

Relations among Invasions of Non-native Earthworms, Forest Floor Habitat, and
Populations of Ground-nesting Songbirds in North Temperate Hardwood Forests

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CHAPTER 1 – INTRODUCTION

Invasions of non-native species pose a significant global threat to native ecosystems and to the persistence of native species (Vitousek et al. 1997). Although not all non-native species are considered to be invasive (i.e., having adverse environmental, social, or economic effects), those that are invasive can have substantial direct and indirect impacts on populations of individual species, richness and diversity of biological communities, and functioning of ecosystems (reviewed by Mack et al. 2000, Mooney and Cleland 2001, White et al. 2006). Invasive species with disproportionately large effects, known as ecosystem engineers, can substantially change the abiotic and biotic structure of ecosystems, affect resource availability for many species, and alter the flow of energy or biomass (Crooks 2002). An important area of ecological research is clarifying linkages between belowground and aboveground biological systems to understand how human-driven changes, such as introduction of invasive species, affect species richness and ecosystem function (Wardle 2004).

Earthworms are ecosystem engineers, and non-native earthworm species are established in nearly every ecosystem in the world (Hendrix et al. 2008). Invasion by non-native earthworms has been identified as a major emerging issue because these invasive species are expected to substantially affect conservation of biodiversity in North America (Sutherland et al. 2010). In north-temperate and boreal regions of North America, European earthworms are colonizing forests that were historically free of earthworms (James 2004) and having substantial effects on soil structure and function that affect aboveground biota (Bohlen et al. 2004). Non-native invasive earthworms,

particularly those in the genus *Lumbricus*, consume leaf litter and organic layers below the leaf litter, mix soil horizons (Alban and Berry 1994, Hale et al. 2005a), reduce abundance of mycorrhizal fungi (Lawrence et al. 2003) and arthropods (Migge-Kleian et al. 2006), and alter nutrient dynamics (Costello and Lamberti 2008). Changes to the soil reduce cover and diversity of understory plants, increase dominance by sedges and grasses (Hale et al. 2006, Holdsworth et al. 2007a, Nuzzo et al. 2009), and lead to extirpations of plants (Gundale 2002).

The effects of invasive earthworms on vertebrates are poorly known; however, earthworm-caused reductions in litter depth and arthropod abundance may result in declines in abundance of ground-dwelling salamanders (Maerz et al. 2009). In the northeastern and midwestern United States, several species of songbirds forage and build their nests on the forest floor. Although earthworm invasions may provide a food source for these birds, a study of the ground-nesting Ovenbird (*Seiurus aurocapillus*) showed increases in nest predation related to decreases in litter thickness that are thought to have been caused by non-native earthworms (Mattsson and Niemi 2006).

Despite the evidence for cascading effects of invasive earthworms on vertebrates, no explicit investigation of earthworm effects on ground-dwelling birds has been conducted. Ground nests are highly prone to predation (Robinson et al. 1995), and the reduced plant cover and litter depth associated with earthworm invasions may increase predation on ground nests. Additionally, reductions of arthropod abundance due to reduced litter depth may deplete food sources for ground-foraging birds. Thus, birds that

inhabit the forest floor may have greater rates of reproductive failure in areas colonized by earthworms.

In chapter 2, I investigate relations between invasive earthworms, attributes of the forest floor habitat, and density and nest survival (i.e., probability that nestlings successfully fledge) of 2 ground-nesting and ground-foraging songbirds, the Ovenbird and Hermit Thrush (*Catharus guttatus*), in hardwood forest stands of the Chequamegon-Nicolet National Forest in northern Wisconsin (U.S.A.). Although both bird species are relatively common and widespread, their abundances have recently decreased substantially in parts of the study area (Lind et al. 2006). It has been hypothesized that songbird populations in this primarily forested region are sources of colonists for populations in fragmented woodlands of the southern Midwest (Robinson et al. 1995). My goal was to test whether earthworm-caused changes to the forest floor may cause declines of Ovenbirds and Hermit Thrushes in these putative source populations. To achieve this goal, I assessed densities of Ovenbirds and Hermit Thrushes in earthworm-invaded and earthworm-free forests and investigated whether invasive earthworms and forest-floor attributes (e.g., litter depth and cover of understory plants) were related to Ovenbird nest survival.

Full clarification of the impacts of invasives to populations and ecosystems requires research to be scaled-up from local sites to regional and continental extents (Crowl et al. 2008). Although regional and continental declines of bird populations have been linked to invasive diseases (Hochachka and Dhondt 2000, LaDeau et al. 2007), studies of bird responses to invasive invertebrates are typically limited in spatial extent to

local study areas covering <100,000 ha (1,000 km²) (Thurber et al. 1994, Allen 1995). Regional-scale studies illustrate that earthworm invasions are widespread in hardwood forests of the north-central U.S., with most of the landscape invaded or susceptible to invasion, even in large unbroken tracts of protected land such as national forests (Gundale et al. 2005, Holdsworth et al. 2007b). However, it is unclear whether earthworms have adverse effects to songbirds at a regional extent.

In Chapter 3, I scale up my investigation of the association between invasive earthworms and populations of ground-nesting songbirds to a regional extent. I investigate relations between earthworm invasions, point and landscape-scale habitat characteristics, and populations of ground-nesting songbirds, at bird survey points scattered across the Chequamegon-Nicolet National Forest and the Chippewa National Forest in north-central Minnesota, an area covering >1.2 million hectares. My primary goal was to determine whether invasive earthworms pose a region-wide threat to the conservation of ground-nesting forest songbirds in the northern Midwest. To achieve this goal I identified the following objectives: (1) compare densities of four ground-dwelling species – the Ovenbird, Hermit Thrush, Veery (*Catharus fuscescens*), and Black-and-white Warbler (*Mniotilta varia*) – between earthworm-invaded and earthworm-free forest stands, and (2) clarify the relative contribution of earthworm invasions compared to point and landscape-scale habitat characteristics for determining density of the Ovenbird, the most common ground-nesting songbird in the region.

Preventing further spread of invasive earthworms and mitigating earthworm impacts to soil, plant communities, and vertebrates requires identification of large-scale

earthworm distributions, remaining earthworm-free areas, and the severity of existing invasions. To achieve these objectives, sampling must be conducted to quantify earthworm populations. Several available techniques allow earthworm populations to be sampled (reviewed by Butt and Grigoropoulou 2010), including removal and hand-sorting of the soil (Raw 1960, Coja et al. 2008), liquid extraction using permanganate (Svendsen 1955), formalin (Raw 1959, Callaham and Hendrix 1997), or a mustard-water mixture (Lawrence and Bowers 2002, Hale et al. 2005a), and electrical extraction (Weyers et al. 2008). However, these methods are time-consuming and effort-intensive, thus precluding rapid sampling of a large number of points. Some of the methods are also physically destructive or require use of environmentally toxic substances. Clearly, development of a tool that provides an environmentally friendly and rapid means for assessing earthworm populations will assist conservation, management, and research projects that require either in-depth mapping of invasion at a fine resolution or of broad invasion patterns at large spatial extents.

In chapter 4, I develop a visual assessment method based on a test of the relations between visual habitat cues and presence and severity of earthworm invasions in hardwood forests. Because the effects of invasive earthworms are highly visible, it may be possible to use visual cues, such as depth of the litter layer, preponderance of sedges, and the presence of earthworm middens and castings to provide a coarse assessment of the presence or severity of earthworm invasions. However, to date no empirical test of the accuracy of such visual cues has been conducted. To provide this test, I: (1) analyzed how accurately a 5-level visual system of invasion classification, based on multiple

characteristics of the soil and forest floor, reflected invasion as quantified by mustard extraction sampling, and (2) used data generated from two different visual sampling protocols to assess relations between individual soil, litter layer, and vegetation variables and presence of different earthworm species, including the species of greatest management concern in North America, *L. rubellus* and *L. terrestris*.

The impetus for this dissertation was not only to further understand the scope of the impact of an invasive ecosystem engineer, but also to inform songbird conservation and management to prevent and mitigate earthworm effects. In addition, I sought to develop a robust method for rapidly assessing earthworm invasions that can provide a blueprint for the development of similar protocols in other regions of North America experiencing earthworm invasion (e.g., the northeastern U.S. and much of Canada), and that is also easily transferrable to multiple user groups (e.g., land managers, biological technicians, researchers, and citizen scientists).

Budgets for management and conservation activities are limited. At the same time, it is becoming increasingly important to clarify earthworm impacts – including impacts to songbirds – and to identify remaining earthworm-free areas in which to target these activities. This dissertation, which investigates impacts of invasive earthworms on ground-dwelling songbirds and also develops a visual method for assessing earthworm invasions, contributes to clarification of earthworm impacts while also providing an efficient method for achieving the above conservation and management objectives.

**CHAPTER 2 - REDUCED DENSITY AND NEST SURVIVAL OF
GROUND-NESTING SONGBIRDS RELATIVE TO EARTHWORM
INVASIONS IN NORTHERN HARDWOOD FORESTS**

European earthworms (*Lumbricus* spp.) are spreading into previously earthworm-free forests in the United States and Canada and causing substantial changes, including homogenization of soil structure, removal of the litter layer, and reduction in arthropod abundance and species richness of understory plants. Whether these changes affect songbirds that nest and forage on the forest floor is unknown. In stands with and without earthworms in the Chequamegon-Nicolet National Forest, Wisconsin (U.S.A.), I surveyed for, monitored nests of, and measured attributes of habitat of Ovenbirds (*Seiurus aurocapillus*) and Hermit Thrushes (*Catharus guttatus*), both ground-dwelling songbirds, and I sampled earthworms at survey points and nests. Bird surveys indicated significantly lower densities of Ovenbirds and Hermit Thrushes in relation to *Lumbricus* invasions at survey point and stand extents (3.1 and 15-20 ha, respectively). Modeling of Ovenbird nest survival (i.e., probability that nestlings successfully fledge) indicated that lower survival probabilities were associated with increased sedge cover and decreased litter depth, factors that are related to *Lumbricus* invasions, possibly due to reduced nest concealment or arthropod abundance. My findings provide compelling evidence that earthworm invasions may be associated with local declines of forest songbird populations.

Introduction

Ecosystem engineers, including non-native invasive species, substantially change the abiotic and biotic structure of ecosystems, affect resource availability for many species, and alter the flow of energy or biomass (Crooks 2002). Clarifying relations between belowground and aboveground biological systems is important for understanding how human-driven changes, such as introduction of invasive species, affect species richness and ecosystem function (Wardle 2004). Earthworms are ecosystem engineers, and non-native earthworm species are established in nearly every ecosystem in the world (Hendrix et al. 2008). Invasion by non-native earthworms is a major emerging issue because these invasive species are likely to substantially affect conservation efforts in North America (Sutherland et al. 2010).

In north-temperate and boreal regions of North America, European earthworms are colonizing forests that were historically free of earthworms (James 2004) and having substantial effects on soil structure and function that affect aboveground biota (Bohlen et al. 2004). Non-native invasive earthworms, particularly those in the genus *Lumbricus*, consume leaf litter and organic layers below the leaf litter, mix soil horizons (Alban and Berry 1994, Hale et al. 2005a), reduce abundance of mycorrhizal fungi (Lawrence et al. 2003) and arthropods (Migge-Kleian et al. 2006), and alter nutrient dynamics (Costello and Lamberti 2008). Changes to the soil reduce cover and diversity of understory plants, increase dominance by sedges and grasses (Hale et al. 2006, Holdsworth et al. 2007a, Nuzzo et al. 2009), and lead to extirpations of plants (Gundale 2002).

The effects of invasive earthworms on vertebrates are poorly known; however, earthworm-caused reductions in litter depth and arthropod abundance may result in declines in abundance of ground-dwelling salamanders (Maerz et al. 2009). In the northeastern and Midwestern United States, several species of songbirds forage and build their nests on the forest floor. Although earthworm invasions may provide a food source for these birds, results of a study of the ground-nesting Ovenbird (*Seiurus aurocapillus*) showed increases in nest predation related to decreases in litter thickness that are thought to have been caused by non-native earthworms (Mattsson and Niemi 2006).

Despite the evidence for cascading effects of invasive earthworms on vertebrates, no explicit investigation of earthworm effects on ground-dwelling birds has been conducted. Ground nests are highly prone to predation (Robinson et al. 1995), and the reduced plant cover and litter depth associated with earthworm invasions may increase predation on ground nests. Additionally, reductions of arthropod abundance due to reduced litter depth may deplete food sources for ground-foraging birds. Thus, birds that inhabit the forest floor may have greater reproductive failure in areas colonized by earthworms.

I investigated relations between invasive earthworms, attributes of the forest floor, and density and nest survival (i.e., probability that nestlings successfully fledge) of 2 ground-nesting and ground-foraging birds, the Ovenbird and Hermit Thrush (*Catharus guttatus*), in hardwood stands of the Chequamegon-Nicolet National Forest in northern Wisconsin (U.S.A.). Although both species are relatively common and widespread, their abundances have recently decreased substantially in parts of the study area (Lind et al.

2006). It has been hypothesized that songbird populations in this primarily forested region are sources of colonists for populations in fragmented woodlands of the southern Midwest (Robinson et al. 1995). My goal was to test whether earthworm-caused changes to the forest floor may contribute to declines of Ovenbirds and Hermit Thrushes in these putative source populations. I assessed densities of Ovenbirds and Hermit Thrushes in earthworm-invaded and earthworm-free forests and investigated whether invasive earthworms and forest-floor attributes (e.g., litter depth and cover of understory plants) were related to Ovenbird nest survival.

Methods

Study Area and Site Selection

My 12 study sites in the Chequamegon-Nicolet National Forest, Wisconsin (46°N, 91°W) were a subset of sites previously sampled for earthworms during a study of the effects of earthworms on plants (Holdsworth et al. 2007a) (Fig. 2.1). The sites were 15-20 ha and at least 2 km from each other (18.7 km apart on average), as measured by distance between site centroids. Sites represented a range of earthworm invasion stages on the basis of biomass data from the earlier study, including earthworm-free ($n=3$; average biomass = 0.05 g/m²), lightly invaded ($n=3$; average biomass = 4.77 g/m²), and heavily invaded ($n=3$; average biomass = 11.03 g/m²) sites and sites containing a leading edge of invasion (i.e., transition from invaded to earthworm-free near middle of site) ($n=3$). All sites were in upland-mesic sugar maple (*Acer saccharum*)-basswood (*Tilia americana*) forests >60 years old, had sandy loam and loamy sand soils, had no timber

removed within the last 40 years, and were adjacent to lakes >4 ha (Holdsworth et al. 2007a). All invaded sites were adjacent to gravel roads that received light automobile traffic. All earthworm-free stands in the study were in remote areas designated as wilderness by the U.S. Forest Service (Holdsworth et al. 2007b); therefore, earthworm-free and leading-edge sites were >1.6 km from the nearest road.

Whereas line transects ranging from 350-500 m in length were used in the original study, I needed 2-dimensional sites centered on transects that were large enough to include several nesting territories. I used Arc Map 9.3 (ESRI 2008) to inspect Landsat images and a spatial land cover layer from the National Land Cover Database (NLCD; Multi-resolution Land Characteristics Consortium 2011), both at 30-m resolution, and to plot the extent of the sugar maple-basswood stand in which each line transect occurred. Plots were then visited to delineate boundaries of sites such that the sites fell entirely within the forest type of the original transect. For the 9 homogeneous sites (i.e., sites that were earthworm-free or invaded, not leading edge sites), I adjusted boundaries to ensure that earthworm invasion status was consistent within each site. I assessed invasion status on the basis of visual estimates of litter depth and cover, sedge and grass cover, and the presence of earthworm castings (C. Hale personal communication).

Bird Point Counts

I established 5 bird-survey points within each site. In the 9 homogenous sites, I randomly located points (100 m in radius [3.1 ha]) on a map. Then I visited point locations and adjusted the location, if necessary, to ensure the entire radius of the point

fell within the forest type and invasion status of the site. Points were spaced evenly throughout each site and point centers were >200 m from each other. In the 3 leading-edge sites, I established 50-m radius (0.8 ha) survey points during site visits; all points were located along transects of the original study. Point centers were >100 m from each other, and each leading-edge site had 2 points in the invaded area, one at the leading edge of invasion, and 2 in the earthworm-free area.

I used the distance method (Buckland et al. 2001) to conduct 10-minute bird surveys. I recorded each Ovenbird and Hermit Thrush that was seen or heard within 50 m and 100 m of point centers in leading-edge and homogeneous sites, respectively, and I estimated distance to each bird. I conducted all surveys between 0.5 and 4 h after sunrise (0530-0900) on relatively calm days with no precipitation. Sites were surveyed 5 times from 1 June-21 July in 2008 and 3 times from 1 June-13 July in 2009, which corresponded to the peak breeding season for both species.

Nest and Vegetation Sampling

I searched for and monitored nests of Ovenbirds in 9 of the 12 sites in 2009 and 2010; the same sub-set of sites was used in both years. Although I also searched for Hermit Thrush nests, I did not find enough to allow for rigorous statistical analysis. I selected sites to maximize logistical efficiency and to represent all invasion categories (3 earthworm-free, 2 heavily invaded, 2 lightly invaded, and 2 leading edge). Trained technicians searched for Ovenbird nests between 0500 and 1400 from 20 May-14 July 2009 and from 17 May-9 July 2010. Nests were monitored every 3-4 days to record the

number of eggs or nestlings present. I monitored nests until they were abandoned, predated, or ≥ 1 nestling had fledged. I classified nests as predated if eggs were destroyed or nestlings were absent before I would have expected them to fledge (i.e., 8 days after hatching [Stodola et al. 2010; S.R.L. unpublished data]). To minimize observer effects and to prevent predators from following human scent trails to nests, nest visits were < 3 minutes and technicians continued past nests in the opposite direction from which they arrived (Martin and Geupel 1993).

I sampled understory vegetation and the litter layer at each nest and at random locations (controls) within 33 m of the nest after nests had ceased to be active in mid-July. I measured litter depth by pushing a metal skewer through the litter until I met resistance from rock or mineral soil. I based average litter-depth calculations on 4 measurements 1 m from the nest or control location in each cardinal direction (Mattsson and Niemi 2006). Within a 2-m square centered on the nest or control location, I estimated percent cover of maple seedlings (< 50 cm tall), all sedges and grasses combined, and all herbaceous and woody plants (< 50 cm tall) combined. Preliminary analyses indicated no significant difference in vegetation between nests and control locations; thus, I assumed nest data were representative of nesting territories.

Earthworm Sampling

I used the hot mustard extraction technique (Lawrence and Bowers 2002) to sample earthworms. I poured a mustard-water mixture (40 g ground yellow mustard dissolved in 4 L water) on the soil surface and collected all emerging earthworms. I

sampled in September, when soil-moisture conditions are favorable for earthworms and when the population contains a high proportion of adults (C. Hale personal communication). In 2008 I sampled earthworms at a subset of bird-survey points to ensure that invasion status was the same as reported by Holdsworth et al. (2007a). I sampled 2 randomly selected survey points at each homogeneous site. In leading edge sites, I randomly selected 3 points, such that samples represented invaded, leading-edge, and earthworm-free portions of the site. For all points, earthworms were sampled from 3 randomly selected 33-cm² plots within 100 m of the center of the point.

Preliminary sampling indicated point-level earthworm biomass was proportional to estimates from the original study. Sites originally classified as earthworm-free were still earthworm-free or were lightly invaded by the small surface-feeding species *Dendrobaena octaedra*. This species is believed to have little effect on soil structure, litter depth, or plant cover in hardwood forests of the region (Hale et al. 2006, 2008); therefore, I still considered these sites earthworm-free. I found no significant difference in total earthworm biomass between lightly invaded and heavily invaded sites, which suggests the lightly invaded sites had become heavily invaded since the original sampling by Holdsworth et al. (2007a) in 2001-2002. Thus, I categorized all these sites as earthworm-invaded. Sampling also revealed that leading edges of invasion had shifted ≤ 40 m; thus, I used these sites in my analysis.

In 2009 and 2010, I sampled earthworms at all nests. In 2009 I sampled 3 sub-plots at one-third ($n=36$) of nests (1 at nest, 2 at random locations ≤ 33 m from nest) and 1 sub-plot at the nest for the remaining two-thirds ($n=76$) of nests because a short period of

favorable sampling conditions prevented us from sampling 3 sub-plots at all nests. Preliminary analyses indicated biomass of different earthworm species did not differ significantly for nests at which 3 versus 1 sub-plot had been sampled; therefore, I sampled a single sub-plot at the nest for all nests in 2010.

I used 70% isopropyl alcohol to preserve earthworms in the field and buffered 10% formalin for long-term storage of earthworms . I used a dissecting microscope to count, identify, and measure the length of earthworms. Adult earthworms were identified to species, whereas most juveniles were identified to genus. I used length measurements and regression equations from allometric relations to estimate biomass (Hale et al. 2004).

Data Analyses

I used distance estimates from point counts and program Distance 6.0 (Thomas et al. 2010) to calculate species-specific detection probabilities and to estimate densities of Ovenbirds and Hermit Thrushes. I used repeated-measures analyses of variance (ANOVA) to compare site-level densities of birds between earthworm-free and invaded sites (the between-subjects factor), which accounted for lack of independence between multiple counts conducted at each point. I used linear regression analyses to assess the relation between biomass of *Lumbricus* spp. and density of each bird species at the point level for homogeneous and leading-edge sites. Because I found a significant difference in Ovenbird and Hermit Thrush density between years in a preliminary analysis and because I wanted to assess how density varied between years, I analyzed density for both species separately for 2008 and 2009.

I used the nest-survival application in program MARK (Dinsmore and Dinsmore 2007) to model the relation between Ovenbird nest survival and nest-site habitat and earthworm invasion variables. This program uses a mark-recapture framework for modeling daily survival rates (DSR) on the basis of a maximum likelihood approach and allows for inclusion of covariates and comparison of a priori candidate models on the basis of Akaike's information criterion corrected for small sample sizes (AIC_c) (Burnham and Anderson 2002).

To test whether Ovenbird DSR varied over the nesting season, I developed 4 temporal models: time-constant DSR, linear increase in DSR as nesting date increased, variation in DSR between 10-day intervals of the nesting season, and variation in DSR between each third (23-day intervals) of the nesting season. The time-constant DSR model was best supported by the data ($\Delta AIC_c = 0.00$, AIC weight [ω_i] = 0.52; Deviance = 270.91); therefore, models used for the remainder of analyses included no temporal variation in DSR.

I included 5 vegetation and 3 earthworm-invasion variables in the nest-survival analysis, which I based on previously published relations and my research objectives. I used Pearson's correlation to assess multicollinearity among variables. I retained 1 variable from highly correlated variable pairs ($r > 0.4$) and thus had 6 candidate variables (Table 2.1).

I ran all single-variable DSR models, an intercept-only (null) model, and a global model containing all 6 variables. Single-variable models with ΔAIC_c values between 0 and 2 and values at least 2 lower than the null model were considered strongly supported.

I combined variables from strongly supported models to produce all possible 2-variable and 3-variable additive models. I based final assessment of covariate importance on the difference in AIC_c values between the top model and each model of interest (ΔAIC_c) and ranking on the relative strength of support for the models (AIC weight – ω_i). I identified variables with strong support (appearing in >1 model with ΔAIC_c between 0 and 2 and single-variable ΔAIC_c between 0 and 2), moderate support (appearing in >1 model with ΔAIC_c between 0 and 2 and single-variable ΔAIC_c between 2 and 4), or weak support (not meeting criteria for strong or moderate support). I conducted univariate linear regression to assess whether there was a relation between *Lumbricus* spp. biomass and vegetation variables that received strong or moderate support in the above analysis. Because normality assumptions were met, transformation of dependent variables was unnecessary.

Results

Ovenbird and Hermit Thrush Density

I observed Ovenbirds and Hermit Thrushes at all sites and across all portions of the leading edges. Nevertheless, most analyses indicated lower density of Ovenbirds and Hermit Thrushes at areas with earthworm invasion. Densities of Ovenbirds (2008 – $F=12.12$, $df=1$, $p=0.01$; 2009 – $F=19.82$, $df=1$, $p<0.01$) and Hermit Thrushes (2008 – $F=6.48$, $df=1$, $p=0.04$; 2009 – $F=9.00$, $df=1$, $p=0.02$) were significantly lower in earthworm-invaded sites than in earthworm-free sites (Fig. 2.2). In homogeneous sites

there was a significant inverse linear relation between *Lumbricus* spp. biomass and Ovenbird density in 2008 and Hermit Thrush density in 2009 at the point level (Fig. 2.3). The inverse relation between *Lumbricus* spp. biomass and Ovenbird density in 2009 increased in strength after removal of an outlier ($r^2=0.48$, $p<0.01$). In leading-edge sites *Lumbricus* biomass and Ovenbird density in 2008 ($r^2=0.37$, $p=0.08$) and 2009 ($r^2=0.40$, $p=0.07$) exhibited an inverse relation at the point extent, although the relation was not significant. *Lumbricus* biomass and Hermit Thrush density across leading edges were not significantly related in either 2008 ($r^2=0.09$, $p=0.43$) or 2009 ($r^2=0.03$, $p=0.65$).

Ovenbird Nest Survival

I found 181 Ovenbird nests during 2009 and 2010. I removed abandoned nests from analysis to focus inference on predation-induced mortality; therefore, I based the nest-survival analysis on a sample of 165 Ovenbird nests. There were more Ovenbird nests in earthworm-free sites than in earthworm-invaded sites. However, after correcting for search effort, the difference was not statistically significant (independent samples t test; $t=2.07$, $df=5$, $p=0.06$).

Presence of *Lumbricus* spp. was not associated directly with Ovenbird DSR; the single-variable *Lumbricus* model had weak support (Table 2.2). However, inclusion in the Ovenbird DSR models of vegetation features known to be influenced by *Lumbricus* spp. was supported. For example, sedge and grass cover was the most strongly supported single variable in the analysis, included in the top 4 models, and displaying an inverse relation with DSR ($\beta = -0.24$ [95% CI ± 0.18]; Fig. 2.4a). Year effect was strong; year

was included in the top 2 models. The year-only model also had strong support (Table 2.2), and Ovenbird DSR was lower in 2009 than 2010 ($\beta = -0.78$ [95% CI ± 0.61]). Litter depth was included in 2 of the strongly supported models and was positively related to Ovenbird DSR ($\beta = 0.16$ [95% CI ± 0.14]; Fig 4b); however, as evidenced by moderate support for the litter depth-only model, overall support for this variable was equivocal.

There was a significant association between *Lumbricus* spp. and the 2 variables with strong or moderate association with Ovenbird DSR. There was a significant positive relation between *Lumbricus* biomass and sedge cover ($r^2 = 0.04$, $\beta = 0.83$, $p = 0.01$), and *Lumbricus* biomass and average litter depth exhibited a significant inverse relation ($r^2 = 0.24$, $\beta = -3.04$, $p < 0.01$).

Discussion

Earthworm Invasion and Songbird Density

I found strong evidence that the presence of invasive earthworms on forest floors in northern hardwood forests affects populations of ground-dwelling songbirds. In general, Ovenbird density increased when progressing from earthworm-invaded portions of sites across the invasion fronts to earthworm-free areas. I found no evidence for a similar fine-resolution pattern with Hermit Thrushes. A potential explanation for the species-specific association with patterns of *Lumbricus* invasion across leading edges is the difference in territory size of Ovenbirds and Hermit Thrushes. In general, Ovenbird territories are smaller (0.26 ha on average) (Smith and Shugart 1987) than Hermit Thrush territories (0.76 ha on average) (Martin 1960). I often found Ovenbird nests <25 m apart,

whereas Hermit Thrush nests were typically spaced >100 m apart (S.R.L. personal observation). Leading edges of invasion are often sharp, with transition from heavily invaded to earthworm-free areas sometimes occurring in ≤ 75 m (Hale et al. 2006). My observation that Ovenbird density varied across leading edges therefore suggests that spatial distribution of Ovenbird territories is close enough to respond to fine-grained earthworm invasion patterns. Hermit Thrush territories are likely too large to result in observed variation in density in association with fine-grained invasion patterns. However, across study sites, fine-grained variation in earthworm invasion appeared to be associated with an overall decrease in Hermit Thrush density.

The site-selection method I used, including controlling for soil type, forest type and age, and history of timber removal, reduced the probability that observed differences in density were caused by factors other than *Lumbricus* invasion. Furthermore, because the study area was located in a primarily forested landscape and sites were far from clearcuts or other forest openings, density of birds was not likely influenced by fragmentation or edge effects, factors known to affect demographics of forest birds, including Ovenbirds (Flaspohler et al. 2001, Lampila et al. 2005). A potential confounding variable was the presence of roads near earthworm-invaded sites but not near earthworm-free sites. Ovenbird habitat quality may be reduced within 150 m of roads within forests (Ortega and Capen 1999). In my study, it is unlikely that the proximity of earthworm-invaded sites to roads was responsible for density differences because most or all portions of invaded sites were >150 m from roads, and all roads <150 m from invaded sites were gravel roads ≤ 8 m across. Thus, roads likely had relatively

small effects (Haskell 2000). In addition, the inverse relation between Ovenbird density and *Lumbricus* biomass was noticeable, although not statistically significant, at leading edge sites, which were all >1.6 km from roads.

Earthworm Invasion and Nest Survival

Results from the nest-survival analyses suggest *Lumbricus* spp. did not directly affect Ovenbird DSR. Nevertheless, because the biomass of *Lumbricus* was associated with sedge cover and litter depth, variables supported in the nest-survival analyses, my findings suggest earthworms indirectly increase rates of Ovenbird nest mortality by increasing dominance of sedges and grasses, and, to a lesser extent, by decreasing litter depth. As evidenced by strong support for the year variable, there also exists substantial unexplained variation in Ovenbird survival. Mechanisms responsible for the above relations remain unknown.

Although the differences in modeled Ovenbird daily survival rates between the upper and lower observed values of the supported variables may appear to be of little biological significance, small differences in DSR translate to substantial disparities in the probability of nest survival across the entire 20-day nesting period of Ovenbirds. For example, at 0% sedge cover, DSR of 0.98 was associated with 74% success probability, whereas at 100% sedge cover, DSR of 0.95 was associated with 37% success probability. Likewise, the upper and lower observed litter depths corresponded to divergent nest-survival probabilities (for 9.7 cm and 0 cm litter depth, survival probability = 84% and 45% respectively). These results indicate habitat changes that are strongly associated

with earthworm invasions have biologically significant effects on Ovenbird nest survival. Not only were fewer Ovenbirds present in earthworm-invaded forests, but the birds that built nests in locations with high sedge cover and thin leaf litter – habitat attributes that are associated with earthworm invasions – appeared to have lower probability of nest survival.

Plant communities on the forest floor in heavily invaded portions of the study region were often characterized by extensive mats of sedges and grasses (dominated by Pennsylvania sedge [*Carex pennsylvanica*]). This species may thrive in response to earthworm invasion because it has a competitive advantage in the absence of other plants, tolerates disturbed soil (Powers and Nagel 2008), and does not depend on mycorrhizal fungi (Brundrett and Kendrick 1998). Nest concealment is an oft-cited driver of songbird nest predation (e.g., Martin and Roper 1988), although results of some studies suggest concealment of Ovenbird nests may be unimportant (King et al. 1998; Flaspohler et al. 2000). In my study, concealment of Ovenbird nests appeared to decrease as sedge cover increased. Because Ovenbird nests are composed primarily of fallen leaves, leaf stems, and dead or decaying sedges and grasses, they often stand out from the primarily green sedge mats (S.R.L. personal observation). Furthermore, sedge mats are characterized by a single homogeneous layer (~15-25 cm height) that provides limited cover for nests. The more diverse ground-layer plant communities characteristic of earthworm-free forests provide multilayered cover from herbs and tree seedlings of various heights. Nests placed in sedge-dominated areas may therefore be less concealed from visual-hunting predators.

My finding of a highly significant inverse relation between biomass of *Lumbricus* and litter depth, the substantial difference in nest-survival probability between locations with thin and thick litter, and the fact that results of previous studies show a positive relation between litter depth and Ovenbird nest survival (Mattsson and Niemi 2006) and pairing success (Van Horn 1990) all suggest that litter depth has a biologically significant effect on Ovenbird nest survival in my study area. Decreased litter depth can result in reduced arthropod abundance (Haskell 2000) and subsequently in reduced food availability for ground-foraging songbirds. Lower food availability can lead to increased time spent foraging and reduced nest attentiveness (Zanette et al. 2003, Pearse et al. 2004). Litter depth and food availability may interact with predator abundance and affect rates of nest survival (Zanette et al. 2003).

Ovenbirds, Hermit Thrushes, and other ground-dwelling bird species have decreased in abundance in national forests of the U.S. northern Midwest in recent decades (Lind et al. 2006). I found strong evidence that the effects of invasive earthworms, particularly those in the genus *Lumbricus* are associated with decreased abundance of Ovenbirds and Hermit Thrushes at extents of 3.1 and 15-20 ha. It remains unclear, however, whether earthworm-driven habitat changes are the primary driver of regional declines across entire national forests that are larger than 5000 km².

There are currently no cost-effective, logistically feasible, and widely effective methods for removing invasive earthworms from forests. Large portions of northern forests are already invaded (Holdsworth et al. 2007b) or likely to be invaded (Gundale et al. 2005). Prevention of the spread of earthworms remains the most effective approach to

preserving remaining earthworm-free areas, especially given the slow natural rate of earthworm colonization (Marinissen and van den Bosch 1992). Even in forests invaded by 1 or 2 earthworm species, preventing introduction of the full complement of earthworm species may avert further changes to the forest floor (Hale et al. 2005a, 2008) and therefore limit earthworm effects on populations of ground-dwelling songbirds.

Table 2.1. Candidate variables and justification for inclusion in analysis of factors influencing Ovenbird nest survival (i.e., probability that nestlings successfully fledge) in Chequamegon-Nicolet National Forest (Wisconsin, U.S.A.), 2009-2010.

Candidate Variables	Justification for Inclusion ^a
Year	annual variation in nest survival due to factors not accounted for by other variables
Sedge	earthworms increase sedge and grass cover (1-3), possibly influencing nest concealment
Maple	earthworms decrease cover of maple seedlings (2,4), possibly influencing nest concealment
Total ground cover ^b	earthworms decrease total cover of ground layer plants (1), possibly influencing nest concealment
Litter depth	earthworms decrease depth of the litter layer (1-3, 5,6), possibly reducing nest concealment or reducing abundance or diversity of arthropod food supplies (7,8)
<i>Lumbricus rubellus</i> biomass	<i>Lumbricus rubellus</i> strongly affects soil, litter layer, and understory plants (1,2,9)
Total <i>Lumbricus</i> biomass	<i>Lumbricus</i> spp. strongly affect soil, litter layer, and understory plants (3,10)
Total earthworm biomass ^c	together all earthworm species have greater effects on soil or forest-floor vegetation than subset of species (2,11)

^a Numbers refer to citations in support of justification: (1) Hale et al. 2006, (2) Hale et al. 2008, (3) Holdsworth et al. 2007a, (4) Lawrence et al. 2003, (5) Maerz et al. 2009, (6) Nuzzo et al. 2009, (7) Mattsson and Niemi 2006, (8) Haskell 2000, (9) Gundale 2002, (10) Gundale et al. 2005, (11) Hale et al. 2005b.

^b Removed from final analysis due to strong correlation with variable maple.

^c Removed from final analysis due to strong correlation with variable total *Lumbricus* biomass.

Table 2.2. Results of model selection for Ovenbird daily nest survival analysis derived from Akaike’s information criteria corrected for small samples (AIC_c).

Model ^a	K^b	ΔAIC_c^c	w_i^d	Deviance ^e
Sedge + year	3	0.00	0.296	376.17
Sedge + year + litter depth	4	1.29	0.155	375.45
Sedge	2	1.76	0.123	379.93
Sedge + litter depth	3	1.81	0.120	377.97
Year	2	1.84	0.118	380.01
Year + litter depth	3	2.11	0.103	378.27
Litter depth	2	3.94	0.041	382.11
Maple	2	5.89	0.016	384.06
Global model (6 variables)	7	6.18	0.013	374.30
Null model	1	6.80	0.010	386.97
<i>Lumbricus rubellus</i> mass	2	8.40	0.004	386.57
Total <i>Lumbricus</i> mass	2	8.48	0.004	386.66

^aCovariates included in each candidate model.

^bNumber of model parameters (including intercept).

^cDifference in AIC_c value between model and the most strongly supported model.

^dThe AIC weight (i.e., relative strength of support for model).

^eModel deviance.

Figure 2.1. Location of the study area in the United States (left), and locations of study sites (squares) within the Chequamegon-Nicolet National Forest (right), Wisconsin (U.S.A.).

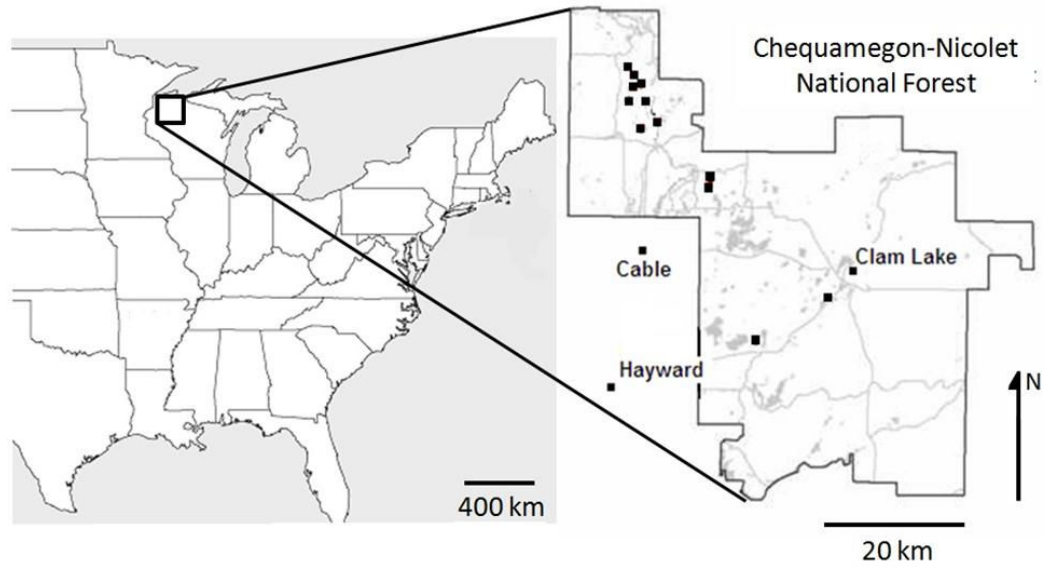


Figure 2.2. Mean (SE) Ovenbird (a, b) and Hermit Thrush (c, d) density in earthworm-free and earthworm-invaded sites in 2008-2009. Units on y-axes are different for each species. Results are from repeated measures analysis of variance.

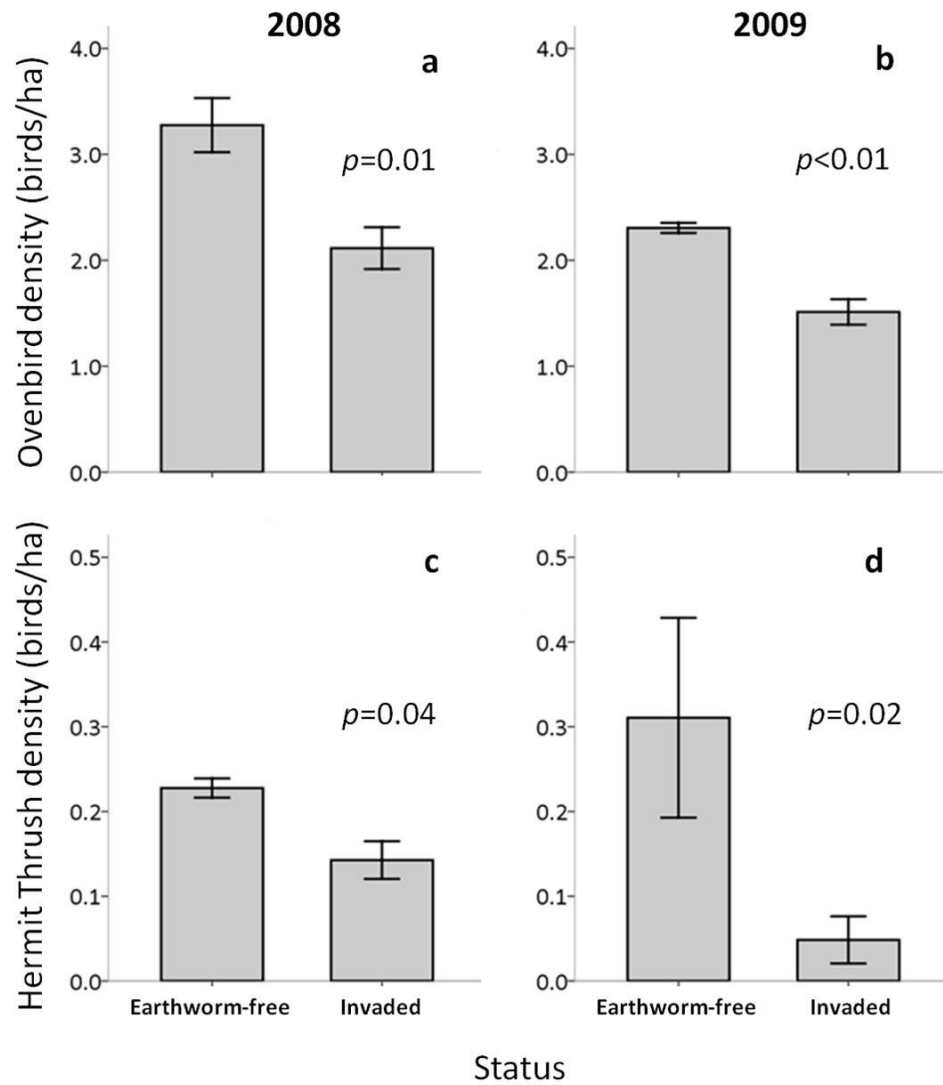


Figure 2.3. Relation between *Lumbricus* spp. biomass and density of Ovenbirds and Hermit Thrushes at the level of survey points (100 m radius) within study sites that were either earthworm-free or earthworm invaded in 2008-2009. With an outlier removed, results for the upper-right graph are statistically significant ($r^2=0.48$, $p<0.01$).

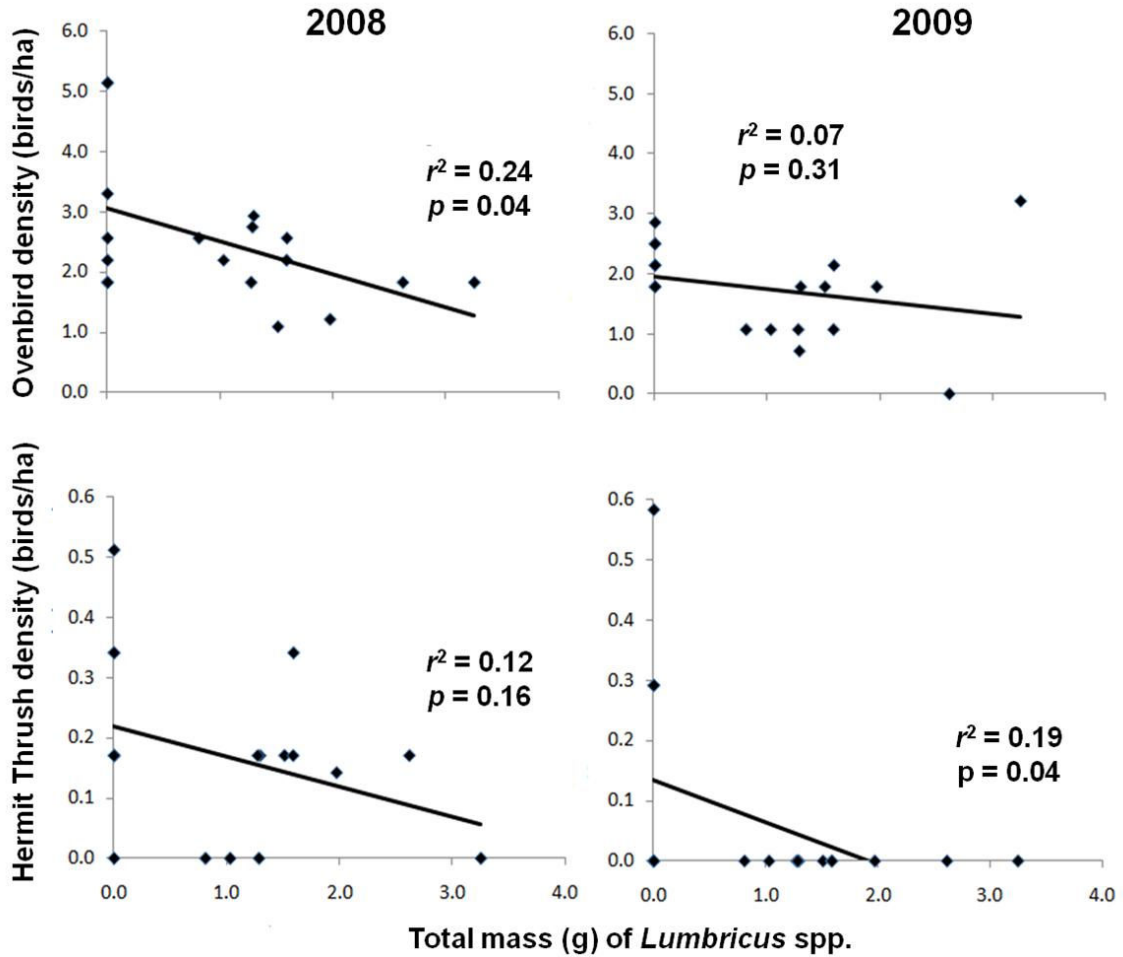
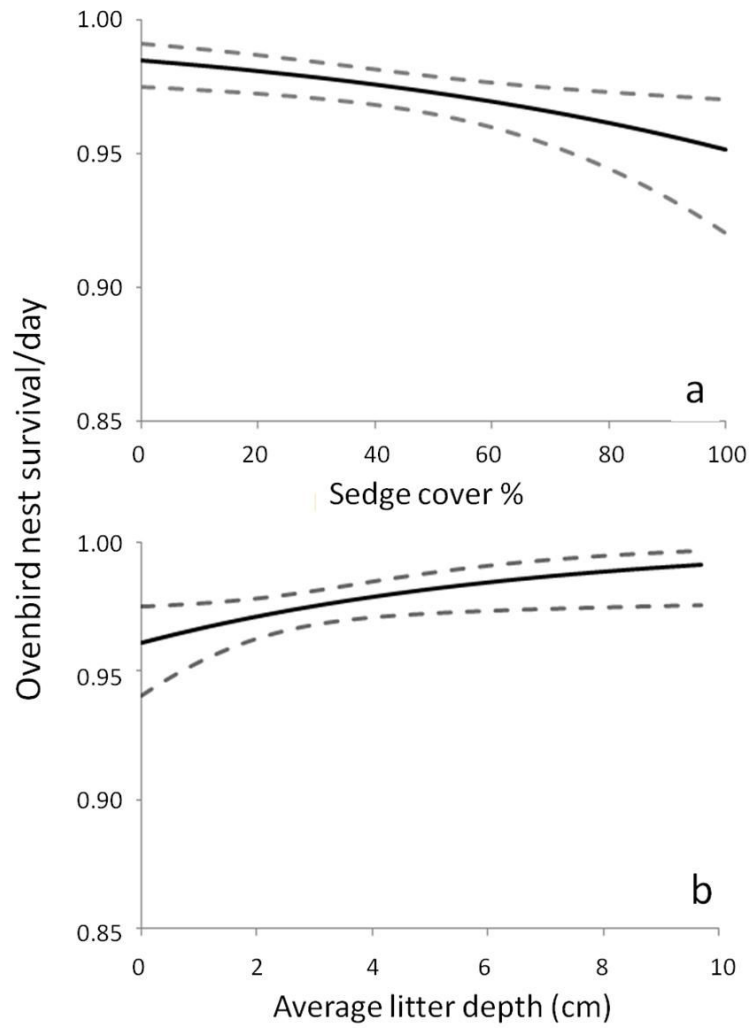


Figure 2.4. Relation between daily nest survival of Ovenbirds and observed values of percent cover of sedge and grass (a) and average litter depth (b) in 2009-2010 (dashed lines, 95% confidence intervals).



CHAPTER 3 - INVASIONS OF NON-NATIVE EARTHWORMS RELATED TO POPULATION DECLINES OF GROUND-NESTING SONGBIRDS ACROSS A REGIONAL EXTENT

Non-native invasive earthworms in the genus *Lumbricus* are causing ecosystem-level changes to previously earthworm-free hardwood forests in North America, including removal of the litter layer and reduction of understory plant richness. These changes have been associated with local population declines of ground-nesting songbirds; however, it is unclear whether earthworms pose a substantial threat to the conservation of songbirds across a regional extent. I investigated relations between *Lumbricus* biomass, point and landscape-scale habitat characteristics, and populations of 4 ground-nesting songbird species at survey points scattered across the Chequamegon-Nicolet (Wisconsin) and Chippewa (Minnesota) National Forests, U.S.A. Of the 4 focal species, the Ovenbird (*Seiurus aurocapilla*) was the only one to display significantly reduced density at *Lumbricus*-invaded points compared to *Lumbricus*-free points; however the effect was only observed in sugar maple (*Acer saccharum*)-basswood (*Tilia americana*) forest. Density of the Hermit Thrush (*Catharus guttatus*) was lower at *Lumbricus*-invaded points; however, the difference was not statistically significant. Among the 13 variables assessed, *Lumbricus* biomass was the variable most strongly related to Ovenbird density, displaying a strong inverse relation in sugar maple-basswood forest. Point and landscape-scale habitat variables received little support as predictors of Ovenbird density in these forests. My results support previous evidence suggesting that earthworm

invasions pose a potential region-wide threat to the conservation of the Ovenbird, and to a lesser extent, the Hermit Thrush, in hardwood forests of the U.S. northern Midwest.

Introduction

Invasions of non-native species are a leading threat to the persistence of native species and to the functioning of ecosystems (Wilcove et al. 1998, Mack et al. 2000). Full clarification of the impacts of invasives to populations and ecosystems requires research to be scaled-up from local sites to regional and continental extents (Crowl et al. 2008). Birds are particularly vulnerable to invasives, with an estimated 68% of population declines of endangered North American species at least partially attributed to invasive species (Gurevitch and Padilla 2004). Regional and continental declines of bird populations have been linked to invasive diseases (Hochachka and Dhondt 2000, LaDeau et al. 2007); however, studies documenting bird responses to invasive invertebrates (Thurber et al. 1994, Allen 1995), plants (Nordby et al. 2009, Rodewald et al. 2010), and predators (Courchamp et al. 2003, van Heezik et al. 2010) are typically limited in spatial extent to local study areas covering <100,000 ha (1,000 km²).

In North America, invasive European earthworms are causing substantial ecosystem-level changes to historically earthworm-free north-temperate and boreal forests (Bohlen et al. 2004) to the extent that earthworm invasions have been recently identified among the top emerging concerns for the conservation of biodiversity (Sutherland et al. 2010). Invasive earthworms, primarily those in the genus *Lumbricus*, alter soil structure and consume the leaf litter layer (Alban and Barry 1994, Hale et al.

2005a, Costello and Lamberti 2008), thus reducing richness of understory plants (Hale et al. 2006, Holdsworth et al. 2007a) and regeneration and growth of trees (Lawrence et al. 2003, Larson et al. 2010). A recent study in the northern U.S. further found that earthworm invasions are related to reduced density of two ground-nesting songbirds and that decline of the Ovenbird (*Seiurus aurocapilla*) is related to deterioration of forest floor habitat by earthworms, which in turn reduces nest concealment and increases predation rates (Loss and Blair 2011; see also chapter 2).

Regional-scale studies illustrate that earthworm invasions are widespread in hardwood forests of the north-central U.S., with most of the landscape invaded or susceptible to invasion, even in large unbroken tracts of protected land such as national forests (Gundale et al. 2005, Holdsworth et al. 2007b). However, it is unclear whether the adverse effect of earthworms to songbirds exists across this entire region. Songbird populations in the primarily forested U.S. northern Midwest are thought to be sources of colonists for smaller populations in fragmented woodlands of the central and southern Midwest (Robinson et al. 1995). It is important to determine the regional extent of the earthworm effect on songbirds because population declines in the putative source populations of the northern Midwest could lead to larger scale population declines across the entire mid-continent (Temple and Cary 1988, Donovan et al. 1995).

I investigated relations between *Lumbricus* invasions, point and landscape-scale habitat characteristics, and populations of ground-nesting songbirds, at bird survey points scattered across two national forests that cover >1.2 million ha, the Chequamegon-Nicolet National Forest in Wisconsin and the Chippewa National Forest in Minnesota,

U.S.A. My primary goal was to determine whether invasive earthworms pose a region-wide threat to the conservation of ground-nesting forest songbirds in the northern Midwest. To achieve this goal I identified the following objectives: (1) compare densities of four ground-dwelling species – the Ovenbird, Hermit Thrush (*Catharus guttatus*), Veery (*Catharus fuscescens*), and Black-and-white Warbler (*Mniotilta varia*) – between stands that were invaded by *Lumbricus* spp. and stands that were either completely earthworm-free or only contained *Dendrobaena octaedra*, a species known to have minimal effect on soil structure, litter depth, and plant cover in forests of the region (Hale et al. 2006, 2008); and (2) clarify the relative contribution of *Lumbricus* invasions compared to point and landscape-scale habitat characteristics for determining density of the Ovenbird, the most common ground-nesting songbird in the region. The impetus for this study was to further understand the scope of the impact of an invasive ecosystem engineer and to inform songbird conservation and management to prevent and mitigate earthworm effects.

Methods

Study Area and Survey Points

The study was conducted at survey points that are part of the Forest Birds of the Western Great Lakes monitoring program (Hanowski et al. 2005, Etterson et al. 2009). All points used for the current study were located in the Chippewa National Forest in north-central Minnesota (47°N, 94°W) and the Chequamegon unit of the Chequamegon-Nicolet National Forest in northern Wisconsin (46°N, 91°W; Fig. 3.1). From an initial

set of 283 survey points that were previously classified as hardwood or mixed hardwood-coniferous forest types, I randomly selected a sub-set of 124 points for this analysis, with roughly half of the points in the Chippewa ($n=59$) and half in the Chequamegon-Nicolet ($n=65$) National Forest. I stratified selection by forest district to ensure that points were spatially scattered across both forests. I also inspected maps of point locations to ensure that points were located varying distances from roadways, because this factor has been shown to provide a reliable first-order predictor of earthworm invasion in the region (Holdsworth et al. 2007b).

Habitat at the points was characterized by varying species and age of canopy trees and different histories of timber management. Hardwood forest types – as classified by dominant canopy tree species – included sugar maple (*Acer saccharum*), sugar maple-basswood (*Tilia americana*), northern red oak (*Quercus rubra*), quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), aspen/paper birch, and ash/elm/red maple (*Fraxinus* spp./*Ulmus* spp./*A. rubrum*). The single mixed hardwood-coniferous forest type was aspen/paper birch/fir (*Abies balsamea*). The above point selection method was designed to provide broad spatial coverage of both national forests and to capture earthworm-free and earthworm-invaded sites.

Bird Point Surveys

Bird surveys were conducted at all 124 points, with the vast majority of points ($n=110$) surveyed once in both 2009 and 2010. However, some points were sampled only once in 2009 ($n=13$) or 2010 ($n=1$), due to logistical constraints and/or prolonged

inclement weather. All surveys were conducted between 1 June-4 July in both years. The minimum distance between points was 220 m; however, because the sub-set of points used for my study was selected to be widely scattered, most points were >0.5 km apart. Because of this large distance between points, and because bird surveyors were trained to avoid double-counting, it is unlikely that individual birds were counted at more than one point.

I coordinated 10-minute bird point surveys according to previously published methods (Howe et al. 1998). Extensively trained bird surveyors (see Hanowski and Niemi 1995 for training procedure) recorded each Ovenbird, Hermit Thrush, Veery, and Black-and-white Warbler that was seen or heard within 10, 25, 50, and 100 m distance bands. All surveys were conducted between 0.5 h before sunrise and 4 h after sunrise (0445–930 local time) on relatively calm days (winds <19 kph) with little or no precipitation. The field crew consisted of 4 surveyors in 2009 and 6 surveyors in 2010 with 2 members conducting surveys in both years. To minimize observer effects within forest types and for individual points, each surveyor sampled the same number of points within each forest type, and no point was surveyed by the same person in subsequent years (Hanowski and Niemi 1995).

Earthworm Sampling

Trained earthworm-sampling technicians collected earthworms at all survey points between 15 September–10 October of 2009 ($n=59$) or 2010 ($n=65$), with roughly half of each year's samples collected in each forest. Sampling dates correspond to a

period of soil moisture conditions favorable for earthworms and in which the population contains a high proportion of adults. Sampling earthworms during bird surveys was not possible due to time constraints related to fieldwork. Moreover, because low soil moisture can result in reduced activity for some earthworm species (C. Hale personal communication), sampling during the relatively dry summer months could have resulted in inaccurate population estimates.

I used the “hot mustard” extraction technique (Lawrence and Bowers 2002) to collect earthworms from three 33 x 33 cm sub-plots randomly located within 100 m of each bird survey point, a radius corresponding to the outermost distance band for bird survey data. I cleared all vegetation and leaf litter from sub-plots, poured a mustard-water mixture (40 g ground yellow mustard dissolved in 4 L water) on the soil surface, and collected all earthworms that surfaced until no earthworms emerged for 2 minutes. I preserved earthworms in the field using 70% isopropyl alcohol, and I stored them in buffered 10% formalin. I used a dissecting microscope to count, identify, and measure length of earthworms. I identified adult earthworms to species, except for *Aporrectodea* spp., which I identified to genus, owing to morphological similarities (Hale 2007). Most juvenile earthworms were only identifiable to genus. I used length measurements and published allometric relationships to estimate earthworm biomass (Hale et al. 2004). I averaged biomass across sub-plots for each survey point.

Quantification of Habitat at Survey Points

Due to the large number of survey points (>1,200) making up Forest Birds of the Western Great Lakes monitoring program and the significant effort required to collect vegetation data at all points, habitat quantification occurs every 3-4 years, and immediately before or after bird surveys are conducted. Thus, for most habitat variables (see exceptions below), the vegetation data I used were collected during 2005, 2006, or 2007. Nonetheless, bird surveyors noted points that had experienced recent timber harvest, had openings created by natural disturbances, or had changed forest type designation. I was able to exclude such points from my analysis; therefore, habitat changes were likely minimal between the 2005-07 vegetation surveys and 2009-2010 bird and earthworm surveys.

Within a 100 m radius centered on each point, I used ocular estimation to quantify (to the nearest 10%) total high canopy cover, understory canopy cover (all vegetation 0.9-3.7 m in height), and ground cover (all vegetation <0.9 m in height). Within a 10 m radius centered on each point, I also used ocular estimation to quantify shrub density as the number of woody plants with diameter at breast height <2.5 cm. In 2009 and 2010, I collected two additional measurements that have been documented to differ significantly between earthworm-invaded and earthworm-free forests (Hale et al. 2006, Holdsworth et al. 2007a) and have been suggested to affect abundance and/or nesting success of the Ovenbird and Hermit Thrush (Loss and Blair 2011, see also chapter 2). These measurements included litter depth and ocular estimation of percent cover of sedges and grasses within 100 m of the point. I based the average litter depth calculation on

measurements from two random locations within 10 m of the point and estimated by pushing a metal skewer through the litter until meeting resistance from rock or mineral soil.

Quantification of Landscape Habitat

I quantified habitat in the landscape surrounding bird survey points using ArcMap (version 9.3; ESRI 2008). The seven landscape variables I selected for my analysis included distance to forest edge, distance to water, and percentage of core (i.e., interior) hardwood and mixed hardwood/coniferous forest in five concentric rings centered on each point. These variables have been suggested to influence density, nesting success, pairing success, and/or territory size of ground-nesting forest songbirds, and of the Ovenbird in particular. For example, the Ovenbird is known to require large tracts of forest for successful reproduction (Van Horn and Donovan 1994) and to experience increased nest failure near forest edges (i.e., an “edge effect”), even in relatively unfragmented forests (reviewed by Manolis et al. 2000).

I analyzed land cover for both national forests using the National Land Cover Database (NLCD; Multi-resolution Land Characteristics Consortium 2011) which provides data at 30-m resolution. I constructed five circular buffers around each survey point (radius = 100 m, 200 m, 500 m, 1,000 m, and 2,000 m) and within each buffer I estimated the percentage of “core” forest (i.e., forest >100 m from open land). For this calculation, I only considered upland hardwood and mixed hardwood/coniferous forest types (NLCD cover types 41 and 43, respectively), because the Ovenbird does not

commonly occur in lowland forests. I defined open habitat to consist of all non-forested cover types, including open water, wetland, grassland, shrub land, and barren, agricultural, and other human-developed areas. I also calculated average distance of survey points to open water and to nearest opening (i.e. forest edge). For the distance to edge calculation, I defined open habitat to consist of the same cover types used for the core forest calculation, including water. The distance variables were calculated as an average value for the 100 m radius circle surrounding survey points.

Data Analysis

I used the distance bands associated with each bird observation to develop species-specific detectability functions and to estimate density for each species using program Distance 6.0 (Thomas et al. 2010). Bird survey data was analyzed separately for 2009 and 2010; however, I focused the remainder of analysis on the average density from 2009 and 2010 surveys.

I used independent samples *t*-tests to compare density of each bird species between sites that were invaded by *Lumbricus* and those that were *Lumbricus*-free, because earthworms in this genus are known to have the most severe impacts to forests in the region (Gundale et al. 2005, Hale et al. 2006, Holdsworth et al. 2007a) and to populations of ground-nesting songbirds at forest stand scales (Loss and Blair 2011, see also chapter 2). I repeated these analyses for: (1) all hardwood and mixed hardwood-deciduous points combined ($n=124$), and (2) points located in sugar maple and sugar maple-basswood forests (hereafter; maple-basswood; $n=53$), because earthworm effects

in the study region have been documented to be most significant in these forest types (Hale et al. 2005a; 2006, Holdsworth et al. 2007a, b).

I analyzed the relative contribution of *Lumbricus* invasion (biomass) and point-scale and landscape-scale habitat characteristics for determining density of the Ovenbird. I focused on the Ovenbird because this was the most commonly recorded species during my bird surveys, and because it is the most common and widespread ground-nesting songbird throughout much of the midwestern and northeastern U.S. The 14 candidate variables (*Lumbricus* biomass, 6 point-scale and 7 landscape-scale variables) were selected based on previously published relations and the questions of the current study. I used Pearson's correlation to identify pairs of highly correlated variables ($r > 0.4$). To reduce multicollinearity within candidate models, I removed one variable from highly correlated variable pairs, resulting in a final set of 13 variables (see Table 3.1 for final list of variables and justification for selection).

I conducted univariate linear regression for each variable with average Ovenbird density as the dependent variable. I also constructed an intercept-only (null) model and a multiple regression model containing all 13 variables (i.e. global model). I compared these models using Akaike's Information Criteria corrected for small sample sizes (AIC_c ; Burnham and Anderson 2002). I considered single-variable models to receive initial support if they had ΔAIC_c values between 0–2 and ΔAIC_c values at least 2 lower than the null model (Loss and Blair 2011). Variables meeting these initial criteria were combined in 2-variable additive models. Final assessment of support for variables was based on the complete set of 1-variable and 2-variable models, the difference in AIC_c

values between the top model and each model of interest (ΔAIC_c), and the relative strength of support for each model (AIC weight – ω_i). I identified variables receiving strong support (appearing in >1 model with ΔAIC_c between 0 and 2 and single-variable ΔAIC_c between 0 and 2), moderate support (appearing in >1 model with ΔAIC_c between 0 and 2 and single-variable ΔAIC_c between 2 and 4), or weak support (not meeting criteria for strong or moderate support) (Loss and Blair 2011). For strongly supported variables, I calculated model-averaged β -coefficients by weighing coefficient estimates from each model containing a variable by the AIC weight for that model (Burnham and Anderson 2002). All AIC_c analyses were conducted for: 1) all 124 survey points, and 2) the sub-set of 53 maple-basswood points.

Results

Comparison of Bird Density Between Lumbricus-invaded and Lumbricus-free Points

Of the 124 survey points sampled for earthworms, I collected *Lumbricus* from 69 (56%) points. Of the 53 maple-basswood points, I collected *Lumbricus* from 35 (65%) points. Average *Lumbricus* biomass was variable (0–1.38 g/sub-plot). I therefore concluded that my point selection approach resulted in samples of both *Lumbricus*-free and *Lumbricus*-invaded points.

Bird surveys indicated that the Ovenbird remains widespread and relatively common across the study region, with all 124 points having at least one individual recorded in either 2009 or 2010. When considering all survey points, there was no

difference in Ovenbird density between *Lumbricus*-invaded points and *Lumbricus*-free points (independent samples *t*-test; $t=-0.42$, $df=122$, $p=0.69$). However, when analyzing maple-basswood points, I found significantly lower density of the Ovenbird at *Lumbricus*-invaded points compared to *Lumbricus*-free points ($t=2.03$, $df=51$, $p=0.05$; Fig. 3.2a).

The other three ground-nesting bird species were less common and widespread than the Ovenbird, with the Veery, Hermit Thrush, and Black-and-White Warbler recorded at 49%, 31%, and 29% of all points, respectively. All three species occurred at a greater proportion of *Lumbricus*-free sites compared to *Lumbricus*-invaded sites (Veery – 55% vs. 45%; Hermit Thrush – 35% vs. 28%; Black-and-white Warbler – 33% vs. 26%); however chi-square tests indicated that these differences were not statistically significant for any species (for each species, $\chi^2 < 0.01$; $df=3$; $p=0.99$). I found no statistically significant difference in density between *Lumbricus*-free and *Lumbricus*-invaded points for Hermit Thrush (all points $t=0.88$, $df=122$, $p=0.38$; maple-basswood points $t=1.42$, $df=51$, $p=0.14$; Fig. 3.2b), Black-and-white Warbler (all points $t=-0.28$, $df=122$, $p=0.82$; maple-basswood points $t=-1.13$, $df=51$, $p=0.27$; Fig. 3.2c), or Veery (all points $t=0.91$, $df=122$, $p=0.36$; maple-basswood points $t=0.54$, $df=51$, $p=0.59$; Fig. 3.2d).

Relative Contribution of Lumbricus Biomass to Influencing Ovenbird Density

When analyzing the 53 maple-basswood points, the univariate *Lumbricus* biomass model received the greatest support for explaining variation in Ovenbird density, ranking above all other candidate models that contained point and landscape-scale habitat

variables (Table 3.2). *Lumbricus* biomass appeared in the top two models and displayed a strong inverse relation with Ovenbird density ($\beta \pm SE = -0.61 \pm 0.29$; Fig. 3.3a). There was also moderate support for models of Ovenbird density that included percent ground cover, with ground cover appearing in two of the top three candidate models and the single-variable ground cover model receiving relatively strong support (Table 3.2). However, because the inverse relation between ground cover and Ovenbird density was weak ($\beta \pm SE = -0.01 \pm 0.004$; Fig. 3.3b), support for this variable is equivocal.

Compared to the analysis of maple-basswood points, model selection results were different when considering all hardwood forest types, with landscape variables receiving more support than the *Lumbricus* biomass and point-scale variables (Table 3.3). The single-variable *Lumbricus* biomass model received the least support of the candidate models. The two strongly supported variables were amount of core forest within 500 m and within 1,000 m. The model containing amount of core forest within 1,000 m received the greatest support. This variable also appeared in two of the top three models, and displayed a positive relation with Ovenbird density ($\beta \pm SE = 0.51 \pm 0.25$). The model containing amount of core forest within 500 m received the next greatest support. This variable also appeared in two of the top three models, but displayed a weak relation with Ovenbird density ($\beta \pm SE = 0.12 \pm 0.13$).

Discussion

I found reduced Ovenbird density in relation to earthworm invasions in maple-basswood forests across two national forests in northern Minnesota and northern

Wisconsin, providing evidence that invasive earthworms may have an adverse effect on populations of ground-nesting songbirds. Among 13 point and landscape-scale factors, *Lumbricus* biomass was the variable most strongly related to Ovenbird density in maple-basswood forests. These results, when taken in the context of the broad and still expanding spatial extent of earthworm invasions in the northern Midwest, suggest that earthworm invasions are a region-wide concern to the conservation of ground-nesting forest songbirds.

Songbird Density in Lumbricus-free vs. Lumbricus-invaded Forests

The Ovenbird was the only species of the 4 I studied to display significantly reduced density at *Lumbricus*-invaded points compared to *Lumbricus*-free points in maple-basswood forest. Furthermore, *Lumbricus* biomass was the most important of all point and landscape-scale habitat variables for predicting Ovenbird density. I observed lower (though non-statistically significant) Hermit Thrush density at *Lumbricus*-free points compared to *Lumbricus*-invaded points; however, whether this difference is biologically significant remains uncertain. Previous research indicates lower populations of the Hermit Thrush in relation to earthworm invasions at forest-stand scales (Loss and Blair 2011, see also chapter 2), and in this study, Hermit Thrush occurrence was lower at invaded points compared to earthworm-free points. Taken together, the above observations suggest that invasive earthworms could have a biologically significant relation with Hermit Thrush populations, despite no statistically significant effect being

evident at the scale of the current study. For the Veery and Black-and-white Warbler, there is no evidence from this work for earthworm-related declines.

Mechanisms for the apparent effect of earthworm invasions on Ovenbird populations remain unclear; however, I hypothesize two potential mechanisms for the observed pattern. First, decreased probability of nest success results from earthworm-caused habitat changes that reduce nest concealment and elevate predation rates (Loss and Blair 2011, see also chapter 2). Increased nest predation at the population level leads to increased cumulative mortality and reduced bird populations over time. Second, earthworm-invaded forests are perceived by the Ovenbird to be sub-optimal habitat, and such habitats are avoided in favor of territories and nest sites located in more favorable earthworm-free habitat.

It is possible that both hypothesized mechanisms operate to result in the declines associated with earthworm invasions; however, the relative importance of each remains unclear. I have previously documented an association of invasive earthworms with Ovenbird nest success via alteration of forest floor habitat; therefore, it seems likely that increased cumulative mortality of nestlings may be at least partially responsible for lower Ovenbird density in relation to earthworm invasions. Likewise, in line with the second hypothesized mechanism, previous research suggests that Ovenbirds select territories and nest sites that have deep leaf litter (reviewed by Van Horn and Donovan 1994, Rodewald and Yahner 2000, Mattsson and Niemi 2006) and at least some ground cover and shrubs for nest concealment (James 1971, Smith and Shugart 1987). These conditions are more

characteristic of earthworm-free or lightly invaded areas than those heavily invaded by *Lumbricus* (Hale et al. 2006, Holdsworth et al. 2007a).

Relative Importance of Lumbricus Invasions for Predicting Ovenbird Density

Across maple-basswood points in north-central Minnesota and northern Wisconsin, *Lumbricus* biomass was the variable most strongly related to Ovenbird density, with greater earthworm biomass associated with reduced density. Unexpectedly, except for ground cover, which displayed a weak inverse relation with Ovenbird density, point and landscape-scale habitat variables received little support. Density, nesting success, and pairing success of the Ovenbird has been previously found to be influenced by local habitat features, such as litter depth (Burke and Nol 1998, Rodewald and Yahner 2000, Mattsson and Niemi 2006), openness of the understory (Crawford et al. 1981, Smith and Shugart 1987, Smith et al. 2008), and canopy closure (Van Horn and Donovan 1994), and the Ovenbird is repeatedly cited as an area-sensitive species, responding to landscape patterns of forest fragmentation and forest edges (Manolis et al. 2000, Flaspohler et al. 2001, Mattsson and Niemi 2006, Smith et al. 2008). Given these documented associations, I find it surprising that *Lumbricus* biomass was the best predictor of Ovenbird density in maple-basswood forests.

Because my study areas were characterized by large areas of un-fragmented upland forest (68% and 64% average forest cover within the 2,000 m buffer for the Chequamegon-Nicolet and Chippewa National Forests, respectively), it is possible that the Ovenbird does not respond to habitat features at this relatively homogeneous scale.

Instead, it is possible that the mechanisms influencing Ovenbird density, including habitat selection and nest predation, operate in response to habitat heterogeneity occurring at finer spatial scales. Such a response of the Ovenbird to the scale of greatest heterogeneity has been previously suggested (Smith et al. 2008). My results suggest that heterogeneity of earthworm invasions from point to point in maple-basswood forests – to a greater extent than fine-scale variation in forest vegetation and large-scale variation in landscape habitat – influences patterns of Ovenbird density.

Unlike at maple-basswood points, I found little support for *Lumbricus* biomass as a predictor of Ovenbird density when considering all 124 hardwood forest points. Instead, I documented a positive relation of Ovenbird density to amount of core forest cover within a 1,000 m radius. This lack of a relation between earthworms and Ovenbirds was not completely unexpected, because the impacts of *Lumbricus* in North America have been most commonly documented in sugar maple and/or basswood forests, both within my study region (Gundale et al. 2005, Hale et al. 2005a, 2006, Holdsworth et al. 2007a, b) and in the northeastern U.S. (Lawrence et al. 2003, Fox et al. 2010). Studies finding *Lumbricus* impacts in other forest types are less common (but see Alban and Barry 1994, Nuzzo et al. 2009). The greater magnitude of earthworm effects on the soil, leaf litter layer, and ground-layer plant community of sugar maple and basswood forests compared to other hardwood forest types is likely caused by preference for the calcium-rich leaf litter characteristic of sugar maple and basswood (Holdsworth et al. 2008). This preference leads to more rapid decomposition and removal of the litter layer (Reich et al.

2005, Heneghan et al. 2007) and a more rapid cascade of effects to understory plant communities in maple-basswood forests compared to other hardwood forests.

Earthworm impacts on Ovenbird populations could be underestimated or completely hidden when simultaneously considering all hardwood forest types. However, as illustrated above, I provide evidence that within maple-basswood forests of my study region, *Lumbricus* invasions appear to have a substantial adverse effect on Ovenbird abundance. Forests dominated by sugar maple and/or basswood comprise a sizeable portion of the region's wooded landscape, including ~620,000 ha (10% of all woodland) in Minnesota (Miles et al. 2004) and ~490,000 ha (8% of all woodland) in Wisconsin (Vissage et al. 2004), and maple-basswood forest is among the preferred habitats of the Ovenbird in the region (Van Horn and Donovan 1994). My results therefore suggest that earthworm invasions may be related to Ovenbird population declines across a substantial portion of the regional Ovenbird population.

Implications for Management and Conservation of Ground-nesting Forest Songbirds

When combining the results of this study with previous work indicating a relation between invasive earthworms and density and nest success of ground-nesting songbirds (Loss and Blair 2011, see also chapter 2), it appears that earthworm invasions have the potential to be a region-wide threat to the conservation of ground-nesting forest songbirds, especially the Ovenbird, in maple-basswood forests of the northern midwest. Despite apparent population decline in relation to earthworm invasions, the Ovenbird remains common and widespread throughout hardwood forests of the region. The

Ovenbird was the most abundant species recorded during point surveys and remains the most commonly recorded species during a long-term study at the same points (Niemi et al. 2011). Nevertheless, there is concern that declines in the northern Midwest could affect continental Ovenbird populations. Breeding Bird Survey data indicate that northern Minnesota, Wisconsin, and Michigan contain the highest abundance of Ovenbirds in the species' range (Sauer et al. 2008). Large songbird populations in this region, including those of the Ovenbird, have been hypothesized to sustain smaller populations in fragmented woodlands of the central U.S. (Robinson et al. 1995). Declines of northern Midwest Ovenbird populations could therefore lead to declines at much larger scales.

Because investigation of relations between earthworms and forest songbirds is a newly emerging area of research, it is unclear how earthworms will affect songbird conservation in other regions. However, it is likely that similar impacts of invasive earthworms exist in sugar maple and basswood forests – and perhaps in other forest types – in the northeastern U.S. and southern Canada, other previously earthworm-free regions currently experiencing earthworm invasions. Evidence for this broader impact is provided by the observation of an absence of ground-nesting songbirds in an earthworm-invaded sugar maple forest in Indiana (Fox et al. 2010) and by a study in the northeastern U.S. documenting salamander declines as result of earthworm-caused removal of the litter layer and associated reduction in invertebrate food supply (Maerz et al. 2009), a mechanism similar to that posited for Ovenbird declines (Loss and Blair 2011, see also chapter 2). Further research in hardwood forests that also contain native earthworms,

such as those in the southern U.S., will clarify whether invasive earthworms pose a threat to songbirds in these regions.

Currently, there is no cost-effective method for removing invasive earthworms across the broad scale of existing invasions. However, because the natural rate of earthworm spread is slow, except in isolated long-distance water-dispersal events (Costello et al. 2010), top priority should be given to preventing introductions into areas that are earthworm-free and to preventing additional earthworm species from being introduced into already invaded areas. Identification of remaining earthworm-free areas is important for prevention efforts that will target at-risk forests. In areas already largely invaded by earthworms, management and conservation plans for the Ovenbird and Hermit Thrush may benefit from recognition and incorporation of potential earthworm effects to habitat selection, nest concealment, and nest predation. Conservation and management of ground-nesting songbirds will be benefited by further research to improve understanding of earthworm impacts on this species group and to clarify the complex mechanisms underlying these impacts.

Table 3.1. Variables, including citations justifying inclusion, used to develop models of Ovenbird habitat relations in 2009-2010 in the Chippewa and Chequamegon-Nicolet National Forests, Wisconsin and Minnesota, U.S.A.

Variables	Citations and Justification for Inclusion ^a
Shrub density	Ovenbird distribution associated with low (1) and/or intermediate (2) shrub density
Canopy cover	Ovenbird habitat characterized by relatively high (i.e., 60-90%) canopy closure (1,3,4,5,6)
Understory cover	Ovenbird density (7) and occupancy probability (8) inversely related to understory cover
Ground cover	Ovenbird distribution generally associated with low ground cover (1), but relation varies by region (9)
Litter depth	Ovenbird selects nesting territories with thick leaf litter layer (5, 10, 11, 12, 13)
Sedge cover	Ovenbird nest success probability inversely related to cover of sedges and grasses (13)
Distance to forest edge	Inverse relation between distance to edge and pairing success (14), availability of suitable nesting sites (10); and nest success (15, 16)
Distance to water ^b	Ovenbird presence inversely related to distance to nearest water (17)
Core forest (w/i 100m)	Strong support for habitat models of Ovenbird occurrence at 3 ha extent = 100 m radius (8)
Core forest (w/i 200m)	Strong support for habitat models of Ovenbird occurrence at 12 ha extent = 200 m radius (8)
Core forest (w/i 500m)	No citation for this scale; corresponds to intermediate landscape scale between other well-supported scales
Core forest (w/i 1000m)	No citation for this scale; corresponds to intermediate landscape scale between other well supported scales
Core forest (w/i 2000m)	Ovenbird nest success positively related to amount of core forest within 2000 m (11)
<i>Lumbricus</i> biomass	Ovenbird density lower in <i>Lumbricus</i> -invaded compared to <i>Lumbricus</i> -free forest stands and Ovenbird nesting success influenced by habitat changes related to <i>Lumbricus</i> earthworm invasion (13)

^a Numbers refer to the following citations: (1) Smith and Shugart 1987, (2) James 1971, (3) Smith 1977, (4) Collins 1983, (5) Van Horn 1990, (6) Thompson and Capen 1988, (7) Crawford et al. 1981, (8) Smith et al. 2008, (9) Van Horn and Donovan 1994, (10) Burke and Nol 1998, (11) Mattsson and Niemi 2006, (12) Fox et al. 2010, (13) Loss and Blair 2011, see also chapter 2, (14) Van Horn et al. 1995, (15) Flaspohler et al. 2001, (16) Manolis et al. 2000, (17) Mitchell et al. 2001.

^b Removed from final analysis due to strong correlation with "Distance to Forest Edge"

Table 3.2. Results of model selection for analysis of Ovenbird habitat relations based on survey points in sugar maple and maple-basswood forest types ($n=53$). Results are derived from Akaike's Information Criteria, corrected for small samples (AIC_c).

Model ^a	K ^b	AIC _c	ΔAIC_c^c	ω_i^d
<i>Lumbricus</i> biomass + ground cover	4	-95.93	0.00	0.277
<i>Lumbricus</i> biomass	3	-95.47	0.46	0.220
Ground cover	3	-93.77	2.16	0.094
Canopy cover	3	-93.01	2.92	0.064
Sedge cover	3	-92.84	3.09	0.059
Null model	2	-92.79	3.14	0.057
Core forest (w/i 500m)	3	-92.00	3.93	0.039
Shrub density	3	-91.68	4.25	0.033
Distance to forest edge	3	-91.29	4.64	0.027
Core forest (w/i 1000m)	3	-91.15	4.79	0.025
Understory cover	3	-90.94	4.99	0.023
Core forest (w/i 2000m)	3	-90.84	5.09	0.022
Litter depth	3	-90.73	5.20	0.021
Core forest (w/i 200m)	3	-90.67	5.26	0.020
Core forest (w/i 100m)	3	-90.56	5.37	0.019
Global model (13 variables)	15	-54.08	41.85	0.000

^avariables included in each candidate model

^bnumber of parameters in the model (including parameters for intercept and overdispersion)

^cdifference in AIC_c between model and the most strongly supported model

^d AIC weight - relative strength of support for model

Table 3.3. Results of model selection for analysis of Ovenbird habitat relations based on survey points in all hardwood forest types ($n=124$). Results are derived from Akaike's Information Criteria, corrected for small samples (AIC_c).

Model ^a	K ^b	AIC _c	ΔAIC_c^c	ω_i^d
Core forest w/i 1000m	3	-225.97	0.00	0.382
Core forest w/i 500m	3	-224.31	1.66	0.167
Core forest w/i 500m + core forest w/i 1000m	4	-223.84	2.14	0.131
Core forest w/i 2000m	3	-222.17	3.80	0.057
Ground cover	3	-221.92	4.06	0.050
Core forest w/i 200m	3	-221.17	4.81	0.034
Canopy cover	3	-220.79	5.18	0.029
Sedge cover	3	-220.71	5.27	0.027
Null model	2	-220.67	5.31	0.027
Distance to forest edge	3	-219.68	6.30	0.016
Core forest w/i 100m	3	-219.46	6.52	0.015
Litter depth	3	-219.41	6.56	0.014
Shrub density	3	-219.35	6.63	0.014
Understory cover	3	-219.13	6.84	0.012
<i>Lumbricus</i> biomass	3	-219.06	6.91	0.012
Global model (13 Variables)	15	-218.98	6.99	0.012

^avariables included in each candidate model

^bnumber of parameters in the model (including parameters for intercept and overdispersion)

^cdifference in AIC_c value between model and the most strongly supported model

^d AIC weight - relative strength of support for model

Figure 3.1. Map of study area in northern Midwest region of the U.S.A. (A), bird survey points (black circles) in the Chippewa National Forest, Minnesota (B), and Chequamegon unit of the Chequamegon-Nicolet National Forest, Wisconsin (C). Black polygons indicate national forest boundaries, dark gray shading indicates open water, and light gray shading indicates forest cover.

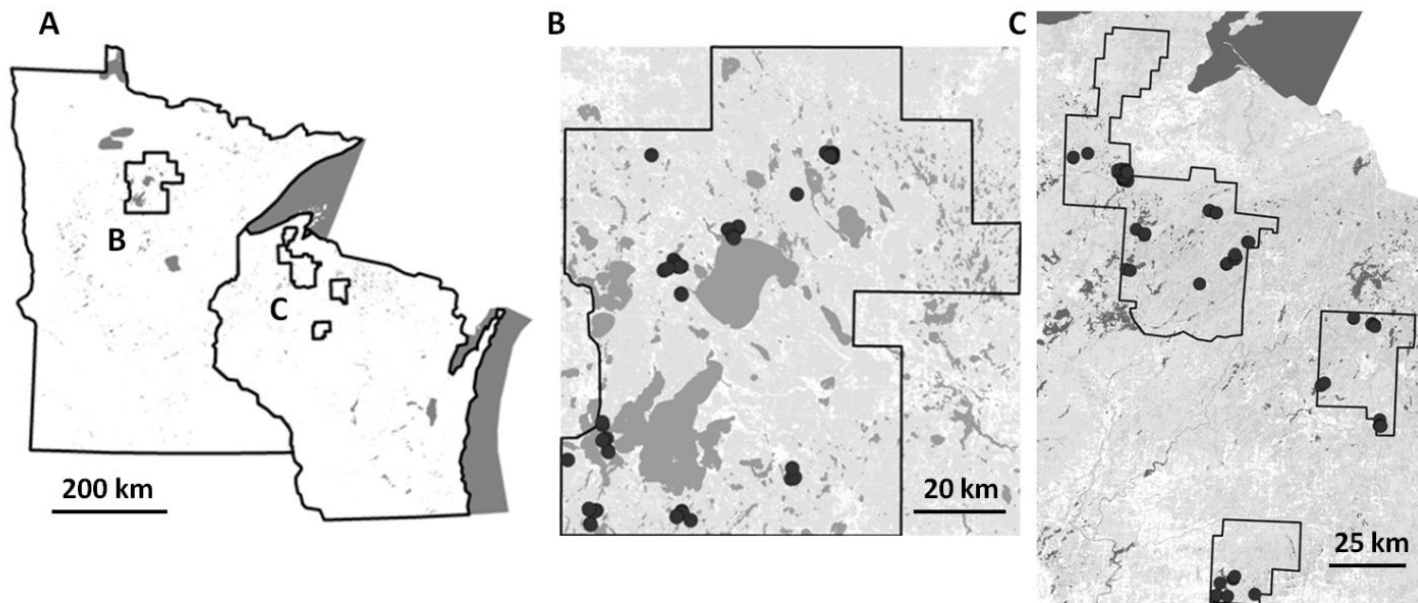


Figure 3.2. Comparison of mean density for Ovenbird (A), Hermit Thrush (B), Black-and-white Warbler (C), and Veery (D) between *Lumbricus*-invaded and *Lumbricus*-free points in sugar maple and maple-basswood forests of the Chippewa and Chequamegon-Nicolet National Forests, Minnesota and Wisconsin, 2009-2010. Note that units on vertical axes are different for each species.

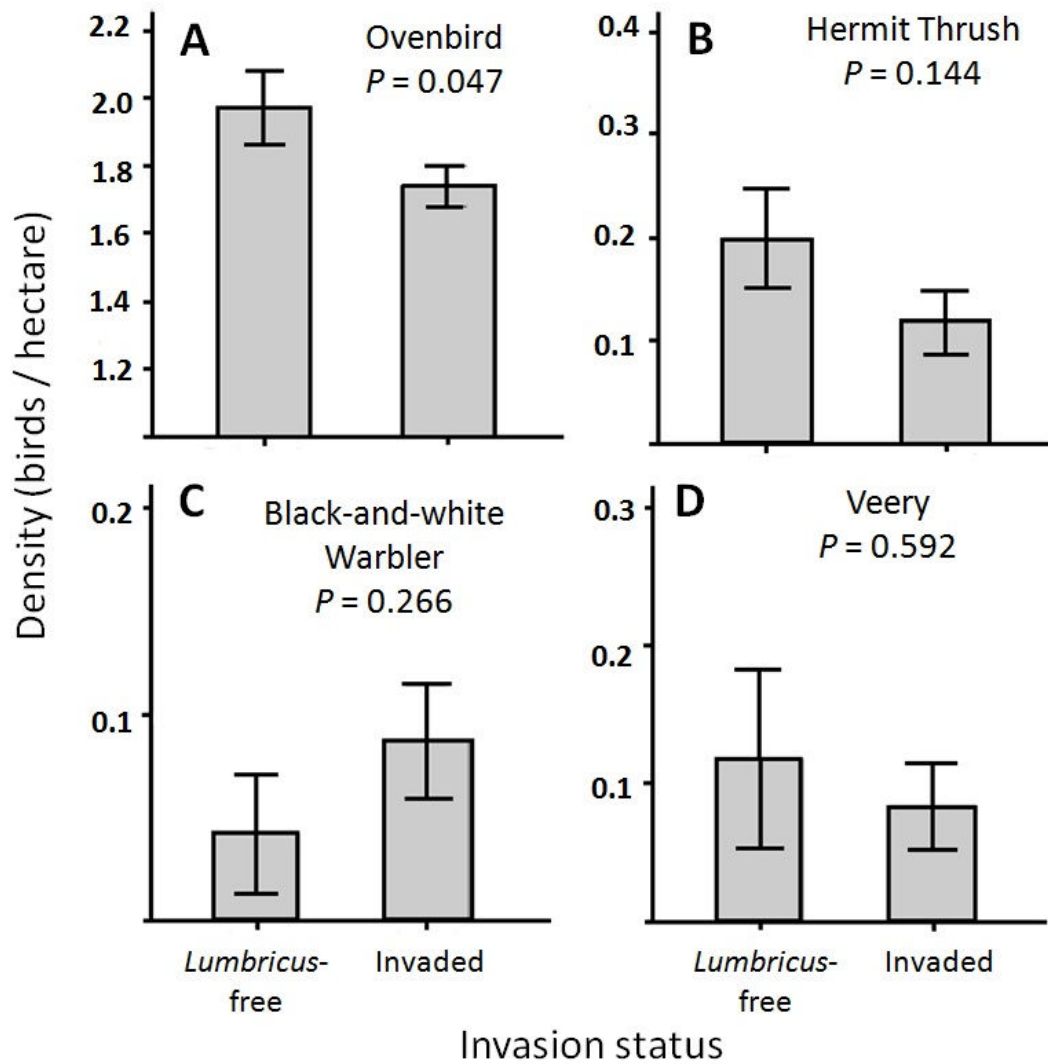
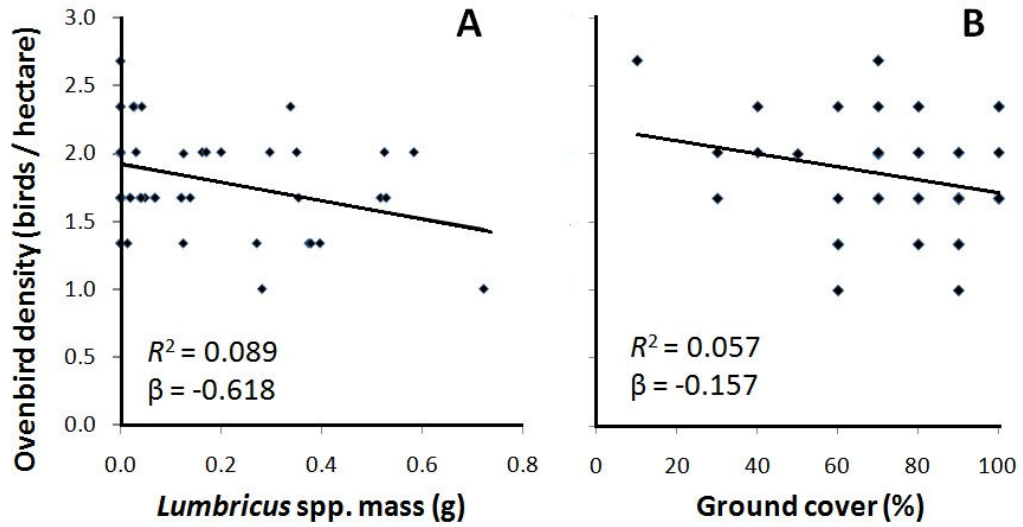


Figure 3.3. Relations between Ovenbird density and biomass of *Lumbricus* (A) and percent ground cover (B) at sugar maple and maple-basswood bird survey points in the Chippewa and Chequamegon-Nicolet National Forests, Minnesota and Wisconsin, U.S.A., 2009-2010.



CHAPTER 4 - A VISUAL METHOD FOR RAPIDLY ASSESSING EARTHWORM INVASIONS IN NORTHERN HARDWOOD FORESTS

Non-native earthworms are invading hardwood forests of boreal and north-temperate North America, with substantial effects to soil, plants, and ground-dwelling vertebrates. Quantifying these invasions is necessary for understanding the scope of earthworm impacts and for identifying remaining earthworm-free areas in which to target prevention measures. Current earthworm sampling methods are effort intensive and/or environmentally damaging, which makes it economically impractical to quantify invasions at high resolution and across broad spatial scales. I developed a visual method for rapidly assessing earthworm invasions by: (1) testing how accurately a 5-level system of visual classification, based on multiple soil and forest floor characteristics, reflected true earthworm assemblages, and (2) using data from 2 visual sampling protocols to assess relations between individual characteristics of the soil, litter layer, and vegetation and presence of different earthworm species. Surveys were conducted along the north shore of Lake Superior, Minnesota, and in the Chequamegon-Nicolet National Forest, Wisconsin (U.S.A.). The 5-level system differentiated among previously proposed stages of earthworm invasion, as indicated by sequential changes in composition and relative dominance of species among levels. With the exception of presence of middens as a predictor of *Lumbricus terrestris* presence, no individual variable appeared to be as useful as the multi-criteria system for rapidly assessing earthworm invasions in the field. I conclude that the 5-level visual method provided an efficient and accurate approach for

rapidly assessing severity of earthworm invasions in hardwood forests of the U.S. northern Midwest region and also provides a foundation for the development of similar visual assessment methods in other regions that are experiencing earthworm invasions.

Introduction

Non-native earthworms are spreading into previously earthworm-free regions of north-temperate and boreal North America, causing significant impacts to hardwood and mixed hardwood-coniferous forests (Frelich et al. 2006) and posing a major concern to the conservation of biodiversity (Sutherland et al. 2010). Invasive earthworms, particularly those in the genus *Lumbricus*, remove organic layers, mix soil horizons (Alban and Barry 1994, Hale et al. 2005a), and alter nutrient dynamics (Burtelow et al. 1998, Costello and Lamberti 2008). Changes to the soil eliminate sensitive plant species (Gundale 2002), reduce cover and diversity of herbaceous plants and tree seedlings, and increase dominance by sedges and grasses (Hale et al. 2006, Holdsworth et al. 2007a). These changes have been related to population declines of salamanders (Maerz et al. 2009) and ground-nesting songbirds (Loss and Blair 2011, see also chapters 2 and 3).

Preventing further spread of invasive earthworms and mitigating earthworm impacts to soil, plant communities, and vertebrates requires identification of large-scale earthworm distributions, remaining earthworm-free areas, and the severity of existing invasions. Several available techniques allow earthworm populations to be sampled (reviewed by Butt and Grigoropoulou 2010), including removal and hand-sorting of the soil (Raw 1960, Coja et al. 2008), liquid extraction using permanganate (Svendsen 1955),

formalin (Raw 1959, Callahan and Hendrix 1997), or a mustard-water mixture (Lawrence and Bowers 2002, Hale et al. 2005a), and electrical extraction (Weyers et al. 2008). However, these methods are time-consuming and effort-intensive, thus precluding rapid sampling of a large number of points. Some of these methods are also physically destructive or require use of environmentally toxic substances.

One of these methods, the mustard extraction technique, has become commonly used in ecological studies of earthworm invasion (e.g., Kourtev et al. 1999, Cameron et al. 2007). Mustard extraction can be conducted comparatively rapidly, is environmentally friendly, and has been found to provide an accurate index of species composition and abundance (Gunn 1992, Lawrence and Bowers 2002, Eisenhauer et al. 2008), especially for the deep-burrowing species *L. terrestris* (Chan and Munro 2001). Nonetheless, the method still requires expenditure of significant time and effort because it requires large quantities of water that often must be transported across long distances into remote areas. For example, in a study of earthworm effects on songbirds, sampling with mustard extraction at 112 points within a small study area (25 km radius) required 80 hours of fieldwork (1.4 points/hr, Loss and Blair 2011, see also chapter 2). In a companion study, sampling of 36 points that were spatially scattered across 2 national forests required 180 hours of fieldwork (0.2 points/hr, Loss et al. *in review*, see also chapter 3). Clearly, development of a tool that provides an environmentally friendly and rapid means for assessing earthworm populations will assist conservation, management, and research projects that require in-depth mapping of invasion at a fine resolution or of broad invasion patterns at large spatial extents.

Previous research in northern Wisconsin and northern Minnesota, U.S.A. suggests that earthworm invasions involve multiple species and that invasion progresses through 5 sequential stages, with earthworm-free conditions in stage 1, and the onset of invasion of by different species or genus in subsequent stages. These stages include invasion by *Dendrobaena octaedra* in stage 2, *Aporrectodea* spp. and *Lumbricus* spp. juveniles in stage 3, *L. rubellus* in stage 4 and *L. terrestris* in stage 5 (Holdsworth et al. 2007b). Because different earthworm assemblages result in varying magnitude of effects to the soil, leaf litter, and plant communities (Frelich et al. 2006), and because earthworm effects are highly visible, it may be possible to use visual cues, such as depth of the litter layer, preponderance of sedges, and the presence of earthworm middens and castings, to distinguish invasion stages and therefore to provide a coarse assessment of earthworm invasion severity.

I sought to develop a visual assessment method based on a test of the relations between visual cues and presence and severity of earthworm invasions in hardwood forests of the U.S. northern Midwest. To achieve this goal, I: (1) analyzed how accurately a 5-level visual system of invasion classification, based on multiple characteristics of the soil and forest floor, reflected true earthworm invasion stage as quantified by mustard extraction sampling, and (2) used data generated from two different visual sampling protocols to assess relations between soil, litter layer, and vegetation variables and presence of different earthworm species, including the species of greatest management concern in North America, *L. rubellus* and *L. terrestris*.

Methods

Study Area and Point Selection

I generated the data used for this analysis from two different earthworm sampling protocols that each included visual sampling and direct earthworm quantification components. One protocol was used at points located in nine state parks along the north shore of Lake Superior in northeast Minnesota (47°N, 92°W to 48 °N, 90°W; hereafter, “Minnesota points”). The other protocol was used at bird nests located in earthworm-free and earthworm-invaded study sites in the Chequamegon unit of the Chequamegon-Nicolet National Forest in northern Wisconsin (46°N, 91°W; hereafter, “Wisconsin points”; see Fig. 4.1 for both study areas).

Loss and Blair (2011, see also chapter 2) reported selection methods for the Wisconsin study sites. The Wisconsin protocol was followed at 271 bird nest locations, primarily of Ovenbirds (*Seiurus aurocapilla*) and Hermit Thrushes (*Catharus guttatus*), that were found and monitored in 2009 ($n=112$) and 2010 ($n=159$). All nests were located in upland-mesic sugar maple (*Acer saccharum*) and sugar maple-basswood (*Tilia americana*) forest stands that were >60 yr old, had no history of timber removal within the last 40 yr, and consisted of sandy loam or loamy sand soils (Loss and Blair 2011, see also chapter 2). In addition, earthworm sampling from a previous study had confirmed that sites containing the nests represented earthworm-free, partially invaded, and completely invaded forest stands (Holdsworth et al. 2007a).

The Minnesota protocol was followed at nine state parks located along the north shore of Lake Superior in the North Shore Highlands subsection of Minnesota’s

Ecological Classification System. I used ArcMap (version 9.3; ESRI 2008) and a geographical information systems layer of forest types from the Minnesota Native Plant Community Classification (Minnesota Department of Natural Resources 2011) to locate 2,000 random points across the parks. The number of points in each state park was proportional to the park's size, and the number of points in each forest type was proportional to the abundance of that forest type. Due to logistical constraints, field sampling was only conducted at 1,127 of the initially selected points; however, these points still proportionally represented park size and abundance of forest types.

The resultant points represented 25 forest types consisting of different combinations of dominant, co-dominant, and sub-canopy tree species. The three dominant canopy species included quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), and sugar maple. Co-dominant and sub-canopy species included balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), black spruce (*P. mariana*), white cedar (*Thuja occidentalis*), white pine (*Pinus strobus*), red pine (*P. resinosa*), yellow birch (*Betula alleghaniensis*), red maple (*Acer rubrum*), red oak (*Quercus rubra*), basswood, black ash (*Fraxinus nigra*), and big-tooth aspen (*Populus grandidentata*). Much of my analysis for Minnesota points focused on points located in aspen-birch and sugar maple forests, the most widespread hardwood forest types in the region.

Visual Assessment Protocols

For the Wisconsin points, I measured understory vegetation and the forest floor litter layer at all 271 nests between 15–31 July of 2009 or 2010. Within a 2 x 2 m square

centered on each nest, I used ocular estimation to quantify percent cover of the litter layer, percent cover of maple seedlings <50 cm tall, percent cover of all sedges and grasses combined, and percent cover of total ground vegetation (i.e., all grasses, sedges, herbaceous plants, and woody plants <50 cm tall). All cover estimates were to the nearest 10%. I also measured average litter depth (O_l , O_e , and O_a horizons combined) based on four measurements taken 1 m from the nest in each of the cardinal directions. I measured litter depth by pushing a metal skewer through the litter until meeting resistance from rock or mineral soil. For litter depth and litter cover estimates, I only considered intact accumulated leaf litter from >1 year and not leaf litter from only the previous autumn. The July time period of vegetation sampling corresponded to the period when all bird nests had become inactive, thus minimizing disturbance to nesting birds and to the habitat surrounding nests.

In addition to the above visual measurements, I also counted earthworm middens, piles of organic material at burrow entrances that can be used to infer the presence of *L. terrestris* (Raw 1959, Butt and Grigoropoulou 2010). I counted middens within 33 x 33 cm sub-plots from which earthworm populations were directly quantified (described in following section). I counted all middens within each sub-plot and counted every other midden that fell roughly 50% within the sub-plot.

For the Minnesota points, visual assessments were conducted between 1 June-31 August 2009. Because the state parks span a broad latitude (>150 km from south to north) and therefore experience differences in seasonal timing of temperature and moisture conditions, parks were surveyed in a random order to minimize confounding

effects of climate. The following data were collected within a 5-m radius centered on each point. Fragmentation of the litter layer was classified using a 3-category system reflecting increasing earthworm decomposition (1 – Intact, layered forest floor, O_l, O_e, and O_a horizons present; 2 – Litter layer partially fragmented, but containing litter from >1 yr; 3 – No intact litter, only freshly fallen leaves from the previous autumn).

Earthworm activity was visually estimated based on an index of both earthworm castings and of *L. terrestris* middens (for both indices: 1 – Absent, 2 – Present, 3 – Abundant).

Soil cores were extracted (6 cm diameter; 15 cm depth) from 3 random locations within the 5 m radius and depth of the litter layer (O_l, O_e, and O_a horizons combined) and of the A-horizon was measured. Soil textural class was determined for the mineral soil component of each core using a manual soil texture key adapted from Brewer and McCann (1982). Finally, a variable radius plot and BAF 10 wedge prism was used to sample tree species and to estimate relative species dominance (i.e., proportional representation by each tree species).

In addition to the above visual sampling, each Minnesota point was broadly classified into 1 of 5 categories (Table 4.1) based on a visual system that was designed to identify increasing levels of earthworm invasion in conjunction with the previously proposed model of multi-species earthworm invasion (Holdsworth et al. 2007), with the following categories: 1 = earthworm-free, 2 = minimally invaded, 3 = moderately invaded, 4 = substantially invaded, 5 = heavily invaded. This classification was based on a combination of measurements including the litter fragmentation index, casting index, and midden index, and from observation of the presence and abundance of fine roots in

the O-horizon, because this parameter has been found to decrease in relation to earthworms (Fisk et al. 2004, Hale et al. 2005a).

Quantification of Earthworm Populations

I quantified earthworm populations using the liquid-mustard extraction technique (Lawrence and Bowers 2002, Hale et al. 2005b), which consists of pouring a mustard-water mixture (40 g of ground yellow mustard dissolved in 4 L of water) on the soil surface and collecting all emerging earthworms. For Wisconsin points, I conducted sampling at all 271 nest locations between 15 September-5 October of 2009 or 2010. For Minnesota points, sampling was conducted at 163 points between 1 September-15 October 2009. This sub-set of points was selected to proportionally represent forest types based on land cover abundance. The seasonal timeframe of sampling corresponds to a period of soil moisture conditions favorable for earthworms and in which the population contains a high proportion of adults.

For Wisconsin points, I sampled one-third of nests surveyed in 2009 ($n=36$) using three 33 x 33 cm sub-plots (1 at the nest, 2 random points ≤ 33 m from the nest), and I sampled two-thirds of nests ($n=76$) using one plot at the nest. Because there was no significant difference in the biomass of different earthworm species between nests sampled with 1 plot versus 3 sub-plots, I sampled all nests in 2010 using only 1 plot at the nest (Loss and Blair 2011, see also chapter 2). For all 163 Minnesota points, I sampled earthworms from 3 randomly selected 33 x 33 cm sub-plots within 5 m plots.

I preserved all earthworms in the field with 70% isopropyl alcohol and later transferred them to buffered 10% formalin for long-term storage. I counted, identified, and measured length of earthworms using a dissecting microscope. Adult earthworms were identified to species when possible, whereas most juvenile earthworms were only identified to genus. I grouped all earthworms in the genus *Aporrectodea* together, due to morphological similarities among *A. caliginosa*, *A. longa*, *A. rosea*, *A. trapezoides*, and *A. tuberculata* (Hale 2007). I estimated earthworm biomass using length measurements and regression equations based on allometric relationships (Hale et al. 2004).

Data Analyses

I averaged earthworm biomass across sub-plots to calculate point-level biomass values for all points. Because I estimated midden counts at Wisconsin points at the sub-plot level, I also averaged these values across sub-plots. Although different visual assessment techniques were used for the Wisconsin and Minnesota points, in all cases, the sampling radius I used for visual assessments corresponded to the radius I used for earthworm sampling. Nonetheless, I conducted statistical analyses separately for Wisconsin and Minnesota points.

I used field-estimated tree dominance values to ground-truth forest types at all Minnesota points. The forest type at some points did not match the type indicated by the GIS-based selection procedure. Therefore, for the following statistical analyses that were conducted separately for different forest types, I based forest type classification on dominance values of canopy trees (i.e., aspen-birch points = combined dominance of all

aspen and birch species ≥ 0.5 ; sugar maple points = dominance of sugar maple ≥ 0.4). I also conducted a preliminary analysis of whether soil texture influenced the relation between visual assessment variables and earthworm variables at Minnesota points. Because I found no significant relation between soil texture and earthworm presence or biomass within the forest types I analyzed, I did not include soil texture as a covariate in remaining analyses.

I compared earthworm biomass among the 5 visual-based categories of invasion at Minnesota points. Because the distribution of biomass values was heavily skewed with zero-values, I was unable to achieve normal distribution of the data. I therefore compared biomass among groups using one-way Kruskal-Wallis non-parametric analysis of variance with pairwise comparisons between group medians using Mann-Whitney U-tests and adjustment for multiple comparisons to reduce probability of Type I error. I conducted analyses separately for 1 earthworm genus (*Aporrectodea*) and 3 species (*Dendrobaena octaedra*, *L. rubellus*, *L. terrestris*). Groupings represent all major earthworm feeding niches and include the most common earthworms in the study area (Tiunov et al. 2006). I conducted these analyses using all 163 points.

I used multivariate logistic regression models to assess the relation between visual assessment variables and presence/absence of the same 3 species and 1 genus of earthworm described in the previous analysis. For the Wisconsin model, continuous independent variables included percentage cover of sedge, maple seedlings, total ground vegetation, and litter, as well as litter depth and midden count. I also included the categorical covariate “year” because temperature and moisture variation between 2009

and 2010 may have affected earthworm sampling results. For the Minnesota models, continuous independent variables included litter depth and A-horizon depth, and categorical variables included litter fragmentation index, casting index, and midden index. For Minnesota points, I used the above variables to construct separate multivariate regression models for aspen-birch forests ($n=79$) and sugar maple forests ($n=42$). For all analyses, I concluded statistical significance when $\alpha \leq 0.10$.

Results

Of the 271 Wisconsin points, 70 (25.8%) had no earthworms detected. All 163 Minnesota points had at least 1 earthworm detected; however, samples from 3 of these points (1.8%) only contained *D. octaedra*. For the Wisconsin and Minnesota points, 174 (64.2%) and 32 (19.6%) points, respectively, had no *L. rubellus* or *L. terrestris* detected but were invaded by *D. octaedra*, *Aporrectodea*, and/or other earthworm species.

Comparison of Earthworm Biomass among the 5 Invasion Levels

The Minnesota points were assigned to all categories of the 5-level visual classification, including earthworm-free ($n=4$; 2.5%), lightly invaded ($n=11$; 6.7%), moderately invaded ($n=72$, 44.2%), substantially invaded ($n=43$; 26.3%), and heavily invaded ($n=33$; 20.2%). Because very few Minnesota points were visually classified as earthworm-free (invasion category 1; $n=4$), I did not include this level in pairwise comparisons. Nonetheless, I found significant differences in *D. octaedra* biomass among the remaining invasion levels ($H= 5.44$, $df=3$, $p<0.01$), and pairwise comparisons

indicated that *D. octaedra* biomass was greater for invasion level 2 compared to levels 3, 4, and 5 (Fig. 4.2a). Biomass of *Aporrectodea* was also different among invasion levels ($H=8.48$, $df=3$, $p=0.04$); however, none of the pairwise contrasts were significant at $\alpha=0.10$ after adjusting for multiple comparisons (Fig. 4.2b). For *L. rubellus* biomass, I found significant differences among invasion levels ($H=22.74$, $df=3$, $p<0.01$), and pairwise comparisons indicated a peak of *L. rubellus* biomass at level 3 with biomass for this level greater compared to level 5 and biomass for level 5 lower compared to all other levels (Fig. 4.2c). For *L. terrestris*, there was a significant difference among invasion levels ($H=49.90$, $df=3$, $p<0.01$), with biomass for level 5 greater compared to all other levels and biomass for level 4 greater than for level 3 (Fig. 4.2d).

Relations between Individual Visual Assessment Variables and Earthworm Presence

I found statistically significant relations between individual visual variables and earthworm presence for each species and genus and for both Wisconsin and Minnesota points (see Tables 4.2 and 4.3 for β -coefficients and p -values). For Wisconsin points, presence of *D. octaedra* was positively related to amount of sedge cover (odds-ratio=1.40) and litter depth (odds-ratio=1.19). For Minnesota points, *D. octaedra* presence was positively related to litter depth in aspen-birch forests (odds-ratio=16.33) and inversely related to litter fragmentation in sugar maple forests (odds-ratio=0.04; Table 4.2). For presence of *Aporrectodea*, the Wisconsin analysis indicated an inverse relation with litter cover (odds-ratio=0.40), and the Minnesota analysis indicated positive relations with A-horizon depth in both forest types (odds-ratio_{aspen-birch}=1.55; odds-

ratio_{sugar maple}=1.57) and with *L. terrestris* midden index in sugar maple forest (odds-ratio=2.89; Table 4.2).

Presence of *L. rubellus* displayed statistically significant relations with several visual measurements at Wisconsin points, including a positive relation with sedge cover (odds-ratio=1.40), total ground cover (odds-ratio=2.10), and *L. terrestris* midden count (odds-ratio=1.49), and an inverse relation with maple seedling cover (odds-ratio=0.71). The Minnesota analysis indicated that *L. rubellus* presence was inversely related to the midden index in sugar maple forests (odds-ratio=0.21) and positively related to the casting index in both forest types, but with a stronger relation in sugar maple forest (odds-ratio=13.61) than aspen-birch forest (odds-ratio=3.73; Table 4.3). For *L. terrestris*, presence at Wisconsin points was inversely related to litter depth (odds-ratio=0.58) and positively related to midden counts (odds-ratio=1.53). Presence at Minnesota points was positively related to midden index in both forest types; however, the relation was much stronger in sugar maple (odds ratio=38.02) than aspen-birch forests (odds ratio=3.67; Table 4.3).

Discussion

I found that the 5-level visual classification system, which is based on combined assessment of the soil, leaf litter, and vegetation, provided a rough approximation of the previously proposed stages of earthworm invasion. Biomass of all earthworm groups except *Aporrectodea* differed as expected among the 5 categories. I also found that the likelihood of earthworm presence was significantly related to at least 1 visual assessment

variable for the 3 species and 1 genus analyzed. Nonetheless, with the exception of midden count and midden index as predictors of *L. terrestris* presence, no single variable appeared to be as useful as the multi-criteria system for rapidly assessing earthworm invasions in the field.

The 5-level Visual System of Invasion Classification

Although greater replication is needed to determine the sensitivity of level 1 of the visual classification system for identifying earthworm-free conditions, levels 2 through 5 appear to differentiate stages of invasion as characterized by changing composition and relative dominance of earthworm species. For example, level 2 (lightly invaded), was characterized by the highest *D. octaedra* biomass and low biomass of *L. rubellus* and *L. terrestris*. Level 3, (moderately invaded) was associated with peak *L. rubellus* biomass and significantly decreased *D. octaedra* biomass. Level 4 (substantially invaded) was characterized by low *D. octaedra* biomass, and high biomass of *L. rubellus* with *L. rubellus* decreasing slightly from level 3. Level 5 (heavily invaded) was dominated by *L. terrestris*, with all other species present, but at low biomass.

The conditions identified with the 5-level visual system are in general agreement with the previously proposed model of multi-species invasion dynamics. This model indicates a predictable invasion sequence in sugar maple forests, with *D. octaedra* typically being the first species to invade, followed by *Aporrectodea* and *L. juveniles*, then *L. rubellus*, and finally *L. terrestris* (Holdsworth et al. 2007b). These different species compositions are thought to be a function of time since original invasion (Hale et

al. 2005b) and rate and mechanism of dispersal (Proulx 2003, Cameron et al. 2007, Costello et al. 2010). The 5-level visual assessment provided a method for approximating these proposed invasion stages, with level 2 corresponding to invasion by *D. octaedra*, level 3 corresponding to invasion by *L. rubellus*, and level 5 corresponding to dominance of *L. terrestris* invasion. Because I found no difference in *Aporrectodea* biomass among levels, this system does not appear to diagnose onset of invasion by this group. Nonetheless, the method detailed in Table 4.1 provides a substantial first step in the development of a visual technique for assessing severity of earthworm invasions and for identifying invasion by *D. octaedra* as well as by *L. rubellus*, and *L. terrestris*, the 2 species that are of greatest management concern in North America, due to the magnitude of their impact to the forest floor.

Relations between Individual Visual Variables and Earthworm Presence

Of the visual assessment variables I analyzed, the midden count and index appeared to be the most useful single metrics for predicting presence of a particular earthworm species. With each additional midden counted, *L. terrestris* was 1.5 times more likely to be sampled in sugar maple forest, and with each stepwise increase in the 3-level midden index, presence of *L. terrestris* in aspen-birch and sugar maple forests was 3.7 and 38.0 times more likely, respectively. For all points combined, sensitivity (i.e., assessment of known presence by midden counts ≥ 1 or index = present or abundant) was 91% and specificity (i.e., assessment of known absence by counts of zero middens or index = absent) was 77%. These results suggest that the presence or absence of middens

is useful for inferring the presence or absence of *L. terrestris*. Furthermore, it is likely that my specificity estimate is conservative, because it does not take into account that this deep-burrowing species likely escaped detection during sampling at some points where it was present and where middens were observed. Although previous studies have suggested a relation between *L. terrestris* and presence of middens (Raw 1959, Butt and Grigoropoulou 2010), no empirical test of this relation had been conducted to date. This study is the first to provide quantitative evidence for the use of midden observations to predict presence and absence of *L. terrestris*.

Although I found significant relations between individual visual assessment variables and presence of each species and genus analyzed, no other single variable appeared to be as useful as midden presence for predicting presence of an earthworm species. I found significant relations between *L. rubellus* presence and several visual assessment variables, with casting index showing the strongest relation. With each stepwise increase in casting index, presence of *L. rubellus* in aspen-birch and sugar maple forests was 3.7 and 13.6 times more likely, respectively. However, it is unclear how useful casting index is for identifying *L. rubellus* presence in the field, because other species also produce casts (Edwards and Bohlen 1996), and because there is no method for distinguishing among casts of different species. Evidence for this limitation is provided by the observation that *L. rubellus* was sampled from only 59% of points classified as having “abundant” casts.

I found greater likelihood of *L. rubellus* presence in relation to reduced cover of maple seedlings, increased sedge cover, and increased total cover. These findings align

with previous research suggesting that *L. rubellus* has significant impacts to plant assemblages, causing reduced species richness and increased dominance by sedges (Hale et al. 2006, Holdsworth et al. 2007a). However, other environmental factors also influence the cover of sedges, including deer herbivory and presence of forest openings (Powers and Nagel 2008). Similarly, fine-grain environmental factors, such as soil productivity and herbivory, likely affect maple seedling and total ground cover. Used by themselves, these vegetation metrics are unlikely to be useful for predicting *L. rubellus* presence. Nonetheless, given the associations between *L. rubellus* presence and forest floor vegetation, further research should address whether incorporation of vegetation measurements into a multi-criteria visual assessment can improve its precision for identifying the onset of *L. rubellus* invasion.

The positive relation I observed between *L. rubellus* presence and midden count at Wisconsin points was unexpected and difficult to explain, given that *L. terrestris* is the only species in my study area that creates middens. However, a possible explanation for this correlation is that more productive forest floor environments favor higher abundance of both *L. rubellus* and *L. terrestris*. Furthermore, in many introduction events, it is likely that both species are introduced together, because fishing bait is a common vector of introduction for each species, and because both *L. rubellus* and *L. terrestris* are usually present in bait that is labeled as containing only one of the species (Keller et al. 2007).

I found that *D. octaedra* was the earthworm species most likely to be present in thick, unfragmented, leaf litter, as evidenced by a positive relation with litter depth and an inverse relation with litter fragmentation. Previous research has indicated a similar

association between *D. octaedra* and thick leaf litter, and this association is thought to result from the relatively minor impact of *D. octaedra* to the forest floor (Hale et al. 2008) and its invasion prior to the arrival of litter-consuming earthworm species that reduce litter depth and subsequently reduce *D. octaedra* abundance (Hale et al. 2005b, Holdsworth et al. 2007b). Even given the minimal impact of *D. octaedra*, it may be possible to distinguish between *D. octaedra*-invaded and earthworm-free forests based on assessment of litter quality (L. Frelich personal communication). However, this differentiation requires much experience with observation of different earthworm invasion stages. In this study, the limited sample size of points categorized in level 1 prevented me from empirically determining whether the 5-class system successfully differentiated between level 1 and level 2.

None of the metrics I investigated was a useful indicator of the presence of *Aporrectodea*. Although presence of this genus was inversely related to litter depth and positively related to A-horizon depth in both forest types, other species co-inhabiting the surface layers of mineral soil – primarily *L. rubellus* – also consume the litter layer, and increase A-horizon thickness by incorporating surface organic matter into the soil. Therefore, inferring presence of *Aporrectodea* based solely on the presence of a thick A-horizon or thin or absent litter layer may not be appropriate. Further empirical investigation of the relation between A-horizon depth and different earthworm species may allow attribution of varying A-horizon depths to particular earthworm species. A possible reason for the relative lack of statistically significant results for *Aporrectodea* – both in the 5-level and individual variable analyses – is that small sample sizes of

individual *Aporrectodea* species required me to combine all members of the genus into 1 group.

Guidelines for Implementing a Visual Earthworm Assessment Method

The 5-level method of visual assessment approximates sequential stages of earthworm invasion, as indicated by changes between levels in composition and relative dominance of different species. Further research is needed to clarify the accuracy of this method for classifying invasion within different forest types and for identifying earthworm-free locations. However, my analysis included sugar maple and aspen-birch forests, which make up a large percentage of all forested land in the northern Midwest, including 51% in Minnesota (Miles et al. 2004) and 29% in Wisconsin (Vissage et al. 2004); therefore, I suggest that the method is useful across the common hardwood forest types in the northern Midwest. I recommend use of the 5-level system in hardwood forests of this region, and I also encourage testing of this and similar visual assessment methods in other regions currently experiencing earthworm invasion (e.g., the northeastern U.S. and much of Canada).

Hot mustard extraction, electrical extraction, and hand-sifting methods for sampling earthworms are necessary in situations requiring high-precision estimates of species richness or biomass, such as in some research projects and management programs. Nonetheless, these methods may result in inaccurate population estimates under unusually dry conditions when earthworms become less active (Edwards 1991). Assessment based on visual characteristics of forest floor vegetation and soil is less

sensitive to temperature and moisture variation. The 5-level visual approach is also less time-consuming (2-5 minutes per point), allowing a large number of points to be sampled in the time it takes to sample a single point using more labor-intensive methods. Depending on management objectives, a large number of points can be sampled in a small area to provide a high-resolution picture of invasion (e.g., in forest stands or state parks and natural areas), or numerous points can be sampled across a large scale to broadly map earthworm invasion patterns (e.g., across watersheds, national forest districts, or entire national forests). Furthermore, the results from the visual assessment technique can be used to indicate locations where rigorous quantitative sampling and monitoring should be conducted.

Use of the multi-criteria visual method requires little training and no previous experience with earthworm invasions. This method can be easily adopted for use by land managers, biological technicians, and researchers, as well as by citizen science monitoring programs. Because earthworm-free and lightly invaded areas generally contain minimally altered plant assemblages, the visual method can be used for targeting botanical surveys of rare and sensitive plants. Ongoing research will determine optimal delivery techniques for the method to these different user groups (R. Hueffmeier and C. Hale *in preparation*).

Budgets for management and conservation activities are limited. At the same, time it is becoming increasingly important to clarify earthworm impacts and to identify remaining earthworm-free areas in which to target these activities. The visual assessment

tool outlined herein shows potential for providing an efficient and effective method for achieving these conservation and management objectives.

Table 4.1. Criteria used for 5-level visual system of assessing earthworm invasion at study sites along the north shore of Lake Superior, Minnesota, U.S.A.

LEVEL 1 – Earthworm-free

- 1) Forest floor and humus fully intact and layered (O₁, O_e and O_a horizons present);
- 2) Roots present in humus and leaf fragments (O_e and O_a horizons);
- 3) Forest floor coherent when picked up with intact recognizable layers;
- 4) No earthworms or earthworm sign present;
- 5) Distinct and rapid transition from forest floor to mineral soil horizon (E horizon, A horizon largely absent).

LEVEL 2 – Minimally Invaded

- 1) Humus present in patches (O_a horizon); may be slightly mixed with mineral soil, the rest of the forest floor is intact and layered (O_e and O_a horizons present);
- 2) Some roots in the forest floor, but not thick;
- 3) Small earthworms or cast material found in the forest floor;
- 4) In mineral soil, earthworm castings present or absent, but not abundant;
- 5) *Lumbricus terrestris* middens absent.

LEVEL 3 – Moderately Invaded

- 1) No or minimal forest floor present; larger, mostly intact leaves from the previous litter fall present (O₁ horizon);
- 2) Also, includes mostly intact, partially decayed leaves from >1 year present (O_e horizon);
- 3) Plant roots absent or sparse in the thin forest floor;
- 4) No humus present (O_a horizon);
- 5) Mineral soil and earthworm castings are present, but not abundant;
- 6) *L. terrestris* middens absent.

LEVEL 4 – Substantially Invaded

- 1) No forest floor; larger, mostly intact leaves from the previous litter fall present (O₁ horizon);
- 2) Also, includes mostly intact, partially decayed leaves from >1 year present (O_e horizon);
- 3) No humus present (O_a horizon);
- 4) Mineral soil and earthworm castings abundant;
- 5) *L. terrestris* middens present.

LEVEL 5 – Heavily Invaded

- 1) No forest floor; only larger, mostly intact leaves from the previous fall present (O₁ horizon);
 - 2) No small leaf fragments from >1 year present (O_e horizon);
 - 3) No humus present (O_a horizon);
 - 4) Mineral soil and earthworm castings abundant;
 - 5) *L. terrestris* middens abundant.
-

Table 4.2. Results of multivariate logistic regression models illustrating relations between visual assessment measurements and presence of *Dendrobaena octaedra* and *Aporrectodea* spp. at Wisconsin points in sugar maple forests in the Chequamegon-Nicolet National Forest and at Minnesota points in aspen-birch and sugar maple forests along the north shore of Lake Superior, U.S.A.

	<i>Dendrobaena octaedra</i>			<i>Aporrectodea</i> spp.		
	Coef.	Odds-ratio	<i>P</i>	Coef.	Odds-ratio	<i>p</i>
<i>Wisconsin points (n=271)</i>						
Intercept	-2.00	0.14	0.13	0.44	1.55	0.80
Year	0.18	1.20	0.55	0.36	1.43	0.40
Sedge cover	0.33	1.40	0.00	0.07	1.07	0.62
Maple cover	0.14	1.15	0.22	-0.23	0.80	0.12
Total ground cover	0.10	1.11	0.64	0.41	1.51	0.17
Litter cover	0.19	1.21	0.18	-0.91	0.40	0.00
Litter depth	0.17	0.84	0.07	0.09	1.09	0.50
Midden count	-0.06	0.94	0.51	0.12	1.13	0.27
<i>Minnesota points</i>						
<i>Aspen-birch (n=79)</i>						
Intercept	-3.12	0.04	0.21	-7.83	0.00	0.02
Litter depth	2.79	16.33	0.06	1.53	4.62	0.19
A-horizon depth	0.09	1.10	0.41	0.44	1.55	0.00
Litter fragmentation	0.13	1.14	0.85	-0.28	0.76	0.71
Casting index	0.59	1.79	0.48	0.28	1.32	0.81
Midden index	0.50	1.64	0.22	0.60	1.83	0.17
<i>Sugar maple (n=42)</i>						
Intercept	10.36	3.15E+04	0.06	-4.28	0.01	0.16
Litter depth	-0.58	0.56	0.17	0.40	1.49	0.28
A-horizon depth	-0.25	0.78	0.28	0.45	1.57	0.01
Litter fragmentation	-3.25	0.04	0.06	-0.24	0.78	0.83
Casting index	-0.20	0.82	0.86	-0.12	0.88	0.90
Midden index	0.91	2.49	0.28	1.06	2.89	0.06

Table 4.3. Results of multivariate logistic regression models illustrating relations between visual assessment measurements and presence of *Lumbricus rubellus* and *L. terrestris* at Wisconsin points in sugar maple forests in the Chequamegon-Nicolet National Forest and at Minnesota points in aspen-birch and sugar maple forests along the north shore of Lake Superior, U.S.A.

	<i>Lumbricus rubellus</i>			<i>Lumbricus terrestris</i>		
	Coef.	Odds-ratio	<i>P</i>	Coef.	Odds-ratio	<i>P</i>
<i>Wisconsin points (n=271)</i>						
Intercept	-3.79	0.02	0.04	-1.33	0.26	0.47
Year	0.13	1.14	0.80	0.30	1.35	0.63
Sedge cover	0.34	1.41	0.04	0.10	1.11	0.60
Maple cover	-0.34	0.71	0.05	-0.13	0.88	0.51
Total ground cover	0.74	2.10	0.04	0.13	1.14	0.73
Litter cover	-0.06	0.94	0.78	-0.39	0.68	0.12
Litter depth	-0.24	0.79	0.22	-0.54	0.58	0.06
Midden count	0.40	0.67	0.00	0.42	1.53	0.00
<i>Minnesota points</i>						
<i>Aspen-birch (n=79)</i>						
Intercept	-1.96	0.14	0.14	-40.35	0.00	1.00
Litter depth	0.54	1.71	0.53	-0.44	0.65	0.79
A-horizon depth	0.11	1.11	0.29	0.05	1.05	0.67
Litter fragmentation	-0.78	0.46	0.18	0.33	1.39	0.65
Casting index	1.32	3.73	0.10	18.23	8.26E+07	0.99
Midden index	-0.48	0.62	0.18	1.30	3.67	0.00
<i>Sugar maple (n=42)</i>						
Intercept	-2.01	0.13	0.47	-21.13	0.00	1.00
Litter depth	0.77	2.17	0.11	-8.82	0.00	0.32
A-horizon depth	0.13	1.14	0.42	-0.12	0.89	0.68
Litter fragmentation	-0.08	0.92	0.93	-1.21	0.30	0.53
Casting index	2.61	13.61	0.06	12.31	2.21E+05	0.99
Midden index	-1.31	0.27	0.09	3.64	38.02	0.03

Figure 4.1. Location of study area in eastern U.S.A. (A), locations of Minnesota study sites along the north shore of Lake Superior (B), and locations of Wisconsin study sites in the Chequamegon-Nicolet National Forest (C). Numbers in (B) refer to the following state parks: (1) Jay Cooke; (2) Gooseberry Falls; (3) Split Rock; (4) Tettegouche; (5) Crosby Manitou; (6) Temperance River; (7) Cascade River; (8) Judge C.R. Magney; (9) Grand Portage.

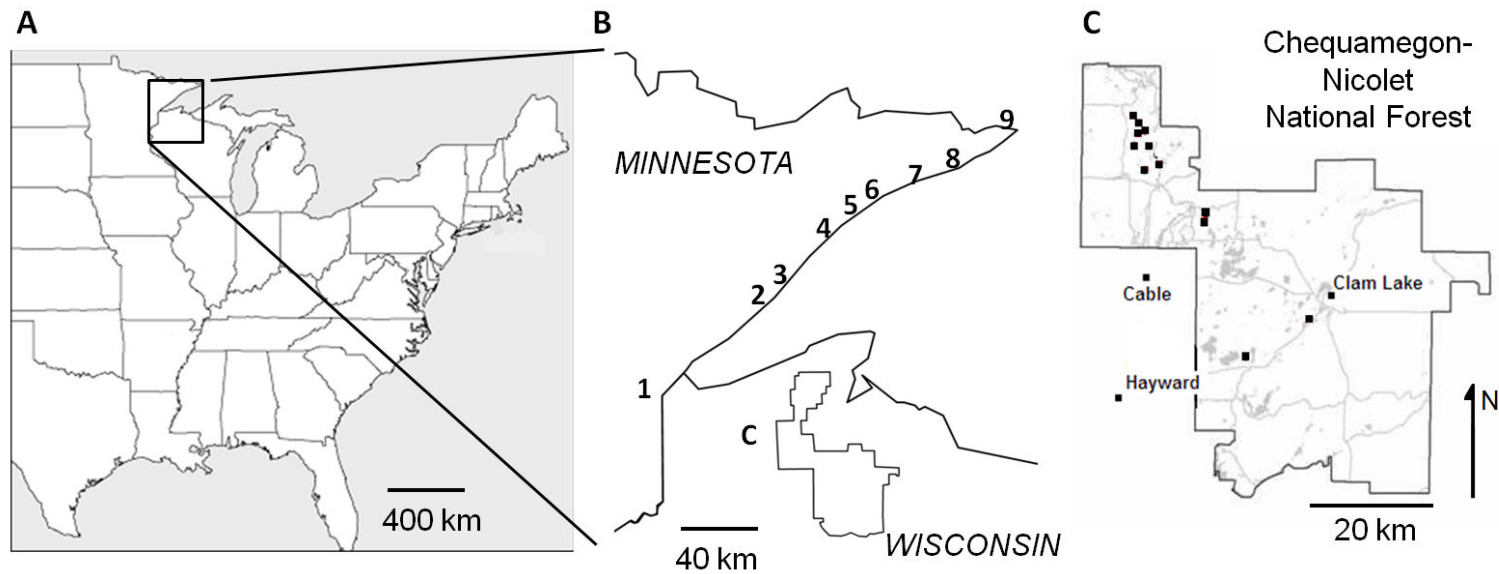
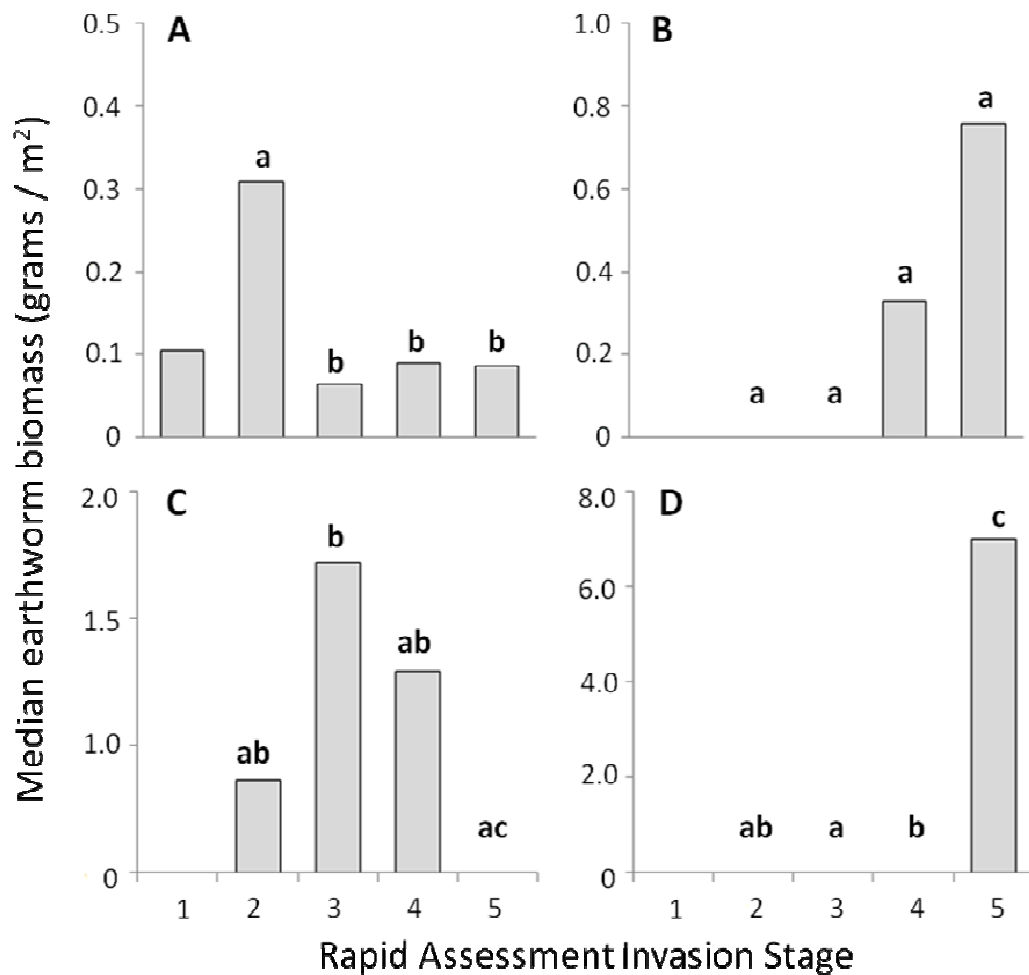


Figure 4.2. Comparison of median biomass of *Dendrobaena octaedra* (A), *Aporrectodea* (B), *Lumbricus rubellus* (C), and *L. terrestris* (D) among 5 visually classified stages of earthworm invasion along the north shore of Lake Superior, Minnesota, U.S.A. Lower-case letters indicate differences between means based on pairwise comparisons. Units on vertical axis are different for each species and invasion stage 1 was not included in pairwise comparisons due to small sample sizes in this stage.



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