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Removal of an invasive shrub (Chinese privet: *Ligustrum sinense* Lour) reduces exotic earthworm abundance and promotes recovery of native North American earthworms

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ABSTRACT

This study investigated the possibility of a facilitative relationship between Chinese privet (*Ligustrum sinense*) and exotic earthworms, in the southeastern region of the USA. Earthworms and selected soil properties were sampled five years after experimental removal of privet from flood plain forests of the Georgia Piedmont region. The earthworm communities and soil properties were compared between sites with privet, privet removal sites, and reference sites where privet had never established. Results showed that introduced European earthworms (*Aporrectodea caliginosa*, *Lumbricus rubellus*, and *Octolasion tyrtaeum)* were more prevalent under privet cover, and privet removal reduced their relative abundance (from >90% to ∼70%) in the community. Conversely, the relative abundance of native species (*Diplocardia michaelsenii*) increased fourfold with privet removal and was highest in reference sites. Soils under privet were characterized by significantly higher pH relative to reference plots and privet removal facilitated a significant reduction in pH. These results suggest that privet-mediated effects on soil pH may confer a competitive advantage to European lumbricid earthworms. Furthermore, removal of the invasive shrub appears to reverse the changes in soil pH, and may allow for recovery of native earthworm fauna.

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1. Introduction

As the second greatest cause of modern extinctions, invasive species are a major threat to biodiversity (Soule, 1990). Early studies of invasive species showed that they can have large effects on ecosystems (Vitousek et al., 1987) and have spurred further research in this area. Unsurprisingly, most of this work has involved species that can be easily seen such as plants, vertebrates, and insects (Hendrix, 2006). This has unfortunately resulted in less attention to some of the more obscure invertebrates, such as earthworms despite their being recognized as very important creatures in the history of the world (Darwin, 1881).

Earthworms have been described as ecosystem engineers due to their proclivity to create long-lasting ecosystem level impacts on the soil environment (Jones et al., 1994). They greatly influence nutrient cycling, soil formation, soil properties, seed germination, microbial communities, soil invertebrate communities, and even aboveground communities (Edwards and Bohlen, 1996; Lavelle, 2001; Lee, 1985). With the ability to produce such broad and fundamental changes, it is likely that invasive earthworms would significantly impact the ecosystems they invade. Indeed, striking alterations to ecosystems have been observed when European earthworms invade regions where there are no native earthworms (Bohlen et al., 2004; Eisenhauer et al., 2007). In North America, areas north of the Wisconsinan glaciation have been free of earthworms for about 12,000 years (Callaham et al., 2006; James, 1995) and introductions of earthworms in this region completely alter conditions within the forest floor, with potential drastic changes to forest species composition over the long term (Bohlen et al., 2004; Eisenhauer et al., 2009; Hale et al., 2006).

Potentially aiding the invasion of exotic earthworms are positive and facilitative relationships with other non-native invasive species. 'Invasional meltdown' is a hypothesis suggesting that one invasive species creates an environment favorable to another, leading to increased or simultaneous invasions (Simberloff and Von Holle, 1999). Many invasive plants are known to change soil properties, often increasing N levels in the soil or altering pH (Asner

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and Beatty, 1996; Ehrenfeld et al., 2001; Vitousek et al., 1987) and increased exotic earthworm densities have been observed under many of these plants, especially those with high quality leaf litter (Aplet, 1990; Heneghan et al., 2007; Kourtev et al., 1999). Furthermore, European buckthorn (*Rhamnus cathartica* L.) and honeysuckle (*Lonicera* × *bella* Zabel) are common invasive plants of the Midwestern United States with high quality leaf litter and their removal led to a decreased abundance of invasive earthworms (Madritch and Lindroth, 2009).

The southeastern United States was not glaciated during the Wisconsinan glaciation and therefore has a native earthworm fauna (Hendrix et al., 2008), but soils are also occupied by exotic European lumbricid earthworms as well as the Asian genus *Amynthas* (Callaham et al., 2003). The region is also host to Chinese privet (*Ligustrum sinense* Lour.), a highly invasive semi-evergreen shrub that was brought to the southeast in 1852 for ornamental purposes (Dirr 1983). Privet escaped cultivation and is now well established in the southeastern U.S. and can be found from Texas to Massachusetts (Miller, 2003). Similar to European buckthorn and honeysuckle, privet is known to have leaf litter of high quality, with lower lignin, cellulose, and C:N ratios relative to native leaf litter. Also, privet litter has been shown to have faster decomposition rates relative to native litters in floodplains of western Georgia, as well as a fivefold increase in soil N mineralization rates (Mitchell et al., 2011). Privet does especially well in floodplains and riparian areas of the Southern Appalachian Piedmont region, where it often creates monotypic stands that crowd out native plants (Brantley, 2008; Hanula et al., 2009). This includes the Upper Oconee River basin in northeast Georgia, where privet now covers at least 59% of the entire floodplain (Ward, 2002). This drastic change of plant species composition in southeastern floodplains would be expected to have significant impacts on these important ecosystems. Land managers are developing methods of privet control to conserve the biodiversity of southeastern floodplains. Although these strategies have proven successful at conserving aboveground biodiversity (Hanula and Horn, 2011a,b; Hanula et al., 2009), our study is the first to investigate the effects of privet and its removal on belowground diversity.

Other than the studies by Brantley (root biomass estimates [2008]) and Mitchell et al. (N mineralization assays and decomposition studies [2011]), there have been no studies of the effects of privet on belowground biotic communities or processes. This leads to the question of how privet invasion and its management via removal may affect soils and influence the earthworm communities in southeastern floodplain habitats. To address these questions, we quantified soil properties as well as earthworm abundance and community composition in sites with privet, sites where privet has been experimentally removed, as well as in sites that have not yet been invaded by privet. Objectives for this study were to evaluate the impact of privet invasion and privet removal on a suite of soil characteristics, and to determine the effects of privet management on the structure of the earthworm community in these riparian forests. We expect these results to provide further insight on the role of facilitation in invasion by specifically addressing the relationships between privet and soil properties and their interactions with native and exotic earthworms.

2. Methods

2.1. Site description

We utilized experimental privet-removal plots established by the U.S. Forest Service in 2005. These plots have been used to study the effects of privet removal on biodiversity in numerous studies. The site and the privet removal process are described in detail in Hanula et al. (2009). Briefly, the study design was a completely randomized block design with four blocks consisting of three experimental treatments. Each block consisted of two privet removal treatments and a control plot where existing privet was not treated in any way. The first privet removal treatment involved mechanical mastication of all vegetation <10 cm dbh with a tractor-mounted Gyrotrac® head (= "mulched" treatment hereafter). The second privet removal treatment involved hand-felling of all privet stems using power saws (= "felled" treatment hereafter), and the resulting slash was brush sawed to achieve a height above soil surface of <1 m. Both removal treatments involved herbicide application (glyphosate) following re-sprouting of privet stumps. The sites were located within the Oconee River watershed in northeast Georgia (Fig. 1). Plots were approximately 2 ha in size and contained similar levels of privet in the shrub and herbaceous plant layers prior to treatment. Three additional plots with minimal privet invasion were used as reference sites, considered to be representative of conditions prior to privet infestation, as well as reflective of the management objective for floodplains without privet. These reference sites were located on the Oconee National Forest near the Scull Shoals and Watson Springs treatment sites. Because of the difficulty associated with finding floodplain forest that has not been invaded by Chinese privet, reference sites were located some distance away from the blocks of treatment plots. As such, these sites may not be directly comparable to the treatment blocks, but we nevertheless included them in our analysis in order to have some form of "reference" to the desired condition for these floodplain forests. Importantly, the plots where privet removal treatments were applied were immediately adjacent to the untreated privet "control" plots, and thus are more comparable due to their proximity, and because they are situated on similar soils, with similar overstory vegetation and similar privet invasion history. Soils at the sites were variously classified as Fluvaquentic Dystrudepts, Fluvaquentic Endoaquepts, and Typic Udifluvents (series names Chewacla, Wehadkee, and Toccoa).

2.2. Earthworm sampling

Sample points were randomly placed in each quadrant of all plots. Earthworm sampling was performed every three months at each sample point beginning September 2010 with the final collection date in July 2011. Earthworms were collected by hand sorting 30 cm × 30 cm × 30 cm soil monoliths and were placed in a 70% ethanol solution to preserve them for later identification. At each sample date, the exact location of sampling pits was moved less than two meters from the original point to avoid sampling soil disturbed by previous digging.

Adult earthworms were identified to species using the keys of Schwert (1990), James (1990), and Reynolds (1978). Aclitellate worms were identified to genus or species based on key characteristics: namely the prostomium type, pigmentation, and setal arrangement. Aclitellate *Amynthas* and *Sparganophilus* could only be identified to genus, whereas aclitellate *Octolasion tyrtaeum*, *Diplocardia michaelsenii*, *Aporrectodea caliginosa*, *Eisenoides lonnbergi*, and *Lumbricus rubellus* could be identified to species.

2.3. Soil sampling

Soil samples were collected in September 2010 at all earthworm sample points to determine pH, mineral N, potential N-mineralization, and soil texture (% sand, silt, and clay). Samples were collected by taking multiple (6 or 8) 10 cm deep and 2 cm diameter soil cores around the earthworm sample points and compositing the cores for each point. Samples were kept in a cooler for transportation and were processed for potential N-mineralization