forests

MDPI

Communication

Non-Native Earthworms Invade Forest Soils in Northern Maine, USA

Joshua J. Puhlick [1,*], Ivan J. Fernandez [1,2] and Jay W. Wason [1]

[1] School of Forest Resources, University of Maine, Orono, ME 04469, USA; ivanjf@maine.edu (I.J.F.); jay.wason@maine.edu (J.W.W.)
[2] Climate Change Institute, University of Maine, Orono, ME 04469, USA
* Correspondence: joshua.puhlick@maine.edu

Abstract: Non-native earthworms can cause abrupt changes in forest ecosystems by altering soil properties and depleting or redistributing soil carbon (C) stocks. The forests of Northern Maine are often perceived as having winters that are too harsh to support earthworm populations and that earthworms are restricted to more southerly regions. In this study, we report the discovery of European earthworms at two research sites in Northern Maine. At one site, earthworms were only found across a portion of the forest, and the median organic (O) horizon C stock in the area with earthworms was 34% less than that of areas without earthworms. At a second site, earthworms were found across the entire 60-ha forest and the median O horizon C stock was 39% less than that of a similar forest without earthworms. Consistent with reports from other regions, areas with earthworms had no or minimal eluvial (E) horizons, while earthworm-free locations always had E horizons. Earthworm presence was always associated with a topsoil (A) horizon, reflecting mechanical mixing and organic matter processing by earthworms. This is one of the first reports of non-native earthworm presence in Northern Maine forests and monitoring changes in soil C will be important for determining rates of C sequestration in these forests. Warmer winter temperatures, particularly winter minimums, and greater annual precipitation will likely increase the success of new earthworm introductions across Northern Maine forests. Management actions that limit the transport of earthworms into earthworm-free areas should be carefully evaluated to minimize the potential for new introductions.

Keywords: biological invasions; climate change; disturbance regimes; forest soil; Maine adaptive silviculture network

check for updates

Citation: Puhlick, J.J.; Fernandez, I.J.; Wason, J.W. Non-Native Earthworms Invade Forest Soils in Northern Maine, USA. *Forests* **2021**, *12*, 80. https://doi.org/10.3390/f12010080

Received: 5 December 2020
Accepted: 11 January 2021
Published: 13 January 2021

1. Introduction

Forests play an essential role in the global carbon (C) cycle because of their ability to sequester large amounts of C from the atmosphere [1–3]. One sizeable forest C pool is that of the soil organic (O) horizon, often referred to as the forest floor. Across forests in the USA, Woodall, et al. [4] estimated that median forest floor C was 25.6 Mg ha^{-1}, which can contain about a third of the amount C that is comparatively stored in live vegetation [1]. Protection and conservation of the O horizon is a focus of forest management [5–7] to improve plant growth by buffering the soil from the temperature extremes, which can limit reductions in soil moisture during summer drought conditions and can minimize the frequency of soil freeze–thaw events in winter [8]. Most tree roots occur within the O horizon [9], which also serves as an important pool of nutrients for plant growth [10]. In forests that are not managed for timber production, strategies for conservation of the O horizon are also important for carbon sequestration as an ecosystem service to reduce greenhouse gas emissions [11–13].

In the area of North America that was glaciated during the Pleistocene Ice Age, which included all of Canada and extended southward to about the Missouri and Ohio Rivers and eastward to Manhattan, earthworms were extirpated from the landscape and

introductions of non-native earthworms likely began with European colonization in the 1500s [14,15]. Today, several species of European and Asian earthworms are present in well-established populations across many regions of this area [16–18], which has led to abrupt changes in forest ecosystems. For example, earthworms alter soil processes such as water regulation and nutrient cycling [19–21], which can in turn influence forest health and biodiversity [22,23]. In the Great Lake States, earthworms have been implicated with sugar maple decline [24] and reductions in sugar maple growth [25]. Earthworms can consume the entire soil O horizon, which increases the likelihood of soil erosion, reductions in soil moisture, and altered freeze–thaw patterns [26]. Additionally, earthworms influence soil systems by altering nutrient cycling, soil structure, water infiltration capacity, root biomass, and mycorrhizal fungi colonization, with impacts that are a function of the species and abundance of earthworms [19,21]. These changes in forest soils can prevent the successful recruitment of native tree species and lead to the establishment of invasive plant species, which can further constrain the recruitment of native tree species [22,27].

Although earthworm invasions into forests are common in areas around the Great Lakes, relatively few instances of earthworm invasions have been reported in Maine [18]. Reynolds [16] reported earthworm detections in Maine by location, habitat, and date. Many of these observations were made by G.E. Gates, his relatives, and students from the 1940s to 1970s. In Maine's northernmost county, three detections (one in 1954 and two in 1979) with habitat information (along a stream bank, under logs in a ditch, and under logs and leaves) were reported in Reynolds [16]. Many of these descriptions do not explicitly state whether observations were made in forests or other ecosystem types. Recent surveys have documented the presence of several invasive earthworms in Maine, but only in the southern regions [27,28] and closely associated with water bodies [29]. In many of Maine's natural areas, earthworms are used as fishing bait, and discarded bait is a common vector for earthworm invasions into forests [30]. In forests managed for wood products, earthworm introductions can also occur when logging equipment is moved from locations with earthworms to earthworm-free locations [30]. In Northern Maine, which is a region that is dependent on the forest industry, early detection and containment of earthworms are critical for reducing their spread and limiting their negative impacts on forest ecosystems.

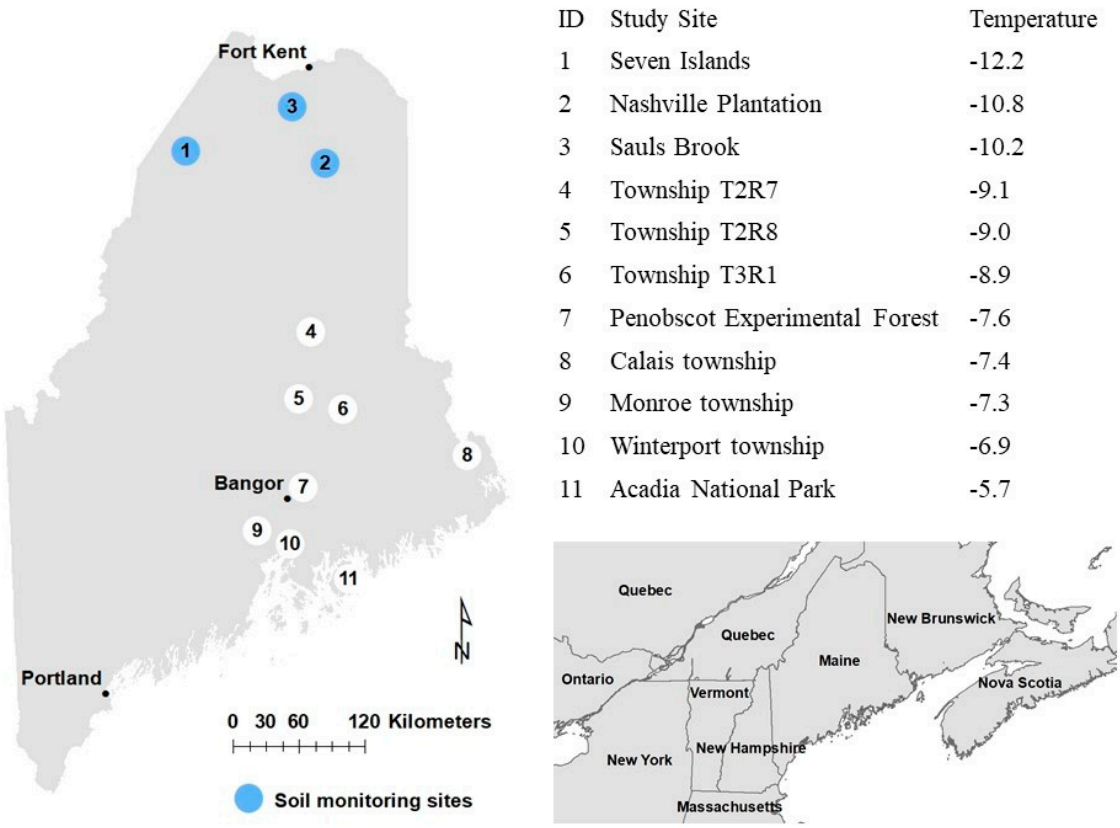
In this study, we report on the recent discovery of non-native earthworms in forests of Northern Maine. The research objectives were to: (1) quantify O horizon C stocks in forests with and without non-native earthworms, (2) report descriptive statistics for O horizon C stocks and soil horizon depths in forests with and without non-native earthworms, and (3) develop pedotransfer functions for predicting O horizon C from O horizon depth. In soil science, pedotransfer functions are used to predict hard-to-measure soil properties from properties that are less difficult to measure or more often available [31–33]. Pedotransfer functions of O horizon C can be used to quantify and monitor O horizon C, while avoiding the high costs that are associated with sampling and processing O horizon samples [9]. Overall, the results of this study are important for understanding changes in C stocks across the Maine landscape, an increasingly important focus for future forest management in the context of climate change.

2. Materials and Methods

2.1. Study System

This study was conducted on a subset of the Maine adaptive silviculture network (MASN) installations in Northern Maine, USA (Figure 1). In 2018 and 2019, three installations were selected for monitoring forest soil C and nutrient stocks over time. The first installation was located west of Eagle Lake near Sauls Brook on the timberlands of J.D. Irving Limited (47°04′ N, 68°73′ W; hereafter referred to as Sauls Brook). The other installations were located on the timberlands of Seven Islands Land Company near the Seven Islands campsite on the Saint John River (46°79′ N, 69°60′ W; hereafter referred to as Seven Islands) and along Route 11 in Nashville Plantation (46°72′ N, 68°46′ W; hereafter referred to as Nashville Plantation). At each installation, the forest area that was assessed was

approximately 60 ha. While collecting baseline data for the monitoring project, earthworms were discovered at the Seven Islands and Nashville Plantation installations. Hence, these discoveries provided a unique opportunity to investigate the impact of earthworms on soil C stocks and other soil attributes.



ID	Study Site	Temperature
1	Seven Islands	-12.2
2	Nashville Plantation	-10.8
3	Sauls Brook	-10.2
4	Township T2R7	-9.1
5	Township T2R8	-9.0
6	Township T3R1	-8.9
7	Penobscot Experimental Forest	-7.6
8	Calais township	-7.4
9	Monroe township	-7.3
10	Winterport township	-6.9
11	Acadia National Park	-5.7

Figure 1. Study sites across Maine cited in this article: Locations 1–3, this study; locations 4–6 and 8–10, Owen and Galbraith [28]; location 7, Puhlick, et al. [9]; location 10, Fisichelli and Miller [27]. Average daily minimum temperature (°C) for the months of November to April from 1981 to 2010 (PRISM Climate Group, http://www.prism.oregonstate.edu/). Earthworms were discovered at locations 1 and 2, which are some of the northernmost reports of earthworms in Maine.

At all three installations, tree species included sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.), yellow birch (*Betula alleghaniensis* Britt.), red spruce (*Picea rubens* Sarg.), and balsam fir (*Abies balsamea* (L.) Mill). The installations also had attributes of old forests such as large tree sizes and abundant standing dead and downed trees. At the Sauls Brook and Seven Islands installations, previous logging occurred between the 1950s and 1970s and was done by hand crews with cable skidders or small bulldozers and horses. During this time period, partial cutting focused on the removal of spruce and possibly balsam fir, and yellow birch for veneer as evidenced by notches in birches that were not cut because of heart rot. At the Nashville Plantation installation, partial cutting occurred in the 1990s, which resulted in a forest with at least two cohorts of trees. An older cohort of trees included large eastern hemlocks (*Tsuga canadensis* (L.) Carrière) and a younger cohort included aspens (*Populus* spp.) and birches (*Betula* spp.). All of the installations had similar average total basal area, but the Nashville Plantation installation had a larger proportion of conifers (Table 1).

Table 1. Mean (and standard deviation) and range of forest attributes by location and earthworm presence. Live tree statistics were derived from measurements of trees ≥ 1.3 cm diameter at breast height. The Nashville Plantation was composed of mixedwood stands, while the other installations were composed of hardwood stands.

	Installation and Earthworm Presence			
Forest Attribute	**Sauls Brook No Earthworms**	**Seven Islands No Earthworms**	**Seven Islands Earthworms**	**Nashville Plantation Earthworms**
Live tree attributes	*N* = 15	*N* = 13	*N* = 2	*N* = 18
Tree density	2476 (513)	994 (231)	1075 (769)	5567 (1582)
(trees ha^{-1})	1483–3249	655-1310	531–1619	3719–9229
QMD	12.3 (1.9)	19.9 (3.1)	19.5 (2.1)	8.9 (1.3)
(cm)	10.4–16.7	16.4–26.7	18.1–21.0	6.4–11.2
Total basal area	28.7 (4.6)	29.7 (4.5)	29.9 (16.3)	33.1 (5.5)
(m^2 ha^{-1})	19.1–36.3	21.4-36.6	18.4-41.4	22.7–42.8
Conifer basal area	22.7 (17.0)	8.0 (5.3)	16.4 (12.2)	34.7 (10.5)
(% of total basal area)	0–48.5	0–20.9	7.8–25.0	16.0–58.3
O horizon attributes	*N* = 36	*N* = 39	*N* = 6	*N* = 47
O_i horizon C stock	4.2 (1.5)	4.6 (1.3)	4.8 (1.0)	4.0 (1.6)
(Mg ha^{-1})	2.2–8.7	2.4–8.0	3.5–5.8	1.5–8.6
O_e + O_a horizon C stock	7.9 (6.3)	5.2 (2.4)	1.5 (2.0)	15.9 (20.7)
(Mg ha^{-1})	1.3–34.5	1.8–12.6	0.2–5.1	0–68.0
Total O horizon C stock	12.1 (6.6)	9.8 (2.9)	6.2 (2.0)	19.9 (21.3)
(Mg ha^{-1})	4.1–37.4	5.7–19.7	3.7–9.0	1.5–73.3
O horizon depth	4.8 (1.7)	4.9 (1.2)	2.9 (1.1)	4.1 (3.4)
(cm)	1.4–9.6	2.6–7.2	1.7–4.9	0.4–12.5

N = number of plots for measuring trees and number of O horizon sample locations. QMD = quadratic mean diameter.

The soils of the Sauls Brook and Seven Islands installations formed in glacial till and included loamy, isotic, frigid Lithic Haplorthods (Monson series); and coarse-loamy, isotic, frigid Aquic Haplorthods (Ragmuff series). The Sauls Brook installation also included the Abram series, which is in the same family as the Monson series. The soils of the Nashville Plantation installation were in a similar catena as the other installations and included coarse-loamy, isotic, frigid Oxyaquic Haplorthods (Plaisted series); coarse-loamy, isotic, frigid Aquic Haplorthods (Howland series); and loamy, mixed, active, acid, frigid, shallow Aeric Endoaquepts (Monarda series). Across installations, soil drainage ranged from excessively (Abram series) to poorly drained (Monarda series) and the soil texture of upper mineral soil horizons tended to be silt loam based on feel and soil taxonomic descriptions. Soil series were determined from field evaluations of soils associated with quantitative soil pits.

2.2. Data Collection

Live trees were measured on permanent sampling plots that consisted of a nested design with 0.08-, 0.02-, and 0.008-ha circular plots sharing the same plot center. Trees ≥ 11.4 cm diameter at breast height (dbh; 1.37 m) were measured on the entire 0.08-ha plot; trees ≥ 6.4 cm dbh were measured on the 0.02-ha plot; and trees ≥ 1.3 cm dbh were measured on the 0.008-ha plot. Tree diameter and species were recorded. Plot locations were selected using a systematic grid. Criteria for plot selection included plots that occurred on the dominant soil series of an installation and that were at least 30 m from the boundaries of the installation. At each installation, 15–18 plots were established (Table 1).

For each permanent plot, soils from a 30 cm × 30 cm quantitative soil pit were collected to a depth of 30 cm below the surface of the B horizon. Organic, eluvial (E), and topsoil (A) horizon depths (when present) were measured at four equally spaced locations along the edge of the pit. An average depth for each horizon was used to report descriptive statistics. The boundaries between horizons were relatively easy to distinguish because of distinctive colors and abrupt boundaries. The O_a horizon, which was black (10YR 2/1) in color and composed of sapric material, was usually present above either a grayish brown (10YR 5/2) E horizon or a very dark grayish brown (10YR 3/2) A horizon. Below the E or A horizon, the B horizon was reddish brown (5YR 4/3) or brown (7.5YR 4/4) in color. The soil pit was located 0° from plot center and 3 m from the perimeter of the plot. If a drainage area occurred at 0°, then locations 120 and 240° from plot center were considered for the pit location.

Three O horizon samples were collected 3 m from the perimeter of each permanent plot. Samples were collected 0, 120, and 240° from the plot center; one of these samples was associated with the quantitative soil pit. At Sauls Brook only one sample was collected from 4 of the 15 plots because of time constraints, and one sample was not used in the analysis because of missing information. At Nashville Plantation, samples of O horizons were not collected from 7 locations because of a high degree of mixing of organic and mineral soil materials by earthworms. Samples were collected using a 30 cm × 30 cm sampling frame after understory plants were clipped from above the forest floor. All woody debris at the surface of the forest floor was also removed from the sampling frame. Organic horizon depth was measured at four equally spaced locations along the edge of the sampling frame, and an average depth was used to report descriptive statistics and in statistical modeling. The portion of the O horizon that could be easily removed using a brush with fine bristles was collected as the O_i horizon. The remaining O horizon was collected as the O_e and O_a horizon using a large serrated knife to separate the bottom of the O_a horizon from the surface of the underlying mineral soil. After separating the O_e + O_a horizon from the mineral soil, any earthworm castings and mineral soil aggregates remaining on the bottom of the sample were removed to avoid their influence on chemical analyses of O_e + O_a horizon fine fractions for the monitoring project.

At Sauls Brook and Nashville Plantation, soil samples were collected from mid-June to early July of 2018 and 2020, respectively. At Seven Islands, samples were collected from mid-July to mid-August of 2018. At each installation, earthworms were identified to genus using a key by Hale [34] and verified by researchers with experience in earthworm identification. Earthworm presence was recorded at each location where O horizon and mineral soil samples were collected. For permanent sampling plots, notes about the visual appearance of the forest floor were also recorded (similar to methods for describing the forest floor in Loss, et al. [35]), and disturbance of the forest floor was always associated with earthworm presence (Figure 2).

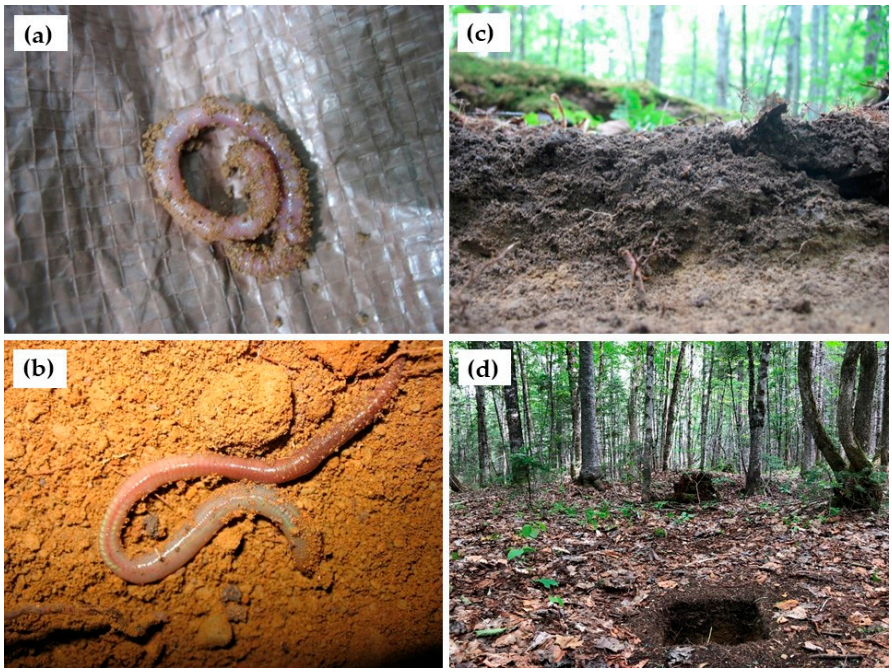


Figure 2. Earthworms in the genera *Aporrectodea* or *Octolasion* (**a**) and *Lumbricus* (**b**). Black A horizon overlying a brown B horizon at the portion of the Seven Islands installation with earthworms (**c**). Forest floor characteristics (mostly fresh leaf litter overlying mineral soil) in an earthworm invaded area at Nashville Plantation (**d**).

2.3. Laboratory Analyses

All samples were air-dried in a greenhouse and then oven-dried. O_i samples were dried to constant mass at 65 °C in a forced hot air oven and woody and non-woody components were weighed separately. The O_e + O_a samples were oven-dried to constant mass at 65 °C and sieved through a 6.4 mm screen to separate fine from coarse fractions. Coarse organic fractions were further sorted into roots, buried wood, and residual organic material. Then, all of the O_e + O_a components were weighed separately. For Sauls Brook and Seven Islands, subsamples of the O_i and O_e + O_a materials were ground to 0.85 mm using a Thomas-Wiley laboratory mill and analyzed for percent total C (TC) by combustion analysis at 1350 °C using a LECO CN-2000 analyzer (LECO Corp., St. Joseph, MI, USA) (Table 2). For each Oi and O_e + O_a component, C stock was calculated by multiplying the component's oven-dry mass by its TC concentration. For each sample location, the O_i and O_e + O_a component C stocks were then summed to derive the total O horizon C stock. Percent total C analysis was not done for samples collected at Nashville Plantation. To derive total O horizon C stocks for Nashville Plantation, weighted average TC concentrations were derived for each O_i and O_e + O_a component using values from mixedwood stands with similar species composition on the Penobscot Experimental Forest (PEF) in central Maine [9].

Table 2. Mean (and standard deviation) of total C (TC, %) concentration by organic (O) horizon component and installation associated with 36, 39, and 6 O horizon samples collected at Sauls Brook, the earthworm-free portion of the Seven Islands installation, and the portion of the Seven Islands installation with earthworms, respectively. The number of samples by component are shown in italics.

	Installation and Earthworm Presence		
O Horizon Component	**Sauls Brook No Earthworms**	**Seven Islands No Earthworms**	**Seven Islands Earthworms**
O_i non-woody materials	*35*	*39*	*6*
	47.2 (1.0)	46.9 (0.7)	43.4 (3.6)
O_i buried wood	*31*	*31*	*6*
	49.0 (1.2)	48.6 (1.4)	47.7 (0.7)
$O_e + O_a$ fines	*36*	*39*	*4*
	44.6 (4.4)	44.9 (2.7)	44.2 (2.8)
$O_e + O_a$ PDL	*36*	*31*	*2*
	46.1 (3.0)	47.6 (1.4)	46.9 (0.8)
$O_e + O_a$ roots	*32*	*21*	*1*
	46.9 (0.9)	46.6 (1.2)	45.4 (NA)
$O_e + O_a$ buried wood	*29*	*36*	*4*
	49.8 (2.2)	49.8 (2.5)	46.8 (1.0)

PDL, partially decomposed litter. NA, not applicable.

2.4. Data Analyses

Descriptive statistics (e.g., mean, SD, and range) for O horizon C stocks and O horizon depth were calculated and reported by installation and earthworm presence. Median values were used to compare O horizon C stocks and depth between areas with and without earthworms because of the highly skewed distributions that are common with O horizon data [4]. Analysis of variance was not used to test for differences in mean C stocks between areas with and without earthworms because of limited replication and differences in tree species composition among installations; specifically, earthworms were only found at two installations, and one of these installations was composed of hardwood stands and the other installation was composed of mixedwood stands. According to Helms [36], mixedwood stands contain hardwoods and softwoods, but neither component is more than 75–80% of the composition. For each installation, pedotransfer functions of total O horizon C that included O horizon depth as a predictor variable were also developed. For the pedotransfer functions, linear models and non-linear models that included power and exponential functions of the predictor variable were evaluated. Non-linear models with a power function of the predictor provided the best fit to the data sets in terms of Akaike's information criteria (AIC) [37], root mean square error, and biological interpretation. Variance weighting functions in the nlme package [38] within R [39] were also used to account for heterogeneity in the standardized residuals of the final pedotransfer functions.

3. Results

At the Seven Islands installation, earthworms in the genus *Aporrectodea* or *Octolasion* were found on two of fifteen permanent sampling plots, and these two plots were in close proximity to one another (Figure 2). Median total O horizon C in the area with earthworms was 34% lower (6.1 Mg ha^{-1}) compared with that of areas of the installation where no earthworms were found (9.3 Mg ha^{-1}; Figure 3). Additionally, the earthworm invaded area had lower median total O horizon C than that of the earthworm-free Sauls Brook installation, which was also composed of hardwood stands. At the Nashville Plantation installation, which was composed of mixedwood stands, earthworms were found on all of the permanent sampling plots. Earthworm species included the genera *Lumbricus* and *Aporrectodea* or *Octolasion* (Figure 2). Since soils in coniferous forests in Maine tend to have greater O horizon C stocks than hardwood forests [40], the median total O horizon C of Nashville Plantation was compared with the median total O horizon C of a similar forest

dominated by mixedwood stands (the PEF) where no earthworms were found [9]. Median total O horizon C at the earthworm invaded Nashville Plantation installation was 39% lower (12.6 Mg ha^{-1}) than in the earthworm-free forest (20.8 Mg ha^{-1}) at the PEF (Figure 3).

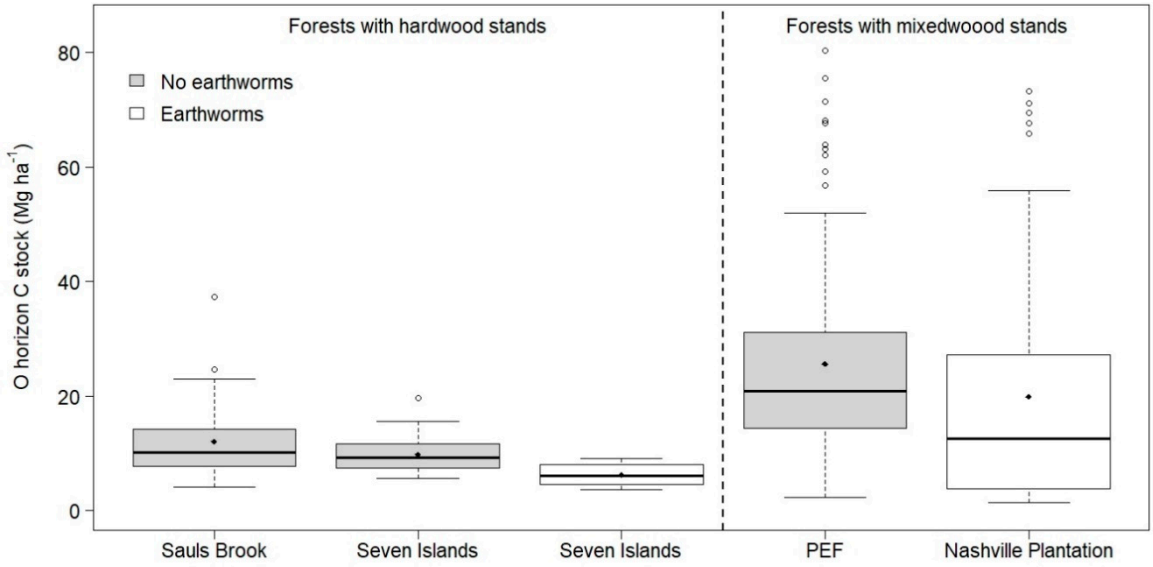


Figure 3. Organic (O) horizon C stocks by location and earthworm presence. The horizontal line and black dot in each box are the median and mean, respectively. The boxes define the hinge (25–75% quartile, and the line is 1.5 times the hinge), and points outside the hinge are represented as dots. Data associated with 124 O horizon samples from the Penobscot Experimental Forest (PEF) were used to present statistics for an earthworm-free forest with mixedwood stands; the O horizon C stocks of the PEF are described in Puhlick, et al. [9].

The pedotransfer functions of O horizon C indicated that O horizon depth explained between 23 and 95% of the variation in O horizon C (Table 3). Additionally, tree species composition and earthworm presence likely influenced the form of curves that were fit to the data (Figure 4). At the quantitative soil pit locations of the Sauls Brook installation and the portion of the Seven Islands installation where no earthworms were found, E horizons were common and there were no A horizons, except at one location (Figure 5). For the portion of the Seven Islands installation with earthworms, soils at the two quantitative soil pit locations had no E horizons and median A horizon depth was 5.5 cm (Figure 5). For the earthworm invaded Nashville Plantation installation, the median and mean O horizon depths at the quantitative soil pit locations were 3.7 and 4.3 cm, respectively (Figure 5). At Nashville Plantation, there also tended to be no E horizons and A horizons were common.

Table 3. Power function parameter estimates (standard errors in parentheses) for predicting O horizon C (Mg ha^{-1}) by installation and earthworm presence. Additionally, shown are the R^2 and root mean square error (RMSE, Mg ha^{-1}) for the respective models.

	Installation and Earthworm Presence			
	Sauls Brook No Earthworms	**Seven Islands No Earthworms**	**Seven Islands Earthworms**	**Nashville Plantation Earthworms**
a	2.679 (0.910)	3.792 (0.998)	2.857 (0.615)	3.200 (0.331)
b	0.951 (0.214)	0.605 (0.170)	0.750 (0.175)	1.238 (0.054)
R^2	0.47	0.23	0.82	0.96
RMSE	2.033	<0.001	0.967	1.804

O horizon C = $a * \text{depth}^b$; depth = O horizon depth (cm).

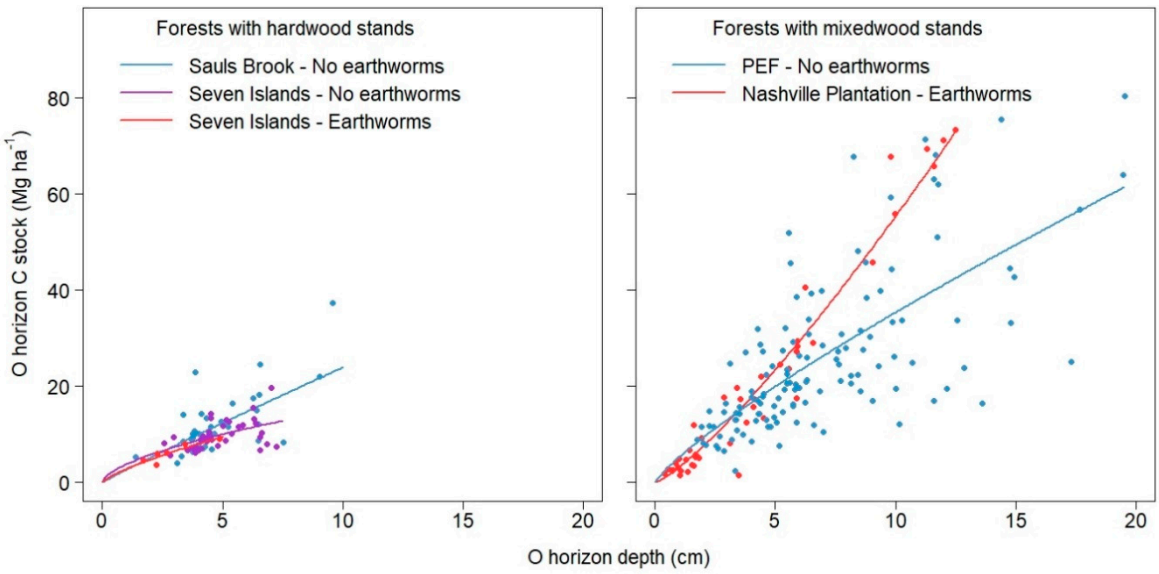


Figure 4. Models of organic (O) horizon C fit to data from organic horizon sample locations by location and earthworm presence (Table 3). The Penobscot Experimental Forest (PEF) model is described in Puhlick, et al. [9].

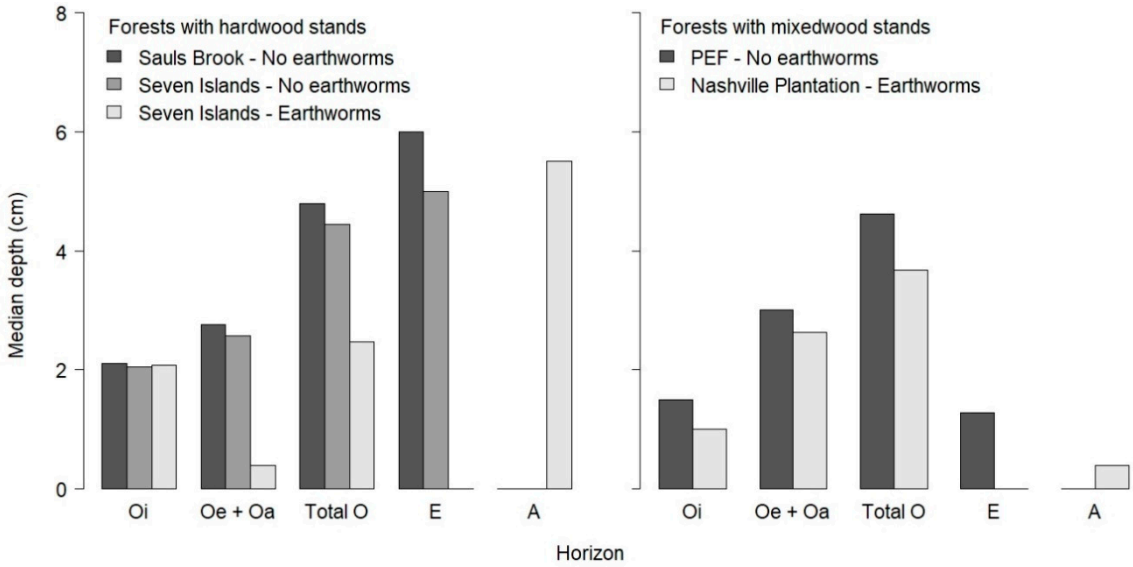


Figure 5. Median depths for operational defined layers of the organic (O) horizon (i.e., O_i and O_e + O_a; see Section 2.2), total O, eluvial (E), and A horizons at quantitative soil pit locations by location and earthworm presence. Data associated with soil core locations at the Penobscot Experimental Forest (PEF) were used to present statistics for an earthworm-free forest with mixedwood stands; the soils of the PEF are described in Puhlick, et al. [9], and Puhlick, et al. [41].

4. Discussion

This study provides evidence that non-native earthworms are already present and may be influencing C dynamics in forest soils of Northern Maine. Earthworms were discovered at two of three forested research sites (each 60 ha), and included a major earthworm invasion at Nashville Plantation. Although our results only include two sites with earthworms, earthworm invaded areas appeared to have lower median O horizon C stocks compared with those of earthworm-free areas with similar tree species composition. However, more replicates of sites with and without earthworms would be needed for a rigorous statistical test of the effect of earthworm presence on O horizon C stocks in Northern Maine. Additionally, many other factors can also influence O horizon C stocks,

such as timber harvesting [42], tree species composition [43], and bryophyte and buried dead wood abundance [9,44]. The relative potential influence of these factors compared to earthworm effects should be investigated in future studies to improve our understanding of factors driving soil C dynamics in this study system.

At sites with earthworms, the initial pattern of change in soil properties appeared similar to that documented in a forest with non-native earthworms in Northern Minnesota [45]. Over a 17-year period since earthworm introduction, Alban and Berry [45] found that earthworms had reduced forest floor biomass and thickness by about 85%, eliminated E horizons, and created A horizons by mixing and modifying the soil. In the present study, the A horizons that were documented also likely developed due to similar modification of the soil by earthworms. While the timing of earthworm introductions at the research sites of the present study is not known, 28% of the O horizon sample locations at Nashville Plantation had thick O horizons (samples with thick O horizons were considered those with depths > 5.9 cm, which was the third quartile of the O horizon depth values for Nashville Plantation). This could suggest that the full assemblage of earthworm species had only been active for a relatively short period of time since earthworms had not yet dramatically reduced O horizon biomass and thickness in all areas of the forest, or that some sample locations had O horizon materials that were not preferred by earthworms (e.g., acidic conifer litter).

The extent of the earthworm invasion at Nashville Plantation was greater than that at Seven Islands, likely because earthworm species within different ecological groups were found at Nashville Plantation. Ecological groups of earthworms include epigeic (surface dwelling), epiendogeic (found in or just beneath litter), endogeic (soil dwelling), and anecic (surfacing to feed) and are known to impact soils differently [46]. Species within the latter three groups were observed at Nashville Plantation and only endogeic earthworms were observed at Seven Islands. Interestingly, the high percentage of conifers at Nashville Plantation did not appear to inhibit earthworm spread across the entire forest. While the severity of the invasions was difficult to assess without repeat measurements, other studies have shown that when species from several ecological groups invade a forest the magnitude of their impact on soils is usually greater than if only species from one group are present [47].

Pedotransfer functions like the one developed for the earthworm invaded Nashville Plantation installation can also be useful for evaluating and monitoring the potential impact of an earthworm invasion on O horizon C stocks. For Nashville Plantation, we found that sample locations with shallow O horizon depths had lower C stocks than might be expected without earthworms and some observations with thick O horizons had higher C stocks than might be expected. As previously mentioned, this could suggest that earthworms had dramatically reduced O horizon C stocks at some locations but had yet to substantially reduce O horizon C at other locations. Alternatively, or in addition to these factors, the spatial variability in the types of trees and their litter inputs could have influenced O horizon C stocks due to litter quality, decomposition rates, and earthworm feeding habits. The curves for forests with hardwood stands were relatively similar to each other, and to the first part of the curve for the non-invaded forest with mixedwood stands at the PEF (i.e., the 0–10 cm depth range). These findings indicate that the selection of appropriate pedotransfer function for estimating O horizon C stocks from O horizon depth may be influenced by the presence of earthworms and their impact on the O horizon, and, tree species composition. Future studies should seek to test these relationships as monitoring earthworm impacts in Northern Maine becomes more important.

In Northern Maine, warming winter temperatures and increasing annual precipitation may be favoring the establishment and expansion of earthworm populations. Fisichelli and Miller [27] showed that warmer air temperatures were correlated with earthworm presence across monitoring plots of the National Park Service in the Northeastern USA. However, the present study includes more northerly locations dominated by hardwoods that contain well-established populations of earthworms. Earthworms also prefer habitats

with high soil moisture [28,29], so increased soil moisture due to increasing precipitation could favor earthworms depending on the amount, timing, and frequency of precipitation during a given year. While the future frequency of droughts in Northern Maine is less certain than predicted temperature and precipitation trends [48], warmer temperatures could favor increased soil drying during droughts. Additionally, the reduction of the O horizon and increase in soil bulk density by earthworms could further intensify soil drying, which can affect tree health and mortality [49].

Regardless of whether forests are managed for timber products or as reserves with no logging, a major pathway for earthworm introductions includes the practice of discarding unused fishing bait near waterbodies within forestlands [30]. In some portions of Northern Maine, agricultural fields (e.g., for growing potato crops) also occur adjacent to forests and some forests have a legacy of previous agricultural use. In Eastern and Central Maine, Owen and Galbraith [28] found that forests that were previously farmed had a greater probability of earthworm presence than forests that were never farmed. In forests managed for timber products, the transportation of logging machinery from harvest areas with earthworms to earthworm-free areas could also result in new earthworm introductions across the landscape [30]. While more surveys should be conducted to estimate the extent of current earthworm invasions in forests of Northern Maine, best management practices to minimize new introductions of earthworms, such as cleaning equipment before transport, should be developed and considered when working in areas known to have earthworms. Developing these practices is crucial because earthworm invasions are almost impossible to eradicate unless earthworms are not well-established or are found in discrete locations [30]. Our study clearly showed that earthworm invasions have already begun in two forested areas of Northern Maine. It is critical for natural resource managers that we identify the extent of these invasions and the potential impacts they may have on ecosystem function.

5. Conclusions

We discovered European earthworms at two of three forested research sites (each 60 ha) in Northern Maine. Although our results only include two sites with earthworms, earthworm invaded areas appeared to have lower median O horizon C stocks compared with those of earthworm-free areas with similar tree species composition. Areas with earthworms also had no or minimal E horizons, while earthworm-free locations always had E horizons. Earthworm presence was always associated with an A horizon, reflecting mechanical mixing and organic matter processing by earthworms. This is one of the first reports of non-native earthworm presence in Northern Maine forests and monitoring changes in soil C will be important for determining rates of C sequestration in these forests. Additionally, best management practices to minimize new introductions of earthworms should be developed and considered when working in areas known to have earthworms.

Author Contributions: All authors contributed substantially to the work reported. Conceptualization, J.J.P., I.J.F. and J.W.W.; methodology, J.J.P. and I.J.F.; software, J.J.P.; validation, J.J.P., I.J.F. and J.W.W.; formal analysis, J.J.P., I.J.F. and J.W.W.; investigation, J.J.P.; resources, J.J.P. and I.J.F.; data curation, J.J.P.; writing—original draft preparation, J.J.P., I.J.F. and J.W.W.; writing—review and editing, J.J.P., I.J.F. and J.W.W.; visualization, J.J.P., I.J.F. and J.W.W.; supervision, J.J.P.; project administration, J.J.P.; funding acquisition, J.J.P., I.J.F. and J.W.W. All authors have read and agreed to the published version of the manuscript.

Funding: Funding related to soil sampling and analyses was provided by the Sustainable Forestry Initiative Inc. and the Cooperative Forestry Research Unit (Orono, Maine). Jay Wason was partially supported by the USDA National Institute of Food and Agriculture, McIntire Stennis Program (Project Numbers ME042121 and ME042007). This is Maine Agricultural and Forest Experiment Publication Number 3790.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy as other reports for funders are currently being drafted with the data.

Acknowledgments: We thank Annise Dobson (Yale University) and Justin Schlawin (Maine Natural Areas Program) for guidance on earthworm species identification and for information about research efforts on earthworms across the northeastern USA. We also thank Shawn Fraver and Aaron Weiskittel (University of Maine), Nancy Sferra (The Nature Conservancy), and Gray Fish (Maine Department of Agriculture, Conservation, and Forestry) for discussions about earthworms at the study areas.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Goodale, C.L.; Apps, M.J.; Birdsey, R.A.; Field, C.B.; Heath, L.S.; Houghton, R.A.; Jenkins, J.C.; Kohlmaier, G.H.; Kurz, W.; Liu, S.R.; et al. Forest carbon sinks in the Northern Hemisphere. *Ecol. Appl.* **2002**, *12*, 891–899. [CrossRef]
2. Pacala, S.W.; Hurtt, G.C.; Baker, D.; Peylin, P.; Houghton, R.A.; Birdsey, R.A.; Heath, L.; Sundquist, E.T.; Stallard, R.F.; Ciais, P.; et al. Consistent land- and atmosphere-based U.S. carbon sink estimates. *Science* **2001**, *292*, 2316–2320. [CrossRef] [PubMed]
3. Pan, Y.; Birdsey, R.A.; Fang, J.; Houghton, R.; Kauppi, P.E.; Kurz, W.A.; Phillips, O.L.; Shvidenko, A.; Lewis, S.L.; Canadell, J.G.; et al. A large and persistent carbon sink in the world's forests. *Science* **2011**, *333*, 988–993. [CrossRef] [PubMed]
4. Woodall, C.W.; Perry, C.H.; Westfall, J.A. An empirical assessment of forest floor carbon stock components across the United States. *For. Ecol. Manag.* **2012**, *269*, 1–9. [CrossRef]
5. Curran, M.P.; Maynard, D.G.; Heninger, R.L.; Terry, T.A.; Howes, S.W.; Stone, D.M.; Niemann, T.; Miller, R.E.; Powers, R.F. An adaptive management process for forest soil conservation. *For. Chron.* **2005**, *81*, 717–722. [CrossRef]
6. Curran, M.P.; Miller, R.E.; Howes, S.W.; Maynard, D.G.; Terry, T.A.; Heninger, R.L.; Niemann, T.; van Rees, K.; Powers, R.F.; Schoenholtz, S.H. Progress towards more uniform assessment and reporting of soil disturbance for operations, research, and sustainability protocols. *For. Ecol. Manag.* **2005**, *220*, 17–30. [CrossRef]
7. Duckert, D.R.; Morris, D.M.; Deugo, D.; Duckett, S.; McPherson, S. Developing site disturbance standards in Ontario: Linking science to forest policy within an adaptive management framework. *Can. J. Soil Sci.* **2009**, *89*, 13–23. [CrossRef]
8. Schaetzl, R.J.; Anderson, S. *Soils Genesis and Geomorphology*; Cambridge University Press: Cambridge, UK, 2005; p. 40.
9. Puhlick, J.J.; Fraver, S.; Fernandez, I.J.; Weiskittel, A.R.; Kenefic, L.S.; Kolka, R.K.; Gruselle, M.-C. Factors influencing organic-horizon carbon pools in mixed-species stands of central Maine, USA. *For. Ecol. Manag.* **2016**, *364*, 90–100. [CrossRef]
10. Brady, N.C.; Weil, R.R. *The Nature and Properties of Soils*, 14th ed.; Pearson Education, Inc.: Upper Saddle River, NJ, USA, 2008; p. 212.
11. Kuehne, C.; Puhlick, J.; Weiskittel, A.; Cutko, A.; Cameron, D.; Sferra, N.; Schlawin, J. Metrics for comparing stand structure and dynamics between Ecological Reserves and managed forest of Maine, USA. *Ecology* **2018**, *99*, 2876. [CrossRef]
12. Luyssaert, S.; Schulze, E.D.; Borner, A.; Knohl, A.; Hessenmoller, D.; Law, B.E.; Ciais, P.; Grace, J. Old-growth forests as global carbon sinks. *Nature* **2008**, *455*, 213–215. [CrossRef] [PubMed]
13. Puhlick, J.J.; Fraver, S.; Fernandez, I.J.; Teets, A.; Weiskittel, A.R.; Kenefic, L.S. Site quality, disturbance, and vegetation effects on carbon storage and accumulation in old, mixed-species stands in central Maine, USA. *Nat. Areas J.* **2019**, *39*, 429–441. [CrossRef]
14. Gates, G.E. Requiem—For megadrile utopias. A contribution toward the understanding of earthworm fauna of North America. *Proc. Biol. Soc. Wash.* **1966**, *79*, 239–254.
15. Reynolds, J.W. *The Earthworms (Lumbricidae and Sparganophilidae) of Ontario*; Life Sci. Misc. Pub.: Toronto, ON, Canada, 1977.
16. Reynolds, J.W. The earthworms (Oligochaeta: Acanthodrilidae, Lumbricidae and Megascolecidea) of Maine, USA. *Megadrilogica* **2008**, *12*, 61–72.
17. Reynolds, J.W. The earthworms (Oligochaeta: Acanthodrilidae, Lumbricidae, Megascolecidea and Sparganophilidae) of the northeastern United States, revisited. *Megadrilogica* **2010**, *14*, 101–157.
18. Reynolds, J.W.; Gorres, J.H.; Knowles, M.E. A checklist by counties of earthworms (Oligochaeta: Acanthodrilidae, Lumbricidae and Megascolecidae) in the states of Maine, New Hampshire and Vermont, USA. *Megadrilogica* **2015**, *17*, 125–140.
19. Blouin, M.; Hodson, M.E.; Delgado, E.A.; Baker, G.; Brussaard, L.; Butt, K.R.; Dai, J.; Dendooven, L.; Peres, G.; Tondoh, J.E.; et al. A review of earthworm impact on soil function and ecosystem services. *Eur. J. Soil Sci.* **2013**, *64*, 161–182. [CrossRef]
20. Dobson, A.M.; Blossey, B.; Richardson, J.B. Invasive earthworms change nutrient availability and uptake by forest understory plants. *Plant Soil* **2017**, *421*, 175–190. [CrossRef]
21. Ferlian, O.; Thakur, M.P.; Gonzalez, A.C.; San Emeterio, L.M.; Marr, S.; Rocha, B.D.; Eisenhauer, N. Soil chemistry turned upside down: A meta-analysis of invasive earthworm effects on soil chemical properties. *Ecology* **2020**, *101*, 12. [CrossRef] [PubMed]
22. Craven, D.; Thakur, M.P.; Cameron, E.K.; Frelich, L.E.; Beausejour, R.; Blair, R.B.; Blossey, B.; Burtis, J.; Choi, A.; Davalos, A.; et al. The unseen invaders: Introduced earthworms as drivers of change in plant communities in North American forests (a meta-analysis). *Glob. Chang. Biol.* **2017**, *23*, 1065–1074. [CrossRef]
23. Moore, J.D.; Gorres, J.H.; Reynolds, J.W. Exotic Asian pheretimoid earthworms (*Amynthas* spp., *Metaphire* spp.): Potential for colonisation of south-eastern Canada and effects on forest ecosystems. *Environ. Rev.* **2018**, *26*, 113–120. [CrossRef]
24. Bal, T.L.; Storer, A.J.; Jurgensen, M.F. Evidence of damage from exotic invasive earthworm activity was highly correlated to sugar maple dieback in the Upper Great Lakes region. *Biol. Invasions* **2018**, *20*, 151–164. [CrossRef]

25. Larson, E.R.; Kipfmueller, K.F.; Hale, C.M.; Frelich, L.E.; Reich, P.B. Tree rings detect earthworm invasions and their effects in northern hardwood forests. *Biol. Invasions* **2010**, *12*, 1053–1066. [CrossRef]
26. Korzekwa, K. Earthworm invaders have huge implications for forest health. *Soil Horizons* **2015**, *56*, 1–3. [CrossRef]
27. Fisichelli, N.A.; Miller, K.M. Weeds, worms, and deer: Positive relationships among common forest understory stressors. *Biol. Invasions* **2018**, *20*, 1337–1348. [CrossRef]
28. Owen, R.B.; Galbraith, W.J. Earthworm biomass in relation to forest types, soil, and land use: Implications for woodcock management. *Wildl. Soc. Bull.* **1989**, *17*, 130–136.
29. Simmons, W. *Non-Native Earthworms in Maine: A review of Ecological Impacts and an Initial Assessment of Maine's Earthworm Community*; A report for the Reptile, Amphibian, and Invertebrate Group of the Maine Department of Inland Fisheries and Wildlife and The Nature Conservancy; Maine Chapter: Brunswick, ME, USA, 2015.
30. Callaham, M.A.; Gonzalez, G.; Hale, C.M.; Heneghan, L.; Lachnicht, S.L.; Zou, X.M. Policy and management responses to earthworm invasions in North America. *Biol. Invasions* **2006**, *8*, 1317–1329. [CrossRef]
31. Cornelis, W.M.; Ronsyn, J.; Van Meirvenne, M.; Hartmann, R. Evaluation of pedotransfer functions for predicting the soil moisture retention curve. *Soil Sci. Soc. Am. J.* **2001**, *65*, 638–648. [CrossRef]
32. Schwarzel, K.; Feger, K.H.; Hantzschel, J.; Menzer, A.; Spank, U.; Clausnitzer, F.; Kostner, B.; Bernhofer, C. A novel approach in model-based mapping of soil water conditions at forest sites. *For. Ecol. Manag.* **2009**, *258*, 2163–2174. [CrossRef]
33. Wosten, J.H.M.; Lilly, A.; Nemes, A.; Le Bas, C. Development and use of a database of hydraulic properties of European soils. *Geoderma* **1999**, *90*, 169–185. [CrossRef]
34. Hale, C. *Earthworms of the Great Lakes*, 2nd ed.; Kollath-Stensaas Publishing: Duluth, MN, USA, 2013.
35. Loss, S.R.; Hueffmeier, R.M.; Hale, C.M.; Host, G.E.; Sjerven, G.; Frelich, L.E. Earthworm invasions in northern hardwood forests: A rapid assessment method. *Nat. Areas J.* **2013**, *33*, 21–30. [CrossRef]
36. Helms, J.A. *The Dictionary of Forestry*; Society of American Foresters: Bethesda, MD, USA, 1998.
37. Akaike, H. Information theory as an extension of the maximum likelihood principle. In *Second International Symposium on Information Theory*; Petrov, B.N., Csaki, F., Eds.; Akademiai Kiado: Budapest, Hungary, 1972; pp. 267–281.
38. Pinheiro, J.C.; Bates, D.M.; DebRoy, S.; Sarkar, D.; R Development Core Team. nlme: Linear and Nonlinear Mixed Effects Models 2014. Available online: http://CRAN.R-project.org/package=nlme (accessed on 30 October 2020).
39. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2014.
40. Fernandez, I.J. *Carbon and nutrients in Maine Forest Soils*; Technical Bulletin 200; Maine Agricultural and Forest Experiment Station, University of Maine: Orono, ME, USA, 2008.
41. Puhlick, J.J.; Fernandez, I.J.; Weiskittel, A.R. Evaluation of forest management effects on the mineral soil carbon pool of a lowland, mixed-species forest in Maine, USA. *Can. J. Soil Sci.* **2016**, *96*, 207–218. [CrossRef]
42. Nave, L.E.; Vance, E.D.; Swanston, C.W.; Curtis, P.S. Harvest impacts on soil carbon storage in temperate forests. *For. Ecol. Manag.* **2010**, *259*, 857–866. [CrossRef]
43. Kulmatiski, A.; Vogt, D.J.; Siccama, T.G.; Tilley, J.P.; Kolesinskas, K.; Wickwire, T.W.; Larson, B.C. Landscape determinants of soil carbon and nitrogen storage in southern New England. *Soil Sci. Soc. Am. J.* **2004**, *68*, 2014–2022. [CrossRef]
44. Hagemann, U.; Moroni, M.T.; Gleissner, J.; Makeschin, F. Accumulation and preservation of dead wood upon burial by bryophytes. *Ecosystems* **2010**, *13*, 600–611. [CrossRef]
45. Alban, D.H.; Berry, E.C. Effects of earthworm invasion on morphology, carbon, and nitrogen of a forest soil. *Appl. Soil Ecol.* **1994**, *1*, 243–249. [CrossRef]
46. Hale, C.M.; Frelich, L.E.; Reich, P.B. Changes in hardwood forest understory plant communities in response to European earthworm invasions. *Ecology* **2006**, *87*, 1637–1649. [CrossRef]
47. Hale, C.M.; Frelich, L.E.; Reich, P.B.; Pastor, J. Effects of european earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. *Ecosystems* **2005**, *8*, 911–927. [CrossRef]
48. Fernandez, I.; Birkel, S.; Schmitt, C.; Simonson, J.; Lyon, B.; Pershing, A.; Stancioff, E.; Jacobson, G.; Mayewski, P. *Maine's Climate Future 2020 Update*; University of Maine: Orono, ME, USA, 2020; Available online: climatechange.umaine.edu/climate-matters/maines-climate-future/ (accessed on 30 October 2020).
49. Frelich, L.E.; Blossey, B.; Cameron, E.K.; Davalos, A.; Eisenhauer, N.; Fahey, T.; Ferlian, O.; Groffman, P.M.; Larson, E.; Loss, S.R.; et al. Side-swiped: Ecological cascades emanating from earthworm invasions. *Front. Ecol. Environ.* **2019**, *17*, 502–510. [CrossRef] [PubMed]