Earthworm Invasions in Northern Hardwood Forests: a Rapid Assessment Method

Author(s): Scott R. Loss , Ryan M. Hueffmeier , Cindy M. Hale , George E. Host , Gerald Sjerven , Lee E. Frelich

Source: Natural Areas Journal, 33(1):21-30. 2013.
Published By: Natural Areas Association
DOI: http://dx.doi.org/10.3375/043.033.0103
URL: http://www.bioone.org/doi/full/10.3375/043.033.0103

RESEARCH ARTICLE

•

Earthworm Invasions in Northern Hardwood Forests: a Rapid Assessment Method

Scott R. Loss[1,4,5]

[1]Conservation Biology Graduate Program
University of Minnesota
1980 Folwell Avenue
St. Paul, MN 55108

Ryan M. Hueffmeier[2]
Cindy M. Hale[2]
George E. Host[2]
Gerald Sjerven[2]
Lee E. Frelich[3]

[2]Natural Resources Research Institute
University of Minnesota – Duluth
5013 Miller Trunk Hwy
Duluth, MN 55811-1442

[3]Department of Forest Resources
University of Minnesota
1530 Cleveland Ave. N
St. Paul, MN 55108

•

Natural Areas Journal 33:21–30

ABSTRACT:
Non-native earthworm invasions in north-temperate North America cause substantial adverse effects to hardwood forest ecosystems. Quantification of invasions is necessary for understanding impacts and identifying remnant earthworm-free areas, but existing sampling techniques are effort-intensive and/or environmentally damaging. We: (1) developed and applied a protocol that allows rapid classification of earthworm invasion into five stages based primarily on visual assessment of the forest floor, (2) sampled earthworms to test whether the protocol's stages can predict invasion by different species, and (3) assessed relationships between individual forest floor characteristics and presence of different earthworm species. Based on differences in biomass among points assigned to different stages, the 5-stage classification protocol accurately identified the onset of invasion by *Lumbricus rubellus* and *Lumbricus terrestris*, the species of greatest management concern in the northern Midwest. Except for middens as a predictor of *L. terrestris* presence, no forest floor variable was useful by itself for assessing invasions. The 5-stage protocol provides an efficient approach for assessing earthworm invasions in hardwood forests of the U.S. northern Midwest, can be implemented with minimal training, and serves as a blueprint for similar protocols in other regions experiencing earthworm invasions.

Index terms: earthworm sampling methods, invasive earthworms, *Lumbricus rubellus*, *Lumbricus terrestris*, northern hardwood forests

INTRODUCTION

Non-native European earthworms are invading previously earthworm-free regions of north-temperate North America, substantially changing hardwood forests (Frelich et al. 2006) and posing a major conservation concern (Sutherland et al. 2010). Invasive earthworms, particularly *Lumbricus* spp., consume organic layers, mix soil horizons (Alban and Barry 1994; Hale et al. 2005b), 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 cover of sedges and grasses (Hale et al. 2006; Holdsworth et al. 2007a). These changes can reduce abundance of salamanders (Maerz et al. 2009) and ground-nesting songbirds (Loss and Blair 2011; Loss et al. 2012).

Preventing further spread of earthworms and mitigating effects to soil, plants, and vertebrates requires identification of remnant earthworm-free natural areas and quantification of invasion across broad spatial scales. Several earthworm sampling techniques exist (reviewed by Butt and Grigoropoulou 2010), including removal and hand-sorting of the soil (Raw 1960; Coja et al. 2008), electrical extraction (Weyers et al. 2008), and liquid extraction with permanganate (Svendsen 1955), formalin (Raw 1959; Callaham and Hendrix 1997), or a mustard-water mixture (Lawrence and Bowers 2002; Hale et al. 2005b). These methods are effort-intensive, which precludes efficient sampling at a large number of sites. Some of the methods are also physically destructive or require use of environmentally toxic substances.

Mustard extraction is commonly used in studies of earthworm invasion (e.g., Kourtev et al. 1999; Cameron et al. 2007). This method is environmentally friendly and provides an accurate index of species composition and abundance (Gunn 1992; Lawrence and Bowers 2002; Eisenhauer et al. 2008), especially for the deep-burrowing *L. terrestris* (Chan and Munro 2001). However, the method requires substantial time and effort because large quantities of water must often be transported long distances into remote areas. In one ecological study, field sampling with mustard extraction at 112 points within a 25-km radius required 80 hours of fieldwork (1.4 points/hr, Loss and Blair 2011); and in another study, sampling at 36 points scattered across two national forests required 180 hours of fieldwork (0.2 points/hr, Loss et al. 2012). In addition, earthworms must be identified and measured to estimate biomass upon returning from the field. Development of a protocol that provides a more efficient means for assessing earthworm invasion will benefit conservation, management, and research that requires mapping of invasion at fine resolution or across broad spatial extents.

---

[4] Corresponding author:
LossS@si.edu: Phone: +1 202 633-1112

[5] Current address:
Migratory Bird Center, Smithsonian Conservation Biology Institute, National Zoological Park, P.O. Box 37012 MRC 5503, Washington, D.C. 20013

Earthworm invasions in the U.S. northern Midwest involve multiple species and are thought to progress through five sequential stages, with earthworm-free conditions in stage 1 and the onset of invasion by different taxa in subsequent stages (stage 2 – *Dendrobaena octaedra;* stage 3 – *Lumbricus* juveniles and *Aporrectodea* spp.; stage 4 – *L. rubellus;* stage 5 – *L. terrestris*) (Holdsworth et al. 2007b). Because invasion by additional species of earthworms compounds effects on the forest floor (Frelich et al. 2006), and because earthworm effects are highly visible, it may be possible to use forest floor characteristics (e.g., litter depth, sedge cover, and earthworm castings and middens) to identify the onset of invasion by these different species.

In hardwood forests of the U.S. northern Midwest, we: (1) developed and applied a protocol that allows rapid classification of earthworm invasion into one of five stages based primarily on visual assessment of the forest floor, (2) directly sampled earthworms to test whether the protocol's stages accurately predicted the onset of invasion by different species, and (3) assessed relationships between several forest floor measurements and presence of different earthworm species, including *L. rubellus* and *L. terrestris*, the species with the greatest impact in northern Midwest forests.

METHODS

Study Area and Point Selection

We collected data from two different study areas, one in northeast Minnesota and one in northwest Wisconsin (Figure 1). Minnesota data were collected in nine state parks along Lake Superior's north shore (47°N, 92°W to 48°N, 90°W; hereafter, "Minnesota points"). Wisconsin data were collected at bird nests in earthworm-free and invaded stands in the Chequamegon-Nicolet National Forest (46°N, 91°W; hereafter, "Wisconsin points").

Loss and Blair (2011) reported detailed selection methods for the Wisconsin points. We collected data at 271 ovenbird (*Seiurus aurocapilla*) and hermit thrush (*Catharus guttatus*) nests that were monitored in 2009 (n = 112) and 2010 (n = 159). All nests were in upland-mesic sugar maple (*Acer saccharum*) and sugar maple-basswood (*Tilia americana*) forest sites that were > 60 years old, on sandy loam or loamy sand soils, and had no timber removed in the last 40 years. Earthworm sampling confirmed that sites represented earthworm-free, partially invaded, and completely invaded forest stands (Holdsworth et al. 2007a; Loss and Blair 2011).

The nine state parks containing the Minnesota points were in the North Shore Highlands subsection of Minnesota's Ecological Classification System. We used ArcMap (version 9.3) (ESRI 2008) and a forest type data layer from the Minnesota Native Plant Community Classification (Minnesota Department of Natural Resources 2011) to locate 2000 random points. Field sampling was conducted at a random subset of 163 of these points. The number of points sampled in each park was proportional to the park's size, and the number of points sampled in each forest type was proportional to its cover on the landscape.

The 163 points represented 25 forest types consisting of different combinations of dominant, co-dominant, and sub-canopy tree species. The dominant canopy species were quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), and sugar maple. The co-dominant and sub-canopy species were 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 the analysis for Minnesota focused on points in aspen-birch and sugar maple forests, the most widespread hardwood forest types in the region.

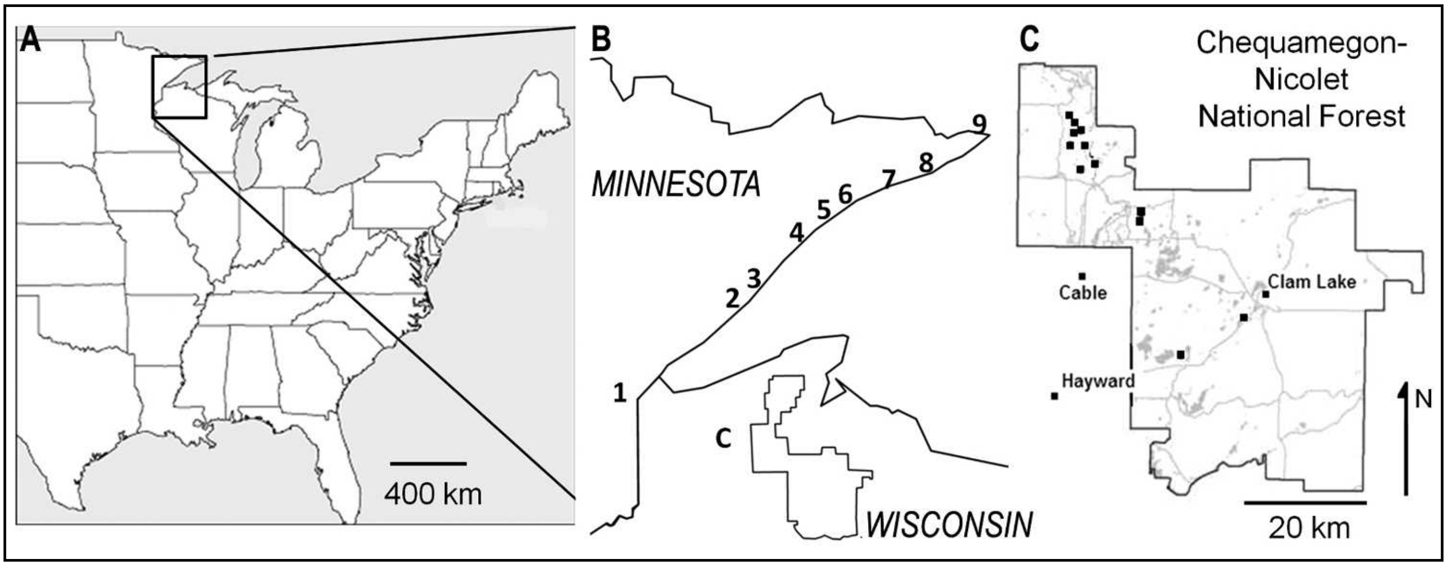

Figure 1. Study area location in the eastern U.S. (A), Minnesota study sites (numbers) along the north shore of Lake Superior (B), and Wisconsin study sites (black squares) in the Chequamegon-Nicolet National Forest (C). Numbers in (B) refer to the following state parks: (1) Jay Cooke; (2) Gooseberry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

Measurement of Forest Floor Variables

We measured the forest floor at all points, but methods and variables measured differed between Wisconsin and Minnesota points. At Wisconsin points, vegetation and the leaf litter layer were measured between 15–31 July of 2009 or 2010. Within a 2-m x 2-m square centered on each nest, we visually estimated percent cover of the litter layer, maple seedlings < 50 cm tall, all sedges and grasses combined, and total ground vegetation (all grasses, sedges, herbaceous plants, and woody plants < 50 cm tall). Cover estimates were to the nearest 10%. Average litter depth (O_i, O_e, and O_a horizons combined) was measured based on four measurements taken 1 m from the nest at each cardinal direction and by pushing a metal skewer through the litter until meeting resistance from rock or mineral soil. For litter depth and cover estimates, only intact, accumulated leaf litter > 1 year old was measured because presence and depth of the uppermost leaves from the previous autumn is independent of earthworm invasions. We also counted earthworm middens, piles of organic material at burrow entrances created by *L. terrestris* (Figure 2) (Raw 1959; Butt and Grigoropoulou 2010), within 33-cm x 33-cm sub-plots from which earthworms were directly sampled (described in later sub-section). We counted every other midden that fell roughly 50% within the sub-plot.

At Minnesota points, the forest floor was measured between 1 June–31 August 2009. Because the state parks span > 150 km from south to north and experience different timing of seasonal temperature and moisture patterns, parks were surveyed in a random order to avoid confounding effects of climate. We collected all data within a 5-m radius centered on each point. Fragmentation of the litter layer was classified into one of three categories that reflect increasing earthworm decomposition (1 – Intact, layered forest floor, O_i, O_e, and O_a horizons present; 2 – Litter layer partially fragmented, but with litter from > 1 yr; 3 – No intact litter, only freshly fallen leaves from the previous autumn). Earthworm activity was visually estimated using an earthworm casting index (1 - Castings absent; 2 - Castings present, ≤ 50% of forest floor covered; 3 - Castings abundant, > 50% of forest floor covered) (see Figure 2 for photograph of casting material) and midden index (1 - Middens absent; 2 - Middens present, ≤ 9 middens in 5-m radius; 3 - Middens abundant, ≥ 10 middens in 5-m radius).We extracted soil cores (6 cm diameter; 15 cm depth) from 3 random locations and used them to measure depth of the litter layer (O_i, O_e, and O_a horizons combined) and A-horizon. Soil textural class was determined for the mineral soil component of each core using a manual texture key adapted from Brewer and McCann (1982). Finally, we used a variable radius plot and BAF 10 wedge prism to sample tree species and estimate relative dominance (i.e., proportional representation by each tree species).

Figure 2. Earthworm casting material (a) and Lumbricus terrestris middens (b).

The 5-Stage Invasion Classification Protocol

At Minnesota points, we used a dichotomous key (Table 1) that incorporated several of the above forest floor measurements to classify points into one of five earthworm invasion stages. The stages were designed to identify the onset of invasion by different species following Holdsworth et al. (2007b) (stage 1 – potentially earthworm-free; stage 2 – *D. octaedra*; stage 3 – *Lumbricus* juveniles and *Aporrectodea* spp.; stage 4 – *L. rubellus*; stage 5 – *L. terrestris*). The dichotomous key was based on casting and midden indices, degree of litter fragmentation, and on observation of fine root presence in the O-horizon, because fine root abundance decreases following invasion (Fisk et al. 2004; Hale et al. 2005b).

Earthworm Sampling

Earthworms were sampled using the liquid-mustard extraction technique (Lawrence and Bowers 2002; Hale et al. 2005a), which consists of pouring a mustard-water mixture (40 g ground yellow mustard, 4 L water) on the soil surface and collecting all emerging earthworms. At Wisconsin points, sampling was conducted between 15 September–5 October of 2009 or 2010. At Minnesota points, sampling was conducted between 1 September–15 October 2009. This sampling timeframe corresponds to a period of soil moisture conditions favorable for earthworms and in which the population contains a high proportion of adults.

At Wisconsin points, we sampled one-third of the 2009 points (n = 36) using three 33-cm x 33-cm subplots (one at the nest, two random points ≤ 33 m from the nest) and two-thirds of points (n = 76) using one plot at the nest. Because there was no significant difference in biomass of different earthworm species between one-plot and 3-subplot points, we sampled all 2010 points with one plot at the nest (Loss and Blair 2011). At all Minnesota points, earthworms were sampled from three randomly selected 33-cm x 33-cm subplots within 5-m radius plots.

Earthworms were preserved in the field with 70% isopropyl alcohol and transferred to buffered 10% formalin for storage. We counted, identified, and measured length of earthworms using a dissecting micro-

Table 1. Dichotomous key for 5-stage rapid classification of earthworm invasion in hardwood forests of the northern Midwest. Details of measurement methods are in the text.

1. Leaf litter greater than one year present (O_i and O_e layers present).
    1a. Yes (go to 2)
    1b. No, leaf litter (O_i) from previous autumn only (go to 6)
2. Small, fragmented, relatively un-decomposed leaves greater than one year present.
    2a. Yes, O_e present (go to 3)
    2b. No, leaf litter (O_i) from previous autumn only (go to 6)
3. Intact, layered forest floor, leaves bleached and stuck together, O_i , O_e , and O_a layers present, fine plant roots in humus (O_a) and leaf fragments (O_e), no earthworms, castings, or middens present.
    3a. Yes (Stage 1 – potentially earthworm-free)
    3b. No (go to 4)
4. Layered forest floor, but leaves loose, O_i, O_e, and patches of O_a layers present. Some small earthworms and/or earthworm castings present in humus (O_a), fine plant roots present.
    4a. Yes (Stage 2)
    4b. No (go to 5)
5. Leaf litter (O_i) from previous autumn and small fragmented leaves (O_e) under intact leaves, no humus (O_a), mineral soil (A-horizon) present, earthworm casting present (≤ 50% of forest floor/mineral soil interface covered), fine plant roots absent.
    5a. Yes (Stage 3)
    5b. No (go to 6)
6. Leaf litter (O_i) from previous autumn, mineral soil (A-horizon) present, earthworm casting *abundant* (> 50% of forest floor/mineral soil interface covered), fine plant roots absent, middens absent or present (≤ 9 middens in 5-m radius).
    6a. Yes (Stage 4)
    6b. No (go to 7)
7. No forest floor (O_i or O_e), humus (O_a) or fragmented leaves present, mineral soil (A-horizon) present, earthworm casting abundant (> 50% of forest floor/mineral soil interface covered), middens abundant (> 9 middens in 5-m radius).
    7a. Yes (Stage 5)

scope. Adult earthworms were identified to species when possible, but most juvenile earthworms were only identifiable to genus. All *Aporrectodea* earthworms were grouped together, because most individuals were juveniles, and adult *A. caliginosa*, *A. longa*, *A. rosea*, *A. trapezoides*, and *A. tuberculata* are morphologically similar (Hale 2007). We used length measurements and regression equations based on allometric relationships (Hale et al. 2004) to estimate earthworm biomass.

Data Analyses

We averaged earthworm biomass (all points) and midden counts (Wisconsin points) across subplots to calculate point-level values and used tree dominance estimates to field-truth forest types at Minnesota points. The forest type at some points did not match the type indicated during point selection; therefore, for statistical analyses conducted separately by forest type, forest types were classified using field-collected dominance estimates (aspen-birch = combined dominance of all aspen and birch species ≥ 0.5; sugar maple = dominance of sugar maple ≥ 0.4). Because different forest floor assessment methods were used for Wisconsin and Minnesota points, all analyses were conducted separately for each state.

For Minnesota points, we compared earthworm biomasses among points classified into the five invasion stages. Because the distribution of biomass values was skewed with zeroes, we were unable to achieve normal distribution of the data. Biomasses were compared with one-way Kruskal-Wallace tests and pairwise comparisons between group medians using Mann-Whitney U-tests. Separate analyses were conducted for *D. octaedra, Aporrectodea* spp., *L. rubellus,* and *L. terrestris*.

Multivariate logistic regression was used to assess relationships between forest floor characteristics and presence of the four earthworm taxa noted above. For Wisconsin points, continuous independent variables were cover of sedge, maple seedlings, total ground vegetation, and leaf litter, as well as litter depth and midden count. A categorical year covariate was also included to account for temperature and moisture variation between 2009 and 2010 that could have affected earthworm sampling results. For Minnesota points, the continuous independent variables were litter depth and A-horizon depth, and the categorical variables were the litter fragmentation, casting, and midden indices. For Minnesota points, regression analyses were conducted separately for aspen-birch (n = 79) and sugar maple forests (n = 42). A preliminary analysis indicated no statistically significant relationships between soil texture and presence of different earthworm species within the above forest types, and soil texture variation was minimal within each type. Therefore, soil texture was likely not a major determinant of earthworm presence within each forest type; and to simplify regression models, we did not include this factor as a covariate.

RESULTS

Of the 271 Wisconsin points, 70 (25.8%) had no earthworms detected. All 163 Minnesota points had at least one earthworm detected; however, samples from three 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.

EFFICIENCY AND ACCURACY OF THE 5-STAGE INVASION CLASSIFICATION PROTOCOL

Characterization of the forest floor using the 5-stage classification protocol required between 5-8 minutes of sampling per point. Minnesota points were assigned to all five stages, including stage 1 (n = 4; 2.5%), stage 2 (n = 11; 6.7%), stage 3 (n = 72; 44.2%), stage 4 (n = 43; 26.4%), and stage 5 (n = 33; 20.2%). Because very few Minnesota points were classified as potentially earthworm-free, we did not include stage 1 in pairwise comparisons of biomass.

D. octaedra biomass was highest at points assigned to invasion stage 2; however, there were no statistically significant biomass differences among stages for this species (H = 5.44, df = 3, p = 0.14) (Figure 3a). *Aporrectodea* biomass was significantly different among stages (H = 8.48, df = 3, p = 0.04), with biomass significantly lower in stage 3 than in stages 4 and 5 but not different between stage 2 and 3 or among stages 2, 4, and 5 (Figure 3b). For *L. rubellus*, we found significant biomass differences among stages (H = 22.74, df = 3, p < 0.01), with biomass in stage 3 significantly greater than all other stages and significant biomass decreases in both stages 4 and 5 (Figure 3c). For *L. terrestris*, there was a significant difference among invasion stages (H = 49.40, df = 3, p < 0.01), with biomass for level 5 greater than all other levels and biomass for level 4 greater than for level 3 (Figure 3d).

Relationships between Forest Floor Variables and Earthworm Presence

We found statistically significant relationships between individual forest floor variables and presence of each earthworm taxa and for both Wisconsin and Minnesota points (see Table 2 for β-coefficients and *p*-values). For Wisconsin points, presence of *D. octaedra* was positively related to sedge cover (odds-ratio=1.40). For *Aporrectodea*, there was an inverse relationship between presence and litter cover at Wisconsin points (odds-ratio=0.40) and a positive relationship between presence and A-horizon depth for Minnesota points in both forest types (odds-ratio$_{\text{aspen-birch}}$=1.55; odds-ratio$_{\text{sugar maple}}$=1.57).

Presence of *L. rubellus* at Wisconsin points was positively related to sedge cover (odds-ratio=1.41), total ground cover (odds-ratio=2.10), and *L. terrestris* midden count (odds-ratio=1.49), and inversely related to

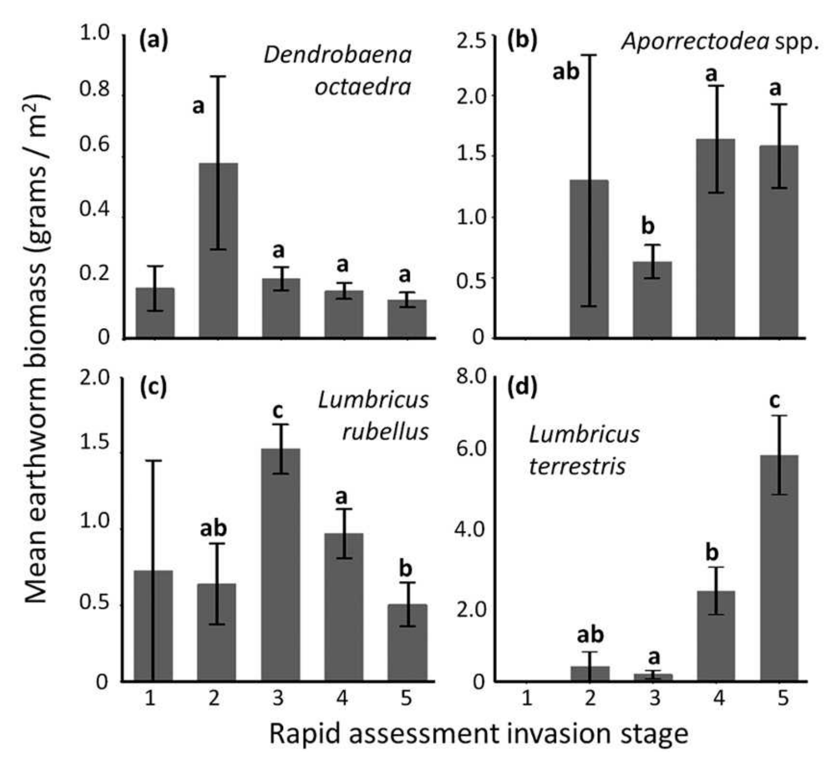

Figure 3. Mean earthworm biomass (± SE) for points classified into 5 earthworm invasion stages along the north shore of Lake Superior, Minnesota: *Dendrobaena octaedra* (a), *Aporrectodea* spp. (b), *Lumbricus rubellus* (c), and *Lumbricus terrestris* (d). Lower-case letters indicate differences among group medians based on Kruskal Wallace and Mann-Whitney U-tests. Units on vertical axis are different for each species; stage 1 was not included in pairwise comparisons due to small sample sizes.

Table 2. Results of multivariate logistic regression models illustrating relationships between forest floor variables and presence of four earthworm taxa at Wisconsin points in sugar maple forest in the Chequamegon-Nicolet National Forest and at Minnesota points in aspen-birch and sugar maple forests along the north shore of Lake Superior.

	Dendrobaena octaedra			*Aporrectodea* spp.			*Lumbricus rubellus*			*Lumbricus terrestris*		
	Coef	Odds-ratio	p	Coef	Odds-ratio	p	Coef	Odds-ratio	p	Coef	Odds-ratio	p
Wisconsin points (n = 271)												
Intercept	-2.00	0.14	0.13	0.44	1.55	0.80	-3.79	0.02	0.04	-1.33	0.26	0.47
Year	0.18	1.20	0.55	0.36	1.43	0.40	0.13	1.14	0.80	0.30	1.35	0.63
Sedge cover	0.33	1.40	<0.01	0.07	1.07	0.62	0.34	1.41	0.04	0.10	1.11	0.60
Maple cover	0.14	1.15	0.22	-0.23	0.80	0.12	-0.34	0.71	0.05	-0.13	0.88	0.51
Total ground cover	0.10	1.11	0.64	0.41	1.51	0.17	0.74	2.10	0.04	0.13	1.14	0.73
Litter cover	0.19	1.21	0.18	-0.91	0.40	<0.01	-0.06	0.94	0.78	-0.39	0.68	0.12
Litter depth	0.17	1.19	0.07	0.09	1.09	0.50	-0.24	0.79	0.22	-0.54	0.58	0.06
Midden count	-0.06	0.94	0.51	0.12	1.13	0.27	0.40	1.49	<0.01	0.42	1.53	<0.01
Minnesota points												
Aspen- birch (n = 79)												
Intercept	-3.12	0.04	0.21	-7.83	<0.01	0.02	-1.96	0.14	0.14	-40.35	<0.01	1.00
Litter depth	2.79	16.33	0.06	1.53	4.62	0.19	0.54	1.71	0.53	-0.44	0.65	0.79
A-horizon depth	0.09	1.10	0.41	0.44	1.55	<0.01	0.11	1.11	0.29	0.05	1.05	0.67
Litter fragmentation	0.13	1.14	0.85	-0.28	0.76	0.71	-0.78	0.46	0.18	0.33	1.39	0.65
Casting index	0.59	1.79	0.48	0.28	1.32	0.81	1.32	3.73	0.10	18.23	8.26E+07	0.99
Midden index	0.50	1.64	0.22	0.60	1.83	0.17	-0.48	0.62	0.18	1.30	3.67	<0.01
Sugar maple (n = 42)												
Intercept	10.36	3.15E+04	0.06	-4.28	0.01	0.16	-2.01	0.13	0.47	-21.13	<0.01	1.00
Litter depth	-0.58	0.56	0.17	0.40	1.49	0.28	0.77	2.17	0.11	-8.82	<0.01	0.32
A-horizon depth	-0.25	0.78	0.28	0.45	1.57	0.01	0.13	1.14	0.42	-0.12	0.89	0.68
Litter fragmentation	-3.25	0.04	0.06	-0.24	0.78	0.83	-0.08	0.92	0.93	-1.21	0.30	0.53
Casting index	-0.20	0.82	0.86	-0.12	0.88	0.90	2.61	13.61	0.06	12.31	2.21E+05	0.99
Midden index	0.91	2.49	0.28	1.06	2.89	0.06	-1.31	0.27	0.09	3.64	38.02	0.03

maple seedling cover (odds-ratio=0.71). At Minnesota points, there were no statistically significant predictors of *L. rubellus* presence. However, there were near-significant ($p \leq 0.10$) positive relationships between *L. rubellus* presence and casting index in both forest types, and odds-ratios for these relationships were relatively high (odds-ratio$_{\text{aspen-birch}}$=3.73; odds-ratio$_{\text{sugar maple}}$=13.61). Presence of *L. terrestris* was positively related to midden counts at Wisconsin points (odds-ratio=1.53) and to the midden index at Minnesota points, but with a much stronger relationship in sugar maple forests (odds ratio=38.02) than aspen-birch forests (odds ratio=3.67).

DISCUSSION

We found that the 5-stage classification protocol identified the onset of invasion by *L. rubellus* and *L. terrestris*, the earthworm species of greatest management concern in forests of the northern Midwest. Biomass of these species differed significantly among points assigned to different stages, with stage 3 characterized by peak *L. rubellus* invasion, and stages 4 and 5 characterized by the onset and eventual dominance, respectively, of invasion by *L. terrestris*. We also found that the presence of each earthworm taxa was significantly related to at least one forest floor variable, but, except for midden count and midden index as predictors of *L. terrestris* presence, no single variable is likely to be useful for rapid assessment of earthworm presence.

Use of the 5-stage Classification Protocol for Predicting Species Invasions

With some exceptions, the differences in sampled earthworm biomasses suggest that the 5-stage classification system identifies the sequential onset of invasion by different species and is, therefore, a useful tool for quickly quantifying earthworm invasions in hardwood forests of the northern Midwest. Holdsworth et al. (2007b) observed a predictable invasion sequence, with *D. octaedra* invading first, followed by *Aporrectodea* and *Lumbricus* juveniles, then *L. rubellus*, and finally *L. terrestris*. Different species compositions are thought to be a function of time since original invasion (Hale et al. 2005a) and rate and mechanism of dispersal (Proulx 2003; Cameron et al. 2007; Costello et al. 2010). Greater replication is needed to determine the accuracy of stages 1 and 2 of our protocol for identifying potentially earthworm-free and *D. octaedra*-invaded forests, respectively. However, even with a small sample of points assigned to stage 2 (n = 11), this stage had greater *D. octaedra* biomass than any other, suggesting the potential for the protocol to accurately identify invasion by this species. Protocol stage 3 corresponds to the onset of *L. rubellus* invasion, and stages 4 and 5 correspond to the onset and eventual dominance of *L. terrestris* invasion, respectively.

Whereas we observed that onset of *L. rubellus* invasion occurred in stage 3 of our protocol, Holdsworth et al. (2007b) first observed this species in a fourth stage, immediately following invasion by *Aporrectodea* and *Lumbricus* juveniles. However, because they observed *Lumbricus* juveniles in the third invasion stage, and because these individuals likely included *L. rubellus*, invasion by this species probably also occurred in Holdsworth et al.'s (2007b) stage 3. The observed differences in *Aporrectodea* biomass among stages were unexpected. Instead of stage 3 being characterized by the onset of *Aporrectodea* invasion, this stage had the lowest observed biomass among stages where it was present, and there were no biomass differences among the other stages. The 5-stage protocol, therefore, does not appear to diagnose onset of invasion by this group. This negative finding may have resulted from our grouping of all *Aporrectodea* spp. in statistical analyses, an approach that may have obscured unique effects of different species to the forest floor.

Relationships between Forest Floor Variables and Earthworm Presence

Our results suggest that observing the presence and abundance of middens on the forest floor is an efficient way to assess whether forests are invaded by *L. terrestris*, and, therefore, whether they have reached the late stages (4 and 5) of earthworm invasion. 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 midden index, sampling 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., accurate assessment of known *L. terrestris* presence by midden counts ≥ 1 *or* index = present or abundant) was 91% and specificity (i.e., correct assessment of known absence by counts of zero middens *or* index = absent) was 77%. Furthermore, the specificity estimate may be conservative because this deep-burrowing species likely escaped detection during mustard sampling at some points where middens were observed.

Although we found significant relationships between individual variables and presence of each earthworm taxa, no forest floor characteristic other than middens is likely to be useful by itself for rapidly assessing invasion by different species. There was a non-significant positive relationship between *L. rubellus* and casting index. However, the utility of this variable for identifying *L. rubellus* presence in the field is uncertain because several earthworm species produce casting material (Edwards and Bohlen 1996), and there is no apparent method for distinguishing among casts of different species. *Lumbricus rubellus* presence was also related to reduced cover of maple seedlings, increased sedge cover, and increased total vegetation cover, in agreement with previous research showing substantial impacts of this species on forest floor plant assemblages (Hale et al. 2006; Holdsworth et al. 2007a). However, other environmental factors also influence understory vegetation cover (e.g., deer herbivory, light availability, and soil productivity) (Powers and Nagel 2008; Reich et al. 2012). Used by themselves, these vegetation cover metrics are unlikely to be useful for predicting *L. rubellus* presence. Further research should address whether incorporation of vegetation measurements into the 5-stage rapid assessment protocol can further improve its identification of *L. rubellus* invasion.

The positive relationship between *L.*

rubellus presence and midden counts at Wisconsin points is unexpected because *L. terrestris* is the only species in the region to create middens. Possible explanations for this correlation are that high-productivity forests favor high abundance of both species or that they are introduced together. The latter explanation is supported by observations that fishing bait is a common vector of introduction for each species and that both species are often present in bait labeled as containing only one or the other species (Keller et al. 2007).

Although presence of *Aporrectodea* 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 – *L. rubellus* in particular – also consume the litter layer and increase A-horizon thickness by incorporating surface organic matter into the soil. Likewise, although *D. octaedra* presence was significantly more likely with increased sedge cover, other earthworm species and environmental factors influence this forest floor variable. Inferring presence of *Aporrectodea* based solely on the presence of a thick A-horizon or thin or absent litter layer and inferring presence of *D. octaedra* based on high sedge cover may, therefore, be inappropriate. Further investigation of relationships with A-horizon depth may allow attribution of varying A-horizon depths to particular earthworm species.

As discussed above, several environmental factors other than earthworms can lead to altered plant communities; and, furthermore, timber management activities can compress the litter layer and cause soil erosion (Yanai et al. 2000). These factors could result in false positive assessments of earthworm invasion. However, *L. rubellus* and *L. terrestris* have substantial effects on multiple aspects of the forest floor. The 5-stage protocol, which includes measurement of several variables, is less likely to result in false positive assessments than a protocol based on one or two forest floor measurements. Classification of points as earthworm-free when they are heavily invaded (i.e., false negatives) is also unlikely given earthworms' substantial effects and that other activities are unlikely to result in forest floors with un-altered soil, extensive plant cover, and a thick, intact litter layer. A limitation of the 5-stage protocol is that accurate assessment of invasion may be difficult when very few individuals of a species are present (e.g., at the invasion's leading edge). This limitation is evidenced by our observation of small numbers and very low biomass of earthworms at points that we classified as potentially earthworm-free.

Recommendations for Implementing the 5-Stage Classification Protocol

The 5-stage classification protocol will be useful across a large proportion of northern Midwest forests. Our analysis focused on sugar maple and aspen-birch forests, which make up a large percentage of forest land in the region, including 51% in Minnesota (Miles et al. 2004) and 29% in Wisconsin (Vissage et al. 2004). Other regions with invasive earthworms (e.g., the northeastern United States and much of Canada) have many of the same European earthworm species, and our protocol may also prove effective for identifying invasions in these areas. Asian earthworms (*Amynthas* spp.) are also invading portions of the eastern U.S., and where they dominate earthworm assemblages, the suite of effects to the forest floor may be different. In these cases, our protocol may be inappropriate; and we encourage development and testing of similar protocols based on assessment of forest floor characteristics.

The 5-stage assessment protocol requires no previous experience with invasive earthworms, and relatively little training. Following a short training session, the method can be easily adopted for use by land managers, biological technicians, researchers, and citizen science monitoring programs. Currently, we regularly conduct two-hour training sessions that prepare surveyors to conduct assessments quickly and independently (see: http://www.nrri.umn.edu/worms/research/IERAT.html*)*; and, in the future, online completion of training will be possible. A preliminary survey indicated that 81% of technicians who had completed training finished each earthworm survey in less than six minutes, and moreover, 90% of surveyors found the training easy to follow and critical for effectively assessing earthworm invasion (R. Hueffmeier and C. Hale, unpubl. data).

Intensive earthworm sampling methods will remain necessary for achieving high-precision estimates of species' composition and biomass. However, these methods are time-consuming and may result in inaccurate population quantification during unusually dry conditions when earthworms are less active (Edwards 1991). Classification based on forest floor characteristics is less sensitive to moisture variation than intensive sampling methods; and, therefore, our protocol can be conducted throughout the summer. The protocol also improves upon other techniques by providing an assessment of the ecological impact of earthworm invasion, rather than simply providing a list of earthworm species present. Results from the rapid assessment protocol can, therefore, be used to indicate locations where rigorous quantitative sampling and monitoring should be conducted or where land protection may be warranted. Furthermore, because earthworm-free and lightly invaded areas generally contain minimally altered plant assemblages, the rapid assessment protocol may be useful for targeting botanical surveys of rare and sensitive plant species. Depending on management objectives, the protocol allows a large number of points to 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 coarsely map invasion patterns (e.g., across watersheds, national forests, and national parks).

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 conservation and management activities. The 5-stage earthworm invasion assessment protocol that we introduce here provides an efficient and effective method for achieving these objectives in hardwood forests of the northern Midwest and a blueprint for the development of protocols in other regions experiencing earthworm invasions.

ACKNOWLEDGMENTS

Research was funded by the American Museum of Natural History, Bell Museum of Natural History, Dayton-Wilkie Foundation, The Explorers Club, Great Lakes Worm Watch, Minnesota Department of Natural Resources, Minnesota Ornithologists' Union, Natural Resources Research Institute, U.S. Forest Service – Chequamegon-Nicolet National Forest, and Wisconsin Society for Ornithology. S.R.L. was supported by a University of Minnesota Graduate School Fellowship and an NSF IGERT grant: Risk Analysis for Introduced Species and Genotypes (NSF DGE-0653827). R.M.H. was supported in part under the Coastal Zone Management Act, by NOAA's Office of Ocean and Coastal Resource Management, in cooperation with Minnesota's Lake Superior Coastal Program, and also by the Legislative-Citizen Commission on Minnesota Resources. We thank C. Hakseth, L. Lambert, J. Mulligan, M. Sharrow, S.S. Loss, L. Olson, E. Wartman, A. Alness, N. Vander Heiden, M. Hueffmeier, E. Feichtinger, Z. Toland, C. Wright, K. Jeager, J. Johnson and the Minnesota Conservation Corps for field assistance. We also thank R.B. Blair, D.E. Andersen, P. Bolstad, H. Hansen, A. Holdsworth, and B. Sietz for guidance, L. Parker and M. Brzeskiewicz for logistical support, and the Cable Natural History Museum and Minnesota North Shore State Parks for housing and office.

Scott Loss completed his Ph.D. degree in the University of Minnesota's Conservation Biology Graduate Program where he studied effects of invasive earthworms on forest songbirds. He is currently a postdoctoral fellow at the Smithsonian Conservation Biology Institute's Migratory Bird Center where he investigates how birds are impacted by direct sources of anthropogenic mortality, including collisions with man-made structures and cat predation.

Ryan Hueffmeier is a junior scientist at the University of Minnesota Duluth's Natural Resources Research Institute where he is program coordinator for Great Lakes Worm Watch. He specializes in providing the tools and resources for citizen scientists to contribute to the development of a database that documents the distribution and impacts of invasive earthworms in the northern Midwest.

Cindy Hale is a research associate at the University of Minnesota Duluth's Natural Resources Research Institute where she directs the Great Lakes Worm Watch and Sustainable Agriculture Programs. She conducts research and education related to forest and ecosystems ecology, plant communities, and soil dynamics, with an emphasis on the impacts of invasive earthworm species.

George Host is a Landscape Ecologist at the University of Minnesota Duluth's Natural Resources Research Institute; he also directs NRRI's Geographic Information System laboratory. His current research includes application of GIS-based spatial models for landscape management, interactions between forest and aquatic systems, and environmental stressor assessments.

Gerald Sjerven is a geographic information technology professional at the University of Minnesota Duluth's Natural Resources Research Institute and is currently on the Minnesota Statewide Geospatial Advisory Council and the Minnesota GIS/LIS Consortium Board of Directors. His research interest is Geographic Information Systems in the Great Lakes region with a focus on GIS coordination in Minnesota.

Lee Frelich is Director of the University of Minnesota Center for Forest Ecology in St. Paul, where he teaches courses in landscape ecology and disturbance ecology. Current research interests include effects of invasive species, large herbivores, large-scale wind and fire, and climate change on temperate and boreal forests.

LITERATURE CITED

Alban, D.H., and E.C. Berry. 1994. Effects of earthworm invasion on morphology, carbon, and nitrogen of a forest soil. Applied Soil Ecology 1:243-249.

Brewer, R., and M.T. McCann. 1982. Laboratory and Field Methods in Ecology. Saunders College Publishing, Philadelphia, Pa.

Burtelow, A.E., P.J. Bohlen, and P.M. Groffman. 1998. Influence of exotic earthworm invasion on soil organic matter, microbial biomass, and denitrification potential in forest soils of the northeastern United States. Applied Soil Ecology 9:197-202.

Butt, K.R., and N. Grigoropoulou. 2010. Basic research tools for earthworm ecology. Applied and Environmental Soil Science 2010:1-12.

Callaham, M.A., and P.F. Hendrix. 1997. Relative abundance and seasonal activity of earthworms (Lumbricidae and megascolecidae) as determined by hand-sorting and formalin extraction in forest soils on the southern Appalachian Piedmont. Soil Biology and Biochemistry 29:317-321.

Cameron, E.K., E.M. Bayne, and M.J. Clapperton. 2007. Human-facilitated invasion of exotic earthworms into northern boreal forests. Ecoscience 14:482-490.

Chan, K-Y., and K. Munro. 2001. Evaluating mustard extracts for earthworm sampling. Pedobiologia 45:272-278.

Coja, T., K. Zehetner, A. Bruckner, A. Watzinger, and E. Meyer. 2008. Efficacy and side effects of five sampling methods for soil earthworms (Annelida, Lumbricidae). Ecotoxicology and Environmental Safety 71:552-565.

Costello, D.M., and G.A. Lamberti. 2008. Non-native earthworms in riparian soils increase nitrogen flux into adjacent aquatic ecosystems. Oecologia 158:499-510.

Costello, D.M., S.D. Tiegs, and G.A. Lamberti. 2010. Do non-native earthworms in southeast Alaska use streams as invasional corridors in watersheds harvested for timber? Biological Invasions 13:177-187.

Edwards, C.A. 1991. The assessment of populations of soil-inhabiting invertebrates. Agriculture, Ecosystems and Environment 34:145-176.

Edwards, C.A., and P.J. Bohlen. 1996. Biology and Ecology of Earthworms. Chapman and Hall Publishing, London.

Eisenhauer, N., D. Straube, and S. Scheu. 2008. Efficiency of two widespread non-destructive extraction methods under dry soil conditions for different ecological earthworm groups. European Journal of Soil Biology 44:141-145.

[ESRI] Environmental Systems Research Institute. 2008. ArcMap 9.3. ESRI, Redlands, Calif.

Fisk, M.C., T.J. Fahey, P.M. Groffman, and P.J. Bohlen. 2004. Earthworm invasion, fine-root distributions, and soil respiration in north temperate forests. Ecosystems 7:55-62.

Frelich, L.E., C.M. Hale, S. Scheu, A.R. Holdsworth, L. Heneghan, P.J. Bohlen, and P.B. Reich. 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biological Invasions 8:1235-1245.

Gundale, M.J. 2002. Influence of exotic earthworms on the soil organic horizon and the rare fern *Botrychium mormo*. Conservation Biology 16:1555-1561.

Gunn, A. 1992. The use of mustard to estimate earthworm populations. Pedobiologia 36:65-76.

Hale, C. 2007. Earthworms of the Great Lakes. Kollath and Stensaas Publishing, Duluth, Minn.

Hale, C.M., L.E. Frelich, and P.B. Reich. 2006. Changes in hardwood forest understory plant communities in response to European earthworm invasions. Ecology 87:1637-1649.

Hale, C.M., L.E. Frelich, and P.B. Reich. 2005a. Exotic European earthworm invasion dynamics in northern hardwood forests of Minnesota, USA. Ecological Applications 15:848-860.

Hale, C.M., L.E. Frelich, P.B. Reich, and J. Pastor. 2005b. Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. Ecosystems 8:911-927.

Hale, C.M., P.B. Reich, and L.E. Frelich. 2004. Allometric equations for estimation of ash-free dry mass from length measurements for selected European earthworm species (Lumbricidae) in the western Great Lakes region. American Midland Naturalist 151:179-185.

Holdsworth, A.R., L.E. Frelich, and P.B. Reich. 2007a. Effects of earthworm invasion on plant species richness in northern hardwood forests. Conservation Biology 21:997-1008.

Holdsworth, A.R., L.E. Frelich, and P.B. Reich. 2007b. Regional extent of an ecosystem engineer: earthworm invasion in northern hardwood forests. Ecological Applications 17:1666-1677.

Keller, R.P., A.N. Cox, C. Van Loon, D.M. Lodge, L.M. Herborg, and J. Rothlisberger. 2007. From bait shops to the forest floor: earthworm use and disposal by anglers. American Midland Naturalist 158:321-328.

Kourtev, P.S., W.Z. Huang, and J.G. Ehrenfield. 1999. Differences in earthworm densities and nitrogen dynamics in soils under exotic and native plant species. Biological Invasions 1:237-245.

Lawrence, A.P., and M.A. Bowers. 2002. A test of the “hot” mustard extraction method of sampling earthworms. Soil Biology and Biochemistry 34:549-552.

Loss, S.R., and R.B. Blair. 2011. Reduced density and nest survival of ground-nesting songbirds relative to earthworm invasions in northern hardwood forests. Conservation Biology 5:983-993.

Loss, S.R., G.J. Niemi, and R.B. Blair 2012. Invasions of non-native earthworms related to population declines of ground-nesting songbirds across a regional extent in northern hardwood forests of North America. Landscape Ecology: in press. DOI 10.1007/s10980-012-9717-4.

Maerz, J.C., V.A. Nuzzo, and B. Blossey. 2009. Declines in woodland salamander abundance associated with non-native earthworm and plant invasions. Conservation Biology 23:975-981.

Miles, P.D., G.J. Brand, and M.E. Mielke. 2004. Minnesota’s Forest Resources 2004. Resource Bulletin NC-262, U.S. Department of Agriculture, Forest Service, North Central Research Station, St. Paul, Minn.

Minnesota Department of Natural Resources. Native Plant Community Classification. Available online <http://www.dnr.state.mn.us/npc/classification.html>. Accessed 2-28-2012.

Powers, M.D., and L.M. Nagel. 2008. Disturbance dynamics influence *Carex pennsylvanica* abundance in a northern hardwood forest. Journal of the Torrey Botanical Society 135:317-327.

Proulx, N. 2003. Ecological risk assessment of non-indigenous earthworm species. Prepared for U.S. Fish and Wildlife Service, International Affairs, Division of Scientific Authority by Minnesota Department of Natural Resources, St. Paul.

Raw, F. 1959. Estimating earthworm populations by using formalin. Nature 184:1661-1662.

Raw, F. 1960. Earthworm population studies: a comparison of sampling methods. Nature 187:257.

Reich, P.B., L.E. Frelich, R.A. Voldseth, P. Bakken, and C. Adair. 2012. Understorey diversity in southern boreal forests is regulated by productivity and its indirect impacts on resource availability and heterogeneity. Journal of Ecology 100:539-545.

Sutherland, W.J., S. Barsely, L. Bennun, M. Clout, I.M. Cote, M.H. Depledge, L.V. Dicks, A.P. Dobson, L. Fellman, E. Fleishman, D.W. Gibbons, A.J. Impey, J.H. Lawton, F. Lickorish, D.B. Lindenmayer, T.E. Lovejoy, R. Mac Nally, J. Madgwick, L.S. Peck, J. Pretty, S.V. Prior, K.H. Redford, J.P.W. Scharlemann, M. Spalding, and A.R. Watkinson. 2010. Horizon scan of global conservation issues for 2011. Trends in Ecology and Evolution 26:10-16.

Svendsen, J.A. 1955. Earthworm population studies: a comparison of sampling methods. Nature 175:864.

Vissage, J.S., G.J. Brand, and M.E. Mielke. 2004. Wisconsin’s Forest Resources in 2004. Resource Bulletin NC-237, U.S. Department of Agriculture, Forest Service, North Central Research Station, St. Paul, Minn.

Weyers, S.I., H.H. Schomberg, P.F. Hendrix, K.A. Spokas, and D.M. Endale. 2008. Construction of an electrical device for sampling earthworm populations in the field. Applied Engineering in Agriculture 24:391-397.

Yanai, R.D., M.A. Arthur, T.G. Siccama, and C.A. Federer. 2000. Challenges of measuring forest floor organic matter dynamics: repeated measures from a chronosequence. Forest Ecology and Management 138:273-283.