

The unseen invaders: introduced earthworms as drivers of change in plant communities in North American forests (a meta-analysis)

DYLAN CRAVEN^{1,2}, MADHAV P. THAKUR^{1,2}, ERIN K. CAMERON^{3,4}, LEE E. FRELICH⁵, ROBIN BEAUSÉJOUR⁶, ROBERT B. BLAIR⁷, BERND BLOSSEY⁸, JAMES BURTIS⁸, AMY CHOI⁹, ANDREA DÁVALOS⁸, TIMOTHY J. FAHEY⁸, NICHOLAS A. FISICHELLI¹⁰, KEVIN GIBSON¹¹, I. TANYA HANDA¹², KRISTINE HOPFENSBERGER¹³, SCOTT R. LOSS¹⁴, VICTORIA NUZZO¹⁵, JOHN C. MAERZ¹⁶, TARA SACKETT⁹, BRYANT C. SCHARENBRUCH¹⁷, SANDY M. SMITH⁹, MARK VELLEND⁶, LAUREN G. UMEK^{18,19} and NICO EISENHAUER^{1,2}

¹German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany,

²Institute of Biology, Leipzig University, Johannisallee 21, 04103 Leipzig, Germany, ³Metapopulation Research Centre,

Department of Biosciences, University of Helsinki, PO Box 65, 00014 Helsinki, Finland, ⁴Center for Macroecology, Evolution and Climate Change, Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, København, Denmark,

⁵Center for Forest Ecology, University of Minnesota, St. Paul, MN, USA, ⁶Département de Biologie, Université de Sherbrooke, Sherbrooke, QC, Canada, ⁷Department of Fisheries, Wildlife, and Conservation Biology, University Of Minnesota, 2003 Upper Buford Circle Suite 135, St. Paul, MN 55108, USA, ⁸Department of Natural Resources, Cornell University, Ithaca, New York, USA, ⁹Faculty of Forestry, University of Toronto, Toronto, ON, Canada, ¹⁰Schoodic Institute at Acadia National Park, Forest Ecology Program, Winter Harbor, ME, USA, ¹¹Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, USA, ¹²Département des sciences biologiques, Université du Québec à Montréal, Montréal, QC, Canada, ¹³Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY, USA, ¹⁴Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK, USA, ¹⁵Natural Area Consultants, 1 West Hill School Road, Richford, NY, USA, ¹⁶Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA, USA, ¹⁷College of Natural Resources, University of Wisconsin – Stevens Point, Stevens Point, WI, USA, ¹⁸Plant Biology and Conservation, Northwestern University, Evanston, IL, USA, ¹⁹Department of Plant Science, Chicago Botanic Garden, Glencoe, IL, USA

Abstract

Globally, biological invasions can have strong impacts on biodiversity as well as ecosystem functioning. While less conspicuous than introduced aboveground organisms, introduced belowground organisms may have similarly strong effects. Here, we synthesize for the first time the impacts of introduced earthworms on plant diversity and community composition in North American forests. We conducted a meta-analysis using a total of 645 observations to quantify mean effect sizes of associations between introduced earthworm communities and plant diversity, cover of plant functional groups, and cover of native and non-native plants. We found that plant diversity significantly declined with increasing richness of introduced earthworm ecological groups. While plant species richness or evenness did not change with earthworm invasion, our results indicate clear changes in plant community composition: cover of graminoids and non-native plant species significantly increased, and cover of native plant species (of all functional groups) tended to decrease, with increasing earthworm biomass. Overall, these findings support the hypothesis that introduced earthworms facilitate particular plant species adapted to the abiotic conditions of earthworm-invaded forests. Further, our study provides evidence that introduced earthworms are associated with declines in plant diversity in North American forests. Changing plant functional composition in these forests may have long-lasting effects on ecosystem functioning.

Keywords: introduced earthworms, plant communities, plant diversity, biological invasions, earthworm invasion, community composition, meta-analysis

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Introduction

Species invasions are among the strongest drivers of environmental change globally (Sala *et al.*, 2000;

Murphy & Romanuk, 2014) and can have large effects on biodiversity and ecosystem processes, functions and services (Wardle *et al.*, 2004; Vilà *et al.*, 2011; Walsh *et al.*, 2016). Understanding the full extent of invasive species impacts is a fundamental challenge in ecology (Simberloff *et al.*, 2013); species invasions can have cascading effects across trophic levels (Estes *et al.*, 2011)

Correspondence: Dylan Craven, tel: +49 341-9733173, fax +49 341-9739358, e-mail dylan.craven@aya.yale.edu

and facilitate future invasions (Simberloff & Von Holle, 1999). While the ecological importance of linkages between above- and belowground communities and ecosystem processes is recognized (de Deyn & van der Putten, 2005), the impacts of introduced belowground organisms, such as earthworms, on aboveground communities are less understood than those of introduced aboveground organisms (Wardle *et al.*, 2004; Cameron *et al.*, 2016).

Introduced earthworms, which have been invading northern North American forests with little or no native earthworm fauna since the time of European settlement (Hendrix & Bohlen, 2002), have a profound influence on ecosystem functioning and services in North American forests. By re-engineering soil physical and chemical characteristics (Bohlen *et al.*, 2004; Hendrix *et al.*, 2008; Eisenhauer, 2010), they accelerate nutrient cycling (Szlavec *et al.*, 2006; Sackett *et al.*, 2013; Ewing *et al.*, 2015) and water infiltration (Eisenhauer *et al.*, 2012; Capowiez *et al.*, 2014) and may increase CO₂ emissions to the atmosphere (Lubbers *et al.*, 2013). Significant impacts of introduced earthworms on ecosystem functioning could also be mediated through changes in biodiversity, given the wide range of above- and belowground organisms with which they interact (Edwards, 2004). Previous studies have documented effects of introduced earthworms on ecological communities in forests (Hale *et al.*, 2006; Eisenhauer *et al.*, 2007; Frelich *et al.*, 2012; Fisichelli *et al.*, 2013), but the generality or variability of such effects has yet to be systematically quantified. Here, we synthesize the impacts of invasive earthworms on the diversity and composition of understory plant communities in North American forests.

Previous studies have documented a range of effects of introduced earthworms on plant communities (e.g., Hale *et al.*, 2006), including changes in both species diversity and composition. Introduced earthworms cause these changes in a number of ways: by predated and ingesting seeds and seedlings (Zaller & Saxler, 2007; Asshoff *et al.*, 2010; Forey *et al.*, 2011; Drouin *et al.*, 2014; Clause *et al.*, 2015; Cassin & Kotanen, 2016), altering seedbank composition (Eisenhauer *et al.*, 2009; Nuzzo *et al.*, 2015), modifying microhabitats where seeds germinate by removing leaf litter (Frelich *et al.*, 2006), and accentuating drought events by accelerating drainage via constructing burrows (Larson *et al.*, 2010; Eisenhauer *et al.*, 2012). Furthermore, disturbances associated with introduced earthworms might confer a competitive advantage to graminoids (Hale *et al.*, 2006; Holdsworth *et al.*, 2007a; Nuzzo *et al.*, 2009; Fisichelli *et al.*, 2013; but see Dobson & Blossey, 2015), or facilitate invasion by other species (e.g., Heneghan *et al.*, 2007; Nuzzo *et al.*,

2009). Other common stressors in North American forests, for example, deer browsing or allelopathic invasive plants, also may interact with introduced earthworms, potentially intensifying compositional changes in plant communities (Fisichelli *et al.*, 2013; Dávalos *et al.*, 2015; Hale *et al.*, 2016). The overall impact of ecosystem engineers on plant diversity is generally considered to be positive (Romero *et al.*, 2015) but has yet to be systematically investigated for earthworms across North American forests.

Variation in the impacts of introduced earthworms on biodiversity could reflect differences in invasion history. Different assemblages of introduced earthworms have been strongly correlated with distinct stages of invasion (Hale *et al.*, 2005; Holdsworth *et al.*, 2007b; Loss *et al.*, 2013). Recently invaded forests are usually dominated by epigeic earthworms, such as *Dendrobaena octaedra*, while forests with longer invasion histories also have endogeic and anecic species where environmental conditions, for example, soil pH, texture and moisture, permit their presence (Hale *et al.*, 2005; Frelich *et al.*, 2006; Holdsworth *et al.*, 2007b; Loss *et al.*, 2013). The combined impact of multiple earthworm ecological groups on forest understories can be dramatic, as these earthworm assemblages can completely remove surface leaf litter, reduce organic matter in upper soil horizons (Hale *et al.*, 2005; Nuzzo *et al.*, 2009; Resner *et al.*, 2015), and cause significant declines in plant diversity (Hale *et al.*, 2006; Holdsworth *et al.*, 2007a). Therefore, accounting for earthworm invasion history is essential for reaching a general understanding of how diversity and composition of plant communities are affected by introduced earthworms.

Changes in biodiversity due to introduced earthworms could lead to significant alterations in the provisioning of vital ecosystem functions in North American forests, such as nutrient and water cycling, which has important implications for the development of future management and conservation strategies. In the present study, we assessed for the first time impacts of introduced earthworms on plant diversity and composition across North American forests using meta-analytic techniques. We predict that the presence and abundance of introduced earthworms (i) decrease plant species diversity (Bohlen *et al.*, 2004) and (ii) systematically favor graminoids (Frelich *et al.*, 2012; Fisichelli *et al.*, 2013) and non-native plant species (Heneghan *et al.*, 2007; Nuzzo *et al.*, 2009). Further, we expect that the magnitude of all abovementioned effects will increase with the number of earthworm ecological groups, reflecting a greater variety of potential disturbance mechanisms likely to result in changes in plant communities (Hale *et al.*, 2006).

Materials and methods

Data selection

To create a database of the effects of earthworm invasion on understory plant communities in North American forests, we performed a search in July 2014 in the ISI Web of Science database using the keywords ('earthworm*') AND ('exotic' OR 'invasive'). These search terms were selected in order to include a wide array of studies that addressed the effects of introduced earthworms on aboveground communities. From the initial list of 359 studies (see PRISMA diagram; Appendix S1), we examined each title and abstract to determine whether they met the inclusion criteria. Our inclusion criteria were that each study: (i) was performed in a North American forest ecosystem, (ii) reported density, biomass, or presence/absence of introduced earthworms that were identified either to species or earthworm ecological group and (iii) reported the cover or presence/absence of plant species (or plant functional groups) in the forest understory. Subsequently, we communicated with authors of the selected studies to obtain raw data for earthworm and plant communities. When raw data were not available, means or effect sizes were extracted directly from figures and tables. Through personal knowledge of the authors, we also obtained data from three Masters theses. In total, we identified 14 unique studies meeting our inclusion criteria (Appendix S2 and Table S1) that allowed us to examine associations of introduced earthworm community properties (density, biomass, and ecological group richness) with understory plant community properties (diversity, species richness, and evenness: 13 studies, 233 effect sizes) and with cover of plant growth forms and native or non-native status (11 studies, 412 effect sizes). As most studies used hierarchical sampling designs, effect sizes were calculated at the site level for each combination of earthworm and plant community measures to capture within-study variation.

Data description and preparation

Studies meeting the inclusion criteria were published between 2006 and 2015 and were predominantly located in the Upper Midwest and Mid-Atlantic regions of the United States, from Indiana, the United States to Alberta, Canada (Table S1). Plant communities were typically assessed using plots (median area = 1.4 m²; range = 0.25–100 m²) in which identity and percent cover were recorded for each species. Most studies assessed plant communities once; for those that did so over multiple years, we calculated the mean percent cover of each plant species over the study period. Species richness (number of species), diversity (Shannon–Wiener diversity), and evenness (Evar; Smith & Wilson, 1996) of the understory plant community (usually all species less than 1 m tall) were calculated at the plot level. Plant growth habits and plant native status were extracted from the USDA plant database (USDA, 2014) for each species and categorized into three functional groups; woody, herbaceous, and graminoid (grasses and sedges), and either native or non-native. Cover of each plant functional group or plant native status was calculated as the summed percent of total cover in a plot.

In general, earthworm communities were sampled in subplots (0.06–0.25 m²) nested within vegetation plots. Earthworms were extracted using a variety of techniques, for example, liquid mustard, cover boards, or formalin (Table S1), sorted to species or earthworm ecological group, counted, and/or weighed. For each plot, we calculated three measures of introduced earthworm communities: total biomass (g m⁻²), total density (number of individuals m⁻²), and richness of earthworm ecological groups (0–3). Introduced earthworm species were categorized into three ecological groups, anecic, epigeic, and endogeic (Table S2), which reflect differences in habitat and feeding preferences (Bouché, 1977; Bohlen *et al.*, 2004). While widely used, this classification system may not be optimal as earthworm ecological groups cover a range of sizes and feeding behaviors (Lavelle, 1983; Brown, 1995). For example, *Lumbricus rubellus* and *Aporrectodea longa* are typically classified as epigeic and anecic, respectively, but their feeding behavior places them between different ecological groups ('epi/endogeic' and 'anecic-endogeic', respectively; Eisenhauer *et al.*, 2008; Ferlian *et al.*, 2014). As earthworm communities were sampled with different frequencies across studies (Table S1), we calculated the mean of each measure of earthworm communities per plot across sampling periods.

Effect sizes

To estimate the direction and strength of the relationships between introduced earthworm and plant communities, Spearman's rank correlation coefficients were calculated because most data were not distributed normally (Myers & Sirois, 2014). Spearman's correlation coefficients were transformed to Pearson's correlations and then converted to Fisher's *z* transformation of *r* for analysis to normalize the distribution of data (Koricheva *et al.*, 2013). Sampling variance for Fisher's *z* transformation of *r* was calculated using unbiased estimates following Hedges (1989). Differences in data collection in particular studies prevented effect sizes from being calculated for all possible combinations of plant and earthworm community measures (Table S1).

Data analysis

We used multilevel, meta-analytic regression models to estimate mean effect sizes of the relationships between introduced earthworm and plant communities in North American forests with the 'metafor' package (Viechtbauer, 2010). These models account for the nonindependence of measures taken from the same study (Nakagawa & Santos, 2012; Koricheva *et al.*, 2013). For all models, we used a random intercept term where 'site' was nested within 'study'. Observations from studies were weighted by the inverse of the sampling variance (Viechtbauer, 2010). Separate models using restricted maximum likelihood estimation were fitted for each measure of plant diversity and each plant functional group or native status. In all models, introduced earthworm community measures were included as a categorical moderator variable to test whether these measures had similar or

contrasting effects. Model assumptions were checked by visually inspecting residuals for homogeneity and Pearson residuals for normality. To assess whether publication bias affected the results of our analyses, we visually inspected contour funnel plots (Koricheva & Gurevitch, 2014). We assessed the sensitivity of our analyses to the type of earthworm sampling technique, for example, formalin, liquid mustard, or cover boards. To do so, we added earthworm sampling technique as a fixed effect and its interaction with earthworm community measures to our original models. All analyses were performed using R 3.2.3 (R Core Team, 2015). Effect sizes and sampling variances used for all analyses are included as Supporting Information (for metadata, see Appendices S3 & S4).

Results

Plant diversity

We found that plant species diversity was significantly and negatively correlated with richness of earthworm ecological groups (Fig. 1; 95% confidence intervals did not overlap with zero), but was not significantly correlated with earthworm biomass or density. Plant species evenness and richness were not significantly correlated with any measure of introduced earthworm communities (Fig. 1). For each plant community variable, effect sizes were similar for earthworm biomass, density, and ecological group richness (Table 1).

Plant community composition

Graminoid cover was significantly positively correlated with introduced earthworm biomass, density, and ecological group richness (Fig. 2). In contrast, cover of neither herbaceous nor woody plants was significantly correlated with any measure of introduced earthworm communities. Non-native plant cover exhibited a significant, positive correlation with introduced earthworm biomass (Fig. 3), but not with introduced earthworm density or richness of earthworm ecological groups. Native plant cover decreased, albeit nonsignificantly (mean effect size = -0.24 ; 95% confidence interval = -0.49 – 0.01), with increasing introduced earthworm biomass.

Effect sizes for the relationships of native and non-native plants with introduced earthworm communities differed significantly depending on the earthworm community measure (Table 1). Native plant cover and non-native plant cover were more strongly related to introduced earthworm biomass than to either earthworm density or richness of earthworm ecological groups (Fig. 3).

Across-study variation

For all models testing the association between plant diversity and community composition with introduced

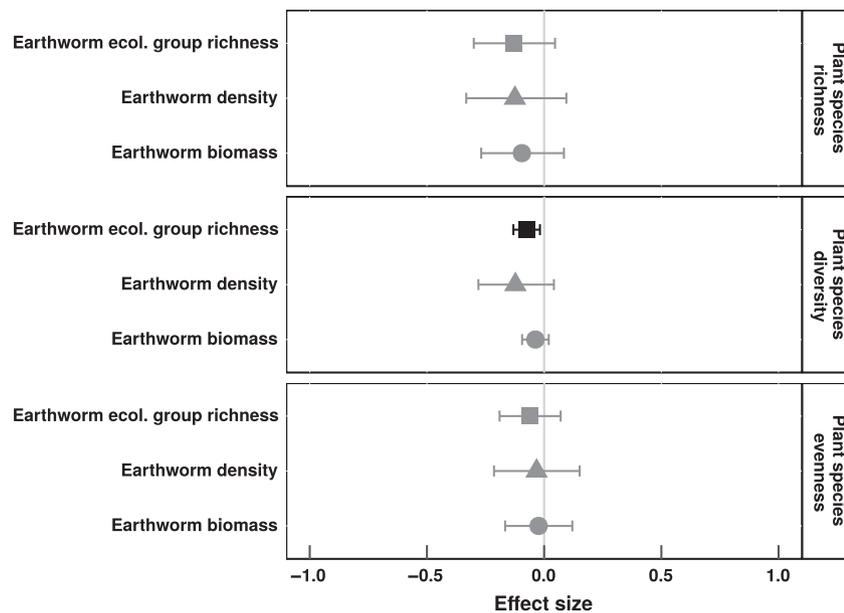


Fig. 1 Mean effect sizes of relationships between introduced earthworm communities and plant species richness, diversity, and evenness of forest understory communities in North America. Whisker bars are 95% confidence intervals: Black whisker bars did not overlap with zero. Mean effect sizes are Pearson's correlation coefficients. Plant species diversity was calculated using Shannon–Wiener diversity, and plant species evenness was calculated using Evar (Smith & Wilson, 1996). Earthworm biomass is biomass of introduced earthworms (g m^{-2}), earthworm density is number of introduced earthworms ($\text{individuals m}^{-2}$), and earthworm ecological group richness is the number of introduced earthworm ecological groups.

Table 1 Summary of meta-analytic mixed-effects models testing the relationships between introduced earthworm biomass, density, and ecological group richness and plant diversity, native and non-native status, and functional group cover of forest understory communities in North America

Response variable	Studies	Observations	AICc	Residual heterogeneity	L
<i>Plant diversity</i>					
Plant species richness	13	83	56.1	209.2	0.4
Plant species diversity	10	75	43.0	112.8	0.4
Plant species evenness	10	75	28.2	98.0	0.5
<i>Plant functional groups</i>					
Herbaceous cover	11	102	35.5	188.8	3.4
Graminoid cover	9	88	37.1	203.9	1.2
Woody cover	11	102	458.6	816.6	3.4
<i>Plant native status</i>					
Native plant cover	10	61	67.1	262.0	7.9
Non-native plant cover	9	59	60.5	221.4	8.7

Meta-analytic mixed-effects models evaluated the size effects representing the association between a measure of introduced earthworm community abundance or structure (density, biomass, richness of earthworm ecological groups) and plant diversity, plant native and non-native status, or cover of plant functional groups. Plant species diversity is Shannon–Wiener diversity, plant species evenness is Smith and Wilson’s evenness measure (‘Evar’; Smith & Wilson, 1996). Residual heterogeneity shows if the variability of the effect sizes not captured by the moderator variables is heterogeneous. The moderator variable in all models was a categorical factor representing measures of introduced earthworm communities. L is the likelihood ratio test statistic for model coefficients. Values of residual heterogeneity and L in black italics indicate statistical significance ($\alpha = 0.05$).

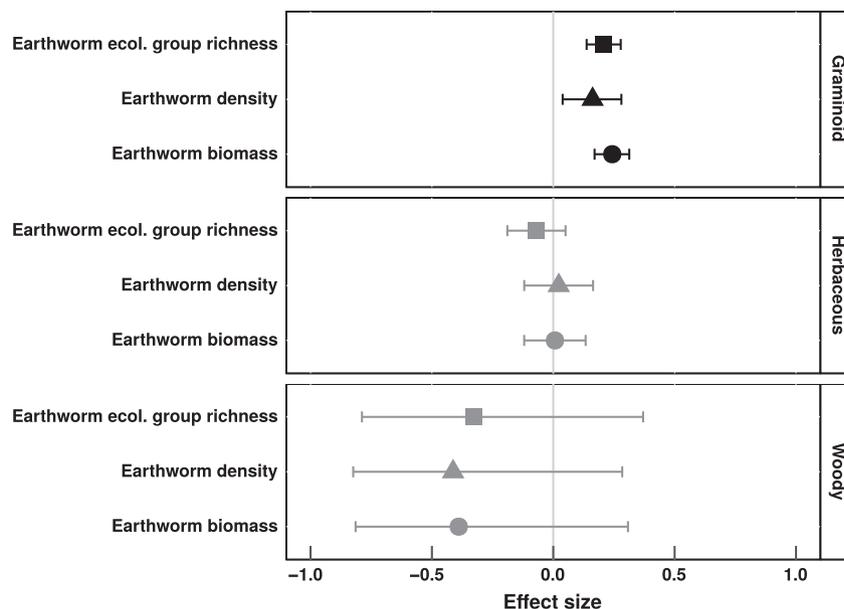


Fig. 2 Mean effect sizes of relationships between introduced earthworm communities and cover of plant functional groups in forest understory communities in North America. Whisker bars are 95% confidence intervals: Black whisker bars did not overlap with zero. Effect sizes are Pearson’s correlation coefficients. Earthworm biomass is biomass of introduced earthworms (g m^{-2}), earthworm density is number of introduced earthworms ($\text{individuals m}^{-2}$), and earthworm ecological group richness is the number of introduced earthworm ecological groups.

earthworm communities, the results showed a consistent and statistically significant amount of residual heterogeneity among studies (Table 1). In other words, our analysis detected that additional, unmeasured variables would be important for explaining the association

between plant and earthworm communities. Visual inspection of contour funnel plots did not reveal publication bias toward over-reporting relationships between plant diversity or community composition with non-native earthworm abundance or community

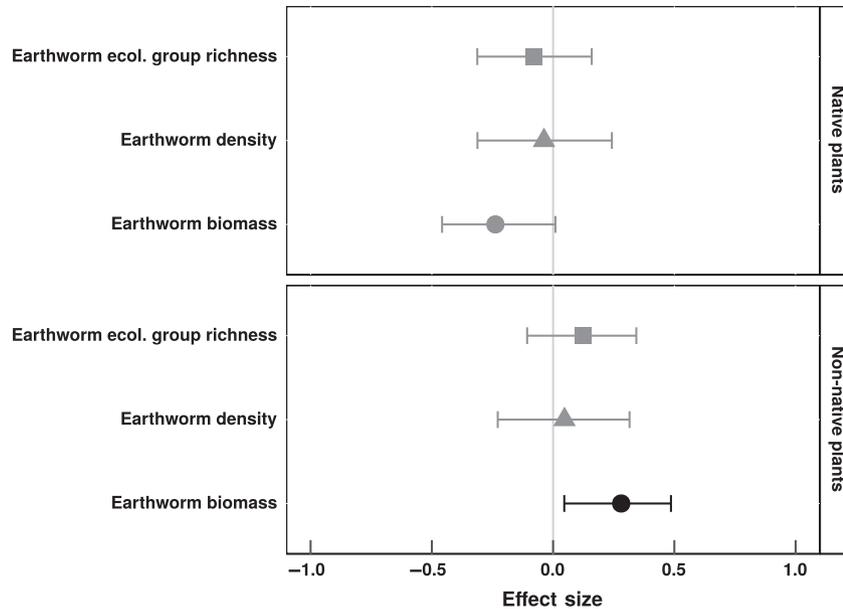


Fig. 3 Mean effect sizes of relationships between introduced earthworm communities and cover of native and non-native plants in forest understory communities in North America. Whisker bars are 95% confidence intervals: Black whisker bars did not overlap with zero. Effect sizes are Pearson's correlation coefficients. Earthworm biomass is biomass of introduced earthworms (g m^{-2}), earthworm density is number of introduced earthworms ($\text{individuals m}^{-2}$), and earthworm ecological group richness is the number of introduced earthworm ecological groups.

composition. Results from the sensitivity analyses indicated that different earthworm sampling techniques did not influence the association between plant diversity and community composition with introduced earthworm communities. For all models, the interaction of earthworm sampling technique and earthworm community measures was not statistically significant ($P > 0.10$).

Discussion

The extensive effects of introduced ecosystem engineers, such as earthworms, in North American forests could have transformative impacts on biodiversity and diversity-dependent ecosystem functions (Ehrenfeld, 2010). Here, we present the first quantitative meta-analysis showing that introduced earthworms are significantly associated with declines in plant species diversity and changes in plant community composition across multiple studies in North American forests.

Changes in plant diversity

Our analysis showed that earthworm invasion significantly influences plant species diversity. Forests with multiple earthworm ecological groups had significantly lower plant diversity than forests with fewer earthworm ecological groups. While particular earthworm species or ecological groups may have specific effects

on seed fate and seedling growth and mortality (e.g., Asshoff *et al.*, 2010; Eisenhauer *et al.*, 2012; Fahey *et al.*, 2013; Drouin *et al.*, 2014; Groffman *et al.*, 2015), our results highlight the combined impacts of multiple earthworm ecological groups on plant communities (Hale *et al.*, 2005). Changes in plant species diversity likely occurred through a number of direct mechanisms, such as seed or seedling ingestion (Eisenhauer *et al.*, 2010; Clause *et al.*, 2015; Cassin & Kotanen, 2016), and indirect mechanisms, including altering water or nutrient availability, mycorrhizal associations, and soil structure (Hale *et al.*, 2005; Holdsworth *et al.*, 2007b; Dobson & Blossey, 2015; Resner *et al.*, 2015; Paudel *et al.*, 2016). While richness of earthworm ecological groups was positively and significantly correlated with earthworm density and biomass (Fig. S1 and Table S3), neither of the latter surrogates for earthworm activity exhibited significant associations with plant species diversity. In contrast to richness of earthworm ecological groups, variation in earthworm density or biomass may not fully capture the progression of earthworm invasion impacts (Loss *et al.*, 2013) and, rather, may reflect changes in dominance of particular earthworm species, such as small-bodied *D. octaedra*, large-bodied *L. terrestris* (Hale *et al.*, 2004) or entire ecological groups (Hale *et al.*, 2005; Eisenhauer *et al.*, 2007; Holdsworth *et al.*, 2007b).

In line with recent global meta-analyses on local-scale changes in species richness (Vellend *et al.*, 2013;

Dornelas *et al.*, 2014; but see Gonzalez *et al.*, 2016), we found that plant species richness was robust to earthworm invasion. Despite no average change in plant species richness or evenness, there were clear changes in plant diversity and community composition. This result also could reflect scale-dependent effects of earthworm invasions on plant diversity (Powell *et al.*, 2013); similar to plant invasions, earthworm invasion may alter the slope and intercept of the species–area relationship by changing species abundance distributions.

Changes in plant community composition: native versus non-native plant species

We provide clear evidence that non-native plant cover is positively associated with biomass of introduced earthworms, thus supporting the ‘invasional meltdown’ hypothesis (Simberloff & Von Holle, 1999). Plant species that coevolved in the presence of earthworms, that is plant species of European and Asian origin, could have adaptations that confer tolerance to the presence of earthworms. Non-native plant species may be adapted to similar soil conditions as those found in earthworm-invaded forests, such as high soil pH and sparse litter cover (Nuzzo *et al.*, 2009; Beauséjour *et al.*, 2014; Whitfeld *et al.*, 2014). The bioturbation of the forest floor associated with earthworm invasion also may favor non-native plant species without obligate mycorrhizal associations (Lawrence *et al.*, 2003; Paudel *et al.*, 2016). Furthermore, introduced earthworms have been found to have positive, direct effects on non-native plant species (Roth *et al.*, 2015) by burying seeds in their burrows, which have high nutrient concentrations and may attenuate drought stress (Migge-Kleian *et al.*, 2006; Eisenhauer & Scheu, 2008). There is also growing evidence of a synergistic effect of introduced earthworms and deer herbivory on non-native plant species (Dávalos *et al.*, 2015). Particularly in newly invaded North American forests, introduced earthworms – in combination with deer herbivory – may be increasing disturbance frequency relative to historical norms (Frelich *et al.*, 2012), which is an important factor in explaining increases in the abundance of non-native species (Moles *et al.*, 2012).

Our analysis showed that cover of native plant species did not change consistently in earthworm-invaded forests. While the trend of decreasing native species cover with increasing introduced earthworm biomass supports the idea that earthworm invasion may lead to declines in native plant species diversity (Nuzzo *et al.*, 2009), high across-study variation reduced the overall strength of this signal. This result suggests that physical disturbance of the forest floor by introduced

earthworms may increase the heterogeneity of regeneration sites, creating a greater diversity of favorable microhabitats for seed germination of both native and non-native plant species (Asshoff *et al.*, 2010; Nuzzo *et al.*, 2015). In addition, we found that total plant cover increased significantly with earthworm biomass (Fig. S2 and Table S4), possibly indicating that earthworm invasion enhances germination of many plant species (Nuzzo *et al.*, 2015). Results from previous studies have shown both positive and negative effects of introduced earthworms on seedling survival of native plant species. Dobson & Blossey (2015) found that twelve of fifteen native species were negatively affected by earthworm invasion, while other microcosm and field studies have reported both types of responses to the presence of introduced earthworms (Holdsworth *et al.*, 2007a; Corio *et al.*, 2009; Drouin *et al.*, 2014). Native species’ responses to earthworms could be driven by tolerance to drought and frost upheaval (Dobson & Blossey, 2015), mycorrhizal associations (Lawrence *et al.*, 2003; Paudel *et al.*, 2016), or tolerance to root herbivory (Cameron *et al.*, 2014).

Changes in plant community composition: graminoids

We predicted and found a positive relationship between graminoid cover and all measures of earthworm invasion. This finding suggests that earthworm invasion acts as a significant ecological filter that appears to drive strong changes in plant community composition. The long-term effects of earthworm invasion on abiotic conditions in the forest understory, for example, rapid soil nutrient release and subsequent depletion, decreased soil water content, and increased surface runoff (Hale *et al.*, 2005; Eisenhauer *et al.*, 2012; Resner *et al.*, 2015), may confer a competitive advantage to graminoids, particularly those with greater drought tolerance (Craine *et al.*, 2013) and persistent bud banks (Bond, 2008; VanderWeide & Hartnett, 2015). Certain graminoids, such as those with greater tolerance of root herbivory (Cameron *et al.*, 2014; Gilbert *et al.*, 2014) or those without obligate mycorrhizal associations (e.g., *Carex pensylvanica*; Holdsworth *et al.*, 2007a), also may respond positively to earthworm invasion. However, positive responses of graminoids to earthworms might be restricted to a subset of species within this functional group (Corio *et al.*, 2009; Dobson & Blossey, 2015). Increasing graminoid cover in North American forests also may be attributable to positive, synergistic interactions with co-occurring disturbances, such as deer browsing, fire history, forest management, and land-use history (Powers & Nagel, 2008; Fisichelli *et al.*, 2013). Given the prevalence of disturbances in northern North American forests that may influence biodiversity

(Murphy & Romanuk, 2014), future studies should account for co-occurring disturbances to add greater precision to estimates of the impacts of earthworm invasion on biodiversity of above- and belowground communities (Cameron *et al.*, 2016).

Variation across studies

Across studies, impacts of earthworm invasion were significantly heterogeneous, likely due to variation in biophysical factors (Hale *et al.*, 2005; Resner *et al.*, 2015), invasion history (Hale *et al.*, 2006), and co-occurring disturbances (Fischelli *et al.*, 2013; Dávalos *et al.*, 2015). Variation and error associated with measurement of earthworm and plant communities also may contribute to this heterogeneity. Estimates of earthworm density or biomass may be imprecise or inconsistent because of the scale of sampling, time of year, and year to year fluctuations in abundance (Callaham & Hendrix, 1997). In contrast, measures of plant communities likely reflect the history of earthworm invasion at a given site (Larson *et al.*, 2010), which may also vary within studies (e.g., Hale *et al.*, 2006; Holdsworth *et al.*, 2007b). However, one-time plant inventories along earthworm invasion fronts have a limited capacity to capture taxonomic and functional turnover of plant communities in response to earthworm invasion; repeated inventories, including those of uninvaded forests to establish pre-invasion baselines, are vital for improving current estimates of biodiversity change (Eisenhauer *et al.*, 2016; Gonzalez *et al.*, 2016). Disentangling the impacts of earthworm invasion from biophysical and other co-occurring disturbances on plant communities may be best addressed by combining field experiments (e.g., Dobson & Blossey, 2015) with quantitative trait and phylogenetic information (e.g., Cassin & Kotanen, 2016; Lemoine *et al.*, 2016). Accounting for such factors in future studies, particularly in regions where plant communities have interacted with native earthworm fauna, would help to clarify the mechanisms through which earthworm invasion affects biodiversity.

Overall, our results show that earthworm invasion is associated with significant changes in the diversity of plant communities in North American forests. By changing the functional composition and facilitating the invasion of non-native plant species, earthworm invasion may have long-lasting impacts on ecosystem functioning and services in these forests. Furthermore, there is growing evidence that terrestrial, invertebrate invaders likely have strong impacts on other trophic levels and associated ecosystem functions (Wardle *et al.*, 2011; Cameron *et al.*, 2016), which may be accentuated with climate change (Eisenhauer *et al.*, 2012). A more holistic approach to assessing the impacts of

earthworm invasion, therefore, will be vital for developing management and conservation strategies that enhance the resilience of North American forests (Nimmo *et al.*, 2015).

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References

- Asshoff R, Scheu S, Eisenhauer N (2010) Different earthworm ecological groups interactively impact seedling establishment. *European Journal of Soil Biology*, **46**, 330–334.
- Beauséjour R, Handa IT, Lechowicz MJ, Gilbert B, Vellend M (2014) Historical anthropogenic disturbances influence patterns of non-native earthworm and plant invasions in a temperate primary forest. *Biological Invasions*, **17**, 1267–1281.
- Bohlen PJ, Scheu S, Hale CM, McLean MA, Migge S, Groffman PM, Parkinson D (2004) Non-native invasive earthworms as agents of change in northern temperate forests. *Frontiers in Ecology and the Environment*, **2**, 427–435.
- Bond WJ (2008) What limits trees in c_4 grasslands and savannas? *Annual Review of Ecology, Evolution, and Systematics*, **39**, 641–659.
- Bouché M (1977) Strategies lombriciennes. *Ecological Bulletins*, **25**, 122–132.
- Brown GG (1995) How do earthworms affect microfloral and faunal community diversity? In: *The Significance and Regulation of Soil Biodiversity*, vol. 63 (ed./eds Collins HP, Robertson GP, Klug MJ), pp. 247–269. Springer, Dordrecht, The Netherlands.
- Callaham MA, Hendrix PF (1997) Relative abundance and seasonal activity of earthworms (Lumbricidae and Megascolecidae) as determined by hand-sorting and formalin extraction in forest soils on the southern Appalachian Piedmont. *Soil Biology and Biochemistry*, **29**, 317–321.
- Cameron EK, Cahill JF Jr, Bayne EM (2014) Root foraging influences plant growth responses to earthworm foraging. *PLoS ONE*, **9**, e108873.
- Cameron EK, Vilà M, Cabeza M (2016) Global meta-analysis of the impacts of terrestrial invertebrate invaders on species, communities and ecosystems. *Global Ecology and Biogeography*, **25**, 596–606.
- Capowiez Y, Sammartino S, Michel E (2014) Burrow systems of endogeic earthworms: Effects of earthworm abundance and consequences for soil water infiltration. *Pedobiologia*, **57**, 303–309.
- Cassin CM, Kotanen PM (2016) Invasive earthworms as seed predators of temperate forest plants. *Biological Invasions*, **18**, 1567–1580.
- Clause J, Forey E, Lortie CJ, Lambert AM, Barot S (2015) Non-native earthworms promote plant invasion by ingesting seeds and modifying soil properties. *Acta Oecologica*, **64**, 10–20.
- Core Team R (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Corio K, Wolf A, Draney M, Fewless G (2009) Exotic earthworms of great lakes forests: a search for indicator plant species in maple forests. *Forest Ecology and Management*, **258**, 1059–1066.
- Craime JM, Ocheltree TW, Nippert JB, Towne EG, Skibbe AM, Kembel SW, Fargione JE (2013) Global diversity of drought tolerance and grassland climate-change resilience. *Nature Clim. Change*, **3**, 63–67.
- Dávalos A, Nuzzo V, Blossey B (2015) Single and interactive effects of deer and earthworms on non-native plants. *Forest Ecology and Management*, **351**, 28–35.
- de Deyn GB, van der Putten WH (2005) Linking aboveground and belowground diversity. *Trends in Ecology & Evolution*, **20**, 625–633.
- Dobson A, Blossey B (2015) Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of north-eastern North America. *Journal of Ecology*, **103**, 153–164.
- Dornelas M, Gotelli NJ, McGill B, Shimadzu H, Moyes F, Sievers C, Magurran AE (2014) Assemblage time series reveal biodiversity change but not systematic loss. *Science*, **344**, 296–299.
- Drouin M, Bradley R, Lapointe L, Whalen J (2014) Non-native anecic earthworms (*Lumbricus terrestris* L.) reduce seed germination and seedling survival of temperate and boreal trees species. *Applied Soil Ecology*, **75**, 145–149.

- Edwards CA (2004) *Earthworm Ecology*. CRC Press, Boca Raton, Florida. 441 pp.
- Ehrenfeld JG (2010) Ecosystem consequences of biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, **41**, 59–80.
- Eisenhauer N (2010) The action of an animal ecosystem engineer: identification of the main mechanisms of earthworm impacts on soil microarthropods. *Pedobiologia*, **53**, 343–352.
- Eisenhauer N, Scheu S (2008) Invasibility of experimental grassland communities: the role of earthworms, plant functional group identity and seed size. *Oikos*, **117**, 1026–1036.
- Eisenhauer N, Partsch S, Parkinson D, Scheu S (2007) Invasion of a deciduous forest by earthworms: changes in soil chemistry, microflora, microarthropods and vegetation. *Soil Biology and Biochemistry*, **39**, 1099–1110.
- Eisenhauer N, Marhan S, Scheu S (2008) Assessment of anecic behavior in selected earthworm species: Effects on wheat seed burial, seedling establishment, wheat growth and litter incorporation. *Applied Soil Ecology*, **38**, 79–82.
- Eisenhauer N, Schuy M, Butenschoen O, Scheu S (2009) Direct and indirect effects of endogeic earthworms on plant seeds. *Pedobiologia*, **52**, 151–162.
- Eisenhauer N, Butenschoen O, Radsick S, Scheu S (2010) Earthworms as seedling predators: Importance of seeds and seedlings for earthworm nutrition. *Soil Biology and Biochemistry*, **42**, 1245–1252.
- Eisenhauer N, Fischelli NA, Frelich LE, Reich PB (2012) Interactive effects of global warming and “global worming” on the initial establishment of native and exotic herbaceous plant species. *Oikos*, **121**, 1121–1133.
- Eisenhauer N, Barnes AD, Cesarz S *et al.* (2016) Biodiversity–ecosystem function experiments reveal the mechanisms underlying the consequences of biodiversity change in real world ecosystems. *Journal of Vegetation Science*, doi: 10.1111/jvs.12435.
- Estes JA, Terborgh J, Brashares JS *et al.* (2011) Trophic Downgrading of Planet Earth. *Science*, **333**, 301–306.
- Ewing H, Tuininga A, Groffman P *et al.* (2015) Earthworms reduce biotic 15-Nitrogen retention in northern hardwood forests. *Ecosystems*, **18**, 328–342.
- Fahey TJ, Yavitt JB, Sherman RE, Maerz JC, Groffman PM, Fisk MC, Bohlen PJ (2013) Earthworm effects on the incorporation of litter C and N into soil organic matter in a sugar maple forest. *Ecological Applications*, **23**, 1185–1201.
- Ferlian O, Cesarz S, Marhan S, Scheu S (2014) Carbon food resources of earthworms of different ecological groups as indicated by ¹³C compound-specific stable isotope analysis. *Soil Biology and Biochemistry*, **77**, 22–30.
- Fischelli NA, Frelich LE, Reich PB, Eisenhauer N (2013) Linking direct and indirect pathways mediating earthworms, deer, and understory composition in Great Lakes forests. *Biological Invasions*, **15**, 1057–1066.
- Forey E, Barot S, Decaëns T *et al.* (2011) Importance of earthworm–seed interactions for the composition and structure of plant communities: A review. *Acta Oecologica*, **37**, 594–603.
- Frelich LE, Hale CM, Scheu S, Holdsworth AR, Heneghan L, Bohlen PJ, Reich PB (2006) Earthworm invasion into previously earthworm-free temperate and boreal forests. *Biological Invasions*, **8**, 1235–1245.
- Frelich LE, Peterson RO, Dovčiak M, Reich PB, Vucetich JA, Eisenhauer N (2012) Trophic cascades, invasive species and body-size hierarchies interactively modulate climate change responses of ecotonal temperate–boreal forest. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **367**, 2955–2961.
- Gilbert KJ, Fahey TJ, Maerz JC *et al.* (2014) Exploring carbon flow through the root channel in a temperate forest soil food web. *Soil Biology and Biochemistry*, **76**, 45–52.
- Gonzalez A, Cardinale BJ, Allington GRH *et al.* (2016) Estimating local biodiversity change: a critique of papers claiming no net loss of local diversity. *Ecology*, **97**, 1949–1960.
- Groffman PM, Fahey TJ, Fisk MC, Yavitt JB, Sherman RE, Bohlen PJ, Maerz JC (2015) Earthworms increase soil microbial biomass carrying capacity and nitrogen retention in northern hardwood forests. *Soil Biology and Biochemistry*, **87**, 51–58.
- Hale C, Reich PB, Frelich LE (2004) Allometric equations for estimation of ash-free dry mass from length measurements for selected European earthworm species (Lumbricidae) in the western Great Lakes region. *The American Midland Naturalist*, **151**, 179–185.
- Hale C, Frelich L, Reich P, Pastor J (2005) Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. *Ecosystems*, **8**, 911–927.
- Hale CM, Frelich LE, Reich PB (2006) Changes in hardwood forest understory plant communities in response to European earthworm invasions. *Ecology*, **87**, 1637–1649.
- Hale AN, Lapointe L, Kalisz S (2016) Invader disruption of belowground plant mutualisms reduces carbon acquisition and alters allocation patterns in a native forest herb. *New Phytologist*, **209**, 542–549.
- Hedges LV (1989) An unbiased correction for sampling error in validity generalization studies. *Journal of Applied Psychology*, **74**, 469–477.
- Hendrix PF, Bohlen PJ (2002) Exotic earthworm invasions in North America: ecological and policy implications: expanding global commerce may be increasing the likelihood of exotic earthworm invasions, which could have negative implications for soil processes, other animal and plant species, and importation of certain pathogens. *BioScience*, **52**, 801–811.
- Hendrix PF, Callahan M Jr, Drake JM, Huang C-Y, James SW, Snyder BA, Zhang W (2008) Pandora’s box contained bait: The global problem of introduced earthworms. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 593–613.
- Heneghan L, Steffen J, Fagen K (2007) Interactions of an introduced shrub and introduced earthworms in an Illinois urban woodland: impact on leaf litter decomposition. *Pedobiologia*, **50**, 543–551.
- Holdsworth AR, Frelich LE, Reich PB (2007a) Effects of earthworm invasion on plant species richness in northern hardwood forests. *Conservation Biology*, **21**, 997–1008.
- Holdsworth AR, Frelich LE, Reich PB (2007b) Regional extent of an ecosystem engineer: Earthworm invasion in northern hardwood forests. *Ecological Applications*, **17**, 1666–1677.
- Koricheva J, Gurevitch J (2014) Uses and misuses of meta-analysis in plant ecology. *Journal of Ecology*, **102**, 828–844.
- Koricheva J, Gurevitch J, Mengersen K (2013) *Handbook of Meta-Analysis in Ecology and Evolution*. Princeton University Press, Oxford.
- Larson E, Kipfmüller K, Hale C, Frelich L, Reich P (2010) Tree rings detect earthworm invasions and their effects in northern Hardwood forests. *Biological Invasions*, **12**, 1053–1066.
- Lavelle P (1983) The structure of earthworm communities. In: *Earthworm Ecology: From Darwin to Vermiculture* (ed./eds Satchell JE), pp. 449–466. Springer, Dordrecht, the Netherlands.
- Lawrence B, Fisk MC, Fahey TJ, Suárez ER (2003) Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). *New Phytologist*, **157**, 145–153.
- Lemoine NP, Burkepile DE, Parker JD (2016) Quantifying differences between native and introduced species. *Trends in Ecology & Evolution*, **5**, 372–381.
- Loss SR, Hueffmeier RM, Hale CM, Host GE, Sjerven G, Frelich LE (2013) Earthworm invasions in northern hardwood forests: a rapid assessment method. *Natural Areas Journal*, **33**, 21–30.
- Lubbers IM, van Groenigen KJ, Fonte SJ, Six J, Brussaard L, van Groenigen JW (2013) Greenhouse-gas emissions from soils increased by earthworms. *Nature Climate Change*, **3**, 187–194.
- Migge-Kleian S, McLean MA, Maerz JC, Heneghan L (2006) The influence of invasive earthworms on indigenous fauna in ecosystems previously uninhabited by earthworms. *Biological Invasions*, **8**, 1275–1285.
- Moles AT, Flores-Moreno H, Bonser SP *et al.* (2012) Invasions: the trail behind, the path ahead, and a test of a disturbing idea. *Journal of Ecology*, **100**, 116–127.
- Murphy GE, Romanuk TN (2014) A meta-analysis of declines in local species richness from human disturbances. *Ecology and Evolution*, **4**, 91–103.
- Myers L, Sirois MJ (2014) Spearman Correlation Coefficients, Differences between. In: *Wiley StatsRef: Statistics Reference Online*. John Wiley & Sons Ltd.
- Nakagawa S, Santos ES (2012) Methodological issues and advances in biological meta-analysis. *Evolutionary Ecology*, **26**, 1253–1274.
- Nimmo DG, Nally RM, Cunningham SC, Haslem A, Bennett AF (2015) Vive la résistance: reviving resistance for 21st century conservation. *Trends in Ecology & Evolution*, **30**, 516–523.
- Nuzzo VA, Maerz JC, Blossey B (2009) Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. *Conservation Biology*, **23**, 966–974.
- Nuzzo V, Dávalos A, Blossey B (2015) Invasive earthworms shape forest seed bank composition. *Diversity and Distributions*, **21**, 560–570.
- Paudel S, Longcore T, MacDonald B, McCormick MK, Szlavecz K, Wilson GWT, Loss SR (2016) Belowground interactions with aboveground consequences: Invasive earthworms and arbuscular mycorrhizal fungi. *Ecology*, **97**, 605–614.
- Powell KI, Chase JM, Knight TM (2013) Invasive plants have scale-dependent effects on diversity by altering species-area relationships. *Science*, **339**, 316–318.
- Powers MD, Nagel LM (2008) Disturbance dynamics influence *Carex pensylvanica* abundance in a northern hardwood forest 1. *The Journal of the Torrey Botanical Society*, **135**, 317–327.
- Resner K, Yoo K, Sebestyen S, Aufdenkampe A, Hale C, Lyttle A, Blum A (2015) Invasive earthworms deplete key soil inorganic nutrients (Ca, Mg, K, and P) in a northern hardwood forest. *Ecosystems*, **18**, 89–102.

- Romero GQ, Gonçalves-Souza T, Vieira C, Koricheva J (2015) Ecosystem engineering effects on species diversity across ecosystems: a meta-analysis. *Biological Reviews*, **90**, 877–890.
- Roth AM, Whitfield TJS, Lodge AG, Eisenhauer N, Frelich LE, Reich PB (2015) Invasive earthworms interact with abiotic conditions to influence the invasion of common buckthorn (*Rhamnus cathartica*). *Oecologia*, **178**, 219–230.
- Sackett TE, Smith SM, Basiliko N (2013) Indirect and direct effects of exotic earthworms on soil nutrient and carbon pools in North American temperate forests. *Soil Biology and Biochemistry*, **57**, 459–467.
- Sala OE, Chapin III FS, Armesto JJ *et al.* (2000) Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770–1774.
- Simberloff D, Von Holle B (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions*, **1**, 21–32.
- Simberloff D, Martin J-L, Genovesi P *et al.* (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution*, **28**, 58–66.
- Smith B, Wilson JB (1996) A consumer's guide to evenness indices. *Oikos*, **76**, 70–82.
- Szlavecz K, Placella SA, Pouyat RV, Groffman PM, Csuzdi C, Yesilonis I (2006) Invasive earthworm species and nitrogen cycling in remnant forest patches. *Applied Soil Ecology*, **32**, 54–62.
- USDA (2014) The PLANTS Database. Available at: <http://plants.usda.gov> (accessed 1 March 2015).
- VanderWeide B, Hartnett D (2015) Belowground bud bank response to grazing under severe, short-term drought. *Oecologia*, **3**, 795–806.
- Vellend M, Baeten L, Myers-Smith IH *et al.* (2013) Global meta-analysis reveals no net change in local-scale plant biodiversity over time. *Proceedings of the National Academy of Sciences*, **110**, 19456–19459.
- Viechtbauer W (2010) Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, **36**, 1–48.
- Vilà M, Espinar JL, Hejda M *et al.* (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, **14**, 702–708.
- Walsh JR, Carpenter SR, Vander Zanden MJ (2016) Invasive species triggers a massive loss of ecosystem services through a trophic cascade. *Proceedings of the National Academy of Sciences*, **113**, 4081–4085.
- Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. *Science*, **304**, 1629–1633.
- Wardle DA, Bardgett RD, Callaway RM, Van der Putten WH (2011) Terrestrial ecosystem responses to species gains and losses. *Science*, **332**, 1273–1277.
- Whitfield TJS, Roth AM, Lodge AG, Eisenhauer N, Frelich LE, Reich PB (2014) Resident plant diversity and introduced earthworms have contrasting effects on the success of invasive plants. *Biological Invasions*, **16**, 2181–2193.
- Zaller JG, Saxler N (2007) Selective vertical seed transport by earthworms: Implications for the diversity of grassland ecosystems. *European Journal of Soil Biology*, **43** Supplement 1, S86–S91.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1.

Appendix S1. PRISMA diagram

Appendix S2. References of studies included in meta-analysis

Appendix S3. Metadata of 'Cravenetal_Earthworms_PlantDiversity.csv'

Appendix S4. Metadata of 'Cravenetal_EffectSizes_Earthworms_PlantFuncGroups.csv'

Table S1. Studies included in meta-analysis and additional information about each study

Table S2. Introduced earthworm species and their corresponding ecological groups

Table S3. Summary of mixed-effects model testing associations between introduced earthworm biomass, density, and ecological group richness.

Table S4. Summary of mixed-effects model testing the relationships between introduced earthworm biomass, density, and ecological group richness and total plant cover.

Fig. S1. Frequency of earthworm ecological group richness and correlations among measures of introduced earthworm abundance.

Fig. S2. Effect sizes of relationships between introduced earthworm communities and total plant cover.

Data S2. Cravenetal_Earthworms_PlantDiversity.csv

Data file containing effect sizes of relationships between introduced earthworm communities and plant species diversity, evenness, and richness of forest understory communities in North America.

Data S3. Cravenetal_Earthworms_PlantFunctionalGroups.csv

Data file containing effect sizes of relationships between introduced earthworm communities and cover of plant functional groups of forest understory communities in North America.