exotic earthworms the soils exhibit a forest floor organic horizon averaging 4 cm thickness. Plots were located in approximately 130-year-old northern hardwood forest stands dominated by sugar maple.

As described in detail in Fahey et al. (2013b), we established nine study plots (20 m × 20 m) arranged in three blocks at the Arnot site based on natural variation in the distribution of earthworms across the landscape. The three blocks were arranged across the topographic sequence from ridgetop to upper and mid slope. Within each block one plot each was established with either no earthworms (No worm) or with earthworm communities dominated by *L. rubellus* or *L. terrestris*. These differences were confirmed throughout the study by field sampling using a “hot” mustard extraction method (Lawrence and Bowers, 2002). Densities of *L. terrestris* ranged from 7 to 10 adults m<sup>-2</sup> in the plots where they were present. *L. rubellus* was present in all earthworm colonized plots in densities ranging from 6 to 19 adults per m<sup>2</sup> with higher densities (14.3–18 adults per m<sup>2</sup>) in the *L. rubellus*-dominated plots than in the *L. terrestris*-dominated plots (5.7–10.7 adults per m<sup>2</sup>) (Table 1). *Octolasion tyrtaeum*, *Apporectoda* spp. and other immature earthworms were also present in all plots that contained earthworms. Paired plots were separated by at least 25 m and were carefully chosen to avoid differences in soils, vegetation, and topography among the plots in each pair.

Plots were treated with litter labeled with  $^{13}\text{C}$  (1.268 atom %) and  $^{15}\text{N}$  (1.5823%) produced in enclosed chambers at the Arnot site using methods described by Horowitz et al. (2009). Eight 0.5-m<sup>2</sup> quadrats were established in each of the nine plots in October 2007. Fresh native litter was removed and replaced (to approximately equal weight, 400 g/m<sup>2</sup>) with labeled litter in October 2007 as described by Fahey et al., (2013a, 2013b).

Estimates of bulk density and background isotope natural abundance were determined in each plot by excavating soil pits (4 per plot) by soil horizon to 20 cm depth at random locations in each

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