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Short communication

Activity of an introduced earthworm (*Lumbricus terrestris*) increases under future rates of atmospheric nitrogen deposition in northern temperate forests

Karl J. Romanowicz[a,*], Donald R. Zak[a,b]

[a] School of Natural Resources & Environment, University of Michigan, Ann Arbor, MI, USA
[b] Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA

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ABSTRACT

When temperate forests of North America are exposed to long-term experimental levels of atmospheric nitrogen (N) deposition that mimic predicted future rates, elevated concentrations of inorganic N suppress microbial decay processes and increase the mass of the forest floor, storing more carbon (C) as soil organic matter. However, when non-native earthworms were serendipitously introduced to forests subjected to long-term experimental atmospheric N deposition, the greater forest floor mass with a higher N concentration had a positive effect on earthworm activity by increasing earthworm abundance and consumption of the forest floor. Here, we present evidence from a long-term experimental N deposition study showing how the abundance of the introduced earthworm, *Lumbricus terrestris,* increased significantly under elevated rates of atmospheric N deposition (+363%) in one of four study sites and contributed to a decline in the forest floor (−50%). In addition, mineral soil C (+97%) and N (+117%) concentrations increased as earthworms redistributed a greater proportion of organic matter belowground under experimental N deposition. We conclude that earthworm-induced changes to the forest floor can supersede a decline in microbial decay under experimental N deposition that has previously increased both forest floor mass and turnover time, thereby potentially negating increases in soil C storage.

1. Introduction

The invasion of non-native earthworms into previously earthworm-free North American forests can have a profound influence on ecosystem function and biodiversity (Bohlen et al., 2004a; Frelich et al., 2006), the nature of which depends on site characteristics and earthworm species present (Burtelow et al., 1998; Hale et al., 2005). Forests recently invaded are typically dominated by epigeic earthworms (i.e., leaf litter feeding, surface-dwelling), with endogeic (i.e., mineral soil feeding and dwelling) and anecic (i.e., leaf litter feeding, vertical burrowing) species establishing as environmental conditions become favorable (Craven et al., 2017). Earthworm communities containing multiple ecological groups can consume seasonal accumulations of surface leaf litter (Hale et al., 2005), reduce organic matter content in upper soil horizons (Nuzzo et al., 2009; Resner et al., 2015), and redistribute surface litter carbon (C) and nitrogen (N) into mineral soil (Bohlen et al., 2004b; Wironen and Moore, 2006; Straube et al., 2009).

In contrast, elevated levels of atmospheric N deposition can foster greater net primary production (NPP) in these N-limited forests (Townsend et al., 1996; Nadelhoffer et al., 1999; Zak et al., 2008) through an increase in N mineralization, nitrification, and plant uptake as the N content of the forest floor increases (Aber et al., 1998; Fenn et al., 1998). Over the past 150 years, rates of atmospheric N deposition have increased an order of magnitude (e.g., from 0.05–0.1 $g\,N\,m^{-2}\,yr^{-1}$ to 1.5–2.0 $g\,N\,m^{-2}\,yr^{-1}$; Galloway et al., 2004), primarily from anthropogenic sources (i.e., fossil fuel burning, agriculture; Socolow 1999; Galloway et al., 2002, 2004), with rates projected to increase by an additional 3 $g\,N\,m^{-2}\,yr^{-1}$ by mid-century in some locations (Galloway et al., 2004). These elevated levels of atmospheric N deposition can also alter microbial mechanisms regulating organic matter decay by repressing the expression of microbial genes that synthesize litter-decomposing enzymes (Edwards et al., 2011; Zak et al., 2011; Freedman et al., 2016). The down-regulation of microbial decay has led to an increase in soil C storage in northern temperate forests (Townsend et al., 1996; Zak et al., 2008).

Over the past two decades, we have been studying a series of northern hardwood forest stands in which experimental N deposition (3 $g\,NO_3^{-}\text{-}N\,m^{-2}\,yr^{-1}$) has altered the biogeochemical cycling of C

* Corresponding author at: School of Natural Resources & Environment, University of Michigan, 440 Church Street, Ann Arbor, MI, USA.
E-mail address: kjromano@umich.edu (K.J. Romanowicz).

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Table 1
Two decades of experimental N deposition has increased the N concentration and mass of forest floor and mineral soil organic matter content in the Michigan Gradient Study. Values represent significant percent change from ambient N deposition treatment ($P < 0.05$).

	% Change	Citation
Plant and Soil		
Net Primary Production (NPP)	+10%	Pregitzer et al., 2008
Leaf Litter N Concentration (mg N g^{-1})	+15%	Zak et al., 2008
Mineral Soil N Concentration (mg N g^{-1})	+60%	Zak et al., 2008
Biogeochemical		
Forest Floor Mass (g m^{-2})	+50%	Zak et al., 2008
Forest Floor Turnover Time (yr^{-1})	+60%	Zak et al., 2008

and N in a manner consistent with the mechanisms described above (Table 1). These changes facilitated the gradual accumulation of forest floor enriched with greater concentrations of N under experimental atmospheric N deposition. However, during the course of our long-term study, serendipitous earthworm invasions have spread into two of our four experimental sites. The extent of earthworm invasion within these sites differs, with earthworms pervasive throughout one site and actively invading another. This provided a novel opportunity to describe the effects that elevated atmospheric N deposition has on the abundance of earthworm species and their feedback on northern temperate forest soils, especially in the context of a long-term field manipulation on atmospheric N deposition.

It has been stated that there is no apparent causal relationship between elevated atmospheric N deposition and introduction as well as activity of non-native earthworm species (Gilliam, 2006). Rather, these two anthropogenic disturbances simply display a high degree of spatial correlation in areas of high densities of human populations (Gundale et al., 2005). Here, we provide evidence that elevated rates of atmospheric N deposition will directly influence the activity of earthworms that invade northern temperate forests, fostering greater earthworm abundance and potentially negating the inhibitory effects of elevated N deposition on forest floor decomposition and soil C storage.

2. Methods and materials

2.1. Study site descriptions

The influence of experimental atmospheric N deposition on earthworm invasion and activity was investigated in four stands of northern hardwood forest in Upper and Lower Michigan, USA (see Fig. S1). The forest stands span the north-south range of sugar maple (*Acer saccharum* Marsh.)-dominated northern hardwood forests in the Great Lakes region of North America (Braun, 1950). The Oi horizon is mainly comprised of sugar maple leaf litter, and the Oe/Oa horizons are interpenetrated by a dense mat of fine roots (~0.5 mm dia.). Soils are sandy (85–90%), well-drained, isotic, frigid Typic Haplorthods of the Kalkaska series. These sites also span a gradient of atmospheric N deposition (0.7–1.2 g N m^{-2} yr^{-1}), of which NO_3^--N composes ~60% of wet-plus-dry deposition (Zak et al., 2008). In 1994, six plots (30 m^2) were established at each site; three receive ambient atmospheric N deposition and three receive experimental N deposition of 3 g NO_3^--N m^{-2} yr^{-1}, a rate approaching that expected in portions of eastern North America by 2050 (Galloway et al., 2004).

2.2. Earthworm abundance and forest floor conditions

Measurements of earthworm abundance were conducted in July 2016 and focused exclusively on the presence of the anecic earthworm species *Lumbricus terrestris* by identifying its middens, which are recognized by the distinct mounding of residual plant material, a central plug composed of leaf litter, an underlying burrow, and castings above the mineral surface. Midden identification is a rapid and non-destructive method to estimate the presence and abundance of *L. terrestris* (Clapperton et al., 2008). At sites B and C, in which *L. terrestris* presence was observed, plot-level surveys were conducted within a 0.5 m^2 quadrat at 20 randomly selected points within each study plot to quantify midden abundance and determine the condition of the forest floor using a 1–5 scale (see Table S1). Sampling efforts to identify and quantify the presence of additional earthworm species were avoided as they would require destructive extraction techniques, thereby negatively impacting the integrity of our long-term experiment. Previous investigations throughout Michigan have confirmed the presence of *Dendrobaena octaedra* (Savigny), *Lumbricus terrestris* (Linnaeus), *L. rubellus* (Hoffmeister), *Aporrectodea trapezoides* (Dugès), and *A. caliginosa* (Savigny; Smith and Green, 1916; Murchie, 1956; Crumsey et al., 2014).

2.3. Field sampling and laboratory analysis

Mineral soil cores (0–20 cm depth) were collected at five randomly selected locations within each plot at sites B and C, composited by plot and homogenized through a 2-mm mesh sieve in the field. In the laboratory, mineral soil subsamples were analyzed for C concentration (mg C g^{-1} soil), N concentration (mg N g^{-1} soil), and C:N ratio in duplicate (0.4 g $replicate^{-1}$) utilizing a TruMac C/N analyzer (LECO, St. Joseph, MI, USA). Soil water content was determined from a plot subsample (1.0 g wet weight) oven-dried overnight at 60 °C and reweighed to calculate the difference from total dry mass. Soil pH was quantified with an Accumet Model 15 pH meter (Fischer Scientific, Waltham, MA, USA) using a mineral soil (1.0 g): H_2O (30 mL) slurry.

2.4. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine if experimental N deposition influenced earthworm abundance (via *L. terrestris* midden counts), forest floor conditions, mineral soil moisture, pH, C and N concentrations, and C:N ratio compared to the ambient treatment. All statistical analyses were considered ecologically significant at $P < 0.10$ due to limited replication of experimental plots under serendipitous earthworm invasion.

3. Results

The presence of *Lumbricus terrestris* was pervasive throughout site B, and there was limited occurrence *L. terrestris* in the other three sites. In Site B, the abundance of *L. terrestris* middens significantly increased from 8 middens m^{-2} in the ambient treatment to 37 middens m^{-2} under experimental N deposition (+363%; $P = 0.01$; Table 2). The forest floor conditions significantly declined from a condition 4 in the ambient treatment, in which earthworms were still present, to a condition 2 under experimental N deposition (-50%; $P = 0.05$; Table 2). Furthermore, soil water content (+58%; $P = 0.02$), C (+97%; $P = 0.10$), and N (+117%; $P = 0.10$) concentration of the mineral soil significantly increased under experimental N deposition concurrent with an increase in earthworm abundance (Table 2).

At site C, the active invasion of *L. terrestris* had only spread across a single experimental N deposition plot at our time of sampling, limiting our ability to detect an effect of experimental N deposition on earthworm abundance and their subsequent feedback on forest conditions at this site ($P > 0.10$, all; Table 2). However, our observations indicated that this invaded plot had the greatest mean abundance of *L. terrestris* of any plot in our study (~51 middens m^{-2}) with the most impacted forest floor condition (1; see Table S2).

4. Discussion

Earthworm activity has clearly increased as invasion spread across