Surveys

Exotic Earthworm Communities Within Upland Deciduous Forests of National Wildlife Refuges in the Upper Midwest

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Abstract

The invasion of exotic earthworms into forests of the Upper Midwest region of the United States is a considerable management issue due to the ability of earthworms to act as ecosystem engineers and modify existing ecosystems. Earthworm taxa differ in their biology and behavior, such that earthworm abundance and community composition can be related to the type and degree of alteration expected to occur. Many National Wildlife Refuges of the Upper Midwest have enabling legislation that identifies migratory birds as a specific management priority, and past studies have indicated that earthworm-modified forests provide lower quality habitat for migratory, ground-nesting birds. We quantified exotic earthworm community composition, abundance, and variability within and among upland deciduous forest stands at six of these refuges (Horicon, Ottawa, Rice Lake, Seney, Shiawassee, and Tamarac), providing a baseline for estimating the status of earthworm invasion. Analyses revealed a significant difference in mean earthworm biomass among upland deciduous forest stands sampled (ANOVA, \( F_{5,52} = 2.81, P = 0.03 \)) and indicated significant differences in earthworm community composition (multiresponse permutation procedure, \( T = -12.57, A = 0.24, P < 0.001 \)) at each refuge. Based upon existing theories regarding earthworm invasion, we suggest that the stage, and impact, of earthworm invasion is varied across the region. Continued research and monitoring of earthworm communities should test this theory and seek to elucidate drivers of earthworm invasion patterns and impacts to forest ecosystems.

Keywords: European earthworms; Great Lakes Region; invasive species; upland deciduous forests; wildlife habitat

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Introduction

Exotic earthworm invasion into deciduous forests in the Upper Midwest region of the United States has been a growing concern for forest managers in recent years. Earthworms are considered to be ecosystem engineers for their ability to modify ecosystems and the resources ecosystems provide (Jones et al. 1994). As ecosystem engineers, earthworms have been shown to have both direct and indirect impacts. Exotic earthworm presence has been linked to decreased plant species richness and changes in plant community composition (Holdsworth et al. 2007). Earthworm activity is also thought to facilitate invasion by exotic plant species (Nuzzo et al. 2009) and could contribute to cases of invasional meltdown (Heimpel et al. 2010), in which multiple exotic species cofacilitate one another. Earthworms also have
the potential to indirectly impact wildlife populations and communities (Migge-Kleian et al. 2006; Hale 2008). Recent research has shown that the decomposition and removal of leaf litter and organic material by earthworms causes declines in ground-nesting songbird abundance, in part due to reduced nest concealment (Loss and Blair 2011). Earthworms have also been shown to impact salamander abundance by decreasing habitat quality and prey abundance (Maerz et al. 2009).

Earthworm taxa differ in their feeding and burrowing activities, and thus differ in the type and degree of impact to forest soils, organic layers, and associated ecosystem components. Litter-dwelling (epigeic) species, such as *Dendrobaena octaedra*, feed on microorganisms and fungi in the surface and upper organic layers. This activity contributes to the breakdown of organic matter and changes in forest microorganism communities (Edwards and Bohlen 1996); however, the overall degree of impact is low (Frelich et al. 2006). Soil-dwelling (endogeic) species, such as *Aporrectodea* spp., feed on organic material in the soil, which creates nonpermanent horizontal burrows. These actions disrupt fungal communities and alter soil nutrient cycling, but the overall impact is also relatively limited (Bohlen et al. 2004). Deep-burrowing (anecic) species, such as *Lumbricus terrestris*, create permanent vertical burrows in the mineral soil, yet feed on fresh surface litter. Their burrows can be identified by the presence of a midden, a mound of residual plant material and earthworm castings (excrement) with a central plug composed of leaf litter. This burrowing and feeding activity slowly increases the mixing of organic matter from the litter into the mineral soil, which results in a large impact over many years (Frelich et al. 2006). Additionally, some earthworm species, such as *Lumbricus rubellus*, live and feed in both the litter and mineral soil (epi-endogeic). The behavior of *L. rubellus*, in particular, has a rapid and large influence on organic matter decomposition, which results in an overall high impact (Frelich et al. 2006). Consequently, the species composition and abundance of earthworms at a site will determine, to a considerable degree, the alteration to forest soils and plant communities that are expected to occur, and indicate potential indirect impacts to other ecosystem components (Bohlen et al. 2004; Frelich et al. 2006).

The composition of exotic earthworm communities within upland deciduous forests of National Wildlife Refuges (hereafter, Refuges) in the Upper Midwest is unknown. Because many of these Refuges have enabling legislation that specifically targets the conservation of migratory birds and have corresponding goals and objectives that may be hampered by exotic earthworms as discussed above, we quantified earthworm species composition, abundance, and variability within and among upland deciduous forest stands of six Refuges. In doing so, we provide a baseline for estimating the status of earthworm invasion and help elucidate potential forest management opportunities and limitations pertinent to wildlife habitat management.

**Study Sites**

This study was conducted within upland deciduous forests of six Refuges located within the Great Lakes Biological Network of the U.S. Fish and Wildlife Service (USFWS) National Wildlife Refuge System. Specifically, sampling took place at Tamarac, Rice Lake, Shiawassee, Seney, Horicon, and Ottawa National Wildlife Refuges (hereafter referred to simply as Refuge(s) or by name). Among these Refuges, considerable variation is found in existing and historical land cover and landscape patterns, with the majority of these Refuges historically dominated by forest ecosystem types (Corace et al. 2009, 2012). Tamarac encompasses 17,770 ha in northwestern Minnesota and is dominated by deciduous forests, which cover 57% of the Refuge (2006 National Land Cover Data [USGS 2011]). Rice Lake (7,316 ha in northeast Minnesota) and Shiawassee (3,882 ha in Michigan’s Lower Peninsula) are dominated by forests; however, much of the forested land is classified as woody wetlands. Deciduous forests at these Refuges make up 22% of the area and 23% of the area, respectively. The largest Refuge in the study, Seney, covers 38,626 ha in Michigan’s eastern Upper Peninsula. Seney is dominated by a mix of woody and emergent herbaceous wetlands, with only 3% of the Refuge being classified as deciduous forest. Horicon covers 8,849 ha in southeast Wisconsin, and Ottawa (including Cedar Point National Wildlife Refuge) covers 3,480 ha in northeast Ohio. Both of these Refuges are dominated by emergent herbaceous wetlands. Deciduous forests at Horicon and Ottawa cover 1% and <1% of the area, respectively.

**Methods**

Earthworm communities were sampled during summer 2010 and 2011 within forest assessment and monitoring plots established systematically within Refuges concurrent to this study (Petrillo and Corace 2011). Within each forest stand, Petrillo and Corace established a linear transect along which they placed sampling plots a minimum of 20 m apart. Transect length was dependent upon stand size, but a minimum of three plots were established per transect. At each plot, they established a 1/100-ha circular sampling area around the center point. Within this plot, they placed three 1-m² quadrat subplots at 0°, 135°, and 225° at randomly assigned distances of 1 m, 2 m, and 4 m from the center point. Petrillo and Corace sampled earthworm communities immediately adjacent (placed randomly on the outside edge) to each of the three subplots. Because studies have shown exotic earthworms in the Upper Midwest to be most common and of greatest concern within upland deciduous forests (Bohlen et al. 2004; Hale et al. 2005; Tiuonov et al. 2006), all earthworm sampling occurred within this forest type.

We quantified earthworm species composition and abundance using the mustard extraction method (Gunn 1992) within an area of 0.11 m² (33 cm × 33 cm). Before sampling earthworms, we cleared the surface litter collecting any earthworms encountered in the litter or on the surface. During clearing, the number of middens present was also recorded to confirm and supplement *L. terrestris* biomass. We made a mustard solution by mixing water and ground yellow mustard (*Sinapis alba*) powder at a ratio of 10 g/L of water. We built a four-
walled box and used it to contain the mustard solution and keep the sampling area consistent. The amount of mustard solution applied varied depending on soil moisture and drainage speed; however, no >3.8 L of solution was used over one 0.11-m² area. We collected all earthworms that emerged within 5 min and preserved them in 70% isopropyl alcohol. We identified earthworms to genus or species (when possible) and measured them within 24 h of collection. We calculated earthworm biomass as ash-free dry mass using earthworm lengths and allometric equations developed by Hale et al. (2004). We used midden counts to supplement biomass data for *L. terrestris* in cases where middens occurred without the collection of *L. terrestris* individuals.

We calculated earthworm biomass by taxa for each subplot and averaged subplots to create plot-level data. We averaged plot-level biomass by transect to compare earthworm communities among forest stands and Refuges. Biomass data by taxa are provided as supplemental material (Table S1, *Supplemental Material*). We used analysis of variance to test for differences in mean earthworm biomass for all upland deciduous forest stands at each Refuge. We used the nonparametric multiresponse permutation procedure (MRPP) to test for differences in earthworm community composition among Refuges and among forest stands within Refuges. The MRPP technique does not require normality or homogeneity of variance, and thus is useful for ecological community data (McCune and Grace 2002). We performed multiresponse permutation procedure with PC-ORD software (McCune and Mefford 1999) using earthworm biomass by taxa, the Euclidean distance measure, and the recommended weighting of groups (n/sum[n]). Pairwise comparisons were assessed for Refuges to describe specific differences and similarities. To compare earthworm community composition by Refuge, we averaged earthworm biomass by taxa for each forest stand, resulting in a sample size of 58 stands across six Refuges. To compare earthworm community composition among forest stands within Refuges, we used plot-level earthworm biomass by taxa (sample sizes are shown in Table 1). For both, a dummy variable called NONE was included to indicate sites without the presence of earthworms.

To further describe and compare earthworm communities among forest stands and Refuges, we also performed nonmetric multidimensional scaling (NMS) and indicator species analysis using PC-ORD. The NMS ordination technique is commonly used with ecological community data, and is appropriate for nonnormal data (McCune and Grace 2002). Ordination using NMS was first performed using autopilot mode to determine dimensionality. Using this result, NMS was then performed using the recommended dimensions (axes), the Sorensen distance measure, a maximum of 200 iterations, a step length of 0.20, and a stability criterion of 0.00001 over 10 iterations. Indicator species analysis is often used in conjunction with MRPP analysis to describe specific species relationships to groups, with perfect indicators (IV = 100 percent perfect indication) always present and exclusive to a group (McCune and Grace 2002). We verified both NMS and indicator species analyses using a Monte Carlo randomization test with 200 runs. For all analyses, we considered results to be statistically significant at a *P*-value of ≤0.05.

### Results

Three-hundred fifty plots within 58 forest stands were sampled across the six Refuges. Earthworms were present at all Refuges in the study and were found at 83% of the total plots and within 93% of the sampled stands. Mean earthworm biomass varied across the six Refuges, with average biomass ranging from 0.54 ash-free dry g/m² at Seney to 2.14 ash-free dry g/m² at Horicon (Table 1). One-way ANOVA revealed a significant difference in mean earthworm biomass among Refuges (*F*$_{5,52}$ = 2.81, *P* = 0.03). Seven distinct earthworm taxa were identified: *Dendrobaena octaedra, Dendrodrilus rubidus, Eiseniella tetraedra, Lumbricus rubellus, Lumbricus terrestris*, a group consisting of *Lumbricus juveniles*, and a genus, *Aporrectodea*. *Lumbricus* juveniles and species in the genus *Aporrectodea* were not identified further due to morphological similarities. All earthworms were of European origin, and no native earthworms were encountered. Earthworm species were similar across Refuges; however, variability in community composition existed (Figure 1). Individuals of *Aporrectodea* and *L. rubellus* were present at all Refuges, *D. octaedra* was absent from Ottawa, *L. terrestris* was absent from Rice Lake, *D. rubidus* was present only at Horicon and Rice Lake, and *E. tetraedra* was present only at Ottawa and Shiawassee.

Results of the MRPP analysis indicated that there was a significant difference in earthworm communities among Refuges (T = −12.57, A = 0.24, *P* < 0.001). Pairwise comparisons also showed significant differences between Refuges; however, earthworm communities at Rice Lake and Seney did not significantly differ (*P* = 0.25, all other comparisons *P* < 0.01). Using NMS ordination, a three-dimensional solution was recommended (Stress = 12.95, Monte Carlo *P* < 0.01). The NMS ordination on axes one and two supports other data that indicate Refuges have distinct earthworm communities, because forest stands within Refuges were more closely grouped with each other, despite all Refuges sharing similar
species (Figure 2). Indicator species analysis found the following taxa to be significant indicators of Refuges: *D. octaedra* (IV = 64.3, $P = 0.01$) and *D. rubidus* (IV = 25.4, $P = 0.03$) at Rice Lake, *L. rubellus* (IV = 34.6, $P = 0.03$) and *Lumbricus* juveniles (IV = 40.2, $P < 0.01$) at Shiawassee, and *L. terrestris* at Horicon (IV = 58.1, $P < 0.01$). No indicator taxa were identified for Ottawa, Seney, or Tamarac. Within Refuges, MRPP analysis indicated that there was a statistically significant difference in earthworm communities among forest stands for all Refuges (Table 2; Figure 3).

### Discussion

Earthworm communities of upland deciduous forests varied among the six Refuges in the Upper Midwest and among the sampled forest stands within Refuges; however, despite earthworm communities being significantly different, a small suite of exotic earthworm taxa were shared. Earthworm taxa identified in this study were similar to those observed in other studies conducted in the Upper Midwest (Gundale et al. 2005; Hale et al. 2005). It is likely that the differences observed in earthworm abundance and community composition among Refuges were influenced by the variation in land cover patterns within and surrounding Refuges (Corace et al. 2009, 2012). In general, those Refuges found within ecoregions more dominated by anthropogenic covers, such as developed land and agriculture (i.e., Horicon and Shiawassee), had greater earthworm biomass than those Refuges with less anthropogenic cover in the surrounding landscape (i.e., Rice Lake, Seney, and Tamarac).

Differences in earthworm communities within Refuges are also likely the result of variation in forest overstory and soil conditions, which are known to influence earthworm taxa in varying ways (Tiunov et al. 2006). Further exploration is needed to determine whether current earthworm communities are the result of stochastic events or driven predictably by dispersal mechanisms, landscape patterns, or habitat conditions.

The patterns of earthworm community composition observed agree with the theory that earthworm invasion proceeds in waves, beginning with epigeic earthworm species such as *D. octaedra* and ending with an increasing abundance of *L. terrestris* (James and Hendrix 2004; Hale et al. 2005). Horicon, the Refuge with the highest mean earthworm biomass, had the highest biomass of *L. terrestris*, which made up 56% of the total Refuge earthworm biomass (not including *Lumbricus* juveniles). In addition, Horicon had low biomass of *D. octaedra* (<1% of the total biomass). In contrast, Seney, which had the lowest mean earthworm biomass, had 2% of its total earthworm biomass in *L. terrestris* (the largest earthworm present) and 22% in *D. octaedra* (the smallest earthworm present). Relating these findings to the theorized progression of invasion (Hale et al. 2005) suggests that Seney is in an earlier stage of colonization, while earthworm communities at Horicon are well-established. Similar trends were seen at Shiawassee, where 16% of the total earthworm biomass was *L. terrestris* and <1% was *D. octaedra*, and Rice Lake, which lacked *L. terrestris* but had the highest biomass of *D. octaedra*, 25% of the total Refuge biomass. By qualitatively assessing invasion progression among Refuges...
based on this theory, we propose that Refuges in the Upper Midwest represent a gradient of invasion stages both among and within Refuges. Using *Lumbricus* spp. biomass as an index of earthworm establishment, the Refuges of study can be ranked from the most poorly established (Seney) to the most well-established (Horicon; Figure 4).

Exotic earthworms have been shown to alter forest stands, causing changes in soil structure, litter content, nutrient availability, microorganisms, and plant communities (Bohlen et al. 2004; Frellich et al. 2006; Nuzzo et al. 2009; Dempsey et al. 2011). These changes have cascading effects within ecosystems (Bohlen et al. 2004; Frellich et al. 2006) and can directly or indirectly affect wildlife and their habitats (Migge-Kleian et al. 2006; Maerz et al. 2009). Earthworm species differ in their type and degree of impact (Bohlen et al. 2004; Frellich et al. 2006), and multiple species tend to work interactively and cause increased alteration (Bossuyt et al. 2006; Asshoff et al. 2010); therefore, our community analysis should elucidate potential forest conservation and restoration constraints for Refuge managers. For instance, the Refuge with the most developed earthworm community (e.g., Horicon) may have more limitations.
imposed on upland deciduous forest management, such as inability to recruit overstory tree species that are inhibited by earthworm activity (Frelich et al. 2006), than would the Refuge with the least developed earthworm community (Seney). In addition, earthworms are expected to act in combination with other drivers of accelerated ecosystem change such as increased white-tailed deer *Odocoileus virginianus* densities, invasive plant species, and climate change (Frelich et al. 2006; Holdsworth et al. 2007).

Although we encountered earthworms at all Refuges, we did not encounter them within all stands. Thus, efforts to prevent further spread of earthworms may still be beneficial. The introduction and spread of earthworms, over both short and long distances, has been

**Table 2.** Multiresponse permutation procedure results for earthworm communities within upland deciduous forests sampled in 2010 and 2011 at Horicon, Ottawa, Rice Lake, Seney, Shiawassee, and Tamarac National Wildlife Refuges in the Upper Midwest region of the United States. A significant difference in earthworm communities among forest stands within the Refuge is indicated by *.

<table>
<thead>
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<th>Refuge</th>
<th>T</th>
<th>A</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Horicon</td>
<td>−6.20</td>
<td>0.20</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Ottawa</td>
<td>−2.87</td>
<td>0.07</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Rice Lake</td>
<td>−6.87</td>
<td>0.19</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Shiawassee</td>
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<td>0.13</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Seney</td>
<td>−10.53</td>
<td>0.18</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Tamarac</td>
<td>−28.62</td>
<td>0.27</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

**Figure 3.** Variation in mean earthworm biomass (ash-free dry mass, AFDg/m²) by taxa for all forest stands sampled in 2010 and 2011 at Horicon, Ottawa, Rice Lake, Seney, Shiawassee, and Tamarac National Wildlife Refuges in the Upper Midwest region of the United States.

**Aporrectodea spp.**

**Dendrobaena octaedra**

**Dendrodrilus rubidus**

**Eiseniella tetraedra**

**Lumbricus juveniles**

**Lumbricus rubellus**

**Lumbricus terrestris**
greatly facilitated by human activity (Bohlen et al. 2004; Cameron et al. 2007; Hale 2008). Earthworms are commonly introduced into new sites by release of fishing bait (Hale et al. 2005; Cameron et al. 2008) and transport in soils, horticultural material, and on vehicles (Bohlen et al. 2004). The discovery that earthworms are still absent from some Refuge forest stands suggests that efforts to limit the movement of soil and equipment between sites, and to restrict the use of earthworms as fishing bait, could prevent introduction or slow further spread of exotic earthworms. It has also been suggested that prevention of additional introductions in invaded sites, which increases species and genetic diversity, could reduce the severity of earthworm impact (Hale 2008). Continued monitoring of earthworm communities in invaded stands, and in those stands presently without earthworms, will be important for predicting potential ecosystem changes and should be an important consideration in habitat management planning.

Supplemental Material

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Table S1. Earthworm biomass by taxa at 350 plots within 58 upland deciduous forest stands (transects) sampled in 2010 and 2011 at Horicon, Ottawa, Rice Lake, Seney, Shiawassee, and Tamarac National Wildlife Refuges, located in the Upper Midwest, United States.

Figure 4. Qualitative ranking (from poorly established to well-established earthworm communities) of six National Wildlife Refuges (Horicon, Ottawa, Rice Lake, Seney, Shiawassee, and Tamarac) in the Upper Midwest region of the United States using the biomass (ash-free dry mass, AFDg/m²) of *Lumbricus* spp. within upland deciduous forests as an index of exotic earthworm establishment.


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