Ecological Predictors and Consequences of Non-native Earthworms in Kennebec County, Maine

Julia A. Rogers[1] and Cathy D. Collins[1,2,*]

Abstract - Non-native earthworms are found throughout much of the United States and southern Canada in areas glaciated during the most recent glaciation. Following invasion, these earthworms altered nutrient cycling, soil structure, and diversity in forests throughout the northern United States. There are no comprehensive studies of earthworm distributions in forested areas of Maine. We surveyed earthworms in forested recreation areas in Kennebec County, ME, and investigated ecological and landscape attributes that may predict their presence. To examine whether the presence of worms modifies forests, we measured environmental variables known from other studies to be affected by worms. We found earthworms at 12 out of 23 sites. Sample sites near roads, in deciduous forests, and in small forests were more likely to have earthworms. We also found that locations with worms have less surface litter and more soil phosphorous, suggesting that earthworms modify soils in Maine forests. Our study is the first to explore the distribution of earthworms in natural forests in Maine, and our findings provide evidence that roads facilitate earthworm invasion, with measurable consequences for soil properties.

Introduction

Earthworms currently found in previously glaciated regions of North America are considered invasive (Bohlen et al. 2004a). Introduced earthworms can have profound impacts on soils, plant communities, and nutrient cycling (Bohlen et al. 2004b, Davalos et al. 2013, Laossi et al. 2009). Humans facilitate the spread of earthworms via road construction, gardening, logging, and fishing (Bohlen et al. 2004a, Hale 2007, Hendrix and Bohlen 2002, Holdsworth et al. 2007, Kalisz and Dotson 1989). In some regions, earthworm invasion is actively monitored (Bohlen et al. 2004a, Hale et al. 2006). However, few systematic earthworm surveys have been conducted in Maine, and the extent to which worms have invaded—and potentially influenced—forested landscapes is unknown.

Predicting the effects of earthworm invasion on forest properties is made challenging by the fact that the magnitude and direction of effects depend on forest composition, land-use history, and soil type (Bohlen et al. 2004a, Frelich et al. 2006, Hendrix and Bohlen 2002). For instance, earthworms alter carbon, phosphorous, and nitrogen levels through their consumption of organic matter and incorporation of this organic matter into the mineral soil (Bohlen et al. 2004b, Frelich et al. 2006, Scheu and Parkinson 1994). However, whether worms increase or decrease soil phosphorous and nitrogen depends on land-use history, the species of earthworms present, and the time since invasion (Bohlen et al. 2004b).

[1]Department of Biology, Colby College, Waterville, ME 04901. [2]Biology Program, Bard College, Annandale on Hudson, NY 12540. [*]Corresponding author - ccollins@bard.edu.

Manuscript Editor: Robert Bertin

By altering nutrients, earthworms indirectly affect the abundance and diversity of belowground microbial communities (Alban and Berry 1994, Bohlen et al. 2004b, McLean and Parkinson 1998), as well as the diversity and invasibility of above-ground plant communities (Bohlen et al. 2004b, Hale et al. 2008). For example, in mixed hardwood forests in Ontario, Canada, earthworms directly alter plant community composition via selective seed consumption (Cassin and Kotanen 2016). Earthworms may also enhance seedling emergence by increasing nutrients available to the seed (Eisenhauer and Scheu 2008, Eisenhauer et al. 2007, Milcu et al. 2006). Given the widespread and inconsistent effects of earthworms on soils and plants, studies from a broad range of ecosystems are needed to better understand and predict the changes to forests following earthworm invasion.

The dramatic influence of earthworms on ecosystem properties has prompted recent efforts to identify environmental factors that predict where earthworms occur and will invade (Cameron et al. 2007, Costello et al. 2011, Gundale et al. 2005, Sackett et al. 2012, Suarez et al. 2006). For instance, forest type is the strongest predictor of earthworm presence in New York, where earthworms were more likely to be found in mixed hardwood forests than in *Fagus* (beech) and *Tsuga* (hemlock) forests (Suarez et al. 2006). Disturbance, too, plays a key role in predicting the earthworm presence. Earthworms are more likely to be found near agricultural clearings (Suarez et al. 2006), close to fishing sites (Cameron et al. 2007), and along roads experiencing regular vehicle traffic (Cameron et al. 2007, Sackett et al. 2012). Additionally, earthworms are associated with non-wilderness sites more than wilderness sites, a pattern likely explained by the presence of roads and logging at the former sites (Gundale et al. 2005).

Little is known about the distribution of earthworms in natural habitats in Maine. Approximately 90% of land in Maine is forested (Huff and McWilliams 2015), much of which is used for logging and recreation and therefore vulnerable to human-mediated earthworm invasion (Gundale et al. 2005). Moreover most of Maine's forests are second-growth forests, where earthworms appear to establish more readily relative to old-growth forests (Simmons et al. 2015). Reynolds (2008) reported that earthworms were present in each of Maine's counties; however his sampling was restricted to backyards, compost piles, and towns. Owen and Galbraith (1989) studied earthworms in relation to *Scolopax minor* Gmelin (American Woodcock) populations in six townships in central and eastern Maine. They found that land-use history and soil type were the best predictors for earthworm presence. Areas that were farmed previously were the most likely to have earthworms regardless of other characteristics. Additionally, earthworms were more abundant in moderately drained fine sandy loamy soils than in other soil types (Owen and Galbraith 1989).

We surveyed forests in Kennebec County, ME, to assess the distributional extent of earthworms and characterize the environmental factors associated with sites where they are present. Our objectives were to (1) record the extent of earthworm presence in Kennebec County, (2) identify landscape and soil factors that predict earthworm presence, and (3) investigate the effects of earthworms on soils in the invaded areas. Based on studies conducted in forests in other states, we expected that distance to roads would be the most significant factor in predicting earthworm presence. Moreover, we expected earthworms to reduce forest litter and alter soil N and P quantities.

Methods

Location and sampling design

We selected 23 study sites in Kennebec County, ME (Fig. 1). Temperatures in this area average -2.4 °C in January and 26.2 °C in June, and the average annual precipitation is 1064 mm (US Climate Data 2016). Earthworm species respond differently to the cold; however, worm species found in the litter layer, which may be more susceptible to freezing than other species, can survive temperatures as low as -14 °C (Greiner et al. 2011, Holmstrup et al. 2007). That threshold is lower than the averarge minimum winter temperature in our study area (United States Climate Data 2016), and though many parts of the state regularly experience winter low air temperatures below -14 °C, the temperatures under the litter and in the ground

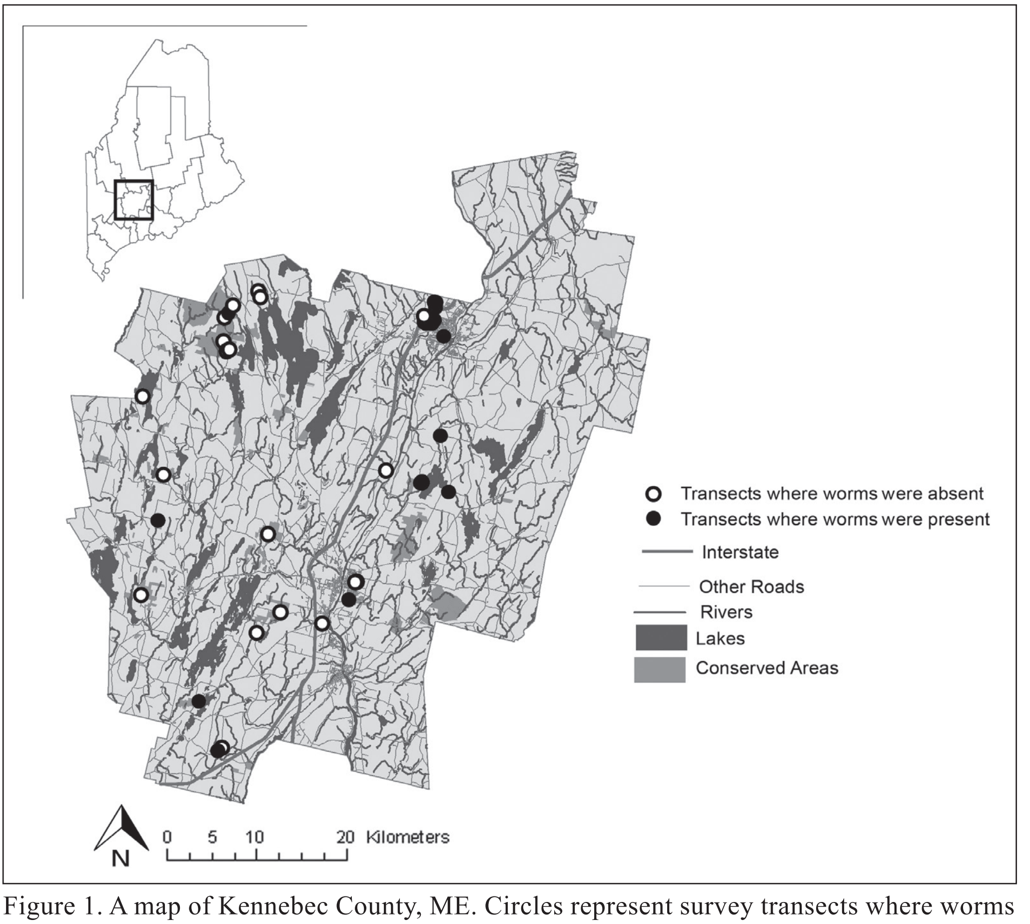

Figure 1. A map of Kennebec County, ME. Circles represent survey transects where worms were present (black circles) and transects where worms were absent (white circles).

where the earthworms live are generally not as extreme and the typical presence of an additonally insulating blanket of snowcover throughout most of the winter in these colder areas suggests the climate throughout Maine is in fact suitable for earthworm survival.

All sample sites were located in forested conservation and recreation areas that included contiguous forest at least 1 ha in size . On each soil type within a site, we haphazardly selected a location for 1 transect. Consequently, the number of transects per site was dictated by the number of soil types per conservation area, resulting in 36 total transects. Across all sites, there were 10 different soil types (Table 1). Transects were each 50 m long and located at least 5 m from human disturbance (e.g. trails), although all transects were within 1 km of a paved road.

Worm sampling

We sampled worms from five 25 cm x 25 cm plots located at 10-m intervals along each of the 36 transects. To minimize heterogeneity due to above- or belowground obstructions, we avoided placing plots within 1 m of any decomposing stumps and trees larger than 10 cm in diameter at breast height (dbh). We first cleared the litter and hand-collected worms on the surface. To stimulate emergence of worms from within the ground, we poured a solution of 3.8 L of water and 40 g of mustard seed powder on the area (Lawrence and Bowers 2002). Though all species of worms may not respond equally to mustard extraction, this sampling technique is as effective as digging and hand-sorting (Hale et al. 2005, Lawrence and Bowers 2002), and causes less disturbance to the forest soils. We standardized sampling effort by collecting emerging earthworms for 10 minutes per plot. Data from 5 plots were compiled such that presence or absence of earthworms was expressed at the transect scale.

We sampled all locations for earthworms between 22 September 2015 and 3 November 2015, as earthworms are known to be most active during the spring and fall months (Gates 1961). A subset of 21 transects was sampled a second time during the latter part of that period following rain and cooler temperatures, which we suspected might alter abundance. Not all sites were re-sampled due to freezing weather that decreased earthworm activity. However, because we did not find earthworms in any places where we had recorded them absent before (nor did we fail to find earthworms in locations where they had been recorded as present), re-sampling increased our confidence that the absence of earthworms from sites during the initial sampling was not caused by lower worm-activity levels or ineffective methods. Abundance varied markedly depending on the time of sampling, thus we use only presence–absence data in our analyses.

Environmental variables

To characterize forest composition, we established belt transects by expanding the 50-m worm-sampling transect to include 2 m on either side. Within the belt (4 m x 50 m, 200 m^2 total), we measured and identified to species all trees larger than 10 cm dbh.

To characterize soil attributes, we collected 6.2 cm^3 of soil from the top 10 cm of soil (after clearing litter) at each sub-plot and combined the 5 sub-plot

Table 1. Location name, soil type, forest size, and latitude and longitude for all transects.

Location	Soil type	Forest size (ha)	Lat., Long. (°)
Runnals Hill, Colby College	Paxton Charlton very stony fine sandy loam	115	44.560, 69.668
Runnals Hill, Colby College	Hollis fine sandy loam	115	44.558, 69.665
Perkins Arboretum, Colby College	Buxton silt loam	76	44.558, 69.657
Perkins Arboretum, Colby College	Buxton silt loam	76	44.560, 69.654
Quarry Road Ski Area, Waterville	Scantic silt loam	81	44.578, 69.654
Quarry Road Ski Area, Waterville	Hollis fine sandy loam	81	44.576, 69.652
Quarry Road Ski Area, Waterville	Buxton silt loam	81	44.573, 69.653
Mount Phillip, Rome	Lyman loam	275	44.586, 69.884
Mount Phillip, Rome	Berkshire very stony fine sandy loam	275	44.579, 69.883
Round Top, Rome	Lyman loam	1019	44.533, 69.928
Round Top, Rome	Berkshire very stony fine sandy loam	1019	44.530, 69.924
Round Top, Rome	Peru fine sandy loam	1019	44.530, 69.923
Sanders Hill, Rome	Lyman loam	294	44.561, 69.930
Sanders Hill, Rome	Berkshire very stony fine sandy loam	294	44.567, 69.923
French Mountain, Rome	Lyman loam	205	44.574, 69.919
Seaward Mills Vassalboro	Buxton silt loam	43	44.400, 69.634
Davidson Nature Preserve, Vassalboro	Hollis fine sandy loam	397	44.453, 69.645
Vassalboro Wildlife Habitat	Scantic silt loam	15	44.409, 69.668
Vassalboro Wildlife Habitat	Buxton silt loam	15	44.409, 69.672
Woodsmen Field, Colby College	Woodbridge very stony fine sandy loam	11	44.565, 69.668
Jamie's Pond WMA, Hallowell	Paxton Charlton very stony fine sandy loam	290	44.286, 69.852
Reynolds Forest, Sidney	Suffield silt loam	223	44.420, 69.715
Oxbow, Waterville	Scantic silt loam	9	44.546, 69.642
Vaughan Woods, Hallowell	Suffield silt loam	98	44.276, 69.797
Woodbury Pond State Park, Litchfield	Paxton Charlton very stony fine sandy loam	38	44.202, 69.957
Mt. Pisgah Conservation Area, Winthrop	Paxton Charlton very stony fine sandy loam	1144	44.301, 70.035
Small-Burnham Conservation Area, Litchfield	Woodbridge very stony fine sandy loam	706	44.157, 69.927
Small-Burnham Conservation Area, Litchfield	Hinckley gravelly sandy loam	706	44.156, 69.933
Parker Pond Headlands, Fayette	Paxton very stony fine sandy loam	136	44.487, 70.036
Torsey Pond, Mt. Vernon	Woodbridge very stony fine sandy loam	92	44.418, 70.000
MacDonald Conservation Area, Readfield	Woodbridge very stony fine sandy loam	424	44.373, 70.013
Hutchinson Pond, Manchester	Woodbridge very stony fine sandy loam	166	44.267, 69.883
Wyman Memorial Forest, Readfield	Hollis fine sandy loam	418	44.360, 69.868
Augusta Arboretum	Hollis fine sandy loam	39	44.299, 69.762
Augusta Nature Center	Suffield silt loam	24	44.315, 69.753
Augusta Nature Center	Hollis fine sandy loam	24	44.315, 69.754

samples into a single sample per transect. Soils were air-dried for 2 weeks prior to analysis. Soil moisture-holding capacity (SMHC; our proxy for soil moisture) was measured by taking the difference in mass between soil wetted to field capacity and soil oven dried at 105 °C for 72 hours (Brudvig and Damschen 2011). Nutrient and texture analyses were conducted by Brookside Laboratories, Inc. (New Knoxville, OH; www.blinc.com). We focused our analyses on soil texture

Table 2. Landscape and local habitat variables that influence and thus serve as predictors in our statistical models) or are influenced by (response variable in our statistical models) earthworm presence.

Variable	Location	System	Study
Predictors of earthworm presence			
Soil pH	Puerto Rico	Forest	González et al. 2007
	Maine	Forest	Owen and Gailbraith 1989
	India	Agricultural area	Singh et al. 2015
	Georgia	Forest	Lobe et al. 2013
	New York	Forest	Homan et al. 2015
	Europe	Forest	Wandeler et al. 2016
Soil texture	Germany	Agriculture areas	Palm et al. 2013
	Ontario, Canada	Forests	Sackett et al. 2012
	Maine	Forests	Owen and Galbraith 1989
Soil moisture	Himalayas	Agricultural field	Kaushal et al. 1999
Distance to roads	Alberta, Canada	Forests	Cameron et al. 2007
	Minnesota and Wisonsin	Forests	Holdsworth et al. 2007
	Ontario, Canada	Forests	Sackett et al. 2012
Distance to water	New York	Forests	Suarez et al. 2006
	Minnesota and Wisonsin	Forests	Holdsworth et al. 2007
Forest composition	New York	Forests	Suarez et al. 2006
	Maine	Forests	Owen and Galbraith 1989
Influenced by earthworm presence			
Soil N	Minnesota	Forests	Alban and Berry 1994
	Alberta, Canada	Forests	Scheu and Parkinson 1994
	New York	Forests	Burtelow et al. 1998
	New York	Forests	Bohlen et al. 2004b
	Minnesota	Forests	Frelich et al. 2006
	Greenhouse	Greenhouse–forests	Hale et al. 2008
	Quebec, Canada	Forests	Wironen and Moore 2006
Soil C	Minnesota	Forests	Alban and Berry 1994
	New York	Forests	Burtelow et al. 1998
	New York	Forests	Bohlen et al. 2004b
	Michigan	Forests	Gundale et al. 2005
Soil P	New York	Forests	Groffman et al. 2004, 2015
	Minnesota	Forests	Frelich et al. 2006
	Michigan	Forests	Hale et al. 2007
Litter depth	Illinois	Forests	Heneghan et al. 2007
	Puerto Rico	Forests	Gonzalez et al. 2003
	Puerto Rico	Forests and fields	Liu and Zou 2002
	Minnesota	Forests	Frelich et al. 2006

(percent silt, sand, and clay), pH, nitrogen levels, organic matter, and phosphorus levels because other studies have shown that they affect or are affected by earthworm presence (Table 2).

Statistical analysis

All statistical analyses were conducted in R version 3.2.2 (R Core Team 2015) and ArcGIS (ESRI, Redlands, CA). We used ArcGIS to determine Euclidean distance to roads and water, and to confirm soil types for each transect based on soil-map data from the USGS Web Soil survey (USDA 2013). Based on previously published studies, we divided the soil variables according to whether they were more likely to influence or be influenced by earthworm presence (Table 2). Using logistic regression, we determined whether earthworm presence was predicted by landscape-level variables (distance to roads, distance to water, and forest size), environmental factors unlikely to be changed by worms (soil texture, SMHC, and pH), and tree composition (specifically the proportion of deciduous trees). Because transect locations signify unique soil types and associated vegetation, we treated each transect independently.

For model selection, we started with a full model including soil pH, soil texture (as percent sand and percent silt), SMHC, distance to roads, distance to water, and forest composition (Table 3). Because some soil variables may be influenced by roads, we included interactions between environmental variables and distance to roads in the initial model (Table 3). We then removed each factor individually in order of least significance and tested for model significance to create the simplest model. We used the Akaike information criterion (AIC) to select the best-fitting model.

To determine the effect of earthworms on soil properties we first used nonmetric multidimensional scaling (NMDS) to visualize the data. We included percent soil

Table 3. Results from the full model, prior to variable selection. All predictors in this model are ecological and landscape level factors previously found to influence the presence of earthworms (Table 2). SMHC is soil moisture holding capacity, DisRoad is the distance to roads, SA.per is the percent sand, Prop.Decid is the proportion of deciduous trees. We included only interactions between variables that may be influenced by distance to roads (see text).

	Estimate	Std. Error	z value	*P*
Intercept	-41.98	45.72	-0.92	0.36
pH	6.23	8.18	0.76	0.05
DisRoad	0.04	0.14	0.24	0.81
SA.per	0.07	0.19	0.35	0.73
SMHC	0.16	0.21	0.75	0.46
Prop.Decid	4.98	10.67	0.47	0.64
Distance to Water	0.002	0.01	0.33	0.74
Forest Size	-0.01	0.01	-1.46	0.15
DisRoad * pH	-0.001	0.03	-0.04	0.97
DisRoad * SA.per	-0.0002	0.001	-0.29	0.77
DisRoad * SMHC	-0.001	0.001	-0.77	0.44
DisRoad * Prop.Decid	0.004	0.05	0.07	0.94

organic matter, average litter depth (cm), estimated nitrogen release via organic matter decomposition (N/ha), and soil phosphorous levels (mg/kg), all of which have previously been shown to be affected by earthworms (Table 2). To test whether the combined effect of soil variables generated statistically significant differences between sites with and without worms, we used a permutation multivariate analysis of variance (PERMANOVA) from the "vegan" package in R. We followed this multivariate approach with 2-sample *t*-tests to identify individual soil properties that differed between sites with and without worms.

Results

Earthworms were found at 12 out of 23 sites (16 out of 36 transects; Fig. 1). Three sites included transects with and transects without worms. In the 22 transects that were re-sampled, all presences and absences were confirmed.

Following model selection, our final model included distance to roads, forest composition, forest size, and pH (AIC final model = 33.61, AIC full model = 45.37; Table 4). Distance to roads, forest composition, and forest size were significant predictors (Table 4). Although not statistically significant, retaining pH improved model fit.

Specifically, invasive earthworms were more likely to be found near roads. Transects varied from 26 m to 870 m from roads; the mean distance from transects to roads was 156 m (± 26 SE) where earthworms were present and 373 m (± 62 SE) where they were absent. The pH ranged from 4.6 to 5.5 across all sites. The mean pH was higher where earthworms were present (5.08 ± 0.06 SE) than where they were absent (4.92 ± 0.05). Forests where earthworms were present were on average smaller (mean ± SE = 145 ± 44 ha), than those without earthworms (413 ± 91 ha). Lastly, the proportion of deciduous trees was slightly higher under the presence of earthworms (mean ± SE = 0.76 ± 0.05), than without earthworms (0.63 ± 0.06).

Table 4. Results from final model with soil pH, distance to roads, the proportion of deciduous trees, and forest size. * indicates significant results.

	Estimate	SE	z value	*P*
Intercept	-23.00	12.96	-1.87	0.08
pH	4.24	2.32	1.83	0.07
DisRoad	-0.01	0.01	-2.07	0.03*
Prop.Decid	7.94	3.32	2.39	0.02*
Forest Size	-0.01	0.002	-2.22	0.02*

Table 5. Results from two sample t-tests for soil factors including nitrogen, phosphorus, litter depth, and percent organic matter. * indicates significant results.

Test	*t*	df	*P*
N~Presence	1.28	34.00	0.21
P~Presence	-2.25	33.78	0.03*
LD~Presence	4.96	20.59	<0.001*
OM~Presence	1.57	32.32	0.13

Transects with and without worms differed with respect to soil attributes previously shown to be influenced by earthworm presence (PERMANOVA: F_{35} = 5.69, P = 0.002; Fig. 2, Table 5). When soil attributes were analyzed separately, transects with earthworms had significantly higher levels of phosphorus and lower litter depths than those without worms (Fig. 3, Table 5). We did not detect a difference in levels of organic matter and nitrogen between sites with and without worms (Fig. 3, Table 5).

Discussion

Non-native earthworms are widespread in Kennebec County forests, though not present at all sites we sampled. Soil texture appeared to have little bearing on the likelihood of worm invasion or persistence relative to landscape attributes, as we found earthworms in distinct soil types even within a single forests. Worms were detected most frequently near roads, in smaller forests with fewer conifers, and in soils with higher pH. Soils where worms were present differed with respect to several variables related to nutrient cycling (litter depth and phosphorous).

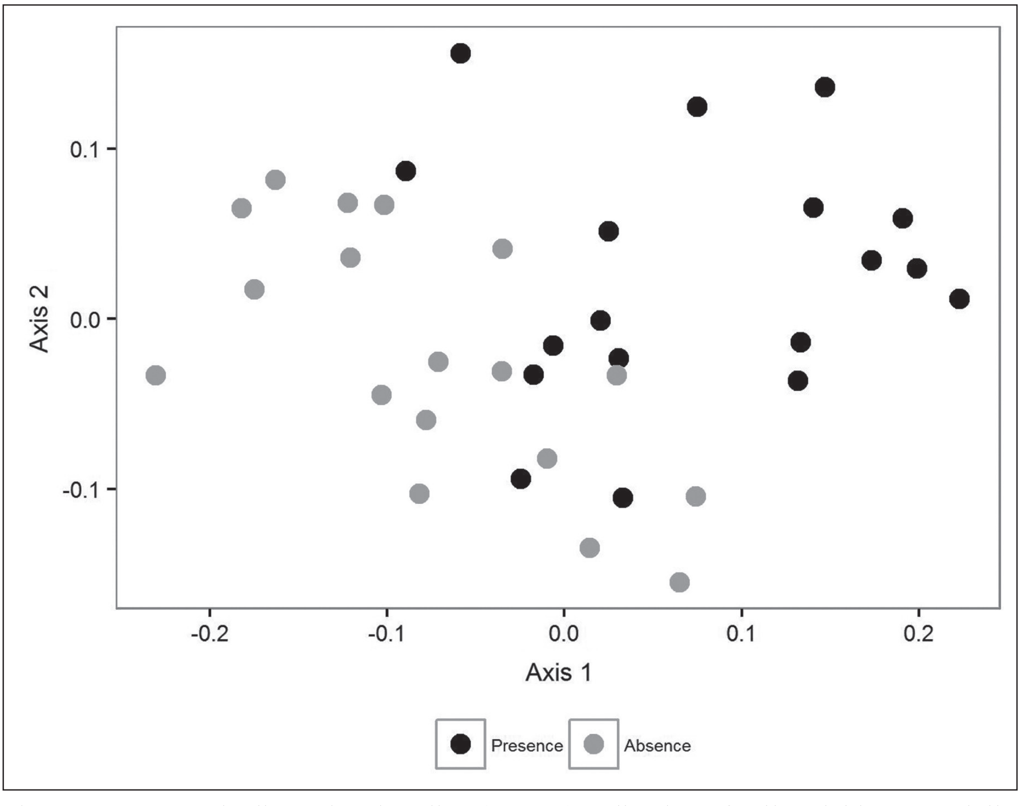

Figure 2. Non-metric dimensional scaling (NMDS) ordination of soil variables potentially impacted by worms (estimated nitrogen release, soil phosphorous, litter depth, and percent organic matter; based on literature in Table 2). Black circles depict sites where worms were detected. Gray circles are sites where worms were surveyed, but not found.

Predictors of earthworm presence

Proximity to roads was a strong predictor of worm presence in Kennebec County, similar to studies from other regions (Cameron and Bayne 2009, 2015; Gundale et al. 2005; Holdsworth et al. 2007; Sackett 2012; Shartell et al. 2015). Roads provide an avenue for earthworm invasion during the construction phase when bulk gravel or other fill is transported from other locations (Cameron et al. 2007, Hendrix and Bohlen 2002). Both during and after road construction, earthworm cocoons can travel along roadways in substrate attached to vehicle tires (Marinissen and van den Bosch 1992). In a sparsely populated state like Maine, lack of roads, or infrequently traveled roads may have slowed the spread of earthworms relative to more-populated states. Nonetheless, introductions through fishing, greenhouses, composting, gardens, road construction, and other factors have led to worms being found in every county in Maine (Reynolds 2008). If road construction, travel, and recreation continue to spread to remote areas in the state (MaineDot 2015), the capacity for earthworms to spread to natural areas will also increase.

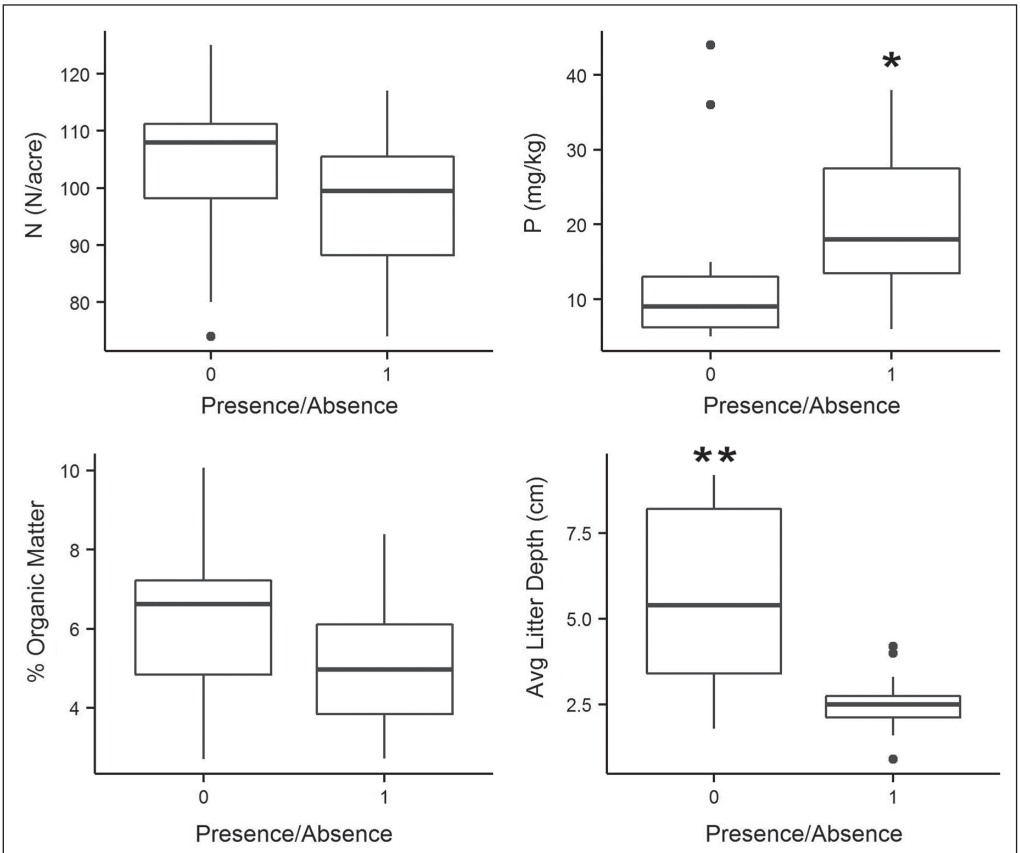

Figure 3. Boxplots of soil factors potentially affected by earthworms. Asterisks denote statistically significant differences (*$P < 0.05$, **$P < 0.001$; see Table 5). For each, whiskers represent max and min values, top of the box is the third quartile, bottom of the box is the first quartile, and the darker line within the box is the median.

Our data may reflect co-occurring disturbances, as many sites with worms were in small forests near the towns of Waterville and Augusta (Fig. 1). Roads and forest fragmentation are typically coupled with other disturbances that facilitate earthworm invasion such as logging (Gundale et al. 2005, Sackett 2012), agricultural fields (Shartell et al. 2015), urban development (Beausejour et al. 2015), and recreational facilities (Bohlen et al. 2004a, Holdsworth et al. 2007). Smaller patches of forests have a greater edge-to-area ratio; closer proximity to anthropogenic activity and disproportionate access for worm introduction on forest edges may be why some species of earthworms occur more frequently on edges of forests relative to the interior (Gibson et al. 2013).

While roads facilitate the spread of worms, and small forests likely increase access for invasion, our data show that not all forest types are equally likely to have worms. Both the proportion of deciduous trees and soil pH played a role in predicting earthworm presence, perhaps in part because conifer-dominated forests have more acidic soils than deciduous forests (Frelich et al. 2006, Suarez et al. 2006). However, it is worth noting that we found earthworms both in sites dominated by conifers and sites dominated by deciduous trees, suggesting that access to sites via roads is a stronger determinant of earthworm presence.

It is certainly plausible that earthworm presence in Kennebec county may reflect factors that we did not measure such as land-use history (Simmons et al. 2015), proximity to wet areas (Suarez et al. 2006), proximity to agricultural fields (Shartell et al. 2015, Suarez et al. 2006), or proximity to logging operations (Costello et al. 2011 Gundale et al. 2005, Sackett et al. 2012). Beginning in the 1800s, logging has occurred in the majority of forests in Kennebec County and elsewhere in the state of Maine (Moore and Whitham 1996). Following the logging in the 1800s, much of the land was used as farmland, both for crops and for sheep farming. Some of these farms were abandoned and returned to forests, while others remain as functional farms (Moore and Whitham 1996). Mapping worm distribution in relation to proximity to agriculture, especially crop farming, and land-use history may help us better interpret current patterns, as well as predict the location of future earthworm invasions (Owen and Galbraith 1989, Suarez et al. 2006).

Effects of earthworms on forest soils

Consistent with other studies (Bohlen et al. 2004b, Burtelow et al. 1998), soil nitrogen levels did not differ between sites with and without earthworms. However, in contrast to studies in forests in New York (Suarez et al. 2004) and Minnesota (Resner et al. 2015), we found that soil phosphorus was higher where earthworms were present. Earthworms may modify phosphorous to varying degrees depending on the species of earthworms present and how long they have been at a site (Bohlen et al. 2004a, Frelich et al. 2006, Resner et al. 2015). For instance, the presence of *Lumbricus terrestris* L. (Nightcrawler) is thought to bring soils from deeper horizons to the surface, increasing the available phosphorous (Frelich et al. 2006). We did not identify worms to the species level, so we cannot say for sure whether species composition explains higher soil phosphorus in our study. Following earthworm invasion, however, soil phosphorous initially increases, then decreases (Bohlen et al. 2004a); it is possible we measured forests during this early phase and that over time, differences will fade.

Our finding that sites with earthworms had less surface litter was expected given that earthworms consume litter (Bohlen et al. 2004a). Litter reduction by earthworms modifies soil nutrient availability and understory plant composition (Frelich et al. 2006, Gonzalezir et al. 2003, Heneghan et al. 2007, Liu and Zhou 2002). It follows that worms invading Maine forests may ultimately have consequences for plant community diversity and ecosystem function. Furthermore, litter consumption and nutrient cycling are not the only mechanisms by which worms may influence aboveground plant communities—including plants of economic importance to the state of Maine. For instance, Lawrence et al. (2003) found that earthworms reduce the colonization and presence of hyphae in mycorrhizal fungi associated with *Acer saccharum* Marsh (Sugar Maple). Moreover, earthworms may create conditions conducive for invasive plant species, including *Rhamnus carthartica* L. (Common Buckthorn), *Alliaria petiolata* M. Bieb. (Garlic Mustard), and *Rosa multiflora* Thunb. (Multiflora Rose) (Clause et al. 2015, Hopfensperger and Hamilton 2015, Nuzzo et al. 2015, Quakenbush et al. 2012, Roth et al. 2015, Whitfeld et al. 2014). Finally, because earthworms consume small-seeded species (Cassin and Kotenen 2016), their presence may influence aboveground plant composition. While we did not address plant communities in this study, the fact that we observed dramatic differences in soil properties in invaded versus uninvaded forests warrants future studies on the aboveground consequences of worms in Maine forests.

Overall, we found that earthworms are present—particularly in small, deciduous forests near roads—and induce measurable changes to soils in Kennebec County forests. Larger-scale systematic surveys are needed to document the extent of the invasion in Maine and better predict the ecosystem consequences for forests that developed in the absence of worms for most of the last 10,000 years.

Acknowledgments

We are grateful to the land owners and conservation organizations that allowed access to their property for this research. We would also like to thank Judy Stone and Herb Wilson for their input on early drafts of this manuscript. This project was supported by funds from the Colby Biology Department and the Dean of Students Special Funds at Colby College.

Literature Cited

Alban, D.H., and E.C. Berry. 1994. Effects of earthworm invasion on morphology, carbon, and nitrogen of a forest soil. Applied Soil Ecology 1:243–49.

Beauséjour, R., I.T., Handa, M.J. Lechowicz, B. Gilbert, and M. Vellend. 2015. Historical anthropogenic disturbances influence patterns of non-native earthworm and plant invasions in a temperate primary forest. Biological Invasions 17:1267–1281.

Bohlen, P.J., S. Scheu, C.M. Hale, M.A. McLean, S. Migge, P.M. Groffman, and D. Parkinson. 2004a. Non-native invasive earthworms as agents of change in northern temperate forests. Frontiers in Ecology and the Environment 2:427–435.

Bohlen, P. J., D.M. Pelletier, P.M. Groffman, T.J. Fahey, and M.C. Fisk. 2004b. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. Ecosystems 7:13–27.

Brudvig, L.A., and E.I. Damschen. 2011. Land-use history, historical connectivity, and land management interact to determine Longleaf Pine woodland understory richness and composition. Ecography 34:257–266.

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

Cameron, E.K., and E.M. Bayne. 2009. Road age and its importance in earthworm invasion of northern boreal forests. Journal of Applied Ecology 46:28–36.

Cameron, E.K., and E.M. Bayne. 2015. Spatial patterns and spread of exotic earthworms at local scales. Canadian Journal of Zoology 93:721–726.

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

Cassin, C.M., and P.M. Kotanen. 2016. Invasive earthworms as seed predators of temperate forest plants. Biological Invasions 18:1–14.

Clause, J., E. Forey, C.J. Lortie, A.M. Lambert, and S. Barot. 2015. Non-native earthworms promote plant invasion by ingesting seeds and modifying soil properties. Acta Oecologica, 64:10–20.

Costello, D.M., S.D. Tiegs, and G.A. Lamberti. 2011. Do non-native earthworms in southeast Alaska use streams as invasional corridors in watersheds harvested for timber? Biological Invasions 13:177–187.

Dávalos, A., V. Nuzzo, J. Stark, and B. Blossey. 2013. Unexpected earthworm effects on forest understory plants. BMC Ecology 13:48.

Eisenhauer, N., and S. Scheu. 2008. Invasibility of experimental grassland communities: The role of earthworms, plant functional group identity, and seed size. Oikos 117:1026–1036.

Eisenhauer, N., S. Partsch, D. Parkinson, S. Scheu. 2007. Invasion of a deciduous forest by earthworms: Changes in soil chemistry, microflora, microarthropods, and vegetation. Soil Biology and Biochemistry 39:1099–1110.

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

Gates, G.E. 1961. Ecology of some earthworms with special reference to seasonal activity. The American Midland Naturalist 66:61–86.

Gibson, K.D., P.M. Quackenbush, N.C. Emery, M.A. Jenkins, and E.J. Kladivko. 2013. Invasive earthworms and plants in Indiana old- and second-growth forests. Invasive Plant Science and Management 6:161–174.

González, G., E. García, V. Cruz, S. Borges, M. Zalamea, and M.M. Rivera. 2007. Earthworm communities along an elevation gradient in Northeastern Puerto Rico. European Journal of Soil Biology 43:24–32.

Gonzalezir, V.G., T.R. Seastedt, and Z. Donate. 2003. Earthworms, arthropods, and plant litter decomposition in Aspen (*Populus tremuloides*) and Lodgepole Pine. Pedobiologia 47:863–869.

Greiner, H.G., A.M.T. Andrew, and S.D. Tiegs. 2011. Cold tolerance among composting earthworm species to evaluate invasion potential. American Midland Naturalist 166: 349–357.

Groffman, P.M., P.J. Bohlen, M.C. Fisk, and T.J. Fahey. 2004. Exotic earthworm invasion and microbial biomass in temperate forest soils. Ecosystems 7:45–54.

Groffman, P.M., T.J. Fahey, M.C. Fisk, J.B. Yavitt, R.E. Sherman, P.J. Bohlen, and J.C. Maerz. 2015. Earthworms increase soil microbial biomass carrying capacity and nitrogen retention in northern hardwood forests. Soil Biology and Biochemistry 87:51–58.

Gundale, M. J., W.M. Jolly, and T.H. Deluca. 2005. Susceptibility of a northern hardwood forest to exotic earthworm invasion: Earthworm invasion susceptibility. Conservation Biology 19:1075–1083.

Hale, C.M. 2007. Evidence for human-mediated dispersal of exotic earthworms: Support for exploring strategies to limit further spread. Molecular Ecology 17:1165–1169.

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

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

Hale, C. M., L.E. Frelich, P.B. Reich, and J. Pastor. 2008. Exotic earthworm effects on hardwood forest floor, nutrient availability, and native plants: A mesocosm study. Oecologia 155:509–518.

Hendrix, P.F., and P.J. Bohlen. 2002. Exotic earthworm invasions in North America: Ecological and policy implications. BioScience 52:801–811.

Heneghan, L., J. Steffen, and K. Fagen. 2007. Interactions of an introduced shrub and introduced earthworms in an Illinois urban woodland: Impact on leaf-litter decomposition. Pedobiologia 50:543–551.

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

Holmstrup, M., J. Overgaard, A.M. Bindesbo, C. Pertoldi, and M. Bayley. 2007. Adaptations to overwintering in the earthworm *Dendrobaena octaedra*: Genetic differences in glucose mobilization and freeze tolerance. Soil Biology and Biochemistry 39:2640–2650.

Homan, C., C. Beier, T. McCay, and G. Lawrence. 2016. Application of lime ($CaCO_3$) to promote forest recovery from severe acidification increases potential for earthworm invasion. Forest Ecology and Management 368:39–44.

Hopfensperger, K.N., and S. Hamilton. 2015. Earthworm communities in previously glaciated and unglaciated eastern deciduous forests. Southeastern Naturalist 14:66–84.

Huff, E.S., and W.H. McWilliams. 2016. Forests of Maine, 2015. Resource Update FS-86. US Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA.

Kalisz, P.J., and D.B. Dotson. 1989. Land-use history and the occurrence of exotic earthworms in the mountains of Eastern Kentucky. American Midland Naturalist 122:288–297.

Kaushal, B.R., B. Kandpal, S.P. Bisht, S. Bora, and R. Dhapola. 1999. Abundance and seasonal activity of earthworms in croplands of Kumaon Himalayas. European Journal of Soil Biology 35:171–176.

Laossi, K.R., D.C. Noguera, A. Bartolomé-Lasa, J. Mathieu, M. Blouin, and S. Barot. 2009. Effects of an endogeic and an anecic earthworm on the competition between four annual plants and their relative fecundity. Soil Biology and Biochemistry 4:1668–1673.

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

Lawrence, B., M.C. Fisk, T.J. Fahey, and E.R. Suarez. 2003. Influence of nonative earthworms on mycorrhizal colonization of Sugar Maple (*Acer saccharum*). New Phytologist 157:145–153.

Liu, Z.G., and X.M. Zou. 2002. Exotic earthworms accelerate plant-litter decomposition in a Puerto Rican pasture and a wet forest. Ecological Applications 12:1406–1417.

Lobe, J.W., M.A. Callaham Jr., P.F. Hendrix, and J.L. Hanula. 2013. Removal of an invasive shrub (Chinese Privet: *Ligustrum sinense* Lour) reduces exotic earthworm abundance and promotes recovery of native North American earthworms. Applied Soil Ecology 83:133–139.

MaineDOT. 2015. Work plan calendar years 2015–2016–2017. Available online at: http://www.maine.gov/mdot/projects/workplan/docs/2015/WorkPlan2015-2016-2017.pdf. Accessed 24 April 2016.

Marinissen, J.C.Y, and F. van den Bosch. 1992. Colonizaiton of new habitats by earthworms. Oecologia 91:371–376.

McLean M.A., and D. Parkinson. 1998. Impacts of the epigeic earthworm *Dendrobaena octaedra* on orbatid mite community diversity and microarthropod abundances in a pine forest floor: A mesocosm study. Applied Soil Ecology 7:125–136.

Milcu, A., J. Schumacher, and S. Scheu. 2006. Earthworms (*Lumbricus terrestris*) affect plant seedling recruitment and microhabitat heterogeneity. Functional Ecology 20:261–268.

Moore, E.H., and J.W. Witham. 1996. From forest to farm and back again: Land-use history as a dimension of ecological research in coastal Maine. Forest History 1:50–69

Nuzzo, V., A. Dávalos, and B. Blossey. 2015. Invasive earthworms shape forest seed bank composition. Diversity and Distributions 21:560–570.

Owen, R.B., Jr., and W.J. Galbraith. 1989. Earthworm biomass in relation to forest types, soil, and land use: Implications for woodcock management. Wildlife Society Bulletin 17:130–136.

Palm, J., N.L.M.B. van Schaik, and B. Schröder. 2013. Modeling distribution patterns of anecic, epigeic, and endogeic earthworms at catchment-scale in agro-ecosystems. Pedobiologia 56:23–31

Quackenbush, P.M., R.A. Butler, N.C. Emery, M.A. Jenkins, E.J. Kladivko, and K.D. Gibson. 2012. *Lumbricus terrestris* prefers to consume Garlic Mustard (*Alliaria petiolata*) seeds. Invasive Plant Science and Management 5:148–154.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at http://www.R-project.org/.

Resner, K., K. Yoo, S.D. Sebestyen, A. Aufdenkampe, C. Hale, A. Lyttle, and A. Blum. 2015. Invasive earthworms deplete key soil inorganic nutrients (Ca, Mg, K, and P) in a northern hardwood forest. Ecosystems 18:89–102.

Reynolds, J.W. 2008. The earthworms (Oligochaeta: Acanthodrilidae, Lumbricidae and Megascolecidae) of Maine, USA. Megadrilogica 12:61–72.

Roth, A.M., T.J.S. Whitfeld, A.G. Lodge, N. Eisenhauer, L.E. Frelich, and P.B. Reich. 2015. Invasive earthworms interact with abiotic conditions to influence the invasion of Common Buckthorn (*Rhamnus cathartica*). Oecologia 178:219–230.

Sackett, T.E., S.M. Smith, and N. Basiliko. 2012. Exotic earthworm distribution in a mixed-use northern temperate forest region: Influence of disturbance type, development age, and soils. Canadian Journal of Forest Research 42:375–381.

Scheu, S., and D. Parkinson. 1994. Effects of earthworms on nutrient dynamics, carbon turnover, and microorganisms in soils from cool temperate forests of the Canadian Rocky Mountains: Laboratory studies. Applied Soil Ecology 1:113–125.

Shartell, L.M., R.G. Corace, A.J. Storer, and D.M. Kashian. 2015. Broad and local-scale patterns of exotic earthworm functional groups in forests of national wildlife refuges of the Upper Midwest, USA. Biological Invasions 17:3591–3607.

Simmons, W., A. Dávalos, and B. Blossey. 2015. Forest successional history and earthworm legacy affect earthworm survival and performance. Pedobiologia 58:153–164.

Singh, S., J. Singh, and A. Pal Vig. 2015. Earthworm as ecological engineers to change the physic-chemical properties of soil: Soil vs vermicast. Ecological Engineering 90:1–5.

Suarez, E.R., T.J Fahey, P.M. Groffman, P.J. Bohlen, and M.C. Fisk. 2004. Effects of exotic earthworms on soil phosphorous cycling in two broadleaf temperate forests. Ecosystems 7:28–44.

Suárez, E.R., G.L. Tierney, T.J. Fahey, and R. Fahey. 2006. Exploring patterns of exotic earthworm distribution in a temperate hardwood forest in south-central New York, USA. Landscape Ecology 21:297–306.

United States Climate Data. 2016. Climate data for Augusta, ME. Available online at: http://www.usclimatedata.com/climate/augusta/maine/united-states/usme0013/2016/1. Accessed 31 December 2016.

United States Department of Agriculture (USDA). 2013. Web soil survey. Available online at http://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm. Accessed 2 September 2015.

Wandeler, H.D., R. Sousa-Silva, E. Ampoorter, H. Bruelheide, M. Carnol, S.M. Dawud, G. Danila, L. Finer, S. Hattenschwiler, M. Hermy, B. Jaroszewicz, F.X. Joly, S. Muller, M. Pollastrini, S. Ratcliffe, K. Raulund-Rasmussen, F. Selvi, F. Valladares, K. Van Meerbeek, K. Verheyen, L. Vesterdal, and B. Muys. 2016. Drivers of earthworm incidence and abundance across European forests. Soil Biology and Biochemistry 99:167–178.

Whitfeld, T.J.S., A.M. Roth, A.G. Lodge, N. Eisenhauer, L.E. Frelich, and P.B. Reich. 2014. Resident plant diversity and introduced earthworms have contrasting effects on the success of invasive plants. Biological Invasions 16:2181–2193.

Wironen, M., and T.R. Moore. 2006. Exotic earthworm invasion increases soil carbon and nitrogen in an old-growth forest in southern Quebec. Canadian Journal of Forest Research 36:845–854.