Investigation of soil and plant characteristics across a continuum of non-native earthworm invasion in hardwood forests, Tettegouche State Park, MN USA

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This thesis is dedicated to Gemma Rose Bennett, my daughter and first born.
May the awe and wonder I see in your eyes never fade.

Abstract

Invasive earthworms cause profound changes in forest floor thickness, soil structure and chemistry, and plant community composition within cold temperate hardwood forests. However, few studies have examined these effects across a continuum of earthworm invasion and in conjunction with canopy disturbance. The research objectives of this thesis were to determine the changes of earthworm invasion on the upper soil horizon's thickness, gravimetric water content, potential horizon field capacity, and available nitrogen and phosphorus; and plant communities in hardwood forest sites within Tettegouche State Park, MN USA. All sites were uneven-aged, unmanaged northern hardwood forests of an approximate age of 225 years. The canopies were dominated by sugar maple and had experienced substantial canopy disturbance (9.7 – 20.5% opening) during an ice storm in spring 2009. Earthworms were sampled in the fall of 2010-2011. Each of the four sites were invaded by differing earthworm assemblages ranging from minimally invaded (1 species and average biomass of 0.1729 AFDgrams/m^2) to heavily invaded (5 species and average biomass of 14.12 AFDgrams/m^2). In the upper soil horizons O horizon thickness decreased and A horizon thickness increased with increasing earthworm richness and biomass. Mineral soil gravimetric water content was measured biweekly (May-August 2011) but did not differ among sites. Total potential horizon field capacity, including the O horizon, determined that 53-59% of the available water in a 12 cm deep core at field capacity is held in the O horizon. Availability of NO_3 was significantly higher in the heavily invaded site compared to all other sites. Plant communities were assessed in the summer of 2009-

2011, nonmetric multidimensional scaling was used to analyze the relationship of herbaceous plant species richness and percent cover to environmental variables and that species richness and diversity indices were positively correlated with O horizon thickness and negatively correlated with earthworm richness and biomass. The main conclusions of this study are that 1) moderate canopy disturbance had no affect on soil characteristics, or earthworms and plant communities; 2) earthworm assemblages (richness and biomass) were strongly correlated with changes in forest floor thickness, moisture holding capacity, nitrogen availability and plant community composition in these sugar maple forests, and 3) traditional exclusion of the O horizon when measuring water holding capacity in forest soils should be reconsidered given the large proportion of potential water holding capacity it provides, and is lost when a site is heavily invaded by earthworms. The implications of the loss of the O horizon and the associated loss of water holding capacity on ecosystem functions and biotic communities of hardwood forest systems need to be more fully explored.

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Chapter 1: Introduction

The introduction and establishment of non-native species is a global problem as habitats are altered, native biota are displaced, and local extinction rates increase (Gurevitch and Padilla 2004). Cold-temperate hardwood forests in the Great Lakes region evolved in an earthworm-free environment as a result of the last glaciation (Gates 1970; Eisenhauer et al. 2007). Following European settlement in the region and with humans serving as the primary vectors of spread, non-native earthworms of the family *Lumbricidae* continue to invade cold-temperate hardwood forests of the Great Lakes region and are leading to changes in soil structure and function, large declines in understory plants, and may threaten the long-term sustainability of these forest systems (Hale 2004; Frelich et al. 2006; Nuzzo et al. 2009).

In earthworm-free hardwood forests distinct upper soil horizons are observed and the native understory plant community is high in richness and diversity. Because decomposition of organic material is a slow process in earthworm-free forests, a thin A horizon may or may not be present, but rests beneath a thick O horizon and atop the mineral soil of the E horizon (Crow et al. 2009). Many native herbaceous plants rely on the thick, organic forest floor for rooting. This O horizon is home to diverse communities of invertebrates, fungi and bacteria that serve as primary decomposers and as such the forest floor is the primary location for nutrient cycling (Li et al. 2002; Ashton et al. 2005; Costello and Lamberti 2009; Burke et al. 2011).

After the introduction and establishment of several earthworm species, the organic horizon is consumed and native soil biota are largely displaced (Eisenhauer et al. 2007;

Gilliam 2007). The consumed organic material is mixed with the E horizon causing the formation of a thick A horizon. There are many proposed mechanisms for plant loss including the loss of or changes in mychorizal communities, exposure of roots, loss of seedling establishment, interactions with deer grazing, and others that may stress native plant communities resulting in a loss of diversity and abundance (Hendrix and Bohlen 2002; Fisk et al. 2004; Groffman et al. 2004; Migge-Kleian et al. 2006; Holdsworth et al. 2007a; Maerz et al. 2009; Asshoff et al. 2010; Szlavecz et al. 2011; Fahey et al. 2012).

Concern among land managers emerged about what how canopy opening in earthworm invaded and earthworm-free sugar maple (*Acer saccharum*, Marshall) stands may differently affect the understory plant communities (Frelich 2002). In the spring of 2009 Tettegouche State Park, MN USA experienced widespread canopy opening due to an ice storm. Previous research in the park indicated areas containing similar sugar maple dominated overstories prior to the ice damage that ranged from being earthworm-free to heavily earthworm invaded (Loss et al. 2013).

The objective of this study was to describe the changes in upper soil horizon properties and plant community composition associated with a continuum of earthworm invasion and canopy disturbance. In chapter 2, changes in soil properties are described in association with a continuum of earthworm assemblages with particular emphasis on upper soil horizon thickness, gravimetric water content, potential horizon field capacity, and available N and P in the forms of NO_3, NH_4, and PO_4. In chapter 3, observed changes in plant community composition associated with a continuum of earthworm assemblages and multiple environmental variables are described.

Chapter 2: Investigation of soil characteristics across a continuum of non-native earthworm invasion in hardwood forests, Tettegouche State Park, MN USA

Introduction

Native earthworms were extirpated from the Great Lakes region of North America during the last glaciation (Gates 1970; Bohlen et al. 2004b; Frelich et al. 2006; Holdsworth et al. 2007b). It is believed that many of the earthworm species now found in this area arrived to the North American continent with European settlers as they are from the family *Lumbricidae* with wide distribution across much of Europe (Gates 1982; Reynolds 1995; Hale 2008). Typical anthropogenic activities like logging, homesteading and road development, movement of soil and mulch, and dumping of earthworms used as fishing bait are the major vectors of spread and establishment of invasive earthworms in previously earthworm-free areas of North America (Hendrix and Bohlen 2002; Cameron et al. 2007). Additionally, patterns of invasion are closely linked with soil and litter properties in habitats associated with this region (Tiunov et al. 2006). In more recent years, spread of Asian earthworms (family *Megascolecidae*) is a growing concern in areas along the southeast and northeastern United States (Zhang et al. 2010). Incipient invasions of Asian earthworms are reported in Minnesota (Hale & Huffmeier, unpublished data), and though this region may provide ideal habitat for these invaders, most areas of invasion to date only consist of European earthworms (Reynolds et al. 2002).

In the Great Lakes region, specifically within Minnesota, there are northern temperate forests that reflect a continuum of earthworm invasion from earthworm-free to heavily invaded conditions (Hale 2004; Holdsworth et al. 2007a). Previous studies in this region show that when studying ecosystem effects of invasive earthworms, biomass and assemblage groups are more informative than richness or density (Hale et al. 2005b, 2006; Holdsworth et al. 2007b). Since earthworm taxa vary in size and density, biomass as a metric proportionally shows the effects of earthworms in a given system. Assemblage groups, such as those used by Hale et al. (2005a, b, 2006a), better describe earthworm populations in relation to the feeding and burrowing behaviors of different earthworm species (i.e. ecological groups). When multiple ecological groups of earthworms are present they have synergistic affects in hardwood forests systems by altering soil characteristics and native biota (Hale et al. 2005b; Frelich et al. 2006; Holdsworth et al. 2007b; Szlavecz et al. 2011).

In undisturbed northern temperate hardwood forests, distinct upper soil horizons are observed. Studies measuring O horizon thickness along leading edges of earthworm invasion show an inverse relationship between O horizon thickness and earthworm biomass and assemblages (Hale et al. 2005b; Frelich et al. 2006; Suarez et al. 2006b). Pigmented epigeic earthworms like *Dendrobaena octaedra* do not consume the leaf litter (O_i) layer directly, but physically separate the organic layers and mix the O_e and O_a layers. Northern temperate forests invaded by only epigeic species display a relatively unaltered soil profile as the mineral soil is left relatively untouched by this species (McLean and Parkinson 1997; Frelich et al. 2006). After the introduction and establishment of epi-endogeic and anecic earthworms (*Lumbricus rubellus* and *Lumbricus terrestris*, respectively) the O horizon is consumed. During digestion the consumed organic material from the O horizon is mixed and redistributed in the earthworm casts within the mineral soil (E horizon), optimizing feeding conditions for endogeic species (e.g. *Aporrectodea* spp. and *Octolasion* spp.) that feed and burrow within this horizon. Effectively, the aforementioned ecological groups enlarge the once thin A horizon by reducing the depth of the O horizon, and this process is accelerated by the increasing presence of multiple ecological groups (Hale et al. 2008).

Agricultural studies of earthworm burrowing patterns and water filtration in crop fields under tillage and no tillage conditions associated earthworms with increased crop productivity via changes in soil moisture and nutrient availability (Brown et al. 2004; Hale et al. 2008). However, agricultural soils behave differently from those in earthworm-free northern temperate forests. For instance, heavy machinery can compact agricultural soils, and earthworm activity is found to loosen soils by decreasing the bulk density through burrowing behaviors. In native forest soils with low bulk density, earthworm invasion may actually increase bulk density by compacting spaces between particles where water would accumulate. Consequently, an increase in bulk density may be followed by the additional effects of water runoff and erosion due to soil manipulation by earthworms and a further decrease in available water to local plant communities (Hale 2004). Soil moisture measured as gravimetric water content directly beneath the O_a horizon in temperate hardwood forests may vary among similar stands due to changes in bulk density.

Measuring the potential horizon field capacity of each soil horizon is critical for understanding the proportion of available water within the soil profile. Since each horizon is a microhabitat for soil flora and fauna, measuring gravimetric water content of each horizon is useful for understanding between-horizon differences in water holding capacity (Migge-Kleian et al. 2006). Additionally, soil moisture is critical to nutrient cycling in forests since water is necessary for the photosynthetic processes and dissolved nutrients critical for plant growth are absorbed by plant roots (Spurr and Barnes 1980; Bockheim 1990; Yahner 2000).

Nutrient availability is also affected by earthworm invasion. Studies in both agricultural settings as well as forest soils have shown an increase in nutrient content and mineralization in fresh earthworm cast material (Alban and Berry 1994; Bohlen et al. 2004b; Hale 2004). However, a net reduction in available nitrogen and phosphorus may occur due to leaching and immobilization (Suarez et al. 2003; Groffman et al. 2004; Hale 2004; Frelich et al. 2006).

The focus of this chapter is to describe the changes in soil properties associated with a continuum of earthworm biomass and assemblage, including horizon thickness of the O, A and E horizons, gravimetric water content and potential soil horizon field capacity, and available N and P at monthly sampling periods throughout the growing season. Under the same level of canopy opening it was hypothesized that:

(1) increasing earthworm species richness and biomass is inversely correlated with O horizon thickness, and positively correlated with A horizon thickness.

(2) increasing earthworm species richness and biomass is inversely correlated with soil gravimetric water content.

(3) measured as the potential horizon field capacity, the O horizon holds a greater proportion of water than the A or E horizons.

(4) increasing earthworm species richness and biomass is inversely correlated with available N and P in the forms of ammonium, nitrate, and phosphate in the soil (NH_4-N, NO_3-N, and PO_4-P).

Methods and Materials

Sites

The study was conducted from 2009-2012 in four sugar maple (*Acer saccharum,* Mar) dominated hardwood stands of Tettegouche State Park, Minnesota USA (Figure 1). Paper birch (*Betula papyrifera*, Mar) and basswood (*Tilia americana*, L) were secondary tree species. Mean annual precipitation is 69 cm, mean temperatures in January and July are -12 and 19° C respectively, and the mean frost-free growing season is 120 days (Minnesota State Climatology Office, 2013). The stands were 1 to 4 km apart and have the same sandy-loam soil texture, unmanaged and uneven age class of approximately 225 years, and primary forest composition (Minnesota Department of Natural Resources, 2013; Table 1). In 2009 a pilot study of these sites provided evidence of similar site characteristics. Previous research conducted in this area documented the existence of sites with a continuum of earthworm invasion from minimally-invaded to heavily-invaded sites (Loss et al. 2013). For this study, each site along the continuum of earthworm invasion was designated by the earthworm assemblages present and from here on will be referred as Minimally Invaded, Moderately Invaded 1, Moderately Invaded 2, and Heavily Invaded.

Within each site, 10 sample points were established in a grid 50 m apart. At each sample point a nested set of plots was established. A 10 m radius subplot was used to sample trees and canopy opening, and within a 5 m radius subplot earthworm biomass and assemblage, upper soil horizons, gravimetric water content, potential horizon field capacity, and available N and P were sampled.

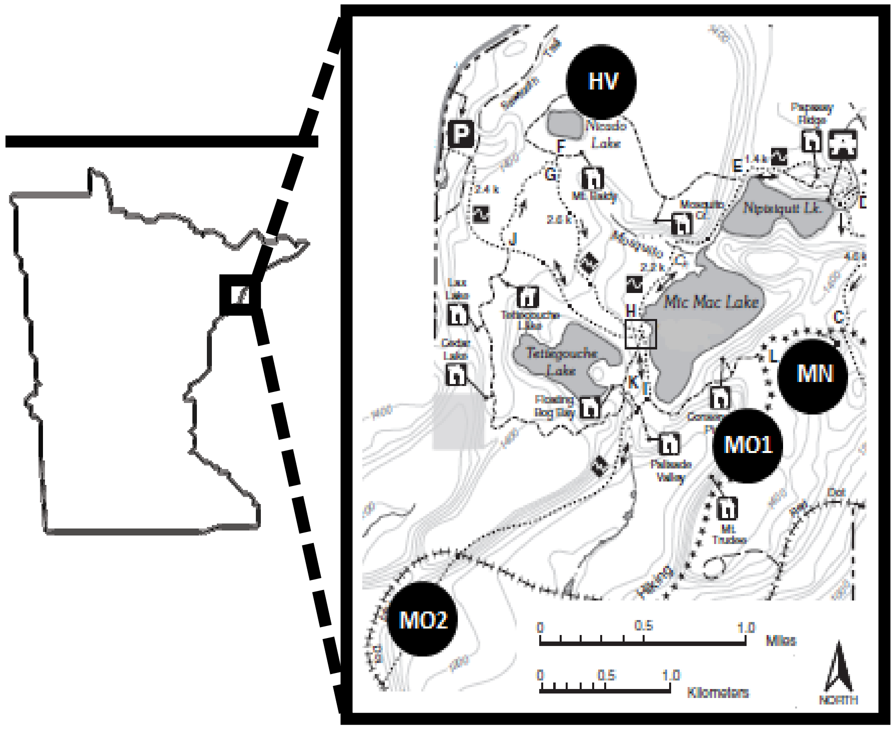

Figure 1. Study sites within Tettegouche State Park, Minnesota USA. MN = Minimally Invaded. MO1 = Moderately Invaded 1. MO2=Moderately Invaded 2. HV=Heavily Invaded.

Table 1. Description of study sites in Tettegouche State Park, MN USA

	Minimally Invaded	Moderately Invaded 1	Moderately Invaded 2	Heavily Invaded
GPS coordinates (NAD 83)	N 47.34534 W 91.24760	N 47.33923 W 91.24850	N 47.32673 W 91.28217	N 47.36193 W 91.25952
[a]Mean DBH of trees ≥ 10cm DBH	27.8 ± 14.3	24.0 ± 11.2	21.8 ± 10.2	23.9 ± 9.2
[b]Mean Canopy opening 2009	17.6% ± 8.8	19.1% ± 10.8	9.7% ± 7.7	20.5% ± 10.9
[a]Mean Basal area (ft^2/acre)	99 ± 28.8	82 ± 25.3	117 ± 41.4	120 ± 35.0

[a]2009 Pilot Study. Trees were defined as ≥ 10cm DBH (*n* = 10 sample points per site). Means ± SD.
[b] *n* = 40 canopy measurements per site. Means ± SD.

Trees and Canopy Opening

Within each 10 m radius subplot (*n* = 10 per site) the diameter at breast height of all tree species (≥ 10cm DBH) was measured. Total percent canopy opening was measured August 29- September 4, 2009 to document the level of canopy disturbance caused by a severe ice storm in these sites in March 2009. Pre-storm canopy closure was assumed to be at 100% from prior knowledge of the sites (Hale, personal observations). The level of canopy opening was measured using the mean of four densiometer readings (a gridded fish-eye mirror) at the four cardinal directions along the perimeter of the 10 m subplot.

Earthworm Sampling

In mid-September to mid October 2009-2011, earthworm populations were quantitatively sampled in each site using liquid mustard extraction in randomly located 0.12 m^2 (33 x 33 cm) subplots (Hale et al. 2005b). In 2009, earthworm extractions were conducted at one subplot per sample point (*n* = 10 per site). In 2010 and 2011, earthworm extractions were conducted at three subplots per sample point (*n* = 30 per site). Specimens were preserved in the field with 70% isopropyl alcohol and later transferred to 10% formalin in the lab before identifying species and measuring lengths for biomass estimations (Hale 2004).

Upper Soil Horizons

In mid-May 2011, three soil cores 5 cm in diameter and 12 cm deep were collected at random locations within a 5 m radius of each sample point (*n* = 30 per site). The thickness of each horizon (O_i, O_e, O_a, O_{Total}, and A) was measured in the field and the presence or absence of the E horizon was recorded.

Gravimetric Water Content

From May 16 through August 30, 2011 soil cores (5 cm in diameter x ~5 cm deep) were collected every 11-14 days just beneath the O_a layer at a random location within a 5 meter radius of each sample point to measure mean gravimetric water content at the site level (*n* = 80 per site per season). Horizon depth and volume was recorded in the field. Cores were stored in Ziplock bags and refrigerated 24-72 hours before measuring the field condition wet weight of soil samples in the lab then oven dried at 60° C for 48 hours and reweighed. Gravimetric water content at field condition was measured using equation 1 (Robertson et al. 1999).

[Equation 1]

$$Gravimetric\ Water\ Content\ = \frac{g\ H_2O}{g\ dry\ material}$$

Potential Horizon Field Capacity

Seven soil cores, 5 cm in diameter and 12 cm in depth, were collected in the Minimally Invaded and Heavily Invaded sites in September 2012 to measure the gravimetric water content at field capacity, bulk density, and potential horizon field capacity in the lab. The potential horizon field capacity is defined as the water content (g H_2O) held in the horizon when saturated and then allowed to gravity drain (Wild 1993). The thickness of the O_{Total} (O_i, O_e, and O_a layers combined), A and truncated E horizons were measured in the field. Each horizon was stored in a PVC pipe carrying case (5 x 9.5 cm), split lengthwise in half and hinged with duct tape, to preserve core integrity. Two layers of cheesecloth were held to the bottom of the case with a rubber band, and then wrapped in cellophane for transport. In the lab a porous foam pad was placed inside a plastic bin of similar dimensions and filled with enough water to saturate the pad. The cellophane was removed from each case and placed cheesecloth side down atop the saturated foam pad. The top of each case was covered with plastic to minimize evaporation. Horizons were allowed to saturate via capillary action until completely saturated (~48 hours), then transferred atop a fine wire mesh screen to gravity drain for 24 hours. After gravity draining, the wet horizons were weighed, oven dried at 60° C for 72 hours and reweighed to calculate the bulk density (dry g/cm^3) and the potential horizon field capacity, which is the gravity drained horizon wet weight minus the horizon dry weight.

<u>Available N and P</u>

Nylon bags (*n* = 36 per site) were each filled with 5 g of Rexyn I-300 (Fischer Scientific, Fairlawn, New Jersey, USA) mixed bed, cation-anion exchange resin beads for measuring available N and P in the forms of NH_4-N, NO_3-N, and PO_4-P throughout the growing season (Binkley 1984). Each site had a total of 36 resin bags buried in groups of

3 or 4 among the 10 sample points. One bag was collected from each sample point at the end of June, July, August and September with a different sample point being systematically skipped each month for a total of 9 resin bags collected per site per month. All bags were buried in sample points within respective sites on the same day in May, and similarly removed from the site on the same day in June, July, August, and September. In mid-May 2011 the resin bags were buried ~30 cm from each other and 10-15 cm deep in the mineral soil at a random compass bearing and distance from the center of each sample point.

After resin bags were harvested from the field, within 48 hours they were rinsed with de-ionized water to remove all sediment, stored in individual Ziplock bags and refrigerated for later processing. At the time of processing, opened resin bags were placed in separate 90 ml paper cups and allowed to air dry in a fume hood for 72 hours. Resin beads were stirred with a sterile mixing rod half way through drying time to ensure even drying. Once resin was dry, 2.5 g resin was weighed from an individual bag and transferred to a 150 ml Erlynmeyer flask. After adding 100 mL aliquot of 1N KCL to each Erlynmeyer flask, the mixture was allowed to sit 18-24 hours until the beads settled, swirling the mixture half way through (Binkley 1984; Pastor personal communication, November 2011). Without disturbing the settled beads, 20 ml of the extract was pipetted into a sample vial and frozen for later analysis. The extracts were homogenized into 3 samples per site per sampling period. The homogenized extracts were analyzed by standard methods for µg of N as NH_4-N or NO_3-N on a Lachat autoanalyzer (Lachat Instruments, Milwaukee, Wisconsin), and P as PO_4-P was measured using the manual ascorbic acid method and a Perkins Elmer Lamda 25 UV/VIS Spectrometer (Ameel et al. 1998; APHA 2005). Resin extract run on the Lachat reported N in the form of NH_4-N and NO_2/NO_3. Representative samples were rerun for NO_2-N only, and the relative proportions of NO_2-N and NO_3-N to NO_2/NO_3 were compared (n = 4, data not shown). Since samples were overwhelmingly NO_3-N, results were reported as μg NO_3-N/gram resin (Rommel 1935; Pastor et al. 1998).

Statistical Methods

For all metrics a site mean was calculated each year from the 10 sample points within each site. All analyses were completed using *JMP® Pro v 9.0* (SAS Inc. 2012). Unless noted, data did not require transformation.

Canopy Opening

One-way analyses of variance (ANOVAs) were used to detect differences in mean percent canopy opening among sites for 2009, 2010, and 2011 (n = 40 per site per year).

Earthworms

Biomass, reported as the ash-free dry grams (AFDg) of individual specimens, was calculated using an allometric equation defined by Hale et al. (2004). For earthworm analyses, a three year weighted mean of AFDg/m^2 was used at the sample point level, and study sites were categorized by earthworm taxonomic and assemblage groups (Tables 2 and 3) as defined by Hale et al. (2005b, 2006). A one-way ANOVA was conducted to detect any significant difference in mean earthworm biomass ($AFDg/m^2$) among the sites.

Table 2. Earthworm taxonomic and ecological groups

Taxonomic Group	Ecological Group	Species Included
Dendrobaena	epigeic	*Dendrobaena octadedra, Dendrodrilus rubidus*
L. rubellus	epi-endogeic	*Lumbricus rubellus* adults
L. juveniles*	epi-endogeic / anecic	*Lumbricus* juveniles
Aporrectodea	endogeic	*A. caliginosa, A. tuberculata, A.* spp.
L. terristris	anecic	*Lumbricus terristris* adults

*Juvenile *L. rubellus* and *L. terrestris* are indistinguishable so are treated as a separate taxonomic group.

Table 3. Earthworm assemblage groups

Earthworm assemblage	Dominant species	Species Included
Group 1	*Dendrobaena octaedra*	*Dendrobaena octadedra, Dendrodrilus rubidus* (rare)
Group 2	*Lumbricus rubellus*	*Lumbricus rubellus* adults, *Lumbricus* juveniles, Group 1
Group 3	*Aporrectodea* spp.	*A. caliginosa, A. tuberculata, A.* spp., Groups 1 & 2
Group 4	*Lumbricus terristris*	*Lumbricus terristris* adults, Groups 1, 2, & 3

<u>Upper Soil Horizons</u>

One-way ANOVAs were used to detect differences among sites in mean thickness of each O_i, O_e, O_a, O_{Total}, A and E horizons for the 2011 cores (n = 30 per site).

Gravimetric Water Content

For gravimetric water content, sampling periods represent the midpoint within a range of sampling days, because sites were sampled 1-6 days apart for each given sampling period. Repeated measures ANOVA was used to test for a time effect among sites at different sampling periods with site as a model effect. Additionally separate one-way ANOVAs were run at each sampling period to test for differences in mean gravimetric water content among sites.

Potential Horizon Field Capacity

It was assumed that the soil horizons did not change significantly at each site between the 2011 and 2012 samplings, thus data from both years was used for the following metrics. The mean gravimetric water content at field capacity was calculated for each of the 2012 soil horizons. The mean dry grams of each 2011 horizon were calculated by multiplying the mean bulk density (g dry matter/cm^3 dry matter) from the 2012 horizons by the mean volume (cm^3) of the 2011 horizons. The mean Potential Horizon Field Capacity of the 2011 samples was calculated by multiplying the mean gravimetric water content at field capacity from the 2012 horizons by the newly calculated mean dry grams of each horizon from 2011. Student's T-test was used to compare means of gravimetric water content at field capacity, bulk density, volume, horizon dry mass, and potential horizon field capacity. Additionally, the relative proportion of Potential Horizon Field Capacity to the total soil core field capacity was compared between the Minimally Invaded and Heavily Invaded sites using Fisher's exact test (Fisher 1922).

Available N and P

For statistical analyses, net flux of available N and P was natural log transformed, $ln(x+1)$ to preserve zero values. Each sampling period was tested separately using a one-way ANOVA to test for differences in site mean µg of NO_3-N, NH_4-N, Total N (i.e. NO_3-N and NH_4-N), and PO_4-P.

Results

Canopy Opening

Moderately Invaded 2 had significantly less canopy opening than the other sites in 2009 and 2010 (Table 4). However, by 2011, the canopy in all sites had closed to an average percent canopy opening of 17.4%, and were not significantly different from each other.

Table 4. Mean annual percent canopy opening per site

Site	^{a}2009	^{b}2010	2011
Minimally Invaded	17.6_A	18.4_A	17.7
Moderately Invaded 1	19.1_A	17.0_A	15.7
Moderately Invaded 2	9.7_B	8.7_B	19.1
Heavily Invaded	20.5_A	12.6_A	17.0

$^{a}F(3,156) = 9.94, p < 0.001$
$^{b}F(3,156) = 10.14, p < 0.001$

Earthworms

The combined presence of multiple earthworm species from different ecological groups indicated the presence of three assemblage groups among the four sites. The Minimally Invaded site was contained assemblage group 1 as all but one earthworm came from the taxonomic group *Dendrobaena*. Moderately Invaded 1 and 2 both contained assemblage group 3, though both may be in the early stages as *Aporrectodea* was not as prevalent as *Lumbricus rubellus* or *Lumbricus* juveniles. Moderately Invaded 1 had slightly more, though not statistically significant, total AFDg/m^2 and a greater proportion of *Aporrectodea* than Moderately Invaded 2. Despite a difference in biomass, the difference in assemblages of both moderately invaded sites are considered intermediates to Minimally Invaded and Heavily Invaded. The Heavily Invaded site contained assemblage group 4 (Table 3), and had significantly more AFDg/m^2 than other sites (Figure 2).

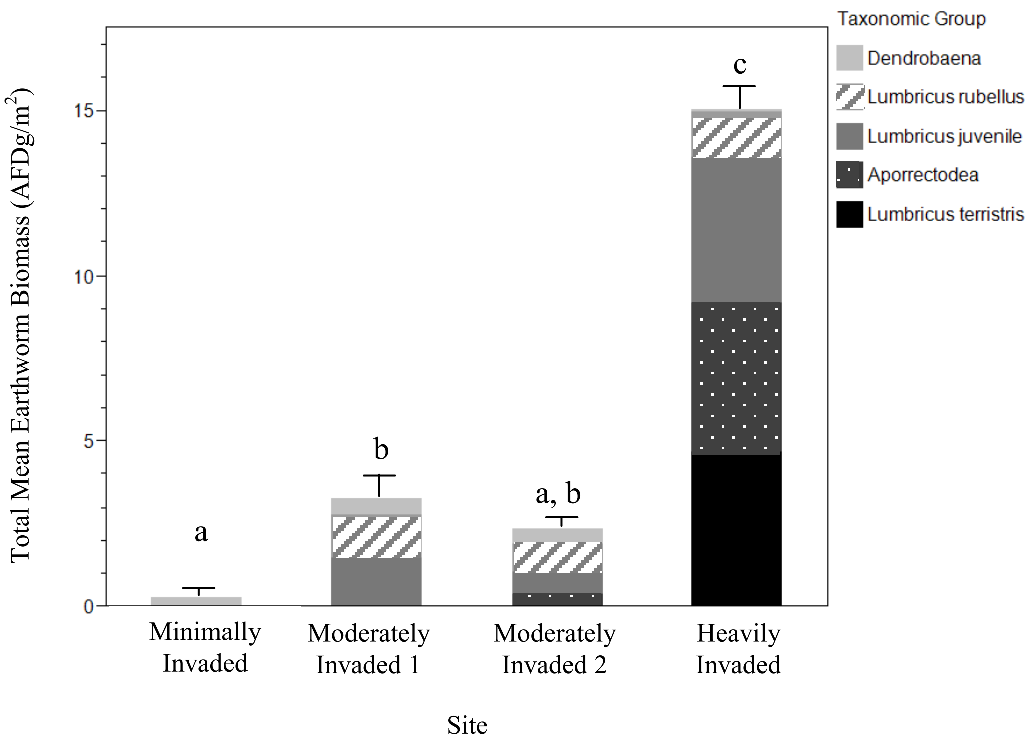

Figure 2. Total mean earthworm biomass (AFDg/m^2) per site. The proportions of each site's total mean AFDg/m^2 is distributed by earthworm taxonomic group. One way ANOVA detected significant differences in total mean AFDg/m^2 at the site level, $F(3, 36) = 75.14$, $P < 0.0001$ ($n = 10$ per site). All comparisons represented by different letters are significant at $\alpha = 0.05$. Error bars represent one standard error from the mean for site total mean AFDg/m^2.

Upper Soil Horizons

The sites varied significantly in mean thickness for mean O_{Total}, O_i, O_e O_a, and A horizons (Table 5). The Minimally Invaded site had on average a significantly thicker O_{Total} horizon and thinner A hoizon than Moderately Invaded 1 and Heavily Invaded sites. The Heavily Invaded site had the thinnest O_{Total} horizon and thickest A horizon, and was significantly different from all sites. The Minimally Invaded site did not differ in mean A horizon thickness from either Moderately Invaded 1 or 2 (Figures 3 and 4).

Table 5. Summary statistics: One-way ANOVAs of horizon thickness among sites

Horizon	[a]*F* ratio	*P* value	R^2
O_{Total}	18.78	< 0.0001	0.33
O_i	12.53	< 0.0001	0.25
O_e	24.43	< 0.0001	0.39
O_a	7.60	< 0.0001	0.16
A	36.15	< 0.0001	0.48

[a]$df_{between}$, df_{within} = 3, 116; *n* = 30 per site

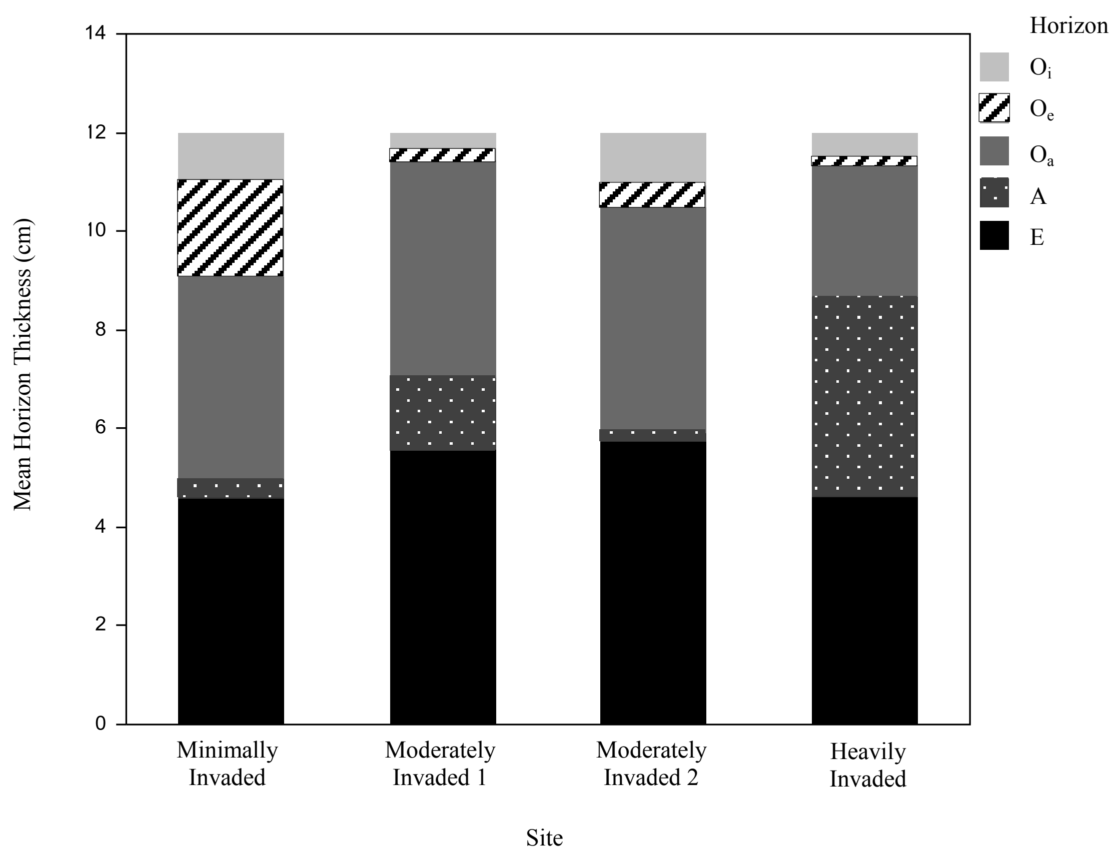

Figure 3. Mean horizon thickness of 12 cm soil cores. Stacked bars represent relative proportion and site mean thickness of O_i, O_e, O_a, and A horizons (*n* = 30 per site). E horizon was truncated at total core depth of 12 cm.

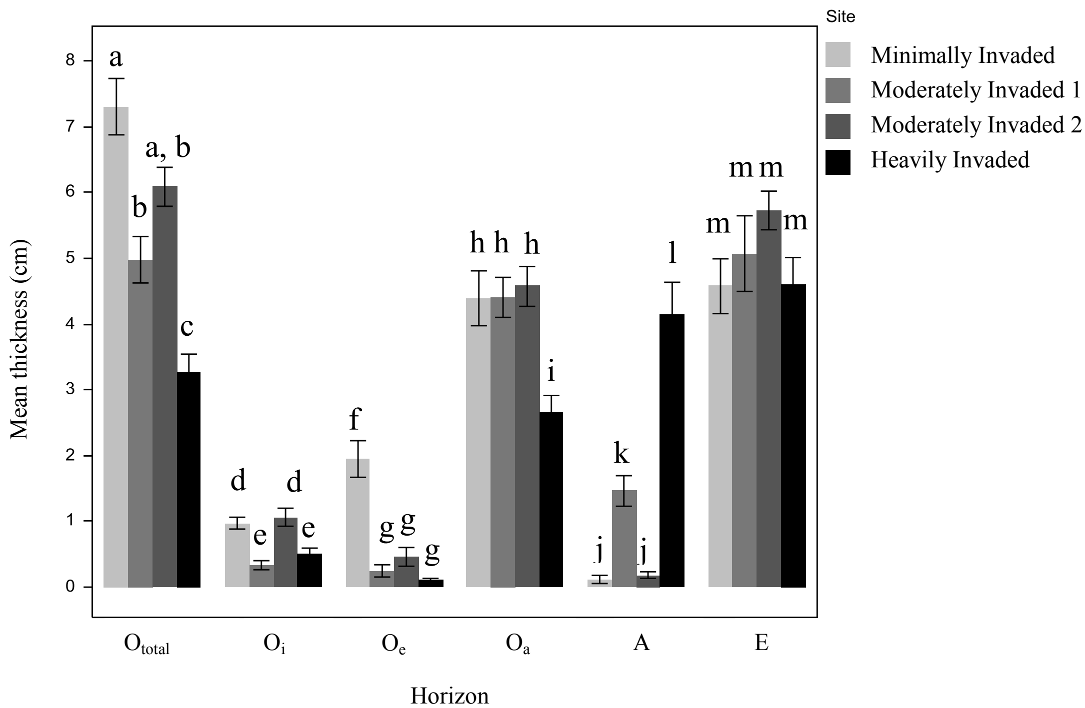

Figure 4. Statistical relationship between like horizons. Bars represent site mean thickness of O_{Total}, O_i, O_e, O_a, A, and truncated E horizons (*n* = 30 per site) of 12 cm soil cores. All comparisons of like horizons represented by different letters are significant at α = 0.05. O_{Total} horizon compared with letters a, b, and c. O_i horizon compared with letters d and e. O_e horizon compared with letters f and g. O_a horizon compared with letters h and i. A horizon compared with letters j, k, and l. Truncated E horizon compared with letter m. Error bars represent one standard error from the mean.

<u>Gravimetric Water Content</u>

Gravimetric water content among sites was not significantly different over the course of the growing season. Repeated measures ANOVA did not indicate a significant effect within sites, but one-way ANOVAs at each sampling period indicated that Moderately Invaded 1 was significantly drier than the other sites at sampling period August 26 ($F(3, 36) = 4.007$, $P = 0.0147$). The Tukey HSD test confirmed that Moderately Invaded 1 had significantly less gravimetric water content than Moderately Invaded 2 (Figure 5).

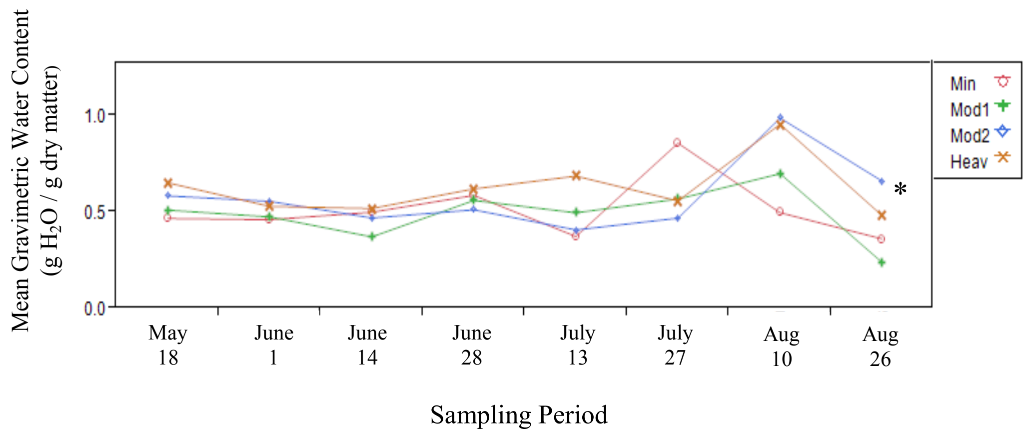

Figure 5. Mean gravimetric water content among sites across all sampling periods. There was no difference among sites overall and at each sampling period with the exception of August 26. One-way ANOVA detected significant differences in mean gravimetric water content at the site level (n = 10 per site), and Tukey HSD test confirms a significant difference between Moderately Invaded 1 and 2 at $\alpha = 0.05$.

Potential Horizon Field Capacity

The mass of water held in the soil depended on the horizon. In both the Minimally Invaded and Heavily Invaded sites, the O_{Total} horizon held on average the majority of the water contained in the saturated core. The Minimally Invaded site held 59% of potential field capacity in the O_{Total} horizon and 41% in the E horizon. The Heavily Invaded site held 53% of potential field capacity in the O_{Total} horizon, 24% in the A horizon, and 23% in the E horizon (Tables 6, 7, and 8). The relative proportions of the Minimally Invaded site's horizons were significantly different from the Heavily Invaded site's horizon proportions ($P < 0.0001$).

Table 6. Mean gravimetric water content at field capacity and bulk density per horizon in Minimally and Heavily Invaded sites

	[a]Mean Gravimetric Water Content at field capacity per horizon (g H_2O/g dry matter)			[a]Mean Bulk Density per horizon (g dry matter/cm^3 dry matter)		
Horizon	Minimally Invaded	Heavily Invaded	*P* value	Minimally Invaded	Heavily Invaded	*P* value
O_{Total}	3.64	3.43	*NS*	0.14	0.42	**
A	not present	1.25	**	not present	0.54	**
E	0.6	0.8	*NS*	0.91	0.68	*
Total	4.24	5.48	*NS*	1.05	1.64	*

[a]n = 7; cores collected in 2012

*$P < 0.05$; **$P < 0.01$; *NS* = Not Significant

Table 7. Mean volume and g dry matter per horizon in Minimally and Heavily Invaded sites

	[a]Mean volume of each horizon (cm^3)			[a]Mean g dry matter		
Horizon	Minimally Invaded	Heavily Invaded	*P* value	Minimally Invaded	Heavily Invaded	*P* value
O_{Total}	148.16	66.07	**	20.74	27.75	**
A	not present	83.91	**	not present	45.31	**
E	92.83	93.23	**	84.48	63.4	**
Total	240.99	243.21	*NS*	105.22	136.46	*NS*

[a]n = 30; cores collected in 2011

**$P < 0.01$; *NS* = Not Significant

Table 8. Mean potential horizon field capacity and proportion of grams H_2O in Minimally and Heavily Invaded sites

	[a]Mean potential horizon field capacity (g H_2O)			[a]Proportion of total core g H_2O at field capacity	
Horizon	Minimally Invaded	Heavily Invaded	*P* value	Minimally Invaded	Heavily Invaded
O_{Total}	75.49	95.18	*	59%	53%
A	not present	56.64	**	not present	24%
E	50.69	50.72	NS	41%	23%
Total	126.18	202.39	**	100%	100%

[a]*n* = 30; cores collected in 2011

* = $P < 0.05$; ** = $P < 0.01$; *NS* = Not Significant

Available N and P

NO_3-N made up over 97% of the available N from the NO_2/NO_3 samples. There was no significant difference among sites at any sampling period for µg PO_4-P and NH_4-N per g resin. The Minimally and Heavily Invaded sites had significantly greater µg NO_3-N per g resin than the moderately invaded sites at every sampling period. Likewise, there was a significant difference in Total N among the sites during sampling period July (Figure 6).

Table 9. One-way ANOVA of available µg N/g resin among all sites at four sampling periods

	NO_3-N			Total N		
[a]Sampling Period	R^2	*F* ratio	*P* value	R^2	*F* ratio	*P* value
June	0.79	10.01	0.0044	0.23	0.84	*NS*
July	0.81	11.52	0.0028	0.69	5.85	0.0205
August	0.88	19.45	0.0005	0.43	1.99	*NS*
September	0.69	5.88	0.0202	0.33	1.33	*NS*

NS = Not Significant

df site, df error (3, 8); n=3

[a]*Sampling periods range from mid-May until the end of the designated month*

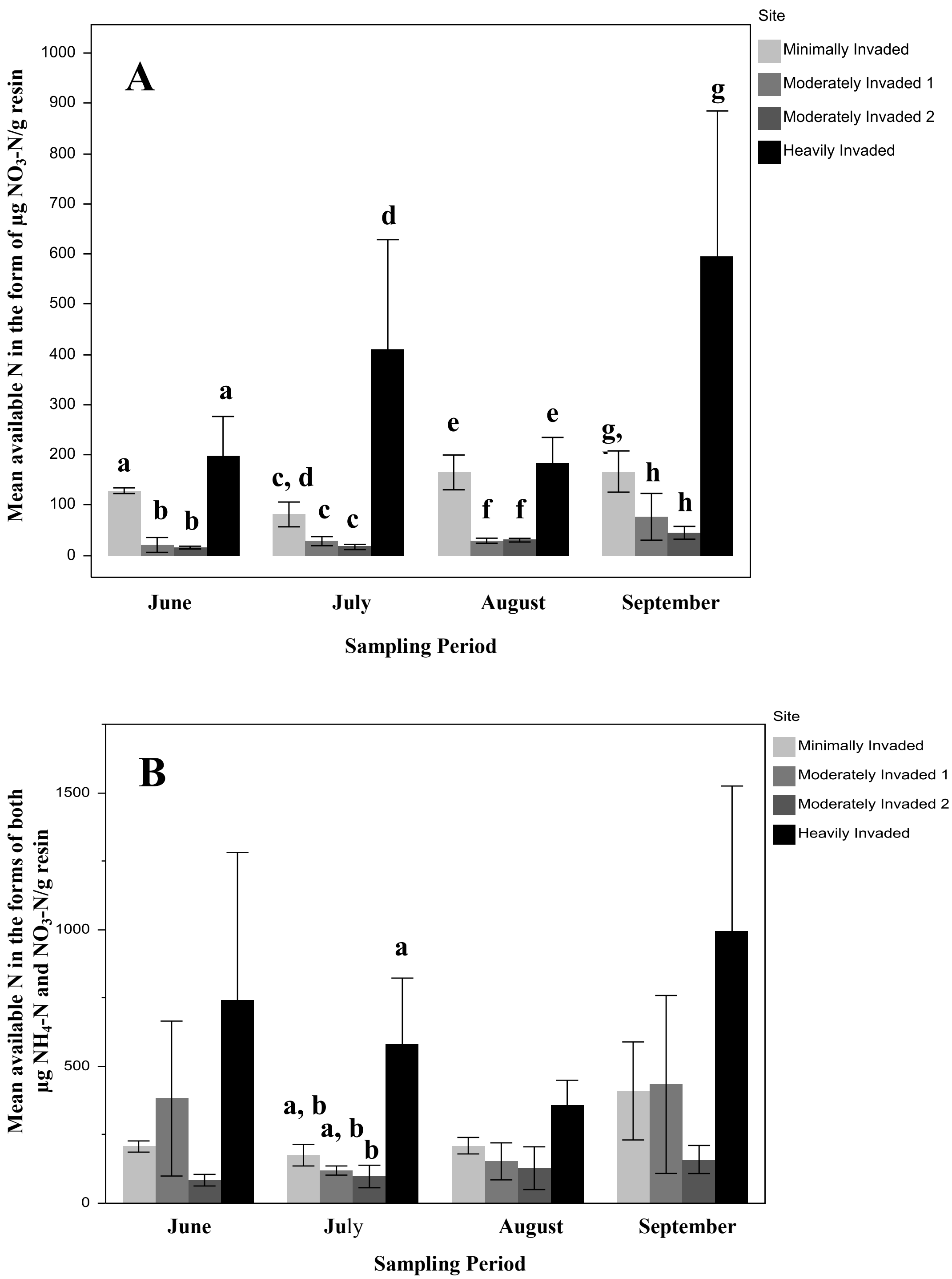

Figure 6. One-way ANOVAs for available N per sampling period. Analyses used natural log transformations $ln(x + 1)$, and figures are constructed using non-transformed data. All comparisons are between sites at different sampling periods, and comparisons indicated by different letters are significantly different at $p \leq 0.02$ ($n = 3$ per site per sampling period). Error bars are constructed using one standard error from the mean.

Discussion

Canopy Opening

Large canopy gaps (> 50%) affect earthworms and herbaceous plant communities (Nachtergale et al. 2002). During the pilot study, the canopy opening appeared to be >50%. However, later quantitative measurements verified that the initial visual estimates of canopy opening were higher than those measured in the first field season. The mean percent canopy opening in the study did not exceed 20% in any site at any year and the degree of canopy opening was not significantly associated with any of the soil, earthworm or plant parameters assessed. Future studies with interest in the interaction of earthworm invasion and canopy opening should implement a pilot study to verify that the opening is at least 50%.

Earthworms, Upper Soil Horizons, and Soil Moisture

The difference in earthworm assemblage groups among sites captured a gradient of soil parameters from minimally to heavily invaded. The soil horizons were more homogenized as earthworm assemblages increased in richness since earthworms have a dominating effect on soil structure compared to native, earthworm-free soils (Edwards et al. 1995; Hale et al. 2005b). Increasing earthworm biomass associated with assemblage groups was correlated with reduced O_{Total} horizon and increased A horizon thickness most likely from a redistribution of organic matter from the O horizon to the A and E horizons. This is consistent with other studies, especially those in northern temperate forests (Alban and Berry 1994; Hale et al. 2005b; Holdsworth et al. 2007b).

The Moderately Invaded sites provided an interesting intermediate between two extremes along a continuum of earthworm invasion. The reduction of the O_e horizon from the Minimally Invaded site to both Moderately Invaded 1 and 2 could be from the presence of the epi-endogeic species *Lumbricus rubellus* and its known consumption of this layer (Zhang and Hendrix 1995; Gundale 2002; Suarez et al. 2006b).

Studies associate a thick A horizon with *Aporrectodea* (Nordstrom et al. 1974; Scheu 1987), however, Moderately Invaded 2 had a thinner A horizon but larger presence of *Aporrectodea* than Moderately Invaded 1. The presence of *Aporrectodea* could possibly have gone largely unnoticed from Moderately Invaded 1 due to sampling technique. In the Moderately Invaded 1 site, many of the sample points were established along hillsides, and if poured too fast the liquid mustard would not soak the soil directly beneath the sample point, but would run downhill before saturation. The topography of Moderately Invaded 2 was much flatter in comparison, and the same sampling challenges were not experienced as in Moderately Invaded 1. Liquid mustard extraction is still the preferred method of sampling compared to alternative methods like hand sampling especially for higher yields of *Aporrectodea* (Zaborski 2003; Hale et al. 2005b; Wironen and Moore 2006).

This study was unable to detect whether earthworms affect the gravimetric water content beneath the O_a horizon. The one difference detected among the sites for sampling period August 26, 2011 could be due to the difference in topography since soil moisture in forests can vary depending on degree of slope and the position on the slope (Yahner 2000). Moderately Invaded 2 was lower in the landscape than the other sites, and the six-day gap between sampling sites was larger than the other sampling periods. Late August 2011 was reportedly dry and no precipitation was recorded during the days between sampling the Moderately Invaded sites (Minnesota State Climatology Office, 2013).

By removing the O_{Total} horizon and measuring gravimetric water content beneath the O_a horizon over half the total water present in soil cores of 12 cm in depth was potentially missed. Since the Minimally Invaded site had an intact O horizon, it serves as an absorbing layer before excess water can seep into the E horizon (Brown et al. 1999). In this study the greatest proportion of Potential Horizon Field Capacity was in the O_{Total} rather than the A or E horizons and may explain in part the results that were not significantly different from the gravimetric water content section. Since the gravimetric water content was measured from samples beneath the O_a horizon, the 2011 samples were not representative of water present within the whole profile of the upper soil horizons. Future studies involving soil moisture levels ought to consider the O_{Total} horizon, and preferably measure each horizon individually for a clearer indication of water presence in the upper soil horizons. Additionally, future studies could use artificial watering to control for varying precipitation.

Available N and P

The hypothesis that available N and P would be inversely correlated to earthworm richness and biomass was not supported. Although N and P levels were generally higher in the Heavily Invaded site than all others, it was only significantly greatest during sampling period July for Total N.

Measuring available N in the form of NO_2/NO_3-N was not problematic for NO_3-N estimates. Given that NO_2-N is more transient in soils, it is not uncommon for soil in maple stands to have 100% of their inorganic nitrogen as NO_3-N.

Moderate invasion might disrupt N cycling until a new equilibrium is established once a site is heavily invaded. Both Moderately Invaded sites had less μg NO_3-N per g resin than the Minimally and Heavily Invaded sites at all sampling periods. This suggests a temporal change in available NO_3-N from early invasion (assemblage group 1) to moderate invasion (assemblage groups 2 and 3) to heavy invasion (assemblage group 4).

The data do not suggest that leaching occurred in the Heavily Invaded site. According to Jeffery (1987) if leaching occurred, then a decrease in NO_3-N in the Heavily Invaded site should have been detected since NO_3-N is more mobile than NH_4-N or PO_4-P. It is possible that the lower levels of NO_3-N detected in Moderately Invaded 1 and 2 were due to chaotic effects of early earthworm invasion through leaching or immobilization (Hale et al. 2005b). A site effect is also possible in Moderately Invaded 2 since it is a younger stand with lower topography, and it experienced less initial canopy opening in the 2009 ice storm (Table 1).

Homogenizing resin extract may account for the wide variances. The rationale for homogenizing the resin extract was to replace a mathematical average of non-homogenized samples (n = 9 per site per sampling period) with a physical average (i.e. physically mixing three samples) of the three homogenized samples. However, instead of flattening variances, large variances were detected in addition to reduced sample size and statistical power.

The variability between sampling periods might be explained by the flux of ions on and off the resin itself (Binkley 1984). A better use of the resin for temporal patterns would be to bury resin bags in all sites on the same day and remove them one month later for analysis. Then new resin bags should be buried in all sites on a new starting day, and removed one month later for analysis. This pattern is appropriate for monthly comparisons of available nutrients using resin bags. Since the resin provides a relative indication of ions within a substrate, future studies that wish to examine with more precision a time component of available nutrients in forest soils ought to consider an alternative, more accurate method.

Chapter 3: Investigation of plant characteristics across a continuum of non-native earthworm invasion in hardwood forests, Tettegouche State Park, MN USA

Introduction

Globally, the loss of biodiversity due to habitat alteration can be attributed in part to invasive species (Madritch and Lindroth 2009). Invasive non-native earthworms spread into previously earthworm-free cold northern hardwood forests, and only in the past two decades has research examined their effects upon native understory plant communities.

There is no evidence of native earthworm populations in the Great Lakes region of North America since the last glaciation (James 2004). Since it can take 100 years for native earthworms to recolonize a distance of < 1 km (Klock et al. 2006), it is widely accepted that human activity has assisted the spread of invasive non-native earthworms into previously earthworm-free northern hardwood forests (Gates 1976; James 1995). Further, the synergistic effects of earthworm assemblage groups can exacerbate changes to soil structure, moisture and chemistry as well as plant communities (chapter 2). As detritivores earthworms depend upon leaf litter and decaying organic material as a food source. Certain earthworm species consume and redistribute the soil O horizon which native herbaceous plants and seedlings have evolved to depend on for rooting, seed bedding, and nutrient cycling (Groffman et al. 2004; Frelich et al. 2006). Earthworms are found to disturb plant fine root mass and displace native mycorrhizal fungi with adverse effects to native plant fitness (Gange 1993; Fisk et al. 2004).

In this chapter plant communities associated with different earthworm assemblage groups are investigated along a continuum of earthworm invasion in sugar maple (*Acer saccharum,* Marshall) stands with moderate canopy disturbance. Under the same level of canopy opening (originally estimated at 50%) this study proposes that increasing earthworm species richness and biomass will be:

(1) correlated with increases in shade intolerant herbaceous plants as well as invasive plants that require higher light levels for germination and seedling establishment.

(2) inversely correlated with herbaceous plant richness and diversity.

Methods and Materials

<u>Sites</u>

The study was conducted from 2009-2012 in four sugar maple dominated hardwood stands of Tettegouche State Park, Minnesota USA. For this study each site along the continuum of earthworm invasion was designated by earthworm assemblages present and from here on referred as Minimally Invaded, Moderately Invaded 1, Moderately Invaded 2, and Heavily Invaded. A descriptive account of the study sites can be found in chapter 2.

Within each site, 10 sample points were established in a grid 50 m apart. At each sample point a nested set of plots was established at radii of 10 m, 5 m, and 2 m. The 10 m radius subplot was used to sample trees and measure canopy opening. Within the 5 m radius subplot earthworm populations, upper soil horizons, saplings and shrubs were assessed. Within the 2 m radius tree seedling and herbaceous plants were surveyed.

Earthworm Sampling and Upper Soil Horizons

Within the 5 m radius earthworm populations and upper soil horizons were sampled. In mid-September to mid October 2009-2011, earthworm populations were quantitatively sampled preserved in the field, and identified in the lab. In mid-May 2011 three soil cores were collected at each sample point and upper soil horizons were measured in the field (chapter 2).

Vegetation Sampling

All vegetation sampling was conducted over three field seasons from mid to late summer of 2009-2011 unless specifically noted. The total cover class of each subplot was measured for each vegetation type.

Trees

Within the 10 m radius subplot all tree species ≥ 10 cm DBH were identified and measured (DBH). Additionally the percent canopy opening caused by an ice storm to these sites in March 2009 (originally estimated at 50%) was measured using four densiometer readings (a gridded fish-eye mirror) at the four cardinal directions along the perimeter of the 10 m subplot.

Saplings and Shrubs

Saplings and shrubs were identified to species and categorized by cover class and stem count. Large saplings included tree species with DBH > 2 cm but < 10 cm. Small saplings included tree species > 0.5 m tall and DBH ≤ 2 cm. Shrub species were defined as all non-tree woody species.

Tree Seedlings and Herbaceous Plants

Tree seedlings, defined as < 0.5 m tall, were identified to species and surveyed for cover class and stem count. Herbaceous plants included all non-woody vascular plants with particular emphasis on forbs, graminoids and fern/fern allies, and were identified to species if possible and classified by cover class.

Statistical Methods

For all metrics and indices, a site mean was calculated each year from the 10 sample points within each site. Tree stem counts, richness, categorical canopy damage, and DBH were calculated for analysis as well as sapling and shrub stem count, total cover class, and richness. Richness, stem count, total cover class, Shannon-Wiener (H') and Simpson's (D) diversity indices were calculated for the tree seedling layer (Magurran 1988). Herbaceous plant community richness, total cover class, Shannon-Wiener (H') and Simpson's (D) diversity indices, as well as forb, fern/fern ally, and graminoid richness were recorded. All percent cover data was converted to ordinal cover classes by midpoint (McCune and Grace 2002). For earthworm analyses, a three year weighted mean of AFDg/m^2 was used at the sample point level, and study sites were categorized by earthworm taxonomic and assemblage groups (chapter 2).

Herbaceous layer species composition of each site and year was analyzed using nonmetric multidimensional scaling (Kruskal 1964) in PC-ORD (McCune and Mefford 2011, v. 6.0). For purposes of analysis, sample units were considered each site per year (e.g. Minimally Invaded 2009). Cover classes of each species were converted into ordinal midpoints, and the mean ordinal midpoint of each species for each sample unit was relativized to the maximum cover attained by each species for that sample unit. A Bray-Curtis ordination was run using Sorensen's distance measure with variance regression as the endpoint selection method, and Euclidean measures used for both axis projection geometry and calculating residual distances. Sorensen's distances were calculated from species' mean cover of the 2 m radius subplot from each of the 10 sample points at each site per year to evaluate compositional differences between sample units. For nonmetric multidimensional scaling (NMS) the output from the Bray-Curtis ordination was used, stepping down from 3 to 1 dimensional solution. Final dimensionality was assessed with 50 runs of real data, and then the final number of dimensions that minimized stress with the fewest dimensions was chosen for further analyses. Environmental variable overlays were used to evaluate relationships between herbaceous layer species composition, earthworm biomass, soil organic horizon thickness, and vegetation environmental variables.

Multi-response permutation procedures (MRPP) was run to test the hypothesis that the overall herbaceous plant communities among the sample units were the same (PC-ORD, McCune and Mefford 2011), and that trajectories of plant communities observed were due to specific categorical environmental variables and not an artifact of site differences. Sample units were categorically tested against each other as canopy opening, earthworm invasion, O horizon thickness, and year. For canopy opening, sample units were categorized into one of two classes where 1 = 0-15% and 2 = > 15% canopy opening. Earthworm invasion was categorized by site ($n = 4$) for one MRPP and in another as one of three invasion degrees denoted as Min, Mod, or Heav ($n = 3$). Additionally, sample units were categorized by O horizon thickness class ($n = 3$) where 1 = < 4 cm, 2 = 4-6 cm, and 3 = > 6 cm. Finally, year ($n = 3$) was tested in MRPP. The MRPP is a nonparametric method for testing the hypothesis of no difference between two or more groups by comparing the observed weighted mean within-group distance (delta) to the expected distribution of delta (Mielke and Berry 2001; McCune and Grace 2002; Hale et al. 2006). An agreement statistic (A) is generated from this procedure, which indicates the degree to which groups differ from that expected by chance, and a p value, indicating the probability that the observed difference is due to chance (McCune and Grace 2002; Hale et al. 2006).

Indicator species analysis (DuFrene and Legendre 1997; PC-ORD, McCune and Mefford 2011) was used to analyze the relationship of earthworm invasion to individual herbaceous species, and was run using each of the categorical environmental variables that produced significant results in MRPP. All herbaceous plant species present in this study were tested for their association with an earthworm invasion degree indicated as Minimally Invaded, Moderately Invaded, or Heavily Invaded. A perfect indicator of a particular group is always present and only present in that group. Based on this standard and the relative species abundance and frequency, this method calculates an indicator value for each species. Tests for statistical significance used 4999 Monte Carlo randomizations (PC-ORD, McCune and Mefford 2011). Statistical significance was evaluated at $\alpha = 0.05$ for NMS, MRPP, and indicator species analysis.

Results

In MRPP categorizing sample units by canopy opening or year did not produce significant results. However, MRPP was significant when sample units were categorized by site ($A = 0.38$, $p = 0.00001$), invasion degree ($A = 0.23$, $p = 0.002$), and O horizon thickness ($A = 0.29$, $p = 0.0003$). The Bray-Curtis ordination produced a 3 dimensional output for all sample units (Table 10). NMS ordination produced a final stress of 7.71103 with zero final instability for a 2-dimensional solution after 46 iterations (Table 11; Figure 7). Additionally, Pearson's correlations of environmental variables were computed in two dimensions with NMS axes for each sampling unit (Table 12). Two environmental variables, earthworm ash-free dry mass (AFDM) and O horizon thickness, had very strong relationships with the herbaceous cover class in the Heavily Invaded and Minimally Invaded sites respectively (Figure 7; Table 12).

Indicator species analysis, when run with site as the categorical variable, produced significant results ($\alpha = 0.05$) for 27 of the 50 herbaceous plant species. However, when the model was rerun with the moderately invaded sites removed, there were no significant indicator species identified. Indicator species analysis was re-run using the invasion degree as the categorical variable, and this produced a shorter list of significant indicator species (Table 13).

Table 10. Bray-Curtis output for herbaceous plant cover class

Sample Unit	Axis 1	Axis 2	Axis 3
Min 2009	0.21026	0.22222	0.41315
Mod1 2009	0.38626	0.56771	0.06225
Mod2 2009	0.18441	0.13662	0.27527
Heav 2009	0.7034	0.17004	0.19701
Min 2010	0.16871	0.11904	0.32441
Mod1 2010	0.41961	0.4151	0
Mod2 2010	0.08464	0.1678	0.18063
Heav 2010	0.58741	0.0499	0.10189
Min 2011	0.33804	0.1719	0.23664
Mod1 2011	0.31812	0.41565	0.09006
Mod2 2011	0	0.17004	0.19701
Heav 2011	0.56622	0	0.06225

Table 11. NMS Ordination Scores for 2D configuration of herbaceous cover class

Site	Axis 1	Axis 2
Min 2009	0.5489	-0.644
Mod1 2009	-1.3032	0.0258
Mod2 2009	0.1583	-0.6157
Heav 2009	-0.1493	1.4128
Min 2010	0.5827	-0.5326
Mod1 2010	-0.7357	0.2993
Mod2 2010	0.1906	-0.9394
Heav 2010	0.1292	1.1524
Min 2011	0.6832	0.1068
Mod1 2011	-0.6076	-0.1074
Mod2 2011	0.2036	-1.2285
Heav 2011	0.2993	1.0705

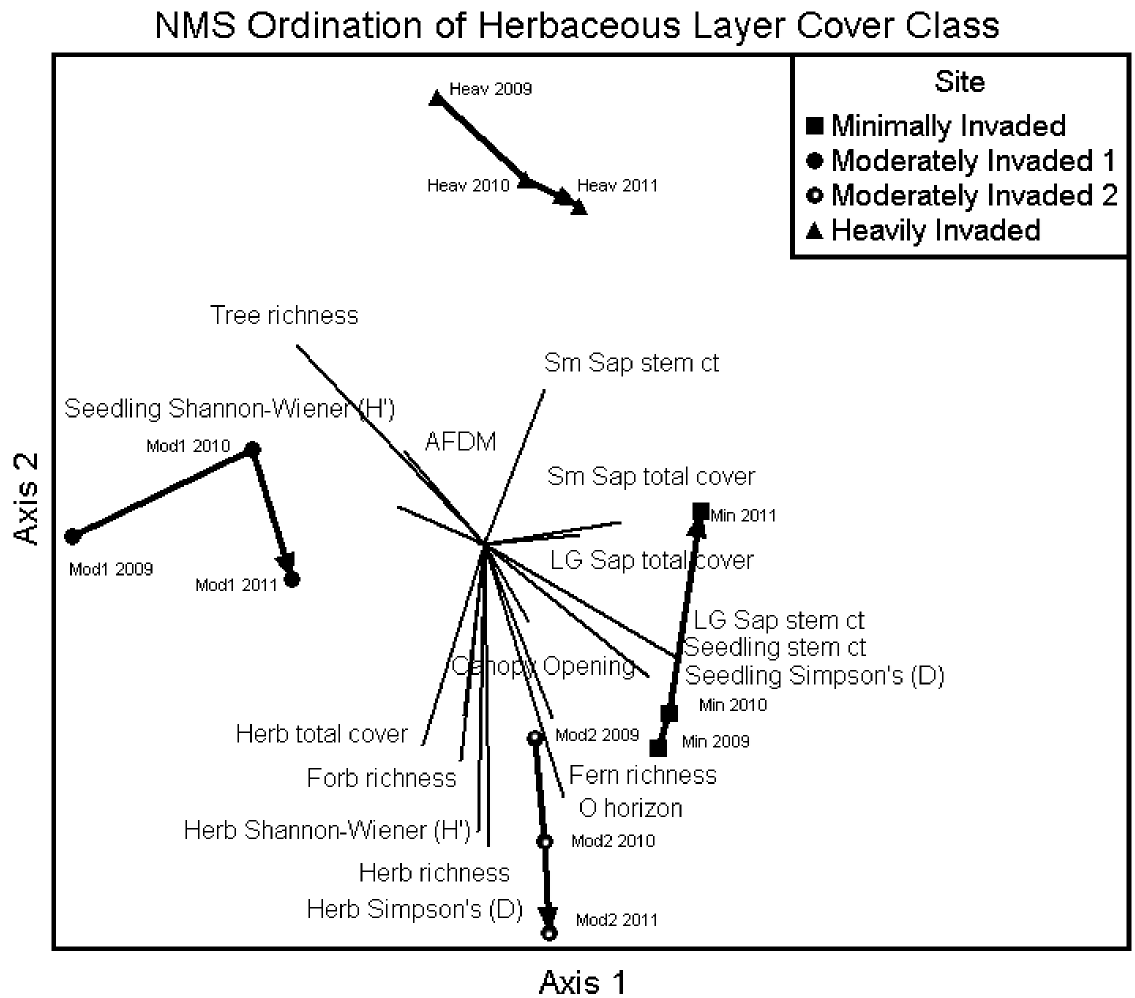

Figure 7. NMS ordination of herbaceous layer cover class. Lines indicate relationship between environmental variables and cover class of individual herbaceous plants per site.

Table 12. Correlation (Pearson's r) of earthworm biomass and other environmental variables with nonmetric multidimensional scaling axes for sites in 2009, 2010, and 2011 in Tettegouche State Park

Environmental Variable	Axis 1	Axis 2
Earthworm Ash Free Dry Mass (AFDM)	-0.438	0.47
Canopy Opening	0.04	-0.464
Herbaceous Total Cover Class	-0.221	-0.638
Herbaceous Simpson's (D)	0.101	-0.85
Herbaceous Shannon-Wiener (H')	-0.234	-0.72
Herbaceous richness	-0.122	-0.83
Forb richness	-0.384	-0.694
Fern richness	0.404	-0.644
Seedling (D)	0.626	-0.564
Seedling (H')	-0.458	0.299
Seedling stem count	0.325	-0.431
Sm sapling total cover class	0.569	0.234
Sm sapling stem count	0.38	0.608
Lg sapling total cover class	0.478	0.15
Lg sapling stem count	0.679	-0.52
Tree richness	-0.67	0.689
O horizon total thickness	0.437	-0.779

Table 13. Herbaceous layer indicator species of sites categorized by earthworm invasion degree

Invasion degree	Herb type	Species	[a]Indicator Value (%)
Minimally Invaded	Forb	*Impatiens capensis*	98**
	Forb	*Circaea alpina*	100*
	Forb	*Smilax herbacea*	100*
Moderately Invaded	Forb	*Galium triflorum*	100**
	Graminoid	*Milium effusum*	81**
	Fern/Fern Ally	*Lycopodium spp.*	85*
	Forb	*Anemone quinquefolia*	59*
Heavily Invaded	Forb	*Actaea spp.*	71**
	Graminoid	*Carex deweyana*	80**
	Forb	*Trientalis borealis*	70**
	Fern/Fern Ally	*Pteridium aquilinum*	100**
	Forb	*Pyrola elliptica*	100**
	Graminoid	*Carex pedunculata*	64*
	Fern/Fern Ally	*Gymnocarpium dryopteris*	81*

[a]Indicator values (% of perfect indication) from indicator species analysis (Dufrene & Legendre 1997). The *p* values are from 4999 Monte Carlo randomizations.
*$p < 0.05$; **$p < 0.01$

Discussion

An increase in earthworm species richness and biomass was not correlated with increases in shade intolerant herbaceous plants nor invasive plants that require higher light levels for germination and seedling establishment; thus the first hypothesis was not supported. Over the course of the study, there were no shade intolerant herbaceous plants nor invasive tree, shrub, or herbaceous plants detected in any site, possibly because the canopy opening was initially not very large and also in part to rapid canopy closure over three years and (Collins and Pickett 1987).

In-depth studies of hardwood herbaceous plant communities in varying intensities of non-native earthworm invasion confirm a decline in many herbaceous species. The resulting plant communities are dominated by a few species with low diversity and relative abundance (Gundale 2002; Hale 2004; Holdsworth et al. 2007a).

The second hypothesis of an inverse correlation between earthworm richness and biomass with herbaceous plant richness and diversity was supported. Though the NMS ordination provided interesting correlations between different environmental variables and the cover class of herbaceous plants within the sites, teasing out definitive causal relationships is beyond the scope of this study. The strong relationship between the herbaceous plant cover class in the Minimally Invaded site and herbaceous plant richness, diversity, and cover supports the hypothesis further. The relationships between the environmental variables and the herbaceous plant cover class in the Moderately Invaded sites responded similarly to the Minimally Invaded site in spite of more earthworm AFDg/m^2 and increased earthworm species richness. It is possible that sampling was not sensitive enough to detect declines in herbaceous plant richness or diversity within these moderately invaded sites. The earthworm assemblages may or may not have been the cause of variation from the Minimally Invaded site. This remains inconclusive since the sites were pseudo-replicated with annual sampling in mid-late summer. Spring ephemerals could be more sensitive to earthworm presence and the combined impacts of epigeic and endogeic species. If *Lumbricus terrestris* is introduced to these areas it is very likely that the earthworm assemblage formed from all ecological groups will work synergistically to remove the forest floor, mix upper soil horizons, and effectively remove the rooting zone and seed bed for native herbaceous plants and tree seedlings (Bormann and Likens 1979; Holdsworth et. al 2012).

Indicator species analysis did not provide any matching species to either Hale (2004) or Holdsworth et al. (2007a). Since the sites of this study were pseudo-replicates, the species list generated by the indicator species analysis was likely of species in these sites before earthworm invasion and not likely due to invasion. In the Minimally Invaded site, three indicator species were detected, but all are inconsistent with both Hale and Holdsworth. For instance, night shade (*Circaea alpina*) and greenbrier (*Smilax herbacea*) were detected as perfect indicators and jewel weed (*Impatiens capensis*) as a near perfect indicator of the Minimally Invaded site. This is problematic as neither Hale nor Holdsworth found this species to be indicative of minimal earthworm impact. Spikenard (*Aralia racemosa*) was not a significant indicator in my study (p = 0.08), but is known to be sensitive to earthworm invasion. Both Hale and Holdsworth found the sedge *Carex pensylvanica* to be indicative of earthworm impacted hardwood forests.

Though *Carex pennsylvanica* was not detected in this study, both *Carex deweyana* and *Carex pedunculata* were detected in all sites with the highest percent cover in the moderately and heavily invaded sites. *Carex* species are commonly found in earthworm invaded sites (Sydes and Grime 1981), but without true replicates of the sites in this study the occurrence of these individual species could be influenced by confounding site characteristics. Future studies ought to consider true replicates of study sites for a clearer picture of plant community trajectories.

Chapter 4: Conclusion

This study found changes in soil characteristics and plant communities along a continuum of earthworm invasion. The four sites spanned an earthworm invasion continuum and provided strong evidence of large changes in soil and plant properties.

Canopy opening was not as catastrophic as originally estimated, nor did it have a strong effect on soil or plant parameters within this study. Future studies with similar research goals would benefit from quantifying canopy disturbance in a pilot study to ensure at least 50% canopy opening.

Upper soil horizons were homogenized as earthworm biomass and richness increased, but gravimetric water content measured beneath the O_a horizon did not vary significantly among sites. Future studies interested in soil moisture ought to consider the entire profile and give particular attention to the O horizon as over half the total moisture of upper soil horizons in the 12 cm deep soil cores was held in the O horizon. Available N and P varied among sites and sampling periods with significantly higher levels of NO_3-N in the Heavily Invaded site. However, with wide variability and a small sample size future studies ought to consider larger sample sizes, time series analysis, and a more definitive mechanism for measuring available nutrients.

Plant communities observed in each site differed in relation to the earthworm assemblage present and thickness of the O horizon. The herbaceous layer richness and diversity decreased as earthworm biomass and richness increased, and increased with O horizon thickness. Indicator species analysis generated a species list indicative of minimally, moderately, and heavily invaded sites. However, only *Carex* in heavily invaded sites matched species lists generated in more in-depth studies (Hale 2004; Holdsworth et al. 2007a). Given the feeding and burrowing patterns of invasive earthworms, it is likely that the observed differences were due to earthworm invasion. Future studies across a continuum of earthworm invasion ought to include true replicates in the study design.

The strong correlations reported in the results suggest that future research could address the specific mechanisms of earthworm impact via elimination of the O horizon, compaction of the mineral soil horizon, and earthworm-grazing in the plant rhizosphere. Additionally, climate change projections for the Great Lakes region suggest that hardwood forests could undergo significant alteration (Pastor and Post 1988; Aber et al. 2001). The combined sources of stress on hardwood forest plant communities could become amplified, and extirpation of vulnerable native herbaceous plants could result (Bohlen et al. 2004). Continued studies of stressors in earthworm impacted hardwood forests may provide better understanding of synergistic effect within these forest systems, and greater insight for land managers to successfully preserve native plant communities for future generations.

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