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Tracking an invasion: community changes in hardwood forests following the arrival of *Amynthas agrestis* and *Amynthas tokioensis* in Wisconsin

Katherine M. Laushman · Sara C. Hotchkiss · Bradley M. Herrick

Abstract Because Upper Midwest temperate forests lack native earthworms, the invasions of European and Asian earthworms can significantly alter soils and understory vegetation. Earthworms' ability to increase leaf litter decay, alter nutrient cycling by mixing the organic layer with mineral soil, and decrease plant species richness leads to concern about the Asian 'jumping earthworm' (*Amynthas agrestis* and *A. tokioensis*) species that were recorded in the University of Wisconsin—Madison Arboretum in 2013. In 2015, we found *A. agrestis* and *A. tokioensis* in a distinct 8-ha region of a 23-ha hardwood forest surveyed in the Arboretum; by 2016 *A. agrestis* and

A. tokioensis had spread over an additional 7 ha. Plots also contained the European earthworm species *Lumbricus terrestris*, *L. rubellus*, and *Apporectodea* spp., whose distributions decreased from 2015 to 2016. While leaf litter, plant species richness, and tree and shrub seedling abundance were generally reduced in areas with European earthworms, they were typically slightly increased in areas with *A. agrestis* and *A. tokioensis* versus those without. Although our results do not show substantial impacts of *A. agrestis* and *A. tokioensis* on vegetation in the initial years of invasion, the rapid replacement of European earthworms by *A. agrestis* and *A. tokioensis* suggests continued monitoring of these new invasive species is important to better understand their potential to change the Upper Midwest's forests.

K. M. Laushman (✉)
Nelson Institute for Environmental Studies, University of Wisconsin – Madison, 550 North Park St., Madison, WI 53706, USA
e-mail: laushman@wisc.edu

S. C. Hotchkiss
Botany Department, University of Wisconsin – Madison, 430 Lincoln Drive, Madison, WI 53706, USA
e-mail: shotchkiss@wisc.edu

B. M. Herrick
University of Wisconsin – Madison Arboretum, 1207 Seminole Hwy, Madison, WI 53711, USA
e-mail: bradley.herrick@wisc.edu

Introduction

Assessing the impacts of invasive species soon after establishment is vital for understanding species' roles in a new ecosystem and can help anticipate changes that will accompany their spread. Because the northern temperate region of the United States lacks native earthworms due to widespread glaciation in the last ice

age (Bohlen and Hendrix 2002; Bohlen et al. 2004, Fig. 1a), the Upper Midwest is an ideal location to study the ecosystem effects of earthworms. Although European earthworms have been present in the Upper Midwest since European settlement (Hale et al. 2005), the ecological impacts of the Asian genus *Amynthas* have not been well studied, representing an important gap in knowledge of invasive earthworm biology (Richardson et al. 2009). The recent arrival of *Amynthas agrestis* and *Amynthas tokioensis* earthworms to the region has allowed for observation of these species' initial effects in Wisconsin forests. Our research observed the understory vegetation and forest floor immediately following the introduction of *Amynthas* earthworms.

European earthworms have been well studied for their effects on the naturally earthworm-lacking hardwood forests of the Upper Midwest (Hale et al. 2006; Holdsworth et al. 2007). Known impacts of earthworms on forest floor structure include increases in leaf litter decay, soil pH, rates of nitrogen transformation and carbon flux, and litter-soil mixing (Burtelow et al. 1998; Bohlen et al. 2004; Drouin et al. 2016). The influence of an earthworm species can depend on its functional group; epigeic species live in and alter the litter layer and upper surface of the soil, endogeic species alter both mineral and organic soil, and anecic species form deep vertical burrows, altering the forest floor and mineral horizons (Bouché 1971; Bohlen and Hendrix 2002; Asshoff et al. 2010). Soil impacts of exotic earthworms include a disruption of native soil biota, including microbes and fungi. For example, earthworms have been found to increase soil microbial biomass (Burtelow et al. 1998; Drouin et al. 2016), and to decrease mycorrhizal associations in forests (Lawrence et al. 2003). Through these abiotic and biotic mechanisms, European earthworms have been found to negatively affect vegetation in invaded regions lacking native earthworms. Native plant species richness is typically negatively correlated with abundance of the European earthworms in the genus *Lumbricus* (Hale et al. 2006; Holdsworth et al. 2007; Gibson et al. 2013), while the presence of multiple non-native earthworm species can lead to an increase in plant mortality, particularly of *Acer* seedlings (Hale et al. 2008).

Unlike European species of the Lumbricidae family, the impacts of invasive *Amynthas* earthworms are not yet thoroughly understood. Native to east-central Asia, the *Amynthas* genus, family Megascolecidae,
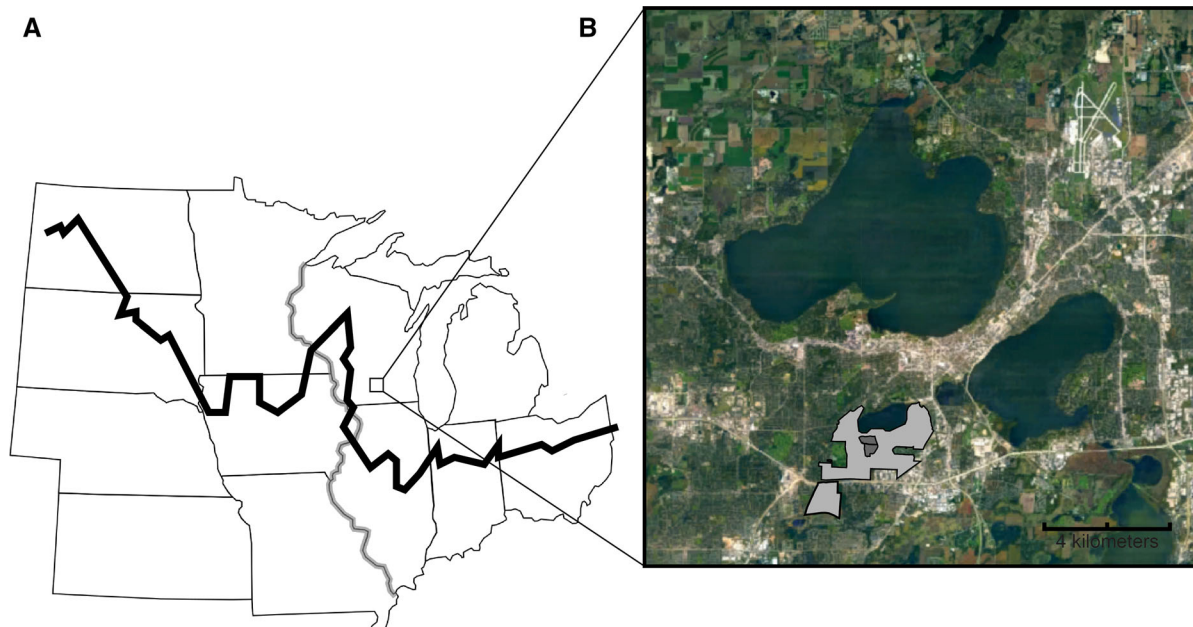

Fig. 1 **a** Location of study area within the Midwest, with bold line representing the southern extent of hypothesized earthworm absence due to glaciation, adapted from Bohlen and Hendrix (2002). **b** Madison, Wisconsin, with the University of Wisconsin—Arboretum in light grey, and the study area in dark grey

has been present in North America since the late 1800s (Snyder et al. 2011) and populations of *A. agrestis* have established in areas of New England (Burtelow et al. 1998; Gorres and Melnichuk 2012; Gorres et al. 2014) and the southern Appalachian mountains (Callaham et al. 2003; Snyder et al. 2011). *A. agrestis* earthworms typically occur in high densities, even in areas with native North American earthworms (Callaham et al. 2003), and their invasions result in a long-lasting granular soil surface structure, a decrease of the organic horizon, and an increase in soil pH and soil microbial biomass (Burtelow et al. 1998; Greiner et al. 2012). Optimum conditions for *A. agrestis* are around 25 °C with high soil moisture (Richardson et al. 2009) and their high potential for invasion has been partially credited to the ability to shift their diet among leaf litter, soil bacteria, and other soil fauna depending on availability (Zhang et al. 2010). Although *A. tokioensis* has not been studied as much as *A. agrestis*, the two are believed to share traits with each other and with others in the genus: they are typically epigeic or epi-endogeic, reproduce parthenogenetically, have faster growth rates than invasive European earthworms, and undergo an annual life cycle, experiencing mortality of adults in the fall and emergence from cocoons in spring (Burtelow et al. 1998; Greiner et al. 2012).

In 2013, the first documentation of *Amynthas* spp. in Wisconsin was recorded at the University of Wisconsin-Madison Arboretum, located in the south-central part of the state. Recent developments in *Amynthas* taxonomy and genetic testing of samples from the Arboretum have confirmed the presence of both *A. agrestis* and *A. tokioensis* (Chang et al. 2016a; Damhnait McHugh, Colgate University, unpublished data and pers. comm. 2017). Both species are colloquially known as 'jumping worms' for the defensive thrashing behavior displayed when handled, and they are thought to have similar ecological impacts. Because the field research in this study occurred before confirmation of the *Amynthas* species present, the two were not differentiated and will be referred to collectively. A recent mesocosm study done at the UW Arboretum found that these two species of *Amynthas* significantly decreased leaf litter and increased the percentage of carbon, nitrogen, and phosphorous in the soil (Qiu and Turner 2016). Because of known vegetation impacts from European species, researchers and managers are concerned with potential negative effects of *A. agrestis* and *A. tokioensis* on

temperate deciduous forests. In an experiment conducted in Michigan, no changes to vegetation biomass were found in treatments with or without *Amynthas* (now *Metaphire*) *hilgendorfi* (Greiner et al. 2012), but observational studies of the potential impacts of *A. agrestis* and *A. tokioensis* on vegetation in the Great Lakes region, in addition to the potential legacy effects of European earthworms, are needed.

Given the opportunity to document a novel invasion at the UW Arboretum, we assessed the changes in forest understory and earthworm communities in areas of *A. agrestis* and *A. tokioensis*' spread within the first 3 years of their confirmed presence. Based on the changes seen in forests after European earthworm introduction, we expected to see decreases in plant species health such as reduced abundance and diversity. We hypothesized that leaf litter would be diminished in areas with high densities of *A. agrestis* and *A. tokioensis* based on the recent work in the Arboretum (Qiu and Turner 2016), and we expected to find European earthworm species presumed to be present pre-*Amynthas* invasion. The goal of this study was to better understand what changes land managers can expect in the initial years of *A. agrestis* and *A. tokioensis* invasion in forests of the Upper Midwest so that priorities for forest management can be set. We hope that these initial surveys will mprove the understanding of invasions by earthworm species into historically earthworm-free areas.

Methods

Study location

Field studies were conducted in Wingra and Gallistel Woods at the University of Wisconsin-Madison Arboretum in Madison, Wisconsin (43.045477°N, − 89.423853°W, Fig. 1b). The area surveyed is a 23-ha sugar maple and oak dominated mesic forest with predominantly Military loam and Dodge silt loam soils at around 260 meters elevation. Gallistel and Wingra Woods are remnant forests on land that was partially cleared but never plowed, and both areas now contain trees that were planted between 1941 and 1964 (UW Arboretum 1981). The two parcels of woods contain hiking trails open to the public and are divided by a two-lane paved road. White-tailed deer are not excluded from the Arboretum, but populations are

culled annually. The hypothesized introduction point of *A. agrestis* and *A. tokioensis* to the Arboretum, a mulch depository, is located just southwest of the survey area, and mulch is frequently spread in horticultural gardens that are located uphill and to the west of the study area.

Selection of plots

71 points were randomly generated using ArcMap for initial placement of surveys in 2015. Each point was located with a Trimble GeoXH unit and represented the southwest corner of a 1-m^2 plot oriented north–south. In 2016, 15 additional plots were added at random locations in the central portion of Wingra and Gallistel Woods to cover an underrepresented area.

Vegetation and forest floor surveys

Each 1-m^2 plot was surveyed for understory vegetation in August of 2015 and August of 2016. Abundances of herbaceous species were recorded in 6 cover classes, adopted from the Daubenmire scale (Mueller-Dombois and Ellenberg 1974): 1 (0–5%), 2 (6–25%), 3 (26–50%), 4 (51–75%), 5 (76–95%), 6 (96–100%). The same cover classes were used to estimate cover of bare soil, coarse woody debris, and total vegetation of each plot. Stem counts and percent cover of woody seedlings and saplings were recorded. Canopy cover was estimated at chest height with a densiometer in 2015, and average leaf litter depth was calculated by measuring depth at the center and two corners of each plot. In addition, leaf litter mass was measured in August 2016 by collecting all leaf litter in adjacent 0.36-m^2 earthworm survey plots and weighing after drying at 60 °C for at least 48 h. In 2016, pH and moisture content of plot soils were measured using a Hannah Instruments 99,121 pH meter and Extech MO750 soil moisture meter. Averages were calculated based on three measurements taken in each vegetation plot for pH in the SW corner, center, and NE corner, and moisture in the SE corner, center, and NW corner.

Earthworm surveys

In August 2015 and 2016, earthworm surveys were conducted at each plot using liquid mustard extraction (Gunn 1992; Hale et al. 2005). Each earthworm plot was 0.36-m^2 and shared one border with a vegetation plot, with the adjacent border selected randomly from one of the four cardinal directions. Earthworm surveys involved removal of leaf litter and debris from the plot area followed by saturating the soil with a solution of 10 L water:100 g (1/3 cup) ground mustard, which agitates earthworms to the surface. After pouring half of the solution, we waited 5 min for epigeic and endogeic earthworms to emerge, and then poured the second half and waited 5 min for anecic earthworms. Earthworms were counted and identified to the genus or species level in the field. Area of earthworm spread was calculated with minimum bounding geometry in ArcGIS using convex hull polygons.

Statistical analysis

Nonmetric multidimensional scaling ordinations built with the Bray–Curtis distance metric were used to visualize the plant and earthworm community. The plant community was represented by midpoints of cover class percentage ranges and included only plants that occurred in at least five plots and only those plots that had at least two plant species. Values were square root transformed and underwent Wisconsin double standardization. Additional ordination axes were retained if reduction in stress was > 5 until final stress values were < 20 (McCune and Grace 2002). Vectors for earthworm abundance were added with the vegan function 'envfit' in R using 1000 permutations (R Development Core Team 2009).

T tests were conducted to compare areas with and without each earthworm taxon for vegetation and forest floor variables. Linear regressions were conducted to compare earthworm abundance to vegetation and forest floor variables, using data only from plots where each earthworm group being examined was present (i.e. data points with zero *A. agrestis* and *A. tokioensis* were excluded for regressions on *A. agrestis* and *A. tokioensis*). Cover classes were converted to midpoints of percentage ranges for use in analyses, and residual plots were assessed for assumptions of normality. Fischer's Exact tests were conducted using presence/absence of individual herbaceous species and presence/absence of earthworm taxa to determine if any herbs were more likely to be present when an earthworm taxon was present. Herbaceous species were also categorized based on traits including: early blooming (before June), late blooming (June and after), high-moisture preference,

low-moisture preference, and mycorrhizal or non-mycorrhizal, and analyzed using Fischer's Exact tests to determine if any traits were more likely to be present when an earthworm taxon was present.

Shannon Diversity Index was calculated for each plot using the midpoint of each species cover class to represent proportional abundance. Shannon Diversity Index was compared with earthworm presence/absence using t tests and with earthworm abundance using linear regression. Herbaceous species were ranked by their Coefficient of Conservatism (CC) as described by the Wisconsin State Herbarium (2015), a scale from 0 to 10 that gives plants with a higher tolerance for site disturbance a lower value and those that have specific habitat requirements a higher value. Average CC per plot was compared with earthworm presence/absence and abundance using t tests and regressions. Paired t tests comparing differences in plot variables between 2015 and 2016 were conducted, in addition to ANOVAs comparing differences in plot variables based on the spread of *A. agrestis* and *tokioensis* between the 2 years. Statistics were performed using R Studio software (RStudio Team 2015).

Results

Earthworm surveys

In addition to *A. agrestis* and *A. tokioensis*, the European earthworms *Lumbricus terrestris*, *Lumbricus rubellus*, and *Apporectodea* spp. were found through mustard pour sampling (Table 1). *Lumbricus terrestris* is a large, pigmented, anecic species, *L. rubellus* is a moderate sized pigmented epi-endogeic

earthworm, and the species of *Apporectodea* surveyed, most likely *A. caliginosa* or *A. rosea*, are smaller than *Lumbricus*, unpigmented, and endogeic (Hale et al. 2006; Bernadette Williams, Wisconsin DNR, *personal communication*).

In 2015, *A. agrestis* and *A. tokioensis* were found in the western half of Wingra and Gallistel Woods, in an area of 8.24 ha (excluding one eastern outlier plot; Table 1, Fig. 2) while *L. terrestris*, *L. rubellus*, and *Apporectodea* spp. were concentrated in the eastern half but widespread throughout the survey area, covering an average area of 14.2 ha (Table 1, Fig. 2). In 2016, *A. agrestis* and *A. tokioensis* were much more widespread throughout the eastern half of the forest, covering an area of 15.58 ha (Table 1, Fig. 2), while the distribution of European earthworm species was reduced to an average area of 1.96 (Table 1, Fig. 2).

Non-metric multidimensional scaling ordinations

A stress value of 12.4 resulted from 20 iterations of a three-dimensional non-metric multidimensional scaling. No significant relationships were found between plant community and earthworm taxa, with all earthworms' $r^2 < 0.05$ (Table 2), but the *A. agrestis* and *A. tokioensis* vector was opposite of the vectors of *L. terrestris*, *L. rubellus*, and *Apporectodea* spp. in vegetation space (Fig. 3). Distributions of plots by sampling year had strong overlap, showing no clear shifts in vegetation between 2015 and 2016.

Table 1 Summary of earthworm distribution in both years of surveys

Earthworm	2015					2016				
	#	%	Av.	Max.	Area	#	%	Av.	Max.	Area
Amynthas spp.	31	43.662	6.478	37	8.24	58	67.442	7.093	49	15.58
L. terrestris	25	35.211	1.154	8	12.44	14	16.279	0.569	7	1.74
L. rubellus	28	39.437	5.366	43	15.43	14	16.279	1.337	23	1.28
Apporectodea spp.	30	42.254	5.08	43	14.69	17	19.767	2.011	35	2.86

Amynthas spp. refers to *A. agrestis* and *A. tokioensis*, *#* number of plots containing worm, *%* percentage of plots surveyed containing worm, *Av* average number of worms per 0.36-m^2 plot, *max* maximum number of worms per 0.36-m^2 plot, *area* area of worm distribution in hectares

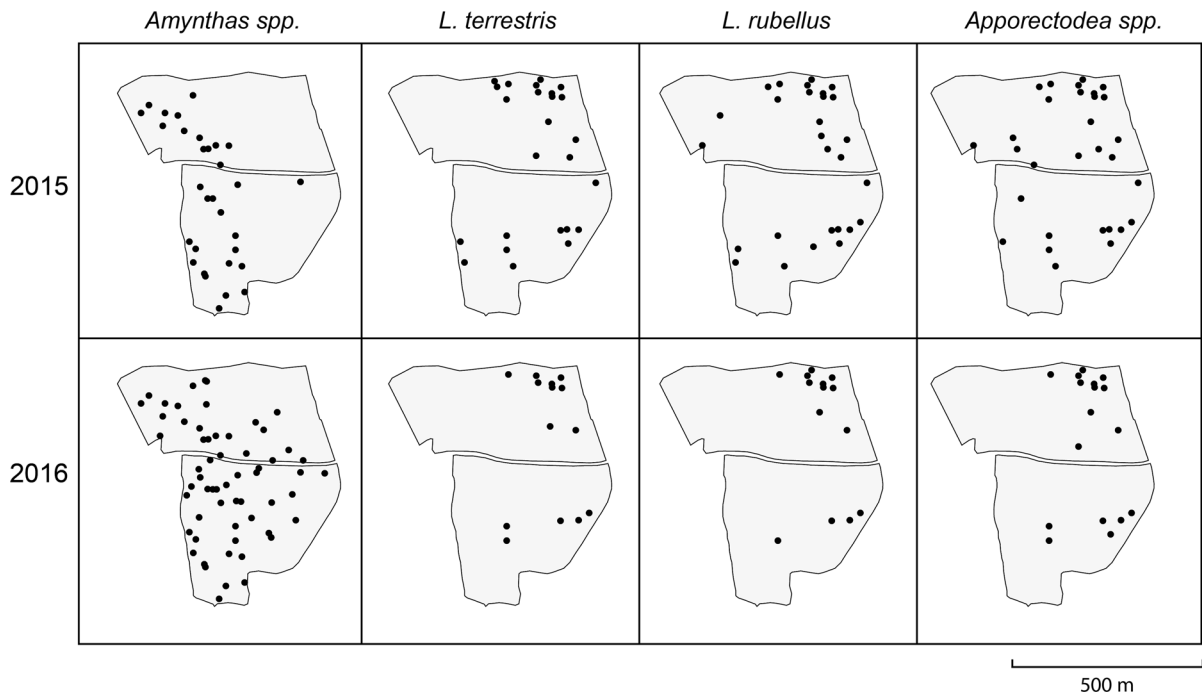| | *Amynthas spp.* | *L. terrestris* | *L. rubellus* | *Apporectodea spp.* |

Fig. 2 Distributions of earthworm presence, represented by black dots from 2015 and 2016 surveys in Wingra and Gallistel Woods. *Amynthas* spp. refers to *A. agrestis* and *A. tokioensis*

Table 2 Correlation coefficients (Pearson's r) of earthworm abundance with ordination axes

	Axis 1	Axis 2	Axis 3
Amynthas spp.	− 0.052	0.083	− 0.046
L. terrestris	0.0198	− 0.187	0.088
L. rubellus	0.1102	− 0.039	− 0.047
Apporectodea spp.	0.0141	− 0.003	0.071

Amynthas spp. refers to *A. agrestis* and *A. tokioensis*

Forest floor characteristics

T tests showed no differences in August leaf litter depth between plots with presence of absence of any earthworm grouping (*A. agrestis* and *A. tokioensis*, *L. terrestris*, *L. rubellus*, *Apporectodea* spp., European earthworms combined and total earthworms combined) in 2015, but in 2016 we found significantly higher August leaf litter depth in plots with *A. agrestis* and *A. tokioensis* present than with *A. agrestis* and *A. tokioensis* absent ($p = 0.013$) and significantly lower depth in plots with *L. terrestris* present ($p = 0.006$), *Apporectodea* spp. present ($p = 0.037$), or European

earthworms present ($p = 0.045$; Table 3). Leaf litter mass reflected these trends in 2016; *t* tests showed significantly lower leaf litter mass in plots with *L. terrestris* present ($p < 0.001$), *L. rubellus* present ($p < 0.001$), or *Apporectodea* spp. present ($p < 0.001$), but no significant difference was detected between plots with and without *A. agrestis* and *A. tokioensis* (Table 3, Fig. 4). Similarly, linear regressions showed weak evidence for a negative relationship between the abundance of *L. terrestris* and leaf litter mass ($p = 0.084$, $R^2 = 0.16$, slope $= − 5.3$), and strong evidence for a negative relationship between the abundance of total earthworms and leaf litter mass ($p = 0.005$, $R^2 = 0.08$, slope $= − 1.11$) and between the abundance of European earthworms and leaf litter mass ($p = 0.030$, $R^2 = 0.21$, slope $= − 0.89$; Online Resource Table S1). Canopy cover was significantly lower in plots containing *L. rubellus*, in plots with any European earthworms compared to plots without, and in plots with any earthworm compared to plots absent of all earthworms (Online Resource Table S2). Average cover of coarse woody debris in vegetation plots did not differ based on earthworm presence or abundance (Online
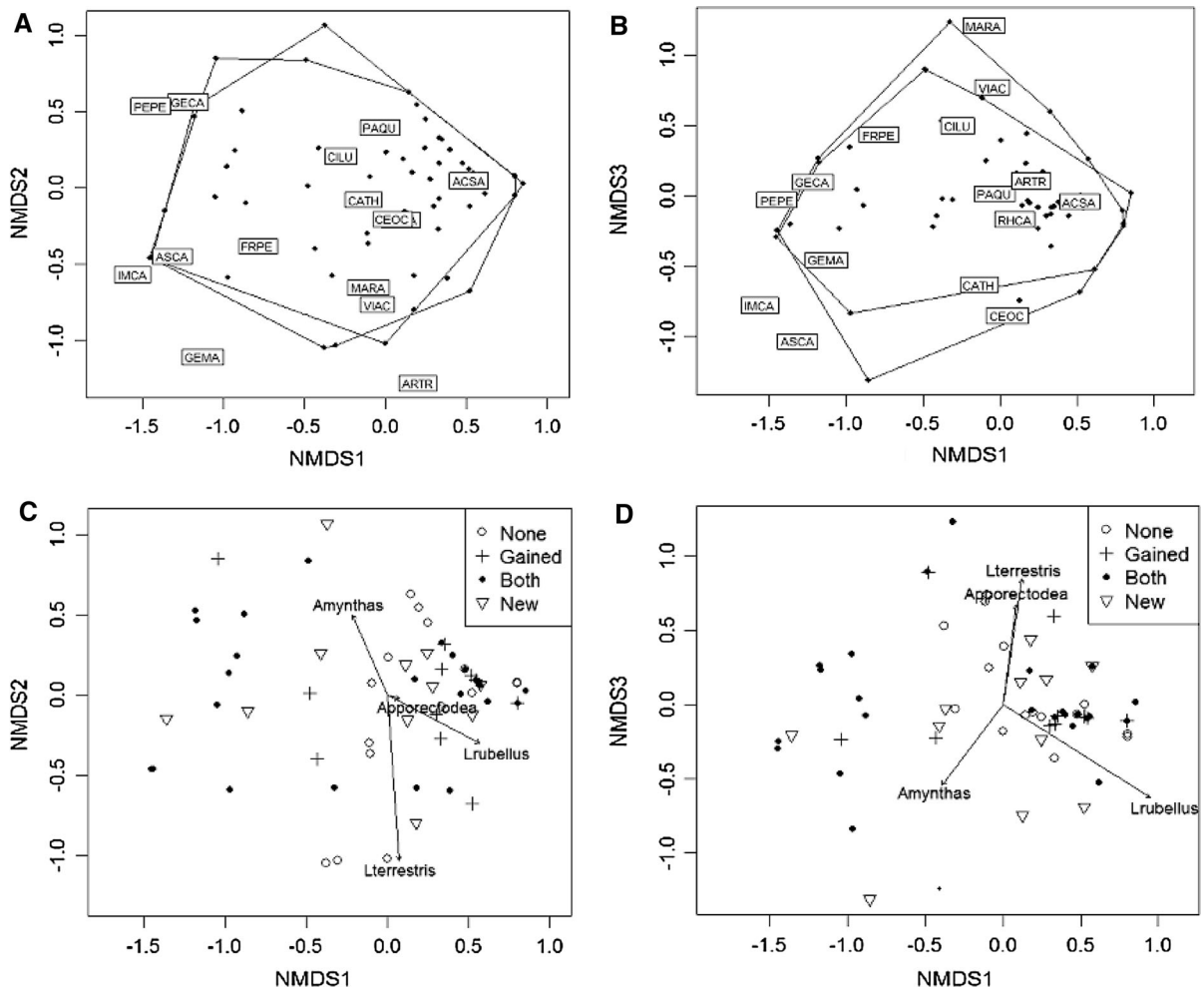
Fig. 3 Nonmetric multidimensional scaling ordination of plots based on plant species percent cover data. Points represent plots analyzed (n = 65), outlines in **a** and **b** enclose sampling years, text represents plant species included (n = 15): RHCA = *Rhamnus cathartica*, ACSA = *Acer saccharum*, PAQU = *Parthenocissus quinquefolia*, VIAC = *Viburnum acerifolium*, CILU = *Circaea lutetiana*, IMCA = *Impatiens capensis*, MARA = *Maianthemum racemosum*, GECA = - *Geum canadense*, CATH = *Caulophyllum thalictroides*, ARTR = *Arisaema triphyllum*, GEMA = *Geranium maculatum*, PEPE = *Persicaria pensylvanica*, ASCA = *Asarum canadense*, CEOC = *Celtis occidentalis*, FRPE = *Fraxinus pennsylvanica*. *Amynthas* spp. refers to *A. agrestis* and *A. tokioensis*. Ordinations **c** and **d** include overlay of earthworm vectors and symbols based on *Amynthas* spp. status of plots: None = plots with no *Amynthas* spp., Gained = plots that did not have *Amynthas* spp. in 2015, but gained *Amynthas* spp. in 2016, Both = plots that had *Amynthas* spp. in 2015 and 2016, New = plots that were added in 2016, all contained *Amynthas* spp.

Resource Table S1 and S2). The average cover of bare soil in plots was significantly higher in areas with each earthworm taxon present compared to areas with the respective earthworms absent, except for *A. agrestis* and *A. tokioensis*, which had a lower cover of bare soil when present (Table 3).

Soil characteristics

The average soil pH across all plots was 5.913. *T* tests based on presence and absence of earthworm groups found higher pH in areas with *A. agrestis* and *A. tokioensis* (*p* = 0.054), but significantly lower pH in areas with *L. terrestris* (*p* = 0.007), *L. rubellus* (*p* = 0.007), and combined European earthworms (*p* = 0.044; Table 3, Fig. 5a). Linear regressions

Table 3 P values of t tests comparing characteristics in areas with each earthworm grouping present versus areas absent

Response variable	*Amynthas* spp.		*L. terrestris*		*L. rubellus*	
	2015	2016	2015	2016	2015	2016
Average bare soil cover	0.696 (−)	**0.015** (−)	**0.014** (+)	**0.009** (+)	**0.013** (+)	**0.014** (+)
Soil moisture (%)	–	**0.009** (+)	–	**0.029** (−)	–	**0.019** (−)
Soil pH	–	0.054 (+)	–	**0.007** (−)	–	**0.007** (−)
Leaf litter mass	–	0.392 (+)	–	**< 0.001** (−)	–	**< 0.001** (−)
Leaf litter depth	0.854 (+)	**0.013** (+)	0.135 (−)	**0.006** (−)	0.296 (−)	0.088 (−)
Canopy cover	0.647 (−)	–	0.961 (=)	–	**0.024** (−)	–
Plant species richness	**0.046** (+)	0.207 (+)	0.901 (+)	0.712 (−)	0.850 (−)	0.438 (−)
Plant SD index	**0.044** (+)	0.171 (+)	0.959 (−)	0.222 (−)	0.606 (−)	0.153 (−)
Av. vegetative cover	0.422 (+)	0.359 (+)	**0.025** (−)	0.323 (+)	0.105 (−)	0.724 (−)
Response variable	*Apporectodea* spp.		All European worms		Total earthworms	
	2015	2016	2015	2016	2015	2016
Average bare soil cover	**0.019** (+)	**0.006** (+)	**0.014** (+)	**0.007** (+)	**0.035** (+)	0.27 (+)
Soil moisture (%)	–	0.151 (−)	–	0.094 (−)	–	0.258 (+)
Soil pH	–	0.063 (−)	–	**0.044** (−)	–	0.320 (+)
Leaf litter mass	–	**< 0.001** (−)	–	**< 0.001** (−)	–	**0.009** (−)
Leaf litter depth	0.103 (−)	**0.037** (−)	0.119 (−)	**0.045** (−)	0.370 (+)	0.844 (+)
Canopy cover	0.126 (−)	–	**0.043** (−)	–	**0.038** (−)	–
Plant species richness	0.867 (−)	0.732 (−)	0.572 (+)	0.633 (−)	0.558 (+)	0.083 (+)
Plant SD index	0.808 (−)	0.432 (−)	0.947 (+)	0.335 (−)	0.763 (+)	0.184 (+)
Av. vegetative cover	**0.047** (−)	0.889 (−)	0.264 (−)	0.974 (+)	0.205 (−)	0.206 (+)

Amynthas spp. refers to *A. agrestis* and *A. tokioensis*, (+) = average is higher when the respective earthworm grouping is present, (−) = average is lower when the respective earthworm grouping is present, (=) = averages are equal in areas where the respective earthworm grouping is present and absent, – = variable not measured in that sampling year, SD = Shannon Diversity. Bolded p values are < 0.05
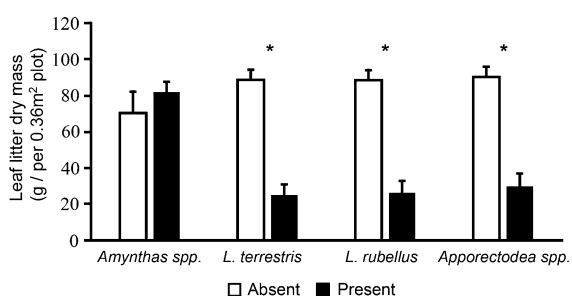

Fig. 4 Average leaf litter mass from plots with and without each earthworm grouping in 2016; t tests on *L. terrestris* ($p < 0.0001$), *L. rubellus* ($p < 0.0001$), and *Apporectodea* spp. ($p < 0.0001$) showed significant differences, indicated by asterisks. *Amynthas* spp. refers to *A. agrestis* and *A. tokioensis*

showed significantly positive relationships between soil pH and the abundance of *A. agrestis* and *A. tokioensis* ($p = 0.035$, $R^2 = 0.05$, slope = 0.01) and

the abundance of *L. terrestris* ($p = 0.0004$, $R^2 = 0.62$, slope = 0.14; Fig. 5b). There was weak evidence for a positive relationship between soil pH and *L. rubellus* abundance ($p = 0.083$, $R^2 = 0.16$, slope = 0.03). No relationships were detected between soil pH and *Apporectodea* spp., total earthworm abundance, or European earthworm abundance (Online Resource Table S1). Moisture was significantly negatively related to the abundance of *Apporectodea* spp. ($p = 0.023$, $R^2 = 0.25$, slope = − 1.6), combined European earthworms ($p = 0.033$, $R^2 = 0.206$, slope = − 2.5) and total earthworms ($p = 0.025$, $R^2 = 0.05$, slope = − 1.02), and was lower on average in areas containing each of *L. terrestris* ($p = 0.029$) and *L. rubellus* ($p = 0.019$). Moisture was significantly higher in areas containing *A. agrestis* and *A. tokioensis* ($p = 0.009$; Table 3).
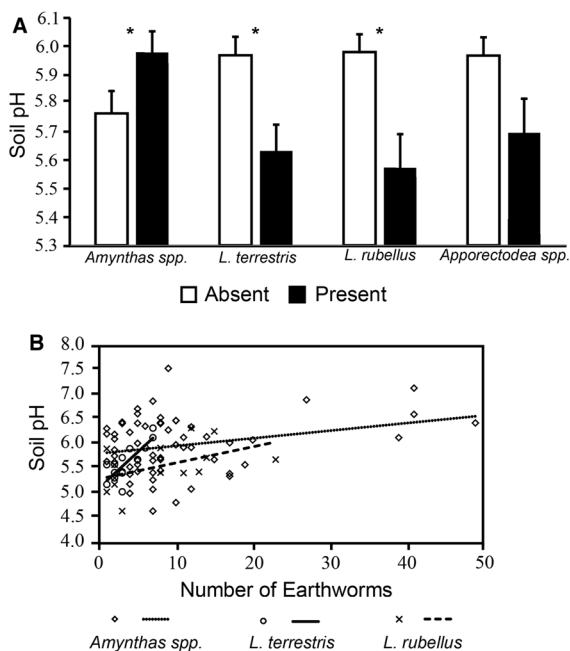
Fig. 5 a Average soil pH from plots with and without each earthworm grouping in 2016. T tests on *A. agrestis* and *A. tokioensis* ($p = 0.054$), *L. terrestris* ($p = 0.007$), *L. rubellus* ($p = 0.007$) showed significant differences, *Apporectodea* spp. ($p = 0.063$) showed weak evidence for significant difference. **b** Linear regressions between soil pH and number of *A. agrestis* and *A. tokioensis* ($p = 0.035$, $R^2 = 0.05$, slope = 0.01), *L. terrestris* ($p = 0.0004$, $R^2 = 0.62$, slope = 0.14), and *L. rubellus* ($p = 0.083$, $R^2 = 0.16$, slope = 0.03) in 2016. Each taxon regression analysis excluded plots with zero earthworms

Vegetation characteristics

Fifteen herbaceous species were found in the vegetation surveys in 2015, and seventeen herbaceous species were found in 2016, all of which were native to southern Wisconsin. The average number of herbaceous species per plot was 1.4 in 2015 (SD 1.59, SE 0.18) and 1.6 in 2016 (SD 1.78, SE 0.19). The most abundant herbaceous species was *Parthenocissus quinquefolia*, found in 60% of plots in 2015 and 2016, followed by *Circaea lutetiana*, found in 20% of plots in 2015 and 25% of plots in 2016. The most abundant tree species was *Acer saccharum*, found in 90% of plots. The only invasive plant species found were honeysuckle (*Lonicera morrowii*) and buckthorn (*Rhamnus cathartica*), found in only 4 and 21% of plots in 2015, respectively, and 2 and 15% of plots in 2016, with no clear spatial patterns.

T tests on a number of vegetation characteristics based on presence and absence of earthworm taxa, including average CC and SD Index, mostly found no significant differences (Table 3, Online Resource Table S3), but trends typically showed increased diversity, richness, and abundance in areas with *A. agrestis* and *A. tokioensis* and in areas without each European earthworm (Table 3, Fig. 6). Linear regressions between each earthworm group's abundance and vegetation response variables largely showed no significant relationships in either year, except for significantly negative relationships in 2016 between plant species richness, herbaceous species richness, *Acer saccharum* abundance and each of *L. rubellus*, *Apporectodea* spp., and total European earthworms (Online Resource Table S4, Fig. 7). No herbaceous species or traits were significantly more or less likely to be present based on earthworm presence using Fischer's Exact tests for 2015 and 2016.

Plots from 2016 were categorized as being completely absent of *A. agrestis* and *A. tokioensis*, being absent of *A. agrestis* and *A. tokioensis* in 2015 but present in 2016, and containing *A. agrestis* and *A.*
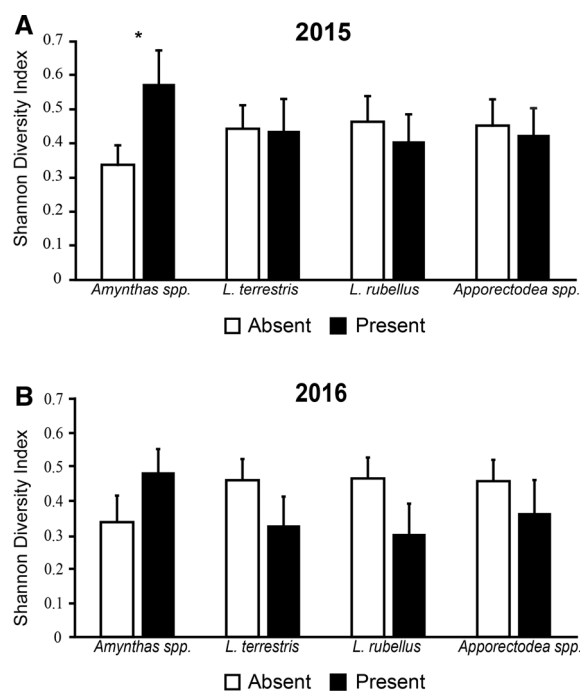

Fig. 6 Average plant Shannon Diversity Index from plots with and without each earthworm grouping in 2015 (**a**) and 2016 (**b**). No groups were significantly different except *A. agrestis* and *A. tokioensis* in 2015 ($p = 0.044$), indicated by asterisk
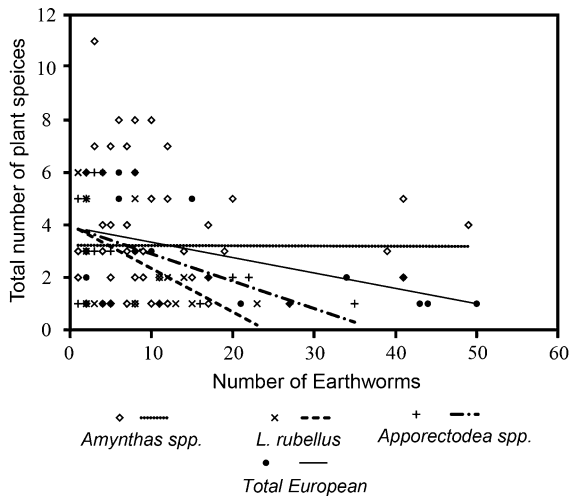
Fig. 7 Linear regressions between plant species richness and number of *A. agrestis* and *A. tokioensis* ($p = 0.977$, $R^2 = -0.01$, slope $= -0.0008$), *L. rubellus* ($p = 0.036$, $R^2 = 0.26$, slope $= -0.166$), *Apporectodea* spp. ($p = 0.015$, $R^2 = 0.27$, slope $= 0.104$), and total European earthworms ($p = 0.026$, $R^2 = 0.22$, slope $= -0.05$) in 2016. Each taxon analysis excluded plots with zero earthworms

tokioensis in both 2015 and 2016. Using these three categories, ANOVAs were conducted on multiple site characteristics, none of which showed significant differences or had large enough sample sizes to be statistically testable. Paired *t* tests comparing leaf litter depth, *Acer saccharum* abundance, Shannon Diversity Index, and plant species richness between 2015 and 2016 showed the same trends for all earthworm taxa, suggesting that earthworm presence did not cause plot-wise temporal changes within the 2 years.

Discussion

Previous research on earthworm invasions has largely found negative impacts to both above- and below-ground communities in forests (Gundale 2002; Hale et al. 2006; Holdsworth et al. 2007; Nuzzo et al. 2009; Craven et al. 2017). This field study provides evidence for a rapid invasion of *A. agrestis* and *A. tokioensis* earthworms and their potential replacement of European earthworms, as has been suggested by previous researchers (Greiner et al. 2012). While much of the understory vegetation did not show detectable changes in relation to the spread of *A. agrestis* and *A. tokioensis*, some forest floor variables such as soil

pH and leaf litter mass reveal differences in areas where *A. agrestis* and *A. tokioensis* were found to be present or absent. Although we were unable to assess their invasion into a completely earthworm-free forest, the changes seen in *A. agrestis* and *A. tokioensis* distribution represent the first observational evidence to track the early invasion of these earthworms in the Upper Midwest, and there are many potential implications of the trends seen in this 2-year study.

Earthworm community changes

The clearest finding of this research was the rapid spread of *A. agrestis* and *A. tokioensis* from 2015 to 2016, gaining approximately 7 ha of the 23-ha study area. This trend was in opposition to that of the three European earthworm taxa found, all of which saw a reduction in distribution. Although the initial distribution of *A. agrestis* and *A. tokioensis* in 2015 was largely in the western half of the study area, many more plots in the eastern half were surveyed with *A. agrestis* and *A. tokioensis* in 2016, and the overlap between *Amynthas* species and European earthworms was reduced (Online Resource Figs. S1 and 2). The high population densities and epi-endogeic niche of *A. agrestis* and *A. tokioensis*, leading to many earthworms and likely cocoons on the upper soil surface, may have led to increased dispersal via human disturbance and precipitation runoff compared to other earthworm groupings. The total precipitation for the spring and summer of 2015 (March through August) was 55 cm, while the same period in 2016 received 68 cm (U.S. Climate Data 2017). This increase in rainfall could explain the increase of plots containing *A. agrestis* and *A. tokioensis* in areas of slightly lower topography in the eastern part of the study area. In the Great Smoky Mountains National Park, Snyder et al. (2011) found that the distribution of an *A. agrestis* population varied monthly with weather conditions, reflected in a receding boundary into lower, moist areas during drought conditions. Our findings suggest *A. agrestis* and *A. tokioensis* may be similarly affected by weather conditions in this study site, since such a dramatic spread was seen after one rainy season and because soil moisture was significantly higher in areas where *A. agrestis* and *A. tokioensis* were found than in areas without them (Table 3). However, this trend is reflected in only two annual August surveys; multiple monthly surveys,

with additional sampling after rain events, would more clearly assess whether the changes in earthworm distribution correlate with increased moisture or heavy rain events as well as if patterns are short-lived, as seen by Gorres et al. (2014). Trends through the early fall should be considered in order to understand how the spread of *A. agrestis* and *A. tokioensis* varies with distribution and survival of cocoons. In addition, long-term trends may reveal important information about how to manage the new invasive species, as earthworm invasion fronts do not always show predictable changes in space and time (Stoscheck et al. 2012).

The opposing patterns in *A. agrestis* and *A. tokioensis* distribution and European earthworm distributions may represent competition between these groups. The high dietary flexibility exhibited by *A. agrestis* in microcosm research by Zhang et al. (2010) included a shift to leaf litter when soil biota was in low abundance, leading to indirect competition with the less flexible litter feeder *L. rubellus*. However, those implications may be dependent on leaf litter quality and plant composition of the study area (Chang et al. 2016c). Earthworm competition for food resources was suggested in a lab mesocosm study by Chang et al. (2016c), which found reduced leaf litter consumption in *L. rubellus* and the European earthworm *Octolasion lactium* when *Metaphire hilgendorfi* was present. The authors also noted reduced *L. rubellus* densities in forests where *M. hilgendorfi* had invaded, but emphasized the importance of differences in species-specific traits such as seasonal versus multi-year life spans as factors that may lead to reduced competition and potential coexistence in the field (Chang et al. 2016c). That research, as well as research focused on changes in carbon and nitrogen associated with earthworm species (Chang et al. 2016b), highlighted the need for considering earthworm species identity rather than functional group to correctly identify their impacts. Our study was limited by not differentiating between *A. agrestis* and *A. tokioensis*, but species identity should be considered for future research on *Amynthas* species in the Upper Midwest.

Changes to the forest floor

Species of Asian earthworms have been shown to have direct effects on soils, with *M. hilgendorfi* increasing NH_4^+ and labile C through granular casts with high

initial microbial activity (Chang et al. 2016b), and *A. agrestis* and *A. tokioensis* increasing mineralization and soil inorganic nitrogen and dissolved organic carbon (Qiu and Turner 2016). Szlavecz et al. (2006) found increased N-mineralization and nitrification in areas containing European earthworms, as well as correlations between those soil characteristics and higher soil pH. Our 2016 sampling found soil pH to be significantly positively related to the abundance of *A. agrestis* and *A. tokioensis*, *L. terrestris*, and *L. rubellus* in areas where each taxon was present (Fig. 5b), but areas without *L. terrestris* and without *L. rubellus* showed significantly higher soil pH on average (Fig. 5a). Increased soil pH in relation to European earthworms has been hypothesized to be due to increased secretion of calcium carbonate by some earthworms (Drouin et al. 2016), or the incorporation of leaf litter into the soil and loss of organic matter causing reduction of organic acids (Burtelow et al. 1998). It is also hypothesized that soil pH may influence earthworm range expansion, based on the tolerance of earthworm species to soil acidity (Stoscheck et al. 2012). It is possible that increases in soil pH could facilitate the invasion of non-native plants, which tend to be less tolerant of acidic soils (Kourtev et al. 1999; Beauséjour et al. 2015), but no changes in exotic plant distribution or abundance were detected to be associated with earthworm distribution in our surveys. However, the differences in the results of the *t* tests and regressions suggest that the overall variability within these plots is too high to conclude that earthworm distribution is determined by soil pH or that earthworm presence and abundance directly influence pH.

One of the most common changes seen in Midwest forests invaded by European earthworms is the reduction of leaf litter on the forest floor through earthworm feeding and soil mixing (Kourtev et al. 1999; Hale et al. 2008; Nuzzo et al. 2009). While our August surveys found significantly less leaf litter mass in areas containing European earthworms compared to areas without European earthworms, litter mass was slightly higher in areas with *A. agrestis* and *A. tokioensis* compared to areas without (Fig. 4). This trend was also reflected in the results of ground cover surveys that showed increased bare soil in plots without *A. agrestis* and *A. tokioensis* and in plots with European earthworms (Table 3). Although *A. agrestis* and *A. tokioensis* create a granular soil signature, we

found individuals predominantly in the upper soil surface under a layer of thick leaf litter, while areas of high European earthworm densities were characterized by exposed roots, middens, and low leaf litter. A preference for soil biota by the *Amynthas* species could explain this initial lack of reduction in leaf litter, as seen by Zhang et al. (2010) with *A. agrestis.* In addition, our sampling timing may have not adequately captured the full pattern of leaf litter depletion; Qiu and Turner (2016) found areas with *A. agrestis* and *A. tokioensis* in the UW Arboretum had higher leaf litter mass than areas without *A. agrestis* and *A. tokioensis* in August, but by September the opposite was true. Different European earthworm species may preferentially feed on older or fresher leaf litter (Hale et al. 2008), suggesting that the timing of sampling may drastically change the observed relationship between earthworm community composition and depth of the forest floor. The full impacts of earthworm invasion, including data on the rate of leaf litter change rather than a static measurement, would be best captured through seasonal or year-round sampling. Furthermore, recent mesocosm work at the Arboretum by Ziter and Turner (unpublished data) suggests that *A. tokioensis* doesn't thrive well in low leaf litter conditions. Leaf litter dynamics represent the complex nature of field studies on earthworm invasion and the uncertainty of the directionality of changes. In this case, it is unclear whether *A. agrestis* and *A. tokioensis* have a smaller impact on leaf litter than other earthworms, or if they preferentially spread to areas with high leaf litter. As with most of the vegetation and forest floor variables in this observational study, both possibilities exist: for earthworm distribution to be dependent on environmental factors or for environmental factors to be dependent on earthworms.

The impact of the study area on changes in vegetation

The trends seen in leaf litter were also seen in analyses comparing vegetation factors such as species richness and Shannon Diversity Index to the presence and absence of *A. agrestis* and *A. tokioensis* and European earthworms. While we did see fewer plant species, less diversity, and less vegetative cover in areas with European earthworms as expected, areas with *A. agrestis* and *A. tokioensis* tended to have slightly higher plant species richness, diversity, and vegetative cover than areas without *A. agrestis* and *A. tokioensis* (Figs. 6, 7), while many vegetation variables such as individual plant species showed no clear differences based on earthworm distributions. One explanation for these findings in comparison to other studies' data is the overall low diversity of plants in this forest, with the majority of vegetation being *Acer saccharum* and *Parthenocissus quinquefolia.* Had the study area been in a forest with higher herbaceous plant diversity or one with a larger number of rare plant species, the patterns seen in the understory vegetation may have been clearer. This low diversity could be attributed to many factors including a legacy effect of European earthworms. Because of the late summer timing of sampling, we also were not able to capture spring ephemeral trends. Our study area was in a smaller patch of forest compared to much of the earthworm research done in national forests of the Upper Midwest, and in a more managed setting. The many trails that run through Wingra and Gallistel Woods as well as the original restoration of this area in the 1940s mean that our results capture a more disturbed setting than the research done in larger and more remote sites. However, because the initial invasion of *A. agrestis* and *A. tokioensis* in Wisconsin has been in urban areas, it is vital to understand how these types of locations respond to their presence.

As an observational field study, this research was influenced by the natural variability of the study area, including the pre-existing populations of earthworms, the state of the understory vegetation, and the health of the overall forest. Results from a study of herbaceous vegetation changes in a Michigan forest using field treatments of *Metaphire hilgendorfi* found similarly inconclusive effects on plants (Greiner et al. 2012). In the present study, as in the work by Greiner et al., the research site was in a forest historically devoid of earthworms, but with European earthworms already present pre-*Amynthas* spp., as opposed to studies of the Upper Midwest investigating the leading edge of initial earthworm invasion (Hale et al. 2006). Greiner et al. suggest that plants in their study site may "have been resistant to earthworm treatments given that earthworm populations were present prior to the start of the experiment." The age of the vegetation can also alter the effects of earthworms: mesocosm research on *L. terrestris* has indicated that established seedlings may not be negatively affected by invasive

earthworms in the same way that seeds can be directly hindered through burial or consumption (Dávalos et al. 2013). Because our research was in a mature forest already invaded by earthworms, our results may not represent the changes that would be seen in an earthworm-free forest invaded by *A. agrestis* and *A. tokioensis*. Although canopy cover differed in areas invaded by different earthworms (Table 3), it is unlikely that earthworms are having an impact on the canopy or that they differentially invade areas with varying shade, but cohorts of maple seedlings currently affected could result in differential canopy cover in the future (Bal et al. 2017). As noted by Hale and Host (2005), the natural variability of areas free of earthworms is crucial to understand but often difficult to determine when studying areas fully invaded.

Another possible explanation for our findings of increased vegetation in areas with *A. agrestis* and *A. tokioensis* is the potential pulse of nutrients that has been documented after some earthworm invasions. Larson et al. (2010) found patterns in tree rings of earthworm-invaded forests that represented a surge in growth due to the increased availability of nutrients caused by earthworm activity, which eventually diminished as nutrients were leached from the soil. More specifically, the rapid mineralization in soils with *A. agrestis* and *A. tokioensis* (Qiu and Turner 2016) suggests more nutrients may be available for plant uptake in areas recently invaded. Asshoff et al. (2010) found an initial positive effect of multiple earthworm groups on seedlings, which they associated with increasing nutrient availability for seed germination in earthworm casts. It is possible that the slight increases in plant species richness and diversity in areas where *A. agrestis* and *A. tokioensis* were found could be due to the initial boost in nutrient availability that the worms provide in the upper soil layers, but this interpretation depends on a more definite arrival timeline of *Amynthas* species in the Arboretum. Furthermore, this hypothesis would suggest that within the next few years, as nutrients become leached from the soils, plants in areas with *A. agrestis* and *A. tokioensis* will experience negative health impacts.

The year-to-year pattern of earthworm distribution in our study shows a shift from European earthworm-dominated areas to *A. agrestis* and *A. tokioensis*-dominated, which raises the possibility that vegetation is being 'released' from effects of multiple European species to those of solely *A. agrestis* and *A. tokioensis*

in the soil system. While the time range of our study was too short to definitively detect any such effects, Hale et al. (2008) found impacts to vegetation and soil nutrients in mesocosms with multiple earthworm species present while little to no changes were seen in some mesocosms with only one earthworm species present, allowing for speculation on the processes that may be at play here. The three European earthworm taxa found in our study area collectively inhabit all three earthworm niches of the soil, whereas *A. agrestis* and *A. tokioensis* do not burrow vertically and typically are not found deeper than 5 cm (Richardson et al. 2009; Qiu and Turner 2016). If the European earthworms were well established prior to *A. agrestis* and *A. tokioensis*' arrival, hindering understory plants, then the shift to soils with no European worms but with *A. agrestis* and *A. tokioensis* may have allowed seedlings and understory plants to rebound, explaining why vegetation was not seen to be negatively impacted in areas with *A. agrestis* and *A. tokioensis*.

The initial invasion dates of both European earthworms and *A. agrestis* and *A. tokioensis* at our study site were unknown, although it is safe to assume that European earthworms have been in the Arboretum on a scale of decades, while *A. agrestis* and *A. tokioensis*' presence is on a scale of years. Knowing the initial distribution of European earthworms pre-*Amynthas* spp. would be immensely helpful in understanding whether patterns seen in this study were pre-existing or new. Our study area differs from the majority of invasive earthworm studies in that the typical invasion front seen starts with epigeic earthworms and over time leads to a complete assemblage of multiple earthworm groups (Hale et al. 2005). Here we have an established community of epi-endogeic and anecic European earthworms with the new addition of the predominantly epigeic *A. agrestis* and *A. tokioensis*. This reverse pattern makes it difficult to predict the time lags and impacts related to *A. agrestis* and *A. tokioensis* as they become established within the Arboretum and Wisconsin (Beauséjour et al. 2015), but emphasizes the importance of tracking this informative invasion.

Future considerations

While *A. agrestis* and *A. tokioensis* differ in behavior compared to many of the European species already in the Upper Midwest, they share similarities with the

European earthworm *Dendrobaena octaedra* found elsewhere in the region, which may be helpful in interpreting our findings. *D. octaedra* has been described as having high cocoon productivity, as do *A. agrestis* and *A. tokioensis*, and the ability to withstand a wider range of environmental conditions than other European species, such as drought and acidic leaf litter (Beauséjour et al. 2015). *D. octaedra* is an epigeic parthenogenetic species that has been found to not reduce the forest floor as other epi-endogeic and anecic species do, but rather thrives in thick leaf litter (Hale et al. 2005; Beauséjour et al. 2015). A study in northern Wisconsin and Minnesota found negative relationships between *Lumbricus* and the depth of leaf litter and richness of plant species, but positive relationships between those variables and *D. octaedra* abundance (Holdsworth et al. 2007). This was attributed to the reduction in *D. octaedra* populations with increasing *L. terrestris* invasions—a pattern we may be seeing in reverse in our study with decreasing *L. terrestris* as *A. agrestis* and *A. tokioensis* invade. Although these newly invasive members of the *Amynthas* genus have additional traits not exhibited by *D. octaedra*, such as dietary flexibility, rapid growth, and the creation of granular soil, the similarities of *Amynthas* spp. and *D. octaedra*, suggest that research done on *D. octaedra* could inform future studies on *A. tokioensis* and *A. agrestis*.

In conclusion, our study provides evidence that *A. agrestis* and *A. tokioensis* have the ability to spread rapidly in deciduous forests of Wisconsin, where they were recently recorded for the first time. The pattern of *A. agrestis* and *A. tokioensis* spread in the UW Arboretum contrasted the shrinking distributions of the three European earthworm groups surveyed, suggesting potential competition or interaction between invasive earthworms. Leaf litter and plant diversity showed increasing trends in areas with *A. agrestis* and *A. tokioensis* compared to areas without, while most traits of the vegetation community did not show conclusive findings. Although there were limitations to this study, including the small survey area and the seasonality of only two annual surveys, we were able to capture a key snapshot of the early years of invasion in a site that exemplifies the areas *A. agrestis* and *A. tokioensis* have begun to invade in Wisconsin, establishing a benchmark for future observations. We recommend a continuation of long-term surveys in both the UW Arboretum and throughout the

state to understand the patterns to the invasion of *A. agrestis* and *A. tokioensis*, their interactions with other earthworms, and the effects on soils and plants to fill the remaining knowledge gaps in the implications for forests of the Upper Midwest.

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