

## EXOTIC EUROPEAN EARTHWORM INVASION DYNAMICS IN NORTHERN HARDWOOD FORESTS OF MINNESOTA, USA

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**Abstract.** European earthworms are invading previously worm-free hardwood forests across Minnesota and the Great Lakes region. In many of these forests, earthworm invasions have been associated with the loss of a previously thick forest floor. The ability of earthworms to alter and control ecosystem processes has been demonstrated in agricultural systems, but the dynamics and impact of these invasions in native forest ecosystems is largely unknown. The impacts of earthworm invasion are expected to be related to the size and species composition of the earthworm population because different species have different habitat and feeding preferences. We identified four sugar maple dominated forests in north central Minnesota in the Chippewa National Forest with active earthworm invasion. In each site a sample grid of 45 points (30 × 150 m) 10 m apart in three parallel transects with 15 points each was established that spanned a visible leading edge of invasion. Over four years earthworm populations and forest floor thickness were sampled across all transects, thus providing both a space-for-time assessment of decadal scale successional dynamics and a four-year window into shorter time changes. We found a succession of earthworm species across the visible leading edge due to different patterns of colonization by different earthworm species. Marked increases in space and time in earthworm biomass were associated with the development of discrete transition zones where forest floor thickness decreases to zero in as little as 75 m from areas that have forest floor layers up to 10 cm thick with advancement of the visible leading edge of up to 30 m in four years at three of the study sites. The epi-endogeic species *Lumbricus rubellus* led to the most rapid removal of forest floor material during initial invasion. Epigeic and epi-endogeic species of earthworms may facilitate the establishment of other species of earthworms leading to the establishment of stable populations of endogeic and anecic species, which prevent recovery of the forest floor.

**Key words:** *Acer saccharum*; earthworm invasion; ecosystem change; exotic species invasion; forest floor; *Lumbricidae*; northern hardwood forests.

### INTRODUCTION

European earthworm invasions have been occurring throughout North America since European settlement (Gates 1976, 1982, Reynolds 1994, James 1995). These invasions could be particularly influential in the many ecosystems in Canada and the northern tier of the United States that have developed in the absence of earthworms (Gates 1977, James 1998). Earthworms have long been recognized as a group of organisms that can exert ecosystem-level control due to their effects on litter decomposition and nutrient dynamics (Lavelle et al. 1997). Because they are powerful detritivores that eat great quantities of leaf litter (Nielsen and Hole 1964), the potential exists for large impacts to occur when European earthworms invade.

Invasions of European earthworms in the Great Lakes region have been in progress since European settlement over a century ago, but have become more numerous during the last few decades due to the widespread use of European earthworms as bait for the recreational fishing industry (Gates 1982, Alban and Berry 1994). Because forested areas in the Great Lakes region contain many lakes used for fishing (>40 000 lakes in Minnesota, Wisconsin, Michigan, and western Ontario), there are many centers of infestation at this time. In Minnesota, it appears that nearly all forests have been invaded in the southern part of the state, where human activity has been most intense for the longest period of time (Reynolds et al. 2002; C. M. Hale, *personal observation*). In contrast, in northern Minnesota, European earthworm invasions in many hardwood forests are recent or just beginning.

Evidence of incipient earthworm invasions in northern Minnesota in the form of leading edges were first noted in a few isolated hardwood stands 10–15 years ago (Mortensen and Mortensen 1998). In many stands there exist discrete transition zones where forest floor

Manuscript received 27 October 2003; revised and accepted 7 July 2004; final version received 16 September 2004. Corresponding Editor: J. Gullledge.

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thickness decreases to zero in as little as 75 m from areas that have forest floor layers up to 10 cm thick (C. M. Hale, *personal observation*). Preliminary investigations indicated a negative correlation between earthworm abundance and forest floor thickness (C. M. Hale, *unpublished data*). The existence of these “leading edges” in northern Minnesota provided a rare opportunity to document the dynamics of incipient earthworm invasions.

In the case of earthworms one generally notes a suite of species invading, each with different habitat and feeding preferences (Smith 1928, Stebbings 1962, Alban and Berry 1994, Bohlen et al. 2004a). Three broad ecological groups have been described (Bouché 1977) including epigeic species, which live and feed exclusively in the litter layer; endogeic species, which live and feed in the mineral soil horizon; and anecic species, which burrow deeply but feed primarily on fresh surface litter. The feeding and habitat preferences of these groups suggest that they may invade ecosystems differently resulting in different spatial distributions during initial invasion (James 1998) and different species assemblages once stable reproducing populations have been established (Hendrix et al. 1999). Additionally, each of these groups of earthworms has different potential to remove the forest floor and is likely to have different impacts on the ecosystem as a whole.

The invasion of North American temperate hardwood forests by European earthworms constitutes the first documented invasion by a group of detritivores (Mooney and Drake 1986, Williamson 1996, Shigesada and Kawasaki 1997) and as such, there are no generalizations that can be made as to the probable outcomes based on current invasion theory. However, the fundamental role that detritivores play in ecosystems and the unique ability of earthworms to rapidly remove surface litter suggests that the impacts could be great. The introduction of powerful detritivores, such as earthworms, where they did not previously exist, results in a shift of ecosystem control from the forest floor community to the invasive detritivore. The ability of earthworms to exert this level of ecosystem control has been demonstrated in other ecosystems (Edwards et al. 1995).

Between 1998 and 2001 a study was conducted to detail earthworm community compositions associated with leading edges in four northern hardwood forests in the vicinity of Leech Lake on the Chippewa National Forest, Minnesota, USA. We hypothesized that (1) the pattern of colonization by different earthworm species would result in different spatial patterns across the leading edge and (2) that there would be species specific effects on forest floor removal and the development of leading edges.

We expected the strictly epigeic species *Dendrobaena octaedra* to be the most common species present in advance of the visible leading edge and exhibit the widest distribution within and among the sites because

it is well adapted to live and feed in thick organic horizons and is reported to be parthenogenetic (Gates 1974). However, *D. octaedra* is expected to have little direct effect on forest floor removal because even at large numbers, this small bodied species has relatively low total biomass and may feed more directly on microorganisms such as fungi (Scheu and Parkinson 1994). *Lumbricus rubellus*, an epi-endogeic species, is also expected to be widely distributed, but to have a large effect on forest floor removal and development of the leading edge because this moderately sized species will have higher total biomass and has a more generalized feeding pattern (Hendrix et al. 1999). Endogeic earthworms, such as *Aporrectodea* species, are expected to be restricted to areas where the forest floor has been largely removed and incorporated into the mineral soil horizon, because they have been shown to feed on soil organic matter as opposed to surface litter (Hendrix et al. 1999). *Lumbricus terrestris*, a very large bodied anecic species, prefers to feed on fresh surface litter (Bouché 1977). While *Lumbricus terrestris* may burrow through a thick organic horizon to access this fresh litter it does not appear to directly feed on the forest floor until all surface litter has been removed (C. M. Hale, *personal observations*). Therefore, *L. terrestris* is not expected to readily invade areas with intact forest floor. However, following removal of the forest floor it is expected to dominate due to its large body size and high total biomass.

## METHODS

### *Study sites and the forest floor leading edge*

The study was conducted over four years (1998–2001) in four mature northern hardwood stands located on the Chippewa National Forest of northern Minnesota (Fig. 1). Four study sites were selected that contained visible leading edges of earthworm invasion radiating from nearby lakeshores and had similar overstory composition, topography, soils, and stand history. Three sites were identified and initially sampled in 1998 (Blackduck, Ottertail, Section 19). A fourth site (Two Points) was later identified and all four sites were sampled during 1999, 2000, and 2001. Sugar maple (*Acer saccharum*, Marshall) is the dominant tree species on the study sites, and secondary species include yellow birch (*Betula alleghaniensis*, Britton), paper birch (*Betula papyrifera*, Marshall), and basswood (*Tilia americana*, Linnaeus). Ironwood (*Ostrya virginiana*, K. Koch) and American elm (*Ulmus americana*, Linnaeus) are common in the subcanopy and sapling layers. The topography in all sites is level to slightly rolling, and the soil is a deep, well-drained and light colored Eutroboralf (Warba series) associated with the Guthrie Till Plain (USDA 1997) that in the absence of earthworms is characterized by a thick forest floor composed of O<sub>1</sub>, O<sub>2</sub>, and O<sub>3</sub> layers. The climate is humid, continental, cold temperate with 65 cm mean annual precipitation.

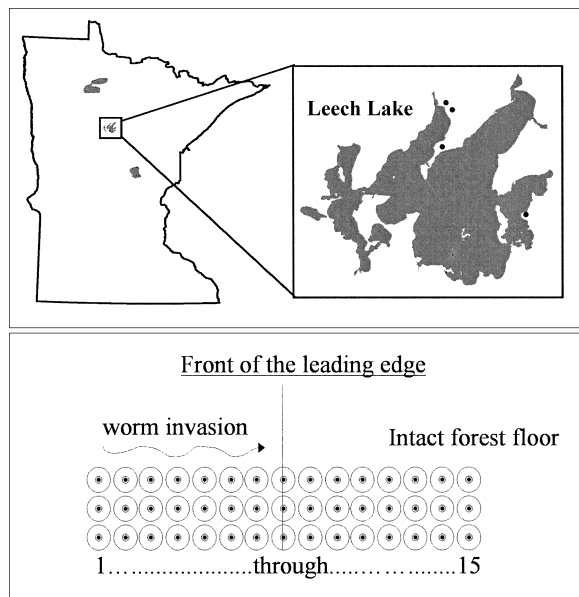


FIG. 1. Locations of four study sites in the Chippewa National Forest near Leech Lake in north central Minnesota, USA, and an illustration of the sample grid ( $30 \times 150$  m) of 45 points laid perpendicular to the leading edge of earthworm invasion in each study site. Sample points are 10 m apart. Earthworm populations were sampled in a randomly located  $35 \times 35$  cm area ( $0.12 \text{ m}^2$ ) 2 m from each sample point. Soil cores 6 cm in diameter and 15 cm deep were collected at randomly selected locations 1 m from each sample point.

The median growing season is 134 days with snow cover from late November to early April. Normal mean temperatures in January and July are  $-15^\circ$  and  $20^\circ\text{C}$ , respectively (Minnesota State Climatology Office 2003).

For the purpose of site selection and sample point placement, a leading edge was defined as a discrete area in the forest where a transition from a thick forest floor to thin forest floor took place within 75–100 m and large patches of bare mineral soil with obvious evidence of earthworm activity were present. At each site, a  $30 \times 150$  m sample grid was established, consisting of 45 sample points 10 m apart in three parallel transects with 15 points each (Fig. 1). Transects were placed perpendicular to the leading edge with sample point 8 located where the forest floor first dropped to zero, the approximate front of the leading edge. Thus, a well-developed forest floor layer existed in front of the leading edge (sample points 10–15) and exposed mineral soil existed behind the leading edge (sample point 1).

In 1999–2001, forest floor thickness was measured at all sites in later summer before leaf fall. Total thickness ( $O_i + O_e + O_a$ ) was measured 1 m from the center of the sample point at a randomly selected cardinal direction. A different cardinal direction was used in each subsequent year. In 1999 and 2000 forest floor thickness was measured in soil cores 6 cm in diameter

and 15 cm deep. In 1999, soil cores were collected adjacent to all odd numbered points and sample point 8 in each site ( $N = 27$  per site). In 2000, soil cores were collected adjacent to all points in each site ( $N = 45$  per site). In 2001, forest floor thickness was measured by using a hand trowel to penetrate and expose the forest floor horizon at each point ( $N = 45$  per site). Under worm-free conditions, the forest floor and mineral soil layers are completely separate. Persistence of dead coarse root material in the forest floor as it was being transformed and consumed by earthworms largely preserved this boundary, facilitating consistent measurement of the forest floor layer.

#### Earthworm sampling

The earthworm populations were censused using a combination of liquid extraction and hand sifting methods (Bouché and Gardner 1984, Lawrence and Bowers 2002). Sampling of earthworm populations was conducted during September and October of each year because at that time soil moisture had recovered from the normal mid-summer dry period and earthworms were active with a relatively high proportion of sexually mature individuals that are easier to identify than juveniles. All earthworms collected were killed in 70% isopropyl alcohol and preserved in 10% formalin for identification in the laboratory. Schwert (1990) and Reynolds (1977) were used as authorities for earthworm species identification. Regression models yielding ash-free dry (AFD) earthworm biomass from the measured length of preserved specimens were determined for each species (Hale et al. 2004). These allometric relationships were used to estimate earthworm biomass of all preserved specimens for the study.

Liquid extraction samples were conducted in  $0.12\text{-m}^2$  ( $35 \times 35$  cm) subplots located 2.0 m from each sample point center at a randomly selected cardinal direction. A different cardinal direction was used in each subsequent year. The liquid extraction solution used was 40 g of ground yellow mustard to 4 L of water. In 1998, liquid extraction sampling was conducted in three sites (Blackduck, Ottertail, Section 19) at a subset of sample points including 1, 8, and 15 in all transects and also points 5–7 and 9–11 in transect B ( $N = 15$  in each site). In 1999, 2000, and 2001, liquid extractions were conducted at all 45 sample points in all four study sites, except that only roughly half the sample points were censused in the Two Points site in 1999 and 2001.

Earthworm populations in a subset of sample points were censused using hand sifting as a control to ensure that the results from liquid extractions were representative. Hand sifted samples were conducted in  $0.12\text{-m}^2$  ( $35 \times 35$  cm) subplots excavated to 30 cm depth. The subplots were located 4.0 m from sample point center adjacent to points 1, 8, and 15 at a randomly selected cardinal direction. All soil and forest floor material from each pit was sifted to collect all worms present.

TABLE 1. Taxonomic grouping of earthworm species used in all analyses.

Taxonomic group	Ecological group	Species included
<i>Dendrobaena</i>	epigeic	<i>Dendrobaena octaedra</i> , <i>Dendrodrilus rubidus</i>
<i>L. rubellus</i> (adults)	epi-endogeic	<i>Lumbricus rubellus</i> (adults)
<i>L. juveniles</i>	epi-endogeic/anecic	<i>Lumbricus juveniles</i>
<i>Aporrectodea</i>	endogeic	<i>A. caliginosa</i> , <i>A. tuberculata</i> , <i>A. trapezoides</i> , <i>A. rosea</i>
<i>Octolasion</i>	endogeic	<i>Octolasion tyrtaeum</i>
<i>L. terrestris</i> (adults)	anecic	<i>Lumbricus terrestris</i> (adults)

Hand sampling of earthworm populations was conducted at Ottertail in 1999 and at all four sites in 2000.

For the purposes of all analysis, closely related earthworm species with similar habitat and feeding preferences were combined into taxonomic groups (Table 1). These taxonomic groups are similar to the ecological groups described by Bouché and Gardner (1984) and Reynolds (1977), with some specific exceptions. *Dendrobaena octaedra* and *Dendrodrilus rubidus* are two very small bodied, pigmented litter dwelling species. *Dendrodrilus rubidus* was a rare species detected in two of the four sites with very low numbers and biomass relative to *Dendrobaena octaedra* so the two were combined into the taxonomic group “*Dendrobaena*.” It is impossible to separate juvenile *Lumbricus rubellus* and *Lumbricus terrestris*, and these specimens are treated as a separate juvenile group called “*Lumbricus juveniles*.” *L. rubellus* (adults) merits a unique category since it is considered an epigeic species by Bouché (1977) but has a much greater ability to consume and mix litter into the upper soil horizon than the other epigeic species (Gundale 2002). *L. terrestris* (adult), the sole anecic species, also merits a unique taxonomic group. A suite of endogeic *Aporrectodea* species including *A. caliginosa*, *A. tuberculata*, *A. trapezoides* and *A. rosea* were combined into the group “*Aporrectodea*.” *Octolasion tyrtaeum* is generally described as an endogeic species, similar to the *Aporrectodea* species, but it was found in only one of the four study sites and in conjunction with a full complement of *Aporrectodea* species. Therefore, *O. tyrtaeum* was treated separately in all analyses.

Many earthworm species enter a state of aestivation under hot and dry soil conditions, which can lead to unrepresentative sampling of earthworm populations. Seasonal moisture deficits (July through September) were calculated (SMD = precipitation – evapotranspiration) in each year of the study. Precipitation and evapotranspiration data were obtained from the State Climatology Office, Minnesota Department of Natural Resources—Division of Waters. Evapotranspiration was estimated by a modification of the Blaney-Criddle procedure (USDA 1967).

#### Analytical methods

For purposes of statistical analysis, earthworm biomass data were log-transformed. A log-transformation

technique described in McCune and Grace (2002) was used. The transformed value ( $b_{ij}$ ) equals

$$b_{ij} = \log_{10}(x_{ij} + d) - c$$

where  $c$  = integer of ( $\log_{10}(\min(x))$ ),  $\min(x)$  = lowest nonzero  $x$ , and  $d$  = inverse  $\log_{10}(c)$ .

This transformation technique was appropriate for this data set because it preserves both zero values and differences in magnitude that exist in the data set.

Total earthworm biomass values from hand samples and liquid samples were compared using paired  $t$  tests for each sample point position across the sample grid, in each site and year where both were collected (SAS Institute 2001). In 15 comparisons, there were no significant differences in mean earthworm biomass between the hand samples and the liquid extraction samples ( $P \geq 0.1$  in four cases,  $P \geq 0.2$  in four cases, and  $P \geq 0.45$  in seven cases). Overall, total earthworm biomass measures from hand sampling and liquid sampling were well correlated (Fig. 2). For all earthworm species groups except *Aporrectodea*, liquid extraction sampling often resulted in higher biomass values than did hand sampling (Fig. 2). Therefore, only the earthworm biomass data collected by liquid extraction were used in subsequent analysis.

The statistical significance of spatial patterns in forest floor thickness and earthworm species biomass across the sample grid were tested using the nonparametric Mantel test in PC-ORD (McCune and Mefford 1999). The Mantel tests the significance of the correlation between two distance matrices by evaluating results from repeated randomizations, where the order of the rows and columns in one of the distance matrices is shuffled (McCune and Grace 2002). If randomizations frequently result in correlations between matrices that are as strong as the correlation with the original data, then we have little confidence that the correlation differs from zero. In this case, forest floor thickness and earthworm biomass distance matrices (Sorensen distance measure) were compared to a geographic distance matrix representing sample point location in the sample grid (Urban et al. 2002) using 1000 randomized runs. The standardized Mantel statistic ( $r$ ) is calculated as the usual Pearson correlation coefficient between the two matrices and is used as a measure of the strength of the relationship between the two matrices. The significance of the relationship is calculated by comparing

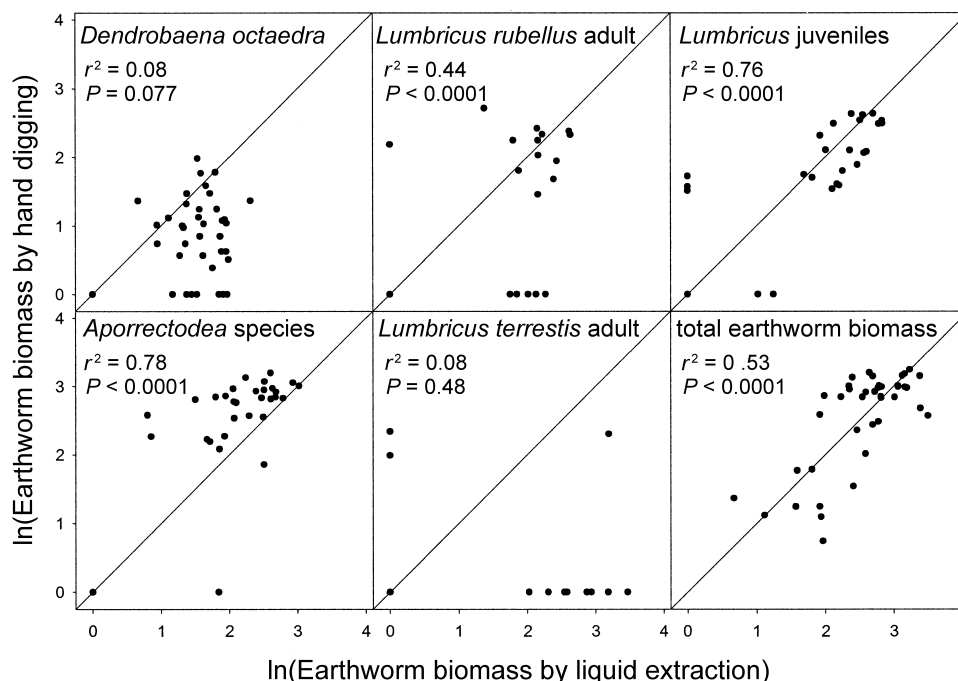


FIG. 2. Correlations of earthworm biomass measures collected using hand sampling and liquid extraction ( $N = 40$ ). The solid line is a 1:1 reference line.

the Mantel Z statistic of the nonrandomized data to the distribution of the Z statistic from the shuffled matrix. A  $P$  value is calculated from the number of randomizations that yielded a test statistic equal to or more extreme than the observed value.

To test for earthworm species effects beyond those associated with increasing biomass, a combination of cluster analyses and indicator species analyses in PC-ORD were used to identify unique earthworm species assemblage groups (McCune and Mefford 1999). Independent analyses were conducted for data from 1999 and 2000. For each year, earthworm sample points from all four study sites were sorted into species assemblage groups by the group average linkage method (a hierarchical, polythetic, agglomerative clustering method) as described by McCune and Grace (2002), using Sørensen distance measures. Six potential earthworm species assemblage groups were initially identified in the cluster analysis process. Then indicator species analysis (McCune and Mefford 1999) was used to select a subset of groups that had unique species assemblages by identifying which subset of groups had both the lowest average  $P$  values and the most significant indicators (Dufrene and Legendre 1997). Monte Carlo randomization tests (1000 runs) were used to assess the significance of indicator values for each species in each potential subset of earthworm assemblage groups.

A subset of sample points for which data existed in both 1999 and 2000 was used to assess the relative effects of earthworm species assemblages and total biomass on changes in forest floor thickness. Using the

1999 species assemblage group designations, ANOVA and paired  $t$  tests were used to test for differences in the mean value of forest floor thickness and total earthworm biomass in each of the earthworm species assemblage groups from 1999 to 2000.

## RESULTS

### *Forest floor and the leading edge in relation to sample point position*

In 1998, at study plot establishment, the location of the front of the leading edge was determined by visual inspection and assigned to sample point 8 on the sample grid. In subsequent years, forest floor thickness was measured directly, and the location of the front of the leading edge was identified as the sample point where forest floor thickness decreased to zero (Fig. 3). In the Blackduck, Ottertail, and Section 19 sites, the leading edge advanced by 20–30 m from 1999 to 2001. In the Two Points site, the leading edge did not appear to advance during the study. However, overall thickness of the forest floor decreased over time.

In all study sites, thickness of the forest floor increased significantly ( $P \leq 0.05$ ) across the sample grid in each year except Section 19 in year 2000 where measurable forest floor existed in only one sample point position (Fig. 3). Overall, mean forest floor thickness ranged from 0 to 7.0 cm. The pattern of change in relation to sample point position was nonlinear, with horizon thickness generally increasing from zero beginning at point 7 or beyond.

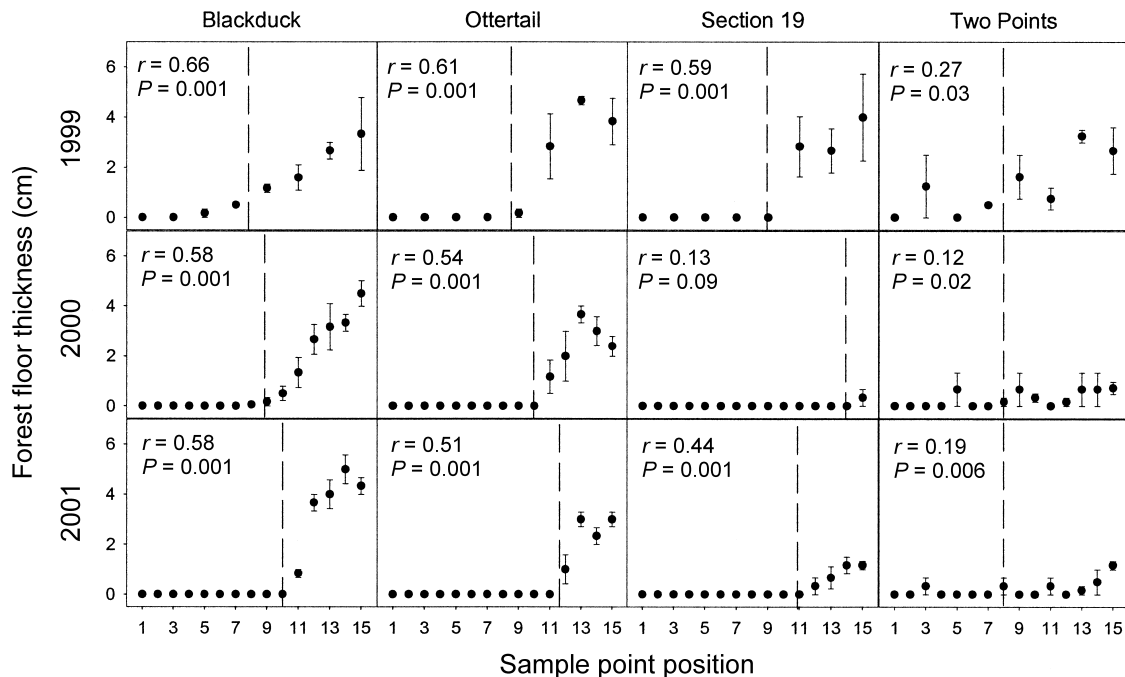


FIG. 3. Scatter plots of forest floor thickness (cm, mean  $\pm$  1 SE) in relation to sample point position across the sample grid in each of four study sites for years 1999 through 2001. Vertical dashed reference lines indicate the position of the leading edge for each site in each year, given that sample point 8 was determined to be the front of the leading edge by visual inspection at establishment of the sample grid. In subsequent years, forest floor thickness was measured directly, and the location of the front of the leading edge was identified as the sample point where forest floor thickness decreased to zero. The standardized Mantel statistic ( $r$ ) indicates the strength of the relationship between sample point position and forest floor thickness, and the associated  $P$  value indicates the significance of that relationship. In 1999,  $N = 24$  sample points per site; in 2000 and 2001,  $N = 45$  sample points per site.

#### *Earthworm composition and biomass variation among study sites*

Earthworm biomass and composition varied substantially among sites (Fig. 4, Appendix A and B). The Blackduck site had the lowest total earthworm biomass of all sites. The community was composed primarily of two species groups, *Dendrobaena* and *Aporrectodea* species, with a few individuals of *Lumbricus* juveniles found in the first and fourth years of the study only. While total earthworm biomass was lowest in the Blackduck site, *Aporrectodea* biomass was higher in Blackduck than in any other site. The Two Points and Ottertail sites had roughly 50% higher total earthworm biomass than the Blackduck site. The Two Points site was composed primarily of four earthworm species groups while the Ottertail site contained five species groups. Section 19 had the highest total earthworm biomass, more than twice that found in Blackduck, and contained all six earthworm species groups.

#### *Interannual variation in earthworm species biomass*

Earthworm biomass varied among years due to interannual variation in seasonal soil moisture conditions (Fig. 4, Appendix C). The mean seasonal rainfall (July through September) at the study sites is 25.9 cm, mean seasonal evapotranspiration is 32.7 cm with a mean

seasonal moisture deficit (SMD = precipitation – evapotranspiration) of –5.5 cm. For 1998 through 2001, the seasonal moisture deficits were –15.1, 9.2, –2.6, and –17.4 cm, respectively.

Large seasonal moisture deficits occurred in 1998 and 2001 and earthworm abundances were correspondingly low (Fig. 4). It is unknown to what degree lower sampled abundances in the drier years represent lower actual populations vs. poorer earthworm capture per unit effort. Moreover, sampling intensity was much lower in 1998 than in other years and sampling was done in only three of the four sites, making analyses across all sites and years unwieldy. For the above reasons, we present results for 1999 and 2000 throughout the rest of this paper. Nonetheless, results for 1998 and 2001 (or for all four years combined) were generally comparable to those in 1999 and 2000, but patterns were muted.

#### *Earthworm biomass in relation to sample point position and the leading edge*

The patterns of species biomass in relation to the direction of invasion were generally similar among sites and years (Appendix B). In multiple regression analyses of the 1999 and 2000 data from all sites, earthworm species biomass had a significant relationship ( $P$

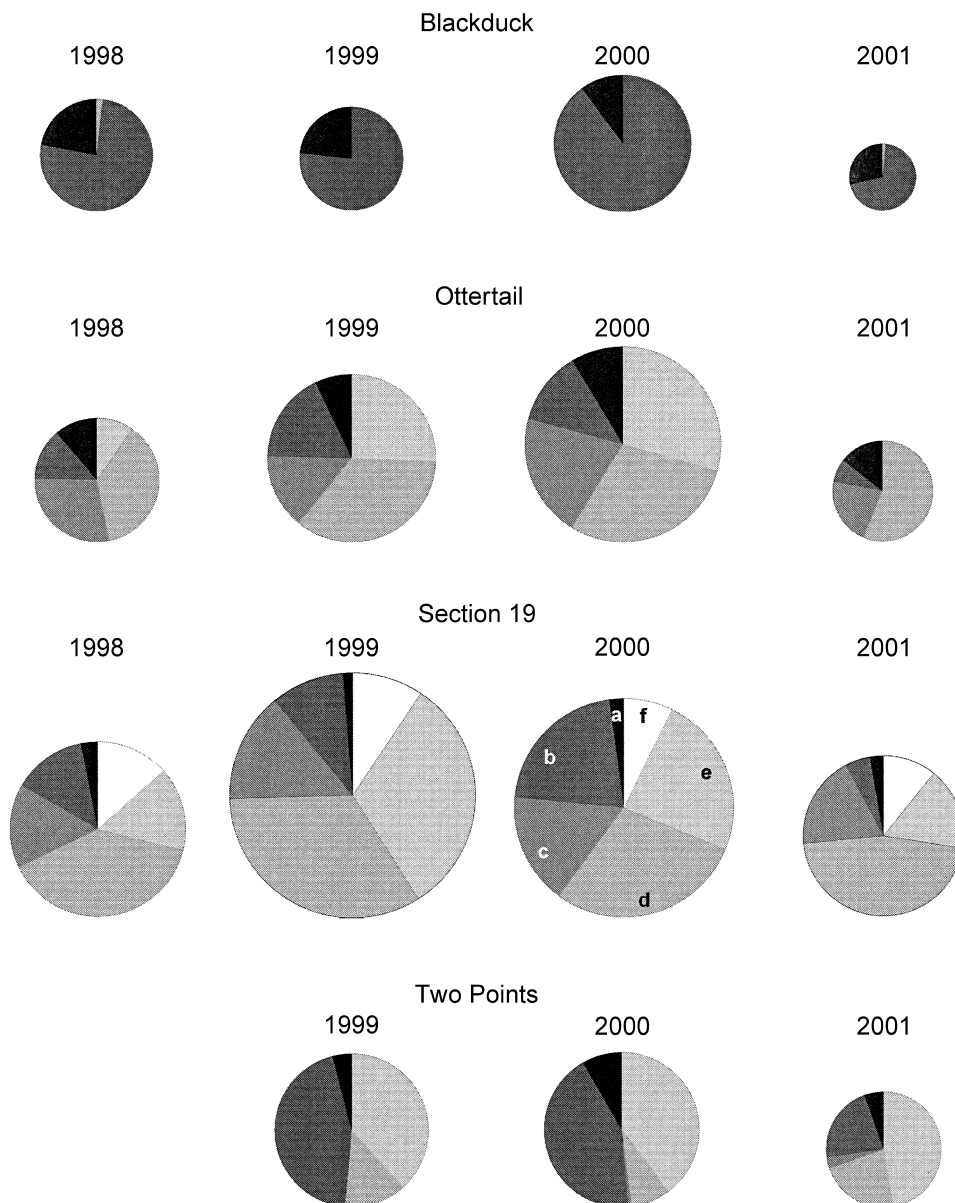


FIG. 4. Pie charts of relative mean earthworm biomass for each species group present in each site, where a = *Dendrobaena*, b = *Aporrectodea*, c = *Lumbricus rubellus* (adult), d = *Lumbricus* juveniles, e = *L. terrestris* (adult), and f = *Octolasion*. The size of the pie charts is proportional to total earthworm biomass among all sites and years (Appendix B) where the maximum in Section 19, year 1999, was 12.3 ash-free dry g/m<sup>2</sup>, and the minimum in Blackduck, year 2001, was 0.92 ash-free dry g/m<sup>2</sup>.

$\leq 0.0001$ ) with sample point "position" across the sample grid for all species groups except *L. rubellus* (Table 2). There were also significant interactions of sample point position with "site" or "year" in many instances (Table 2), indicating some temporal and spatial variation in these relationships.

The patterns of species specific earthworm biomass varied across the sample grids. However, they were typically characterized by a succession of earthworm species relative to the leading edge as shown using data from 2000 (Figs. 4 and 5) to illustrate the general pat-

terns seen across all years in each study site. For the *Dendrobaena* group, biomass increased across the sample grid from sample points 1 to 15 in three of four sites, reaching its maximum 20 m or more in advance of the leading edge (Table 2, Fig. 5).

For all other groups with a significant relationship, biomass decreased across the sample grid reaching their maximums at or behind the leading edge of invasion (Table 2, Fig. 5). The biomass of the *Aporrectodea* species group decreased significantly across the sample grid in three of the four sites. In these sites, its

TABLE 2. Summary statistics: regression results illustrating significant and nonsignificant relationships of earthworm species biomass to sample point position, site, and sample year.

Taxonomic groups	Position	Site	Year	Position $\times$ site	Site $\times$ year
<i>Dendrobaena</i>	+	yes	no	yes	yes
<i>Aporrectodea</i>	—	yes	no	yes	yes
<i>L. rubellus</i> (adults)	NS	yes	no	yes	yes
<i>L. juveniles</i>	—	yes	no	yes	no
<i>L. terrestris</i> (adults)	—	yes	no	yes	no
<i>Octolasion</i>	—	yes	no	yes	no

Notes: Symbols — or + indicate the direction of the relationship of earthworm biomass to increasing sample point number ( $P \leq 0.0001$ ). NS indicates no significant relationship. Yes and no in subsequent columns indicate significant or nonsignificant main and interaction effects, respectively, at  $P \leq 0.05$ .

biomass reached its maximum 20–40 m behind the leading edge and the group was scarce in front of the leading edge. The relationship of *L. rubellus* (adults) biomass to sample point position was inconsistent, and when present, it was detected up to 40 m or more in advance of the leading edge (Fig. 5). *Lumbricus* juvenile biomass decreased significantly across the sample grid in two of the three sites where detected and was rarely found more than 10 m in front of the leading edge. *L. terrestris* (adults) biomass decreased significantly across the sample grid in all three sites where it was detected and was rare in front of the leading edge. *Octolasion tyrtaeum* biomass decreased significantly across the sample grid in the one site where it was detected and was present only 30 m or more behind the leading edge.

#### Earthworm species assemblages

Independent cluster and indicator species analyses of 1999 and 2000 earthworm species data resulted in the same species assemblage groupings. For 1999 and 2000, the minimum average  $P$  value and maximum number of significant indicator species was achieved by splitting the dendrogram resulting from the cluster analysis into four groups (Appendix D, Table 3). Group 1 contained sample points with *Dendrobaena* only. Group 2 contained sample points identified by the presence of *Lumbricus rubellus* (adults) and *Lumbricus* juveniles but also contained *Dendrobaena* and *Aporrectodea* species. Group 3 contained sample points with *Aporrectodea* species and *Dendrobaena* as the dominant species. Group 4 contained all six species groups but was identified by the presence of *Lumbricus terrestris* (adults) and *Octolasion tyrtaeum*.

Using the 1999 earthworm species assemblage group designations and sample points where forest floor thickness and earthworm data were both collected in 1999 and 2000 ( $N = 82$ ), mean forest floor thickness and total earthworm biomass were compared. Sample points in worm Group 1 (*Dendrobaena* only) had very low worm biomass and high forest floor thickness, which did not change from 1999 to 2000 (Fig. 6). At the other extreme, sample points in worm Group 4

(dominated by adult *Lumbricus terrestris* and with all six species group present) had very high worm biomass in both years and negligible forest floor. Forest floor thickness decreased markedly ( $P \leq 0.005$ ) from 1999 to 2000 in sample points with worm groups 2 or 3 (Fig. 6), decreasing from 2.8 to 0.8 cm in group 2 and from 0.5 cm to near zero in group 3. Total earthworm biomass (AFDg/m<sup>2</sup>) also increased significantly ( $P \leq 0.01$ ) from 1999 to 2000 with sample points in group 3 (Fig. 6).

#### DISCUSSION

##### *Development of the leading edge and earthworm population dynamics*

In sugar maple dominated forests undergoing initial invasion by European earthworms, discrete leading edges characterized by rapid decreases in forest floor thickness develop in association with rapid increase in earthworm biomass. The most rapid development and advance in the leading edge occurred with the appearance of *Lumbricus rubellus* and *Lumbricus* juveniles (Fig. 4), and species assemblages dominated by *Lumbricus rubellus* and *Lumbricus* juveniles led to larger decreases in forest floor thickness than might be predicted by biomass alone (Fig. 6). In the two sites where both of these taxonomic groups were present (Ottetail and Section 19) the leading edge advanced a minimum of 30 m over four years, an average of 7.5 m or more per year (Fig. 3). The combination of *Aporrectodea* species and *Dendrobaena octaedra* alone also resulted in the formation a leading edge; however the rate of advance was less than that seen when *L. rubellus* and *L. terrestris* were present. In the Blackduck site, which contained only *Aporrectodea* (endogeic species) and *Dendrobaena* (strongly epigeic species), the leading edge advanced a total of 20 m in four years, a rate comparable to that reported in aspen and pine forests of Alberta, Canada (Dymond et al. 1997). *Dendrobaena* was present in all sites but was not associated with decreases in forest floor thickness (Fig. 6). *L. terrestris* developed large populations behind the leading edge but does not seem to be important in the development or advancement of discrete leading edges in these for-



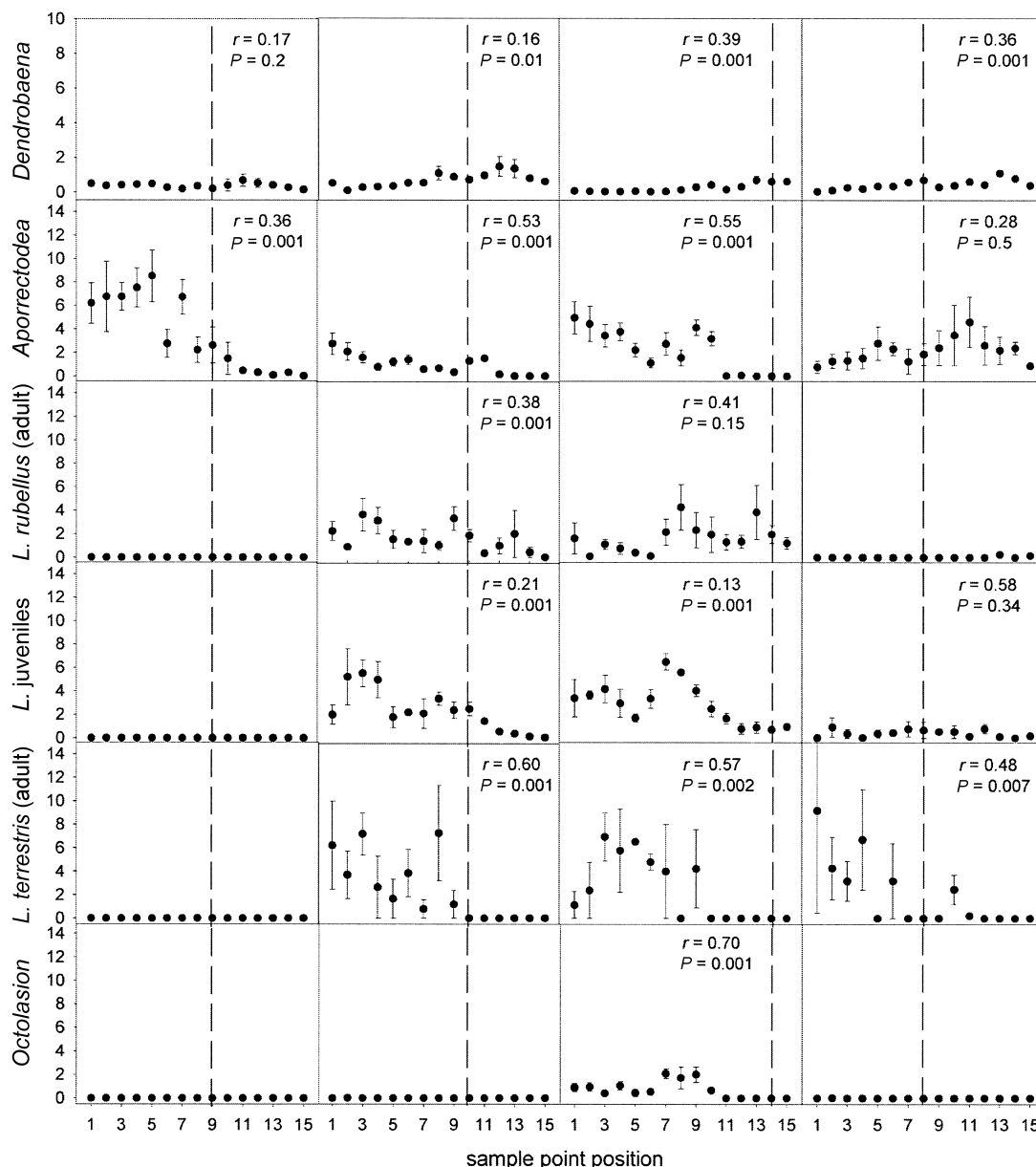


FIG. 5. Earthworm species biomass data from year 2000 illustrating the site-specific patterns in species composition and biomass in relation to the leading edge (see Fig. 4 for pie charts). Scatter plots of earthworm biomass (ash-free dry g/m<sup>2</sup>, mean  $\pm$  1 SE) are shown for each of six species groups in relation to sample point position and the leading edge in each study site. Vertical dashed reference lines indicate the position of the leading edge for each site in year 2000. The standardized Mantel statistic ( $r$ ) indicates the strength of the relationship between sample point position and earthworm species biomass, and the associated  $P$  value indicates the significance of that relationship.  $N = 45$  sample points per site.

ests. However, it may be responsible for the development of diffuse leading edges in situations where it is present but *L. rubellus* is not. For example, in the Two Points site a discrete leading edge did not advance, but rather, the forest floor was removed in a patchy distribution reflecting the distributional pattern and feeding behavior of *L. terrestris*, the dominant species in that site. Patchy removal of the forest floor associated with leading edges also explains the small increase in 2001 of forest floor thickness measures for Section 19.

Qualitatively across the leading edge we observed changes in the relative proportions of  $O_i$ ,  $O_e$ , and  $O_a$  horizons. Immediately ( $\sim 20$ – $30$  m) in advance of the visible leading edge (where the forest floor thickness first declines to zero) there was a transition from a thick  $O_e$  and a thin  $O_a$  horizon to a thin  $O_e$  and a thick  $O_a$  horizon as the epigeic species *Dendrobaena* and the epi-endogeic *L. rubellus* and *Lumbricus* juveniles began transforming the organic horizon. However, this change was relatively short lived as the  $O_a$  appeared

TABLE 3. Indicator values for earthworm species in species assemblage groups as determined by cluster analysis (Appendix D).

Taxonomic groups	Group, 1999				Group, 2000			
	1	2	3	4	1	2	3	4
<i>Dendrobaena</i>	32	30	25	7	28	30	27	9
<i>Aporrectodea</i>	0	10	41	40	1	7	40	40
<i>L. rubellus</i> (adults)	0	11	1	44	0	43	0	34
<i>L. juveniles</i>	0	43	0	56	0	40	2	51
<i>L. terrestris</i> (adults)	0	1	2	44	0	0	1	64
<i>Octolasion</i>	0	0	0	56	0	0	0	56

Notes: Indicator values are significant ( $P \leq 0.01$ ) for all taxonomic groups in each year. For 1999, numbers of sample points in groups 1, 2, 3, and 4 were 11, 22, 24, and 27, respectively. For 2000, numbers of sample points in groups 1, 2, 3, and 4 were 16, 41, 67, and 51, respectively.

to be rapidly consumed by endogeic species. All forest floor measurements were made in late summer, prior to leaf fall so the only remaining  $O_i$  was that which had persisted from the previous growing season. In areas with large worm populations this often meant that all surface litter from the previous year had been consumed (Fig. 3). Such conversion from a mor-moder to mull humus conditions may have significant effects on the nutrient dynamics, microbial biomass and function, and plant root and seedling dynamics of these forests (Scheu and Parkinson 1994, Bohlen et al. 2004b, Fisk et al. 2004, Groffman et al. 2004, Suárez et al. 2004).

The spatial distribution of earthworm species in relation to the leading edge (Fig. 5) reflects the habitat preferences and feeding behaviors of the different species groups (Bouché 1977, Hendrix et al. 1999). Strictly epigeic, litter dwelling, species such as *Dendrobaena octaedra* and *Dendrodriulus rubidus* are present in large numbers well in advance of the leading edge, where the biomass of this species group reaches its maximum. Behind the leading edge, where the forest floor layer has been largely removed, their biomass declines to low levels. Epi-endogeic species were represented in this study by a single species, *L. rubellus*. It was present in two of the four study sites and was distributed nearly uniformly across the sample grid, both in advance of and behind the leading edge. As a generalist feeder (Hendrix et al. 1999), this species has the ability to persist and thrive whether or not a forest floor is present. In this study, the endogeic species *Aporrectodea* and *Octolasion* were generally found behind the leading edge (i.e., in worm-invaded territory) and were never found alone. Their preference for feeding on soil organic matter (Bernier 1998, Hendrix et al. 1999) suggests that the establishment of endogeic populations occurs as a result of prior alterations of forest floor material by epigeic and epi-endogeic species making this pool of organic material more accessible (Shakir and Dindal 1997). The sole anecic species represented in this study, *L. terrestris*, was rarely present in advance of the leading edge and reached its population maximum 20 or more meters behind the leading edge. While this species can colonize areas with intact forest floor, it prefers to feed on fresh surface litter and begins to

degrade intact forest floor only after most of the surface litter has been consumed (C. M. Hale, *personal observation*). The net result of the differences in feeding and habitat preference of different earthworm species is the successive appearance of these species across the leading edge of earthworm invasion in these northern hardwood forests.

The possibility that different rates of colonization by different species or ecological groups could result in something analogous to a chromatic fractionation of

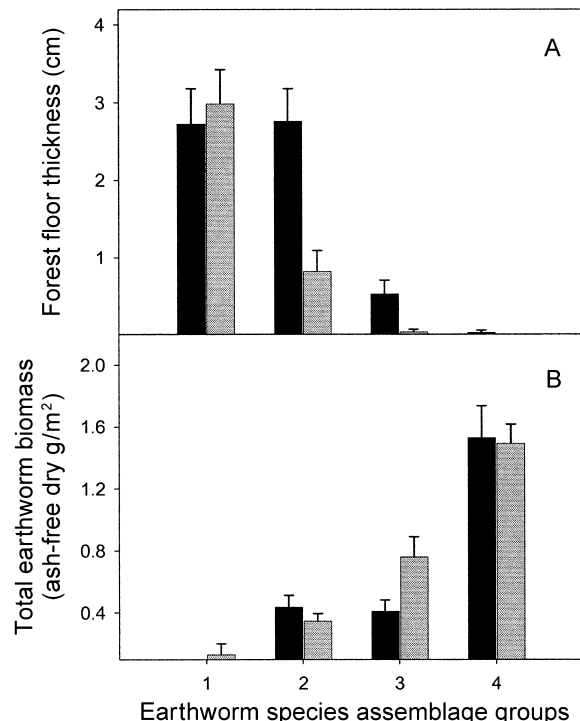


FIG. 6. Comparisons of change in (A) forest floor thickness and (B) total earthworm biomass in each of four earthworm species assemblage groups from 1999 (black bars) to 2000 (gray bars). Group 1 contains *Dendrobaena* only ( $n = 10$ ), group 2 is dominated by *Lumbricus* juveniles and *L. rubellus* (adults) ( $n = 22$ ), group 3 is dominated by *Aporrectodea* and *Dendrobaena* ( $n = 24$ ), and group 4 is indicated by the presence of *L. terrestris* (adults) and *Octolasion* ( $n = 27$ ).

earthworm faunas was proposed by James (1998). While James's reference was to the potential distribution of species based on climatic tolerances as these various species move north, such a pattern describes what we see at the leading edges of invasion, but on a much smaller spatial scale. Rather than a pattern distributed over many miles or even regions, we see this pattern over 150 m within forest stands.

#### *Long vs. short term effects of earthworm invasion*

The development of stable reproductive populations of *Aporrectodea* species and *L. terrestris* after initial invasion prevents recovery of the forest floor because their populations can rapidly adjust to consume all annual litter inputs (Nielsen and Hole 1963, Cothrel et al. 1997). These species also tend to dominate earthworm population assemblages after initial invasion (Fig. 5, Cuendet 1984, Shakir and Dindal 1997, Bohlen et al. 2004a) so *Aporrectodea* species and *L. terrestris* (adults) may have the most profound long-term impacts on forested ecosystems. However, epi-endogeic species (*Lumbricus rubellus* [adults] and *Lumbricus* juveniles) are associated with the most rapid loss of the forest floor during initial invasion and may also facilitate the establishment of the endogeic and anecic species. Therefore, the magnitude of their effects may be much greater than initially assumed by observing only late-stage stable populations of earthworms compared to worm-free conditions. Under this scenario, the overall impacts of invasion by European earthworms is the result of rapid conversion of the forest floor to an alternate state by epigeic and epi-endogeic species and the persistent changes resulting from the establishment of large populations of endogeic and anecic species that follow.

#### *Factors determining earthworm species assemblages and relative abundance*

In northern hardwood forests of Minnesota, the overall assemblage of earthworm species found in any given microsite is related to the time since initial invasion, the probability that any given species is present in the source population, and the ability of a given species to survive and reproduce in northern climates. The dynamics of earthworm invasion are not expected to be uniform in all forest types. The size and species composition of earthworm populations, and therefore the effects exotic earthworms have on a particular forest, will be dependent upon soil texture, moisture conditions, and the palatability and quantity of litter (Ponge and Delhay 1995, Dymond et al. 1997, Lavelle 1997, Bohlen et al. 2004a).

#### *Time since invasion and source populations*

In areas with only incipient invasions, *Dendrobaena* is often the only species present (Fig. 5) since it is capable of rapidly colonizing the intact forest floor. Early stage invasions that contain more than one spe-

cies generally contain both *Dendrobaena* and *L. rubellus*. While *L. rubellus* is clearly capable of colonizing intact forest floor, it appears to do so at a slower rate than *Dendrobaena*. In sites that have been invaded for long periods of time (multiple decades), earthworm populations often contain a full suite of species including epigeic, epi-endogeic, endogeic, and anecic species. However, some sites contain a more limited set of species simply because all species have not yet gained access to the site. In this study, the Blackduck site is an example of a site that is relatively remote with poor road access. As a result, only two species of earthworms have reached that site as compared to the Ottertail and Section 19 sites, which have direct access from a heavily traveled, paved road and are adjacent to a large fishing resort established in the 1950s. The Two Points site is on a moderately well maintained gravel road with low traffic but is close to a small fishing resort where, subsequent to site selection, we found that the owner has spent a great deal of time and energy establishing night crawler populations (*L. terrestris*) for his clients. As a result this site is dominated by *L. terrestris* with other opportunistic species, but does not include *L. rubellus*, the other common bait species used in the area. The red wiggler, *Eisenia fetida* and an Asian genus of earthworms, *Amyntas*, are sold and transported all across the state for vermi-composting (C. M. Hale, *personal observation*). However, the inability of this species to survive Minnesota winters outside of a compost heap appears to have prevented it from colonizing adjacent natural habitats.

#### *Climate and soil moisture conditions*

Depending on soil moisture conditions, the relative abundance of earthworms in any given site can vary greatly from year to year (Fig. 4, Appendix C). In this study, 1998 and 2001 had larger than average seasonal moisture deficits. The relative abundance of *Aporrectodea* species and *L. terrestris* (adults) were more negatively affected by drought conditions than the other species groups (Fig. 4), although we can not be sure whether our sampling reflects changes in abundance or their position in the soil profile. In 2001, *L. terrestris* was not detected in the Ottertail site, illustrating that extreme drought conditions can lead not only to underestimation of the abundance of species but may result in missing drought sensitive species altogether. When quantitatively assessing earthworm populations, the local climate and soil moisture conditions must be considered to evaluate the accuracy and reliability of the data.

The timing of earthworm sampling should also be directed by typical seasonal moisture patterns for the area. For example, the mean seasonal moisture deficit at our study sites is  $-5.50$  cm. During July and August soil moisture conditions normally decline in these areas and rebound again when fall rains begin in late August. For this reason we conducted earthworm sampling late

in September when soil moisture conditions have increased, providing us with the most accurate measure of earthworm populations.

In conclusion, we find that declines in the forest floor thickness near leading edges develop in response to rapid increase in earthworm biomass associated with a succession of earthworm species. Different species had different abilities to remove forest floor material resulting in different patterns and rates of loss of the forest floor during initial invasion. The establishment of stable reproducing populations of earthworms behind the leading edge of invasion prevents recovery of the forest floor.

#### ACKNOWLEDGMENTS

This research was supported by a grant from the National Science Foundation (DEB-0075236) and the Lydia P. Anderson Fellowship, the Wood-Rill Fellowship from the University of Minnesota Center for Hardwood Ecology, and a University of Minnesota Doctoral Dissertation Fellowship. Thanks to Dr. John Pastor and the Natural Resources Research Institute, University of Minnesota Duluth for providing office and laboratory support. The useful comments of J. Gullledge, T. Fahey, and two anonymous reviewers helped to improve the manuscript.

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#### APPENDIX A

A figure showing mean and standard error scatter sample points of earthworm biomass (ash-free dry biomass) by species in relation to sample point position for each study site in each sample year is available in ESA's Electronic Data Archive: *Ecological Archives* A015-023-A1.

#### APPENDIX B

A table showing range and median (in parentheses) of total earthworm biomass and species specific biomass (ash-free dry g/m<sup>2</sup>) per sample point for each study site is available in ESA's Electronic Data Archive: *Ecological Archives* A015-023-A2.

#### APPENDIX C

A table showing climate data for study sites from the State Climatology Working Group is available in ESA's Electronic Data Archive: *Ecological Archives* A015-023-A3.

#### APPENDIX D

A cluster analysis dendrogram of sample points based on earthworm species biomass in 1999 is available in ESA's Electronic Data Archive: *Ecological Archives* A015-023-A4.