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Application of lime ($CaCO_3$) to promote forest recovery from severe acidification increases potential for earthworm invasion

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ABSTRACT

The application of lime (calcium carbonate) may be a cost-effective strategy to promote forest ecosystem recovery from acid impairment, under contemporary low levels of acidic deposition. However, liming acidified soils may create more suitable habitat for invasive earthworms that cause significant damage to forest floor communities and may disrupt ecosystem processes. We investigated the potential effects of liming in acidified soils where earthworms are rare in conjunction with a whole-ecosystem liming experiment in the chronically acidified forests of the western Adirondacks (USA). Using a microcosm experiment that replicated the whole-ecosystem treatment, we evaluated effects of soil liming on *Lumbricus terrestris* survivorship and biomass growth. We found that a moderate lime application (raising pH from 3.1 to 3.7) dramatically increased survival and biomass of *L. terrestris*, likely via increases in soil pH and associated reductions in inorganic aluminum, a known toxin. Very few *L. terrestris* individuals survived in unlimed soils, whereas earthworms in limed soils survived, grew, and rapidly consumed leaf litter. We supplemented this experiment with field surveys of extant earthworm communities along a gradient of soil pH in Adirondack hardwood forests, ranging from severely acidified (pH < 3) to well-buffered (pH > 5). In the field, no earthworms were observed where soil pH < 3.6. Abundance and species richness of earthworms was greatest in areas where soil pH > 4.4 and human dispersal vectors, including proximity to roads and public fishing access, were most prevalent. Overall our results suggest that moderate lime additions can be sufficient to increase earthworm invasion risk where dispersal vectors are present.

1. Introduction

Acidic deposition has resulted in the acidification and depletion of base cation nutrients from poorly-buffered forest soils across much of northeastern North America (Driscoll et al., 2001). Although sulfur and nitrogen oxide emissions, and the resulting deposition of strong acids, have sharply decreased since the passage of the Clean Air Act Amendments of 1990 the recovery of forest ecosystems from acidification has been slow (Lawrence et al., 2012). Impacts of acidic deposition have been particularly pronounced in the Adirondack Mountains of New York State (USA), a region of northern hardwood and mixed conifer forests with naturally acidic soils due to base-poor surficial geology (Driscoll et al., 1991, 2001). Application of crushed limestone, which is primarily calcium carbonate ($CaCO_3$; lime), has been used to hasten recovery of the acid neutralizing capacity (ANC) of surface waters. ANC is strongly associated with important aquatic species such as *Salvelinus fontinalis,* brook trout (Schofield and Keleher, 1996; Driscoll et al., 1996). With lower acidic deposition loads and increases in rainwater pH, liming has become an increasingly feasible approach for restoration of acid-impaired forests (Lawrence et al., 2011, 2012). Lime additions have been used to promote the productivity and health of *Acer saccharum,* sugar maple (Long et al., 2011; Moore et al., 2012, 2015). Sugar maple is a valuable tree that experiences decreased crown vigor, recruitment, radial growth and foliar nutrition in chronically acid-impaired forest soils where biological Ca availability is low or negligible (Sullivan et al., 2013).

Although forest liming may support valuable species such as sugar maple, and more broadly foster the recovery of acid-impaired ecosystems (Battles et al., 2014), liming can also have unintended consequences that include deleterious impacts on beneficial invertebrate fauna (Fisk et al., 2006; Pabian et al., 2012; McCay et al., 2013), as well as facilitation of non-native and

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invasive earthworms. Earthworms as a functional group have higher abundance, survival, biomass and fecundity in forest soils treated with lime, but responses differ among species with varying pH tolerance and nutritional demands (Springett and Syers, 1984; Chan and Mead, 2003; Raty, 2004; Potthoff et al., 2008; Bernard et al., 2009; Moore et al., 2013, 2015).

Among the acid-intolerant earthworms that might be most facilitated by forest liming, *Lumbricus terrestris*, known commonly as the 'night-crawler', is noteworthy for both its ecological impact and likelihood of introduction. This species feeds almost exclusively on leaf litter from the soil surface (Edwards and Lofty, 1972) and in high densities will reduce litter volume on the forest floor (Hale et al., 2005, 2008). In experimental studies, the application of lime and addition of *L. terrestris* caused reductions in soil nitrogen, leaf litter volume and enchytraeid abundance (Raty, 2004; Moore et al., 2013). Moreover, *L. terrestris* is commonly used for fishing bait and may be found in commercial sources of potting soil, compost and mulch, resulting in a high likelihood of dispersal (Reynolds, 1977; Hale, 2008). If acidic soils prevent invasions of *L. terrestris*, then large-scale applications of lime ($CaCO_3$) could improve habitat suitability for these potentially destructive invaders of northern hardwood forests.

We present experimental and observational evidence of the effects of forest liming on earthworms – with a focus on *L. terrestris* – in the Adirondack Mountains, New York (USA). An experimental microcosm study was used to determine potential effects of a current ecosystem-scale liming treatment on the survivorship and biomass growth of *L. terrestris* in acidified soils (pH < 3.5; base cation saturation < 12%) where this species has currently not been observed. We also conducted earthworm surveys, using soil extraction and timed search methods, in upland hardwood forests across the Adirondacks to evaluate earthworm abundance and species composition across a wide gradient in soil pH and base cation availability. We then evaluated the potential consequences of liming in Adirondack hardwood forests with respect to earthworm invasion risk.

2. Methods and materials

2.1. Liming experiment

We conducted a microcosm experiment to evaluate the potential effects of a whole-ecosystem liming treatment on *L. terrestris* survivorship and growth, but without introducing these potentially invasive earthworms *in situ*. The whole-ecosystem experiment is being conducted in two small first-order catchments on the northwestern end of Honnedaga Lake, located in the southwestern Adirondacks, in Herkimer County, NY. Soils are spodosols with very poor buffering capacity that were formed in thin glacial till (Josephson et al., 2014) and due to their location have received among the highest chronic N and S deposition inputs in North America (Driscoll et al., 2001). As a result, the soils at our study sites are some of the most acidic that can be found in upland hardwood forests in the region (Sullivan et al., 2006, 2013). In October 2013, 136 metric tons (150 U.S. tons) of pelletized Ca limestone was applied by helicopter, equivalent to a dosage of 1.4 Mg Ca ha^{-1}, to the 30 ha treatment watershed within the drainage of Honnedaga Lake. The high Ca pelletized lime originated from Shelburne, Vermont and was comprised of 36.5% Ca, 0.5% Mg, and 91.25% $CaCO_3$, with the pellet binder material being a highly soluble sodium salt lignin. The goal of the watershed treatment was to increase Ca availability in the soils and stream of this watershed to accelerate recovery from effects of acidic deposition. A nearby catchment of similar size, with similar biogeochemical and vegetation characteristics, was used as a reference.

We used a 3×3 factorial design with three levels of soil lime additions: 4.85 g, 9.7 g and 0 g (control), which were approximately equivalent to the *in situ* dosages of 1.4 Mg Ca ha^{-1}, 4.8 Mg Ca ha^{-1}, and 0 Mg Ca ha^{-1}, respectively. The second experimental factor was exposure duration, i.e., the amount of time earthworms in microcosms were left in the growth chamber before destructive harvesting. The levels of this factor were 14, 28 and 42 days. We established 54 microcosms with 6 replications per treatment. Microcosms were plastic greenhouse containers (24×10 cm) filled with soil and covered with leaf litter. Soil from the lower O, A, and upper B horizons was removed from the reference watershed at Honnedaga Lake in August 2014. Soil samples were mixed together, dried, sieved and added to microcosms to a height of 20 cm. Pelletized limestone from the same batch used in the Honnedaga Lake study was dissolved in deionized water and added as a slurry to the soil surface. Leaf litter (10 g) collected from the Honnedaga Lake reference watershed was added to each microcosm following lime slurry application and was replaced every two weeks (old leaf litter was removed prior to adding new leaf litter). Two individuals of *L. terrestris* were weighed and added to each microcosm. Mesh screens and cheesecloth were secured on the top and bottom of the containers. Live earthworms were obtained from Carolina Biological Supply Company. Microcosms were placed in a growth chamber with 14 h of daylight, a fluctuating temperature regime (21 °C during daylight hours and 19 °C during night), and were watered 2–3 times per week.

At the end of each exposure duration period (14, 28 and 42 days), we measured survivorship and live biomass of *L. terrestris* in a group of randomly selected microcosms. Microcosms were destructively harvested and were not repeatedly measured. Survivorship at the microcosm level was recorded if both earthworms were alive and present at the sampling date. Mortality was recorded if one or both earthworms were dead or, in the case of the lime treatments only, escaped from the microcosm. To estimate the biomass change per microcosm, we subtracted the average initial live mass from the average final live mass (at the removal date) for each microcosm. We only used live earthworms to estimate biomass changes.

After the experiment concluded, we analyzed soil from microcosms to estimate the effects of the liming treatment. Soils were air dried at room temperature, subsampled and measured for Ca and Al using inductively coupled plasma optical emission spectrometry after extraction with 1 M NH_4Cl and 1 M KCl, respectively. Percent organic matter was estimated using the loss on ignition method, and pH was measured in 0.01 M $CaCl_2$. All soil analyses were conducted at the USGS Water Science Center in Troy, NY. Further details on soil analysis can be found by referencing the methods utilized in Lawrence et al. (2012).

We used the nonparametric Fisher's Exact Test (Fisher, 1922) to identify effects of lime and exposure duration on earthworm survivorship, and two-way ANOVA to measure treatment effects on biomass change. The effects of our soil liming treatments on pH, percentage of organic matter and exchangeable [Ca] and [Al] were assessed using a one-way ANOVA. The Tukey method was used for all pairwise means comparison (Tukey, 1953). We used residual analysis to examine assumptions of normality and Levene's test for homogeneity of variance. All tests were conducted with an $\alpha = 0.05$.

2.2. Field survey

We conducted sampling for earthworms at 14 upland hardwood forests that had been previously studied (Beier et al., 2012). Together these sites represented a regional gradient in soil pH (2.80–5.04) and exchangeable [Ca] (Oa horizon 3.7–53.89 $cmol_c\ kg^{-1}$; B horizon 0.35–7.73 $cmol_c\ kg^{-1}$ for upland