University of Alberta

Above- and below-ground effects of an exotic ecosystem engineer in the boreal forest

by

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ABSTRACT

Species invasions are increasing worldwide and are impacting populations, communities, and ecosystems. Non-native species that are ecosystem engineers, such as earthworms, may be particularly likely to have large impacts due to their ability to modify both biological and physical characteristics of their environment. Using a combination of field and laboratory studies, I examined above- and below-ground effects of non-native earthworms in the boreal forest of Alberta. I found no evidence that earthworm species facilitate each other's invasions or have synergistic effects, as would be expected in an invasional meltdown. In a mesocosm experiment, the litter-dwelling earthworm *Dendrobaena octaedra* and the deep-burrowing species *Lumbricus terrestris* did not facilitate each other's survival or reproduction. Similarly, although the two earthworm species significantly influenced microarthropod abundance, oribatid assemblage structure, and leaf litter depths, they did not have synergistic effects. Further, white spruce (*Picea glauca*) growth and colonization of white spruce roots by mycorrhizal fungi were not affected. Earthworms impacted other taxa via both direct trophic interactions and ecosystem engineering. In laboratory experiments, changes in soil structure associated with earthworm burrowing had strong effects on both microarthropod movement and plant root growth. In a field study, distributions of American robins and earthworms were strongly correlated, suggesting the two groups are linked via predation of earthworms by robins. Although this research indicates non-native earthworms are affecting a variety of taxa in the boreal forest, there is limited awareness of earthworm invasions among the public. Consequently, a combination of research, public education, and regulations will likely be needed to effectively manage earthworm invasions in Alberta.

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CHAPTER 1. INTRODUCTION[1]

1.1 Interactions among non-native species

Species are establishing in areas beyond their native geographic ranges at unprecedented rates worldwide (Ricciardi 2007). Non-native species are defined as invasive if they have demonstrable economic or ecological impacts (Lockwood et al. 2007). Invasive species can cause changes to native populations, communities, and ecosystems, leading to substantial economic costs. For example, losses due to non-native species are estimated to total $7.5 billion per year in the Canadian agricultural and forestry sectors alone (Dawson 2002). Research on biological invasions has increased in recent years partly in response to these significant costs but also as a result of the opportunity this broad-scale rearrangement of species provides for new insights into community ecology (Shea & Chesson 2002).

The growing number of invasions is resulting in greater potential for novel interactions between non-native and native species and even new interactions among different non-native species. Interactions among non-native species can lead to combined effects that are subadditive, additive, or superadditive (Figure 1.1; Rauschert & Shea 2012). Subadditive effects result from competitive interactions between non-native species. Depending on whether both species impact each other negatively, this has variously been termed invasional interference (-/-; Yang et al. 2011), antagonism (+/-; La Pierre et al. 2010), or amensalism (-/0; Griffen et al. 2008). If there is little impact of the species on each other or negative and positive impacts are balanced, their overall effect is instead additive (Rauschert & Shea 2012). In contrast, superadditive or synergistic effects are produced by facilitative interactions between non-native species, in which at least one participant benefits and neither is harmed (+/+ or +/0; Bruno et al. 2003). Facilitation can result in greater establishment, population growth, spread, or impacts of invasive species (Simberloff & Von Holle 1999). In some cases, facilitation between non-native species can lead to an invasional meltdown, in which there is an accelerating increase in the number of species invading or their impacts (Simberloff & Von Holle 1999).

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[1] The research in Chapters 2-8 was collaborative and therefore I use the pronoun “we” throughout those chapters. Chapters 1 and 9 represent my own thoughts.

Non-native species that are ecosystem engineers may be particularly likely to be involved in facilitative interactions with other species, due to their ability to modify both biological and physical characteristics of the environment (Gonzalez et al. 2008). Ecosystem engineers can directly cause population and community level changes similar to non-engineering species (Strayer 2012). However, they can also directly affect ecosystem function, rather than only indirectly via transmission of effects through population and community level changes, as is the case for non-engineering species. Changes to ecosystems caused by engineers can then feedback to cause population and community changes, or even impact the invader itself. Such ecosystem level impacts appear to be more common than previously thought (Simberloff 2011).

1.2 Study species

Earthworms are ecosystem engineers that are currently invading forests across North America (Hendrix & Bohlen 2002; Bohlen et al. 2004b; Frelich et al. 2006). Native earthworms were extirpated from most of Canada and the northern United States during the Pleistocene glaciations (Gates 1970, 1982). Following deglaciation approximately 10,000 years ago, recolonization by native species from unglaciated areas has been slow (James 2004). However, European earthworms were introduced to North America with the arrival of European settlers (Gates 1982, James 2004). Introductions likely occurred accidently with dry ship ballast, plants, and soils, as well as intentionally to improve soil conditions in agricultural areas (Gates 1970, 1982; Hendrix & Bohlen 2002). In western Canada, earthworms were first documented in forests in the mid-1980s, near Kananaskis (Scheu & Parkinson 1994; McLean & Parkinson 1997). In Alberta’s boreal forest, invasions may have begun during the 1950s and 1960s in some forest stands based on surveys of the spatial distribution of earthworms (Cameron & Bayne 2009). Anecdotal reports further suggest earthworms were present in agricultural areas within the boreal ecozone of northern Alberta by the early 1900s (R. Piquette, personal communication).

Earthworms have a limited ability (~2-4 m/year) for active dispersal and therefore passive dispersal via natural or anthropogenic vectors is thought to be important in their spread (Marinissen & van den Bosch 1992). In northern forests, bait abandonment by anglers and transport of earthworms or their cocoons (egg cases) by vehicles are key mechanisms of introduction (Gundale et al. 2005; Cameron et al. 2007; Holdsworth et al. 2007). Earthworms may also be transported along waterways (Schwert & Dance 1979; Costello et al. 2010).

In forests where native earthworms are absent, substantial impacts on populations, communities, and ecosystems can occur when earthworms invade (Figure 1.2; after Bohlen et al. 2004a; Bohlen et al. 2004b). Invasions by non-native earthworms often result in changes to soil structure including decreased leaf litter thickness and greater mixing of the organic and mineral soil horizons (Alban & Berry 1994; Gundale 2002; Hale et al. 2008). Increases in nitrogen leaching (Costello & Lamberti 2008) and changes in carbon storage (Alban & Berry 1994; Wironen & Moore 2006) are also frequently observed. These changes in ecosystem structure and function can lead to cascading effects on other organisms, including shifts in microbial communities (McLean & Parkinson 1997), decreases in richness and abundance of herbaceous plants (Frelich et al. 2006), and declines in forest songbird populations (Loss et al. 2012). The major mechanisms involved in most of these changes are not well understood.

Earthworms often have differing effects depending on the ecological group to which they belong (Bouché 1977): 1) Epigeic species live and feed in the leaf litter layer; 2) Endogeic species live and feed in the mineral soil layer; and 3) Anecic species live in deep burrows but feed on surface leaf litter. A fourth ecological group that is intermediate between the epigeic and endogeic species, the epi-endogeic earthworms, is also considered in some studies (e.g., Hale et al. 2005). These species live and feed in the leaf litter and the top several centimetres of mineral soil. Epigeic species are small-bodied and quick moving, with high reproductive rates. They are also more likely to be transported by humans or other vertebrates (e.g., deer) than the other groups because of their presence near the surface of the leaf litter. Endogeic and anecic species are larger but have lower dispersal and reproductive rates. They are more often used as fishing bait and are introduced via bait abandonment by anglers (Cameron et al. 2007, 2008).

In northern forests invaded by earthworms, field observations indicate epigeic and epi-endogeic species are often widespread, whereas endogeic and anecic species tend to occur only in areas where litter-dwelling species are also found (Hale et al. 2005; Gundale et al. 2005; Suarez et al. 2006; Wironen & Moore 2006; Cameron et al. 2007). This has led to the suggestion that earthworm invasions may be an example of an invasional meltdown, in which epigeic and epi-endogeic species cause decreases in leaf litter thickness and mixing of soil horizons and thereby promote the establishment of endogeic and anecic species (Hale et al. 2005; Suarez et al. 2006; Hendrix et al. 2008). However, it is unclear whether facilitative interactions do occur among earthworm species and can result in synergistic effects on other organisms.

1.3 Study area

Although most of this dissertation involves laboratory experiments, I obtained the soil for the experiments, conducted the field surveys, and focused on species occurring in the boreal forest of northern Alberta. This region is experiencing high levels of anthropogenic disturbance due to the cumulative effects of energy, forestry, agricultural, and recreational development (Schneider et al. 2003). Forested areas consist of a mosaic of uplands and lowlands. Upland forests are dominated by trembling aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), white spruce (*Picea glauca*), and jack pine (*Pinus banksiana*), and most often have luvisolic soils (Natural Regions Committee 2006). Wild rose (*Rosa acicularis*), low-bush cranberry (*Viburnum edule*), beaked hazelnut (*Cornus cornuta*), dogwood (*Cornus stolonifera*), and saskatoon (*Amelanchier alnifolia*) are common understory species. Lowland stands are dominated by black spruce (*Picea mariana*) and tamarack (*Larix laricina*), and most commonly have mesisolic soils. Mean annual temperature in northern Alberta is -1.4 ºC, while average total annual precipitation is 469 mm (Natural Regions Committee 2006).

Six species of earthworms are known to occur in northern Alberta, including two epigeic species (*Dendrobaena octaedra* and *Dendrodrilus rubidus*), one epi-endogeic species (*Lumbricus rubellus*), two endogeic species (*Aporrectodea tuberculata* and *Aporrectodea turgida*), and one anecic species (*Lumbricus terrestris*) (Cameron et al. 2007). No native species have been found. Some previous research has examined effects of exotic earthworms in the hemi-boreal in southern Alberta (e.g., Scheu & Parkinson 1994; Eisenhauer et al. 2007) and spread of earthworms in the boreal forest (Cameron et al. 2007, 2008; Cameron & Bayne 2009), but little to no previous information is available on the effects of non-native earthworms in the Canadian boreal forest.

1.4 Thesis overview

The overall objectives of my dissertation are to: 1) Examine the effects of exotic earthworms in the boreal forest, in particular whether facilitative interactions or synergistic effects may occur when multiple species invade; and 2) Investigate both ecosystem engineering and direct trophic (e.g., as prey) impacts. Thus, through the following seven chapters and a concluding chapter, I examine the above- and below-ground effects of earthworm invasions in the boreal forest (Figure 1.3):

Chapter 2: An experimental test of facilitation between non-native earthworms

Using a greenhouse mesocosm experiment, I tested whether epigeic (*D. octaedra*) and anecic (*L. terrestris*) earthworm species facilitate each other's invasions and have synergistic effects on leaf litter depth, as would be expected in an invasional meltdown.

Chapter 3: Influence of earthworms on abundance and composition of boreal microarthropods

Employing the same greenhouse experiment as described in chapter 2, effects of non-native earthworms on microarthropod abundance and oribatid community composition were studied. In particular, I evaluated whether there was evidence of synergistic effects of the two earthworm species on microarthropods.

Chapter 4: Earthworm effects on below-ground movement of microarthropods

Earthworms have been suggested to influence movement of microarthropods, thereby leading to changes in microarthropod community composition. Here, I investigated the effects of earthworms (the epi-endogeic species *L. rubellus*) on microarthropod movement by conducting a microcosm experiment.

Chapter 5: Earthworm influences on ectomycorrhizal colonization and growth of white spruce

I tested the effects of epigeic and anecic earthworms on growth of white spruce (*Picea glauca*) and colonization of white spruce roots by ectomycorrhizal fungi using the same experiment as in chapters 2 and 3. It has been proposed that changes in plant community composition following earthworm invasions may be related to physical disruption of mycorrhizal fungi by earthworm burrowing.

Chapter 6: Effects of earthworm burrowing on plant root growth

This chapter investigated the effects of *L. terrestris* burrowing on plant biomass and root distribution of two herbaceous perennial species (*Achillea millefolium* and

Campanula rotundifolia) that are native to the boreal forest. Differences in foraging precision (ability to proliferate roots in nutrient patches) between plant species might provide one potential explanation for species' varying responses to earthworm invasions.

Chapter 7: Earthworm effects on distribution of a native predator, the American robin

Here, I studied the effects of earthworms on habitat selection by the American robin (*Turdus migratorius*), a generalist predator. I also investigated whether robins may act as vectors of spread for earthworm cocoons (egg cases) by conducting a captive feeding experiment.

Chapter 8: Evaluation of an invasive earthworm education program

Because of the substantial effects non-native earthworms can have on native species, I created an education program, involving print, television, radio, and internet media, to increase awareness about earthworm invasions and to discourage anglers from dumping earthworm bait. In this chapter, I used pre- and post-surveys to evaluate the program's success in reaching its target audience and in changing knowledge and behaviour.

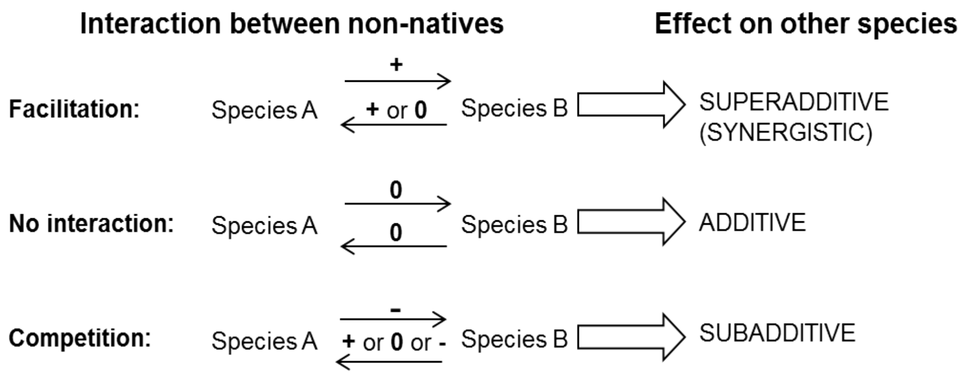

Figure 1.1. Combinations of positive, negative, or neutral interactions between non-native species leading to superadditive, additive, or subadditive effects on native species or ecosystems.

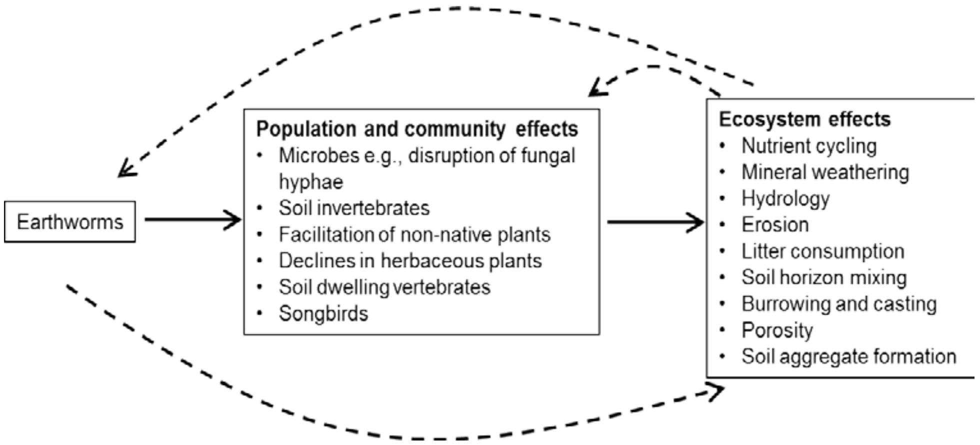

Figure 1.2. Potential effects of earthworm invasions on populations, communities, and ecosystems (after Bohlen et al. 2004a, 2004b; Strayer 2012). Direct effects are represented by solid lines and ecosystem engineering effects are represented by dashed lines.

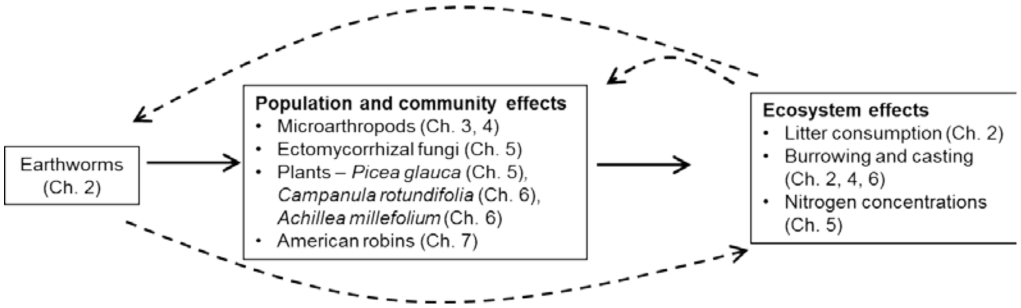

Figure 1.3. Effects of earthworm invasions on populations, communities, and ecosystems examined in this thesis (after Bohlen et al. 2004a, 2004b; Strayer 2012). Direct effects are represented by solid lines and ecosystem engineering effects are represented by dashed lines.

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CHAPTER 2. AN EXPERIMENTAL TEST OF FACILITATION BETWEEN NON-NATIVE EARTHWORMS[2]

2.1 Introduction

The frequency and geographic extent of biological invasions are increasing worldwide, to the detriment of many native taxa (Cohen & Carlton 1998; Ricciardi 2001; Lockwood et al. 2007). With higher densities of non-native species present in many ecosystems, there is growing potential for novel interactions to occur among species. Most research on interspecific interactions of non-native species has focused on the competitive effects occurring between native and non-native species (Bruno et al. 2005). However, there is increasing evidence that interspecific facilitative interactions are occurring among non-native species in their invaded ranges (Simberloff 2006). Of greatest concern is the possibility of an invasional meltdown, whereby non-native species facilitate each other’s invasions in such a way that increases the likelihood of introduction, reproduction, survival, and/or ecological impact (Simberloff & Von Holle 1999).

As ecosystem engineers, non-native earthworms have caused dramatic changes to invaded systems, particularly North American forests (Hale et al. 2005; Frelich et al. 2006; Wironen & Moore 2006; Eisenhauer et al. 2007). There are at least ten species of non-native earthworms known to occur in northern forests of North America (Hale et al. 2005; Cameron et al. 2007). Field observations from forests in Alberta (Cameron et al. 2007), Quebec (Wironen & Moore 2006), Minnesota (Hale et al. 2005), Michigan (Gundale et al. 2005), and New York (Suarez et al. 2006) indicate that where one species is found, other earthworm species also often occur. In these systems, epigeic and epi-endogeic species (litter-dwelling) typically have broader distributions than endogeic (mineral soil-dwelling) and anecic (deep-burrowing) earthworms, which tend to occur only in areas that have been colonized by epigeic or epi-endogeic species. This has led to the suggestion that facilitation is occurring among non-native earthworm species (Hale et al. 2005; Suarez et al. 2006; Hendrix et al. 2008). However, a correlative pattern does not prove causation, as the two groups may simply be exploiting

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[2] A version of this chapter has been published. Cameron, E. K., and E. M. Bayne. 2011. Canadian Journal of Zoology 89:1223-1230.

similar environmental conditions. To effectively show that two species facilitate each other requires evidence of establishment, dispersal, or reproductive patterns that differ when both non-native species are together versus when they are alone. Demonstrating such effects and identifying causation typically requires manipulative experiments.

Endogeic and anecic earthworms are hypothesized to be less capable of invading intact forest floors and their establishment, reproduction, or spread may therefore be facilitated by decreases in organic horizon depth and mixing of organic and mineral horizons caused by litter-dwelling earthworms (Hale et al. 2005; Suarez et al. 2006). This consumption of organic matter and mixing of soil horizons is suggested to make the soil organic matter and fresh litter more accessible as food resources for the endogeic and anecic earthworms (Hale et al. 2005). Once established, the activities of the endogeic and anecic species are believed to prevent forest floor recovery, thereby contributing to an invasional meltdown. Consequently, the patterns seen in the field could be due to: (1) facilitation of endogeic and anecic species through habitat modification caused by epigeic and epi-endogeic species; or (2) the greater reproductive and/or dispersal abilities of epigeic and epi-endogeic species resulting in a wider distribution of these species which by chance overlaps the distribution of the more slowly dispersing endogeic and anecic species.

We used a greenhouse mesocosm experiment to investigate whether *Dendrobaena octaedra* Savigny, 1826 (an epigeic species) facilitates *Lumbricus terrestris* L., 1758 (an anecic species) and whether they have synergistic effects on their habitats, as would be expected in an invasional meltdown. Our previous research in the boreal forest of northern Alberta shows that the epigeic species *D. octaedra* is common and widely distributed, while the anecic species *L. terrestris* is present but restricted to sites near boat launches (Cameron et al. 2007). Our experiment examined facilitation and impacts under the scenario of simultaneous colonization of adult earthworms from the two species. Such a scenario could occur if earthworms were introduced via either bait abandonment or soil transport. *Lumbricus terrestris* appears to be introduced to northern forests mainly via bait abandonment by anglers (Cameron et al. 2007), and therefore it is likely not often introduced as juveniles or cocoons (egg cases). *Dendrobaena octaedra* is commonly introduced via vehicular transport but has also been found as a contaminant in bait (Tiunov et al. 2006). Based on the field evidence described above, we hypothesized that *L. terrestris* would experience increased growth, survival, and reproduction in the presence of *D. octaedra* if facilitation was occurring. We also predicted *L. terrestris* and *D. octaedra* would have synergistic effects on the litter depth and bulk density of boreal forest soils if an invasional meltdown was occurring.

2.2 Methods

We conducted a greenhouse mesocosm experiment from 09-May-2009 to 20-Oct-2009 to test our hypotheses. The experiment included five treatments, with 20 replicate mesocosms randomly assigned to each treatment: 1) no earthworms, 2) 30 *D. octaedra*, 3) two *L. terrestris*, 4) three *L. terrestris*, and 5) two *L. terrestris* and 30 *D. octaedra*. The density of earthworms in each treatment was chosen based on field densities from invaded sites in northern forests (field averages between study areas range from 0 to 7.6 (*L. terrestris*) and 0 to 40 (*D. octaedra*) for areas the size ($0.07m^2$) of our mesocosms (Hale et al. 2005; Cameron et al. 2007; Eisenhauer et al. 2007; Hale et al. 2008)). The decision to use realistic field densities resulted in different total biomasses per mesocosm for the two species. In our samples, *L. terrestris* weighed ~5.3 g each and *D. octaedra* weighed ~0.15 g each. Treatment 4 (containing three *L. terrestris*) was included in the experiment because it had a similar total biomass to treatment 5 (both species), which allowed us to test whether effects observed in treatment 5 were related to its higher total earthworm biomass rather than synergism between species. All earthworms used were adults. *D. octaedra* was collected from a field site in northern Alberta which was known to contain only that species, while *L. terrestris* was purchased from a local bait shop.

The mesocosms were constructed in 30 cm diameter by 38 cm height buckets, which had five 2.2 cm diameter drainage holes drilled in the bottom. Landscape fabric was hot-glued in the bottom of each bucket to prevent soil loss and earthworm escape. We also placed a 5 cm strip of coarse sand around the top of each bucket using white glue to prevent escape. Soil was collected from a trembling aspen (*Populus tremuloides* Michx.) and white spruce (*Picea glauca* (Moench) Voss) forest stand with gray luvisolic soil in northern Alberta (54°36’N, 110° 59’W) where sampling over several years indicated that earthworms were absent (Cameron et al. 2007; Cameron & Bayne 2009). We removed roots and rocks from the mineral soil and filled the buckets to a depth of 18 cm. A 4 to 6 cm thick disc of intact forest floor (FH layers) the same diameter as the bucket and 3 cm of aspen leaf litter was placed on top of the mineral soil. In order to more closely mimic natural conditions, plants germinating from the soil cores during the experiment were not removed. A white spruce (*P. glauca*) seedling was also planted in the centre of each mesocosm for a concurrent experiment examining white spruce growth (Chapter 5).

The mesocosms were randomly placed in a greenhouse in which air temperature was maintained between 14.5°C to 19°C. We initially watered 0.33 L every two days (Belote & Jones 2009), which was reduced to 0.33 L every three days beginning June 17 and 0.33 L every four days starting on July 13. Earthworms were added to half of the pots two weeks after the mesocosms were initially placed in the greenhouse. We added worms to the remaining pots two weeks later because data collection at the end of the experiment was expected to take a month. This allowed all of the mesocosms to be exposed to the treatments for similar amounts of time. During the experiment, we checked for signs of earthworm activity when watering and also maintained an additional replicate which was periodically disturbed to determine if earthworms were active beneath the litter layer.

At the end of the experiment, we harvested above-ground vegetation from the mesocosms and oven-dried it (48 h at 60ºC) to determine dry biomass of forbs and grass. A bulb corer (6 cm base diameter, 7.4 cm top diameter, x 10.8 cm height) was used to take four soil cores from each mesocosm. In each core, we measured the depths of the L and FH layers. One of the cores was then oven-dried to determine bulk density of the organic and mineral horizons, while the remaining three cores were hand-sorted to determine abundances of cocoons and earthworms. we also sieved the remaining soil from each mesocosm to find additional earthworms and *L. terrestris* cocoons. The small size of *D. octaedra* cocoons did not allow them to be separated via sieving, and consequently their abundances were determined only via hand-sorting of the three soil cores. Earthworms were weighed to determine wet biomass and killed using 70% ethanol. Ash-free dry biomass was calculated by inputting preserved lengths (mm) into allometric regression equations developed by Hale et al. (2004). Because wet biomass and ash-free dry biomass were highly correlated ($r^2 = 0.95$) and analyses of final wet biomass using initial biomass as a covariate produced similar results, we report only ash-free dry biomass results.

We used t-tests and one-way ANOVAs to test for differences in dry mass per earthworm, reproduction (number of cocoons per earthworm), bulk density of organic and mineral soil, biomass of grass and forbs, and depth of the L horizon. An ANCOVA was used to examine depth of FH horizon, because the initial FH depths varied, unlike the initial L horizon depths. Shapiro-Wilk's W statistic and Levene's test were used to assess the residuals of data for normality and homogeneity of variances (Levene 1960; Shapiro & Wilk 1965). Data that failed to meet these assumptions were tested using Kruskal-Wallis or Wilcoxon rank-sum tests. When tests were significant overall, differences between treatments were identified using Bonferroni multiple comparison tests or a non-parametric equivalent (Dunn 1964; Zar 1999).

To examine survival of *L. terrestris* and juvenile occurrence, we used exact logistic regression. Exact logistic regression uses the conditional distribution of the parameter sufficient statistics to estimate the logistic model parameters and can produce more reliable results than logistic regression for small sample sizes or unbalanced data (Cox & Snell 1989; Hilbe 2009). In our study, there were few *L. terrestris* juveniles observed and deaths were rare for *L. terrestris*. Negative binomial regression was used to test for differences in juvenile abundance and survival of *D. octaedra* between treatments. This type of regression is based on the negative binomial distribution and is used to model count data which is overdispersed (i.e. the variance is greater than the mean) (Hilbe 2008). All statistical analyses were carried out using Stata 11 (StataCorp, College Station, TX, USA). As in many earthworm experiments (Fründ et al. 2010), some individuals escaped from their original mesocosms, resulting in final sample sizes of 16, 20, 17, 19, and 19 for treatments 1 to 5 respectively. Results were similar regardless of whether these replicates were included in the analyses, and we therefore report results for only the uncontaminated mesocosms.

2.3 Results

2.3.1 Interactions between species

Average ash-free dry mass did not vary among treatments for either *L. terrestris* (Figure 2.1; $\chi^2_2 = 1.938$, $P = 0.3795$) or *D. octaedra* ($z_{19, 20} = -0.857$, $P = 0.3914$). There was also no significant difference in survival between treatments for *D. octaedra* (incidence rate ratio (IRR) = 0.95; $P = 0.820$). *Lumbricus terrestris* survival did not differ among treatments but was closer to statistical significance (model score = 5.3836, $P = 0.0618$). As compared to the treatment containing both species where survival was 100%, survival was slightly lower in the treatment containing only two *L. terrestris* (survival rate = 82%, OR = 0.2100, $P = 0.191$) and lowest in the higher density three *L. terrestris* treatment (survival rate = 73%, OR = 0.1208, $P = 0.0463$). Cocoon production did not differ significantly among treatments for *L. terrestris* ($\chi^2_2 = 0.659$, $P = 0.719$), but it was significantly lower for *D. octaedra* when *L. terrestris* was present versus absent (Figure 2.1; $z_{19,20} = -2.558$, $P = 0.0105$). There were no significant differences among treatments for *L. terrestris* juvenile occurrence (model score = 0.0085735, $P = 1.000$) and *D. octaedra* juvenile abundance (IRR = 2.85, $P = 0.079$).

2.3.2 Impacts

The depth of the FH layer did not differ significantly between treatments after exposure to earthworms (Figure 2.2; $F_{4,86} = 1.13$; $P = 0.346$), while the L layer depth was significantly different ($F_{4,86} = 13.98$; $P < 0.0001$). As compared to the control, the three *L. terrestris* ($P < 0.001$), two *L. terrestris* ($P = 0.003$), and both species together ($P < 0.001$) treatments had significantly lower litter depths, while the *D. octaedra* treatment did not differ from the control ($P = 1.000$). Bulk density of the organic horizons also varied between treatments ($\chi^2_4 = 18.151$, $P = 0.0012$), with the treatment containing both species having a significantly higher bulk density than the control ($P = 0.024$) and *D. octaedra* ($P = 0.006$) treatments. There were no significant differences in mineral soil bulk densities between treatments ($F_{4,86} = 0.49$; $P = 0.744$). Above-ground biomass was not significantly different between treatments for forbs ($\chi^2_4 = 5.427$, $P = 0.2462$) or grass ($\chi^2_4 = 6.411$, $P = 0.170$).

2.4 Discussion

Dendrobaena octaedra did not facilitate the reproduction, survival, or growth of *L. terrestris* in the short-term in our experimental design. Although endogeic and anecic earthworms are thought to have some difficulty colonizing intact forest floors (Hale et al. 2005; Suarez et al. 2006), *L. terrestris* was able to successfully establish and reproduce in our mesocosms when introduced as adults, regardless of whether or not *D. octaedra* was present.

Our experiment showed no evidence of facilitation in the specific case of simultaneous colonization of adults. Thus, field observations of invasions where *D. octaedra* is at the leading edge, with *L. terrestris* farther behind, should not be viewed as strong evidence of facilitative interactions but instead may be caused by differences in reproductive ability or vectors of spread. *Dendrobaena octaedra* reproduces asexually via parthenogenesis and therefore requires only a single individual to establish a population, whereas *L. terrestris* reproduces sexually (Gates 1974; Jaenike et al. 1982). *Lumbricus terrestris* also produces fewer cocoons per year than *D. octaedra* (25.3-36.9 as compared to 46.3 per year at 15°C) and has lower cocoon viability (70-83% vs. 90%) (Butt 1991; Butt et al. 1992; Bindesbøl et al. 2007). In terms of dispersal, *D. octaedra* is present near the surface of the leaf litter and deposits its cocoons there, whereas *L. terrestris* spends most of its time in deep burrows and deposits its cocoons both near the surface and at depths of up to 40 cm (Butt 2002). As a result, *D. octaedra* and its cocoons likely have an increased probability of passive dispersal via human activities, wind, water, or animals as compared to *L. terrestris* (Terhivuo & Saura 1997; Cameron et al. 2007). However, the occurrence of facilitation under a scenario of sequential colonization, in which *D. octaedra* establishes first and alters soil structure before *L. terrestris* is introduced, cannot be excluded by our experiment and warrants further investigation.

Lumbricus terrestris appeared to have a weak negative impact on its own survival and a strong negative effect on *D. octaedra* reproduction. All *L. terrestris* individuals survived in the treatment containing *D. octaedra*, but only 73% of replicates had all earthworms surviving in the three *L. terrestris* treatment. While not quite significant, this difference suggests that intraspecific competition may have affected *L. terrestris* survival. In terms of *D. octaedra,* it produced significantly fewer cocoons when *L. terrestris* was present. No previous research has examined interactions between *D. octaedra* and *L. terrestris*, but interactions between earthworm species in different ecological groups are common (Uvarov 2009). Furthermore, negative interactions appear to predominate, likely as a result of competition for resources (Uvarov 2009). Because *L. terrestris* feeds on the leaf litter where *D. octaedra* feeds and lives, it may negatively affect *D. octaedra* by removing food or habitat resources. Alternatively, *L. terrestris* may consume *D. octaedra* cocoons, either by chance or as a result of having actively sought them out. Another species of earthworm, *Aporrectodea longa* Ude 1885 has been reported to consume the cocoons of the smaller *Microscolex dubius* Fletcher 1887 (Dalby et al. 1998). *Lumbricus terrestris* can consume seeds at least as large as 1.7 mm in diameter and 5.5 mm in length (Eisenhauer et al. 2010), which are similar in diameter and longer in length than *D. octaedra* cocoons, the latter being approximately 2 mm by 2.5 mm in length.

Besides facilitation between invading species, invasional meltdowns can involve an accelerating increase in the impacts of non-native species, such that invasions of multiple species result in synergistic rather than additive effects on the invaded system (Simberloff 2006). Because *D. octaedra* and *L. terrestris* belong to different ecological groups and have different feeding habits, it could be expected that they would have larger impacts together than separately. In fact, field research does indicate that impacts of earthworm invasions are particularly large when multiple species invade (Hale et al. 2005). In our experiment, both species combined had a greater effect on litter depth and bulk density than either species had by itself. However, these effects were additive rather than synergistic (i.e. the effect observed in the treatment containing both species was not larger than either the effect of the single species treatments added together, or the higher biomass *L. terrestris* treatment). Similar results were found in a mesocosm experiment examining the impacts of *D. octaedra*, *L. terrestris*, and *Lumbricus rubellus* Hoffmeister, 1843 on hardwood forests (Hale et al. 2008). Each of the three species significantly reduced organic horizon thickness and had the largest impacts when combined, but the effect was only additive.

Our results may have been affected by the fact that, by necessity, mesocosm experiments lack some of the realism of natural ecosystems. In particular, our mesocosms contained intact litter cores, but the cores were only 30 cm in diameter and therefore may not have been recognized as intact by the earthworms. However, we did not observe increased earthworm activity at the edges of the cores as compared to the middle, as would be expected if this had been the case. Secondly, although we began our experiment with initial earthworm densities similar to those found in natural systems, survival of *D. octaedra* was low. This likely did not strongly influence the results, as most deaths appeared to occur within approximately the last month of the experiment in the additional mesocosm which we maintained to monitor survival. Thirdly, our experiment was carried out over a relatively short period of time (4.5 months), which may have reduced our ability to detect effects. However, earthworms are capable of causing substantial changes to ecosystems within a single growing season (e.g., Gundale 2002), and we did observe significant effects on leaf litter depth, organic horizon bulk density, and *D. octaedra* reproduction. Continuation of our experiment through the winter would have provided longer-term information on the success of *L. terrestris* establishment and reproduction as a function of habitat alteration by *D. octaedra*.

Effective control of a facilitator species may aid in the management of the species that it facilitates (Heimpel et al. 2010). In the case of earthworms, there is no known method of removal from forest stands that have been invaded, meaning that management must focus instead on preventing introductions. However, vectors of introduction and spread differ depending on the species of earthworm and, as such, management resources could be targeted towards groups of higher concern. Because our results suggest that *D. octaedra* does not facilitate *L. terrestris*, resources could be used to preferentially manage *L. terrestris*, which has larger ecological impacts. *Dendrobaena octaedra* appears to be spread primarily by vehicular traffic, while *L. terrestris* is likely introduced largely via bait abandonment, making prevention of *L. terrestris* invasions more straightforward and feasible (Cameron et al. 2007). It should be noted that our study does not provide any information on facilitation between earthworms and other groups such as non-native plants (Belote & Jones 2009; Madritch & Lindroth 2009; Nuzzo et al. 2009) or the occurrence of extensive invasional meltdowns such as that proposed by Heimpel et al. (2010). The potential for these types of interactions should also be considered prior to implementing any management strategy.

Our study did not find evidence to support the ideas that non-native earthworms facilitate each other’s invasions or have synergistic effects on their habitats as would be expected in an invasional meltdown. This conclusion is similar to that of Wonham and Pachepsky (2006), who created null models and examined empirical datasets to investigate increases in non-native species richness over time. This latter study demonstrated that constant rates of introduction can generate exponential trends in species richness and it was therefore concluded that such trends may not be indicative of increased invasion success, nor of invasional meltdown. Our findings likewise suggest that a purely descriptive approach is not sufficient to assess whether facilitation or invasional meltdowns are occurring in an area.

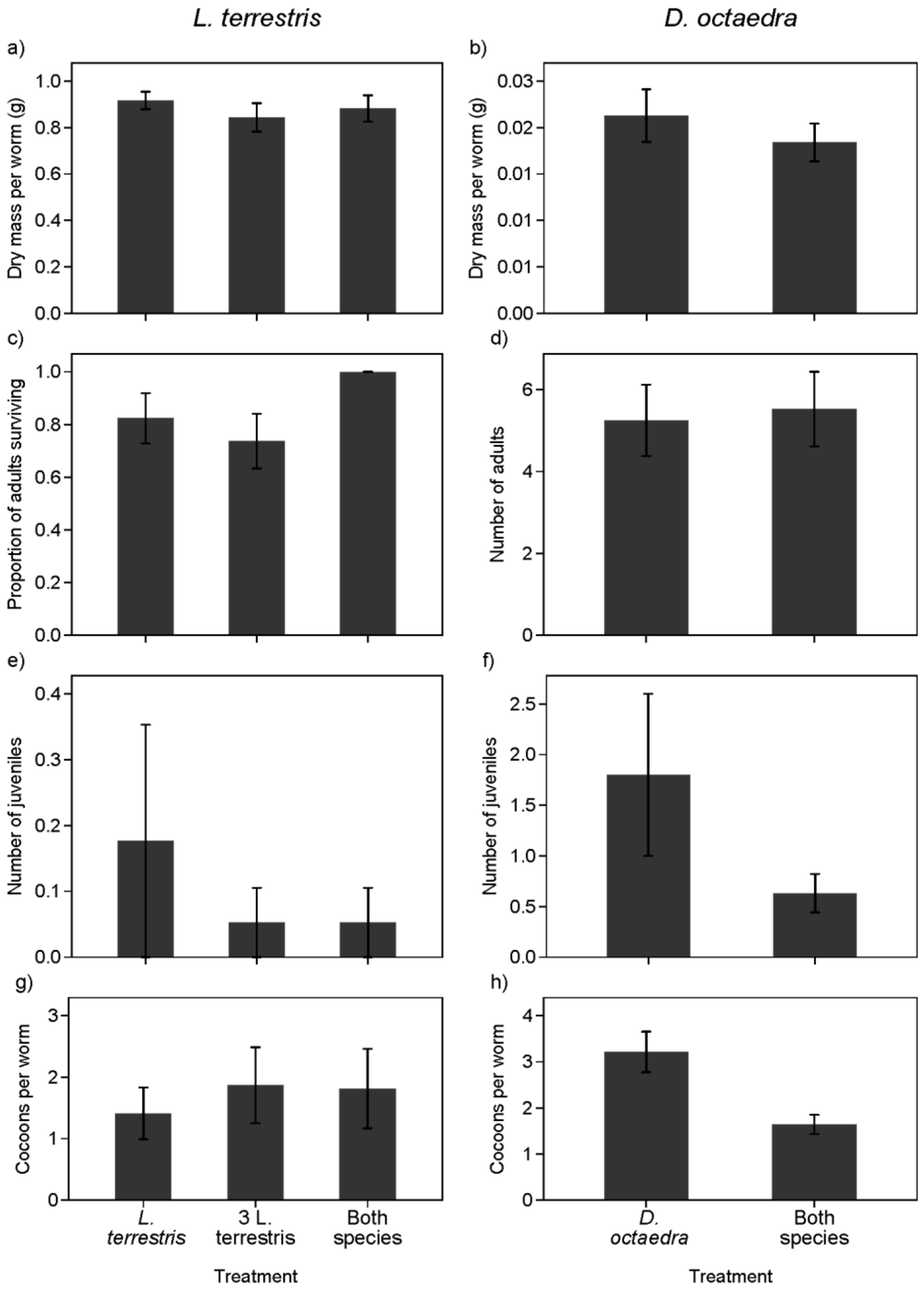

Figure 2.1. Means (± 1 SE) of a) dry mass per *Lumbricus terrestris* worm (g), b) dry mass per *Dendrobaena octaedra* worm (g), c) proportion of replicates with all *L. terrestris* surviving, d) number of adult *D. octaedra* surviving, e) number of juvenile *L. terrestris,* f) number of juvenile *D. octaedra*, g) cocoons per *L. terrestris,* and h) cocoons per *D. octaedra* in the four earthworm treatments (*D. octaedra, L. terrestris,* 3 *L. terrestris,* both species together).

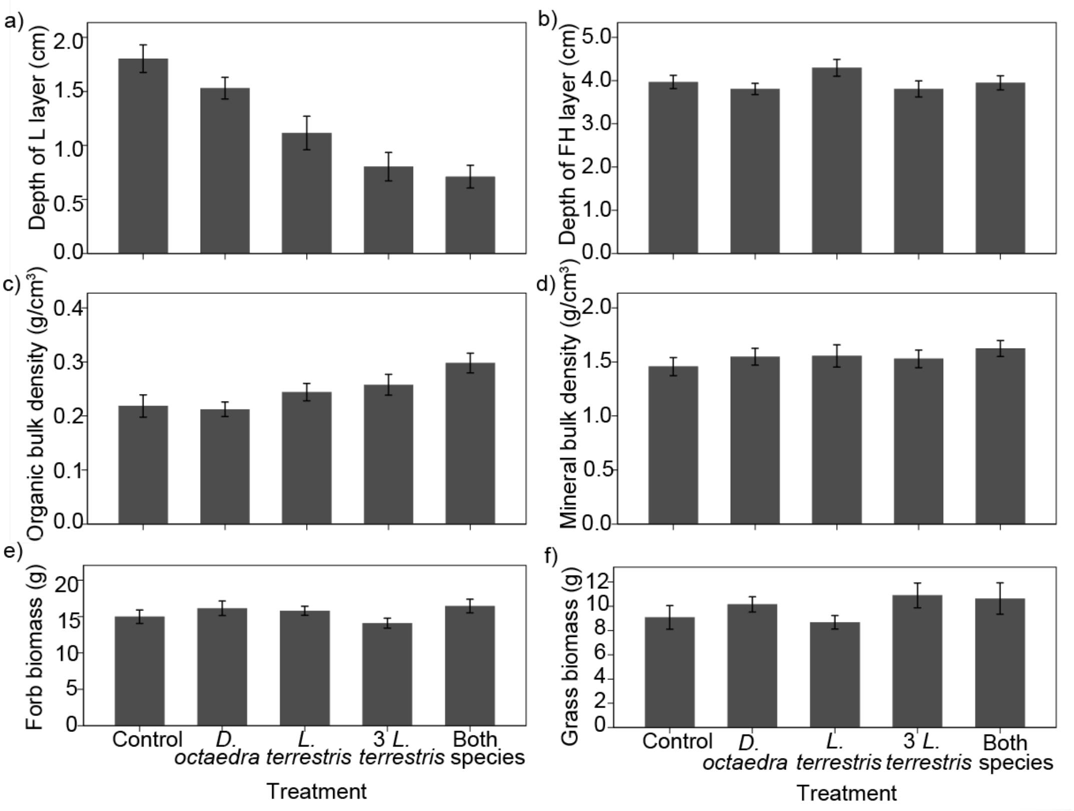

Figure 2.2. Means (± 1 SE) of a) L layer depth (cm), b) FH layer depth (cm), c) organic horizon bulk density (g/cm^3), d) mineral horizon bulk density (g/cm^3), e) forb biomass (g), and f) grass biomass (g) in the five treatments (control, *Dendrobaena octaedra*, *Lumbricus terrestris*, 3 *L. terrestris*, both species together).

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CHAPTER 3. INFLUENCE OF EARTHWORMS ON ABUNDANCE AND COMPOSITION OF BOREAL MICROARTHROPODS[3]

3.1 Introduction

Positive interactions among non-native species, such as mutualism or facilitation, can result in larger effects on native species than would otherwise be expected (Simberloff & Von Holle 1999; Simberloff 2006). Conversely, negative interactions among invading species, including competition or predation, can decrease their success and may lead to reduced effects on native species (e.g., Yang et al. 2011). Non-native species that act as ecosystem engineers may have particularly strong effects on the species with which they interact, due to their ability to modify biological and physical characteristics of the environment (Lavelle et al. 1997).

Non-native earthworms can affect other groups of soil invertebrates such as millipedes (Snyder et al. 2011) and microarthropods (Acari and Collembola) (McLean & Parkinson 1998, 2000; Eisenhauer et al. 2007; Straube et al. 2009). Microarthropod densities may exceed 100,000/m^2 of soil (Coleman et al. 2004), and changes in their abundance and diversity can have strong effects on nutrient cycling (Wickings & Grandy 2011) and decomposition (Eisenhauer & Schaedler 2011). Microarthropods may be affected by earthworms via changes in the physical structure of the soil, by predation, or by competition for food (McLean & Parkinson 1998, 2000; Eisenhauer 2010). However, the response of microarthropods to earthworms in invaded forests varies substantially across studies (Eisenhauer 2010). Increases in microarthropod richness and abundance due to earthworm invasions tend to be observed primarily over short time periods (e.g., several months) or at low earthworm densities, while non-significant or negative effects are more commonly observed over longer time periods (McLean & Parkinson 1998; Migge-Kleian et al. 2006; Eisenhauer 2010).

Effects of earthworms on soil communities can differ depending on the earthworm species' foraging strategies. Microarthropod abundances are typically much higher in the organic than mineral layers (e.g., Battigelli et al. 2004), and

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therefore earthworms that are active in the organic soil might be expected to have a particularly strong effect on microarthropod populations. In invaded forests, the deep-burrowing anecic species *Lumbricus terrestris* L., 1758 has either negative effects (Migge 2001; Burke et al. 2011) or no significant effects on microarthropods at the habitat-scale (Migge 2001; Eisenhauer et al. 2007; Straube et al. 2009). Mineral soil-dwelling endogeic species such as *Octolasion tyrtaeum* Savigny, 1826 generally cause decreases in abundance of microarthropods in forests (Migge 2001; Eisenhauer et al. 2007), although there are exceptions (Straube et al. 2009). Considerably less research has examined effects of epigeic earthworms on microarthropods. Microarthropod abundance decreased in the presence of the epigeic species *Dendrobaena octaedra* Savigny, 1826 in a field study conducted in a pine forest in Alberta, Canada (McLean & Parkinson 2000). In the only other study on exotic epigeic earthworm effects on microarthropods, which was conducted in a laboratory using pine forest soil, microarthropod abundance increased in the presence of *D. octaedra* after 3 months, but declined significantly after 6 months (McLean & Parkinson 1998).

In northern forests in Canada and the United States, non-native earthworm species often co-occur (Gundale et al. 2005; Hale et al. 2005; Suarez et al. 2006; Cameron et al. 2007). The frequent co-occurrence of multiple earthworm species raises the possibility that their effects on native forest biota could be strongly affected by facilitative or competitive interactions between earthworm species. Despite this, manipulative experiments have not typically examined how effects of single earthworm species differ from effects of multiple species together.

We used a greenhouse mesocosm experiment to investigate the effects of the non-native European earthworms *D. octaedra* (epigeic) and *L. terrestris* (anecic) on microarthropods in boreal forest soil. We predicted that the epigeic *D. octaedra* would cause a larger decrease in microarthropod abundance and diversity in the organic soil horizons than the anecic *L. terrestris*. *Lumbricus terrestris* may have fewer direct interactions with microarthropods in the organic layers because it feeds in those layers, but lives in deep burrows. Due to the two species' differing feeding strategies and effects on soil structure, a treatment containing both species was expected to disturb all soil layers and have the largest impact on microarthropods. However, *L. terrestris* can negatively affect the reproduction of *D. octaedra* (Chapter 2; Cameron & Bayne 2011) suggesting the effect of both earthworm species when together might be lower than what would be expected by simply adding together individual effects. We also predicted that Mesostigmata (predatory mites) would be less negatively affected by earthworms than groups that are more detritivorous or microbivorous (Collembola, Prostigmata, and Oribatida including Astigmata) due to competition for food resources or greater disruption of these resources (Salmon et al. 2005; Eisenhauer 2010). Because oribatid mites are a highly diverse group and tend to be sensitive to disturbances (Wallwork 1983; Maraun & Scheu 2000), we further examined the effects of earthworms on oribatids at the genus/family level.

3.2 Methods

3.2.1 Experimental design

We used the mesocosm experiment described in Chapter 2 to test the effects of non-native earthworms on microarthropods in boreal forest soil collected from an earthworm-free area. Microarthropod abundance was examined in four of the five treatments discussed in the previous chapter: 1) no earthworms (control); 2) two *L. terrestris*; 3) 30 *D. octaedra*; and 4) both species (two *L. terrestris* and 30 *D. octaedra*). There were ten replicate mesocosms in each treatment. Mesocosms consisted of 30 cm diameter by 38 cm high plastic buckets, filled with 18 cm of mineral soil, a 4 to 6 cm thick disc of intact forest floor (FH layers), and 3 cm of aspen leaf litter. The experiment took place from 9 May 2009 to 20 October 2009 in a greenhouse at the University of Alberta. For a more detailed explanation of the experimental design, see Chapter 2.

3.2.2 Microarthropod sampling

At the end of the experiment, a bulb corer (6 cm base diameter, 7.4 cm top diameter, 10.8 cm height) was used to collect soil for microarthropod sampling. We divided the soil cores into mineral and organic soil and sorted through them for earthworms and cocoons. 250 mL of organic soil from each mesocosm was then live-extracted for microarthropods using a Tullgren funnel (Macfadyen 1961). To separate microarthropods from residual soil after live extraction, the soil was preserved in 80% ethanol and a kerosene floatation was performed modified from that described in Krantz and Walter (2009). The ethanol-preserved sample was mixed with equal parts kerosene, gently shaken, and left to sit until the chemicals separated. The kerosene layer was then decanted and put through a 45 μm sieve to collect arthropods. The sieve was washed with 80% EtOH and the recovered residue was scanned for the presence of microarthropods under a dissecting microscope. Microarthropods were removed, preserved in 80% ethanol, and later sorted into the coarse categories of Oribatida, Prostigmata/Astigmata, Mesostigmata, and Collembola. Prostigmata and Astigmata were grouped together by the student technicians due to the large number of small immature individuals that would have required slide-mounting to differentiate. Although Astigmata are phylogenetically a subset of Oribatida (Krantz & Walter 2009), non-astigmatan oribatids are traditionally analyzed separately from Astigmata as they have a much different life-history than the faster-developing and more rapidly dispersing astigmatans. Adult Oribatida were identified to genus using keys constructed by D. E. Walter from the Royal Alberta Museum (http://www.royalalbertamuseum.ca/natural/insects/research/research.htm) except for the very small-bodied Brachythoniidae, which were left at the family level.

3.2.3 Statistical analysis

We used one-way ANOVAs to examine the effects of the treatments on microarthropods (all groups combined), Oribatida, Mesostigmata, Astigmata/Prostigmata, Collembola, and oribatid taxon richness. Shapiro-Wilk's W statistic and Levene's test were used to assess residuals for normality and homogeneity of variances (Levene 1960; Shapiro & Wilk 1965). A Bonferroni multiple comparison test was used for post-hoc comparisons between treatments.

Non-metric multidimensional scaling (NMDS) was used to qualitatively examine the effects of each treatment on oribatid assemblages at the family/genus level, as this method is appropriate for data containing multiple zeroes (McCune et al. 2002). In the NMDS, replicates were coded by treatment and positioned on three axes, which provided a less stressed configuration than two axes. We assessed the significance of the effect of the treatments on oribatid assemblage structure using a permutational MANOVA, which is a non-parametric form of MANOVA (Anderson 2001). Bray-Curtis was used as the distance metric in both of these tests. Indicator taxon analysis was also used to examine the responses of individual oribatid taxa to the treatments (Dufrene & Legendre 1997). This analysis examines the degree of treatment specificity of taxa based on their densities. Indicator values range from zero (no predictive ability) to one (perfect prediction of a treatment). Univariate analyses were conducted in Stata 11 (StataCorp, College Station, TX, USA), while multivariate analyses were conducted using R version 2.14.0 (R Foundation for Statistical Computing, Vienna, Austria). Tests were deemed significant if $P < 0.05$.

3.3 Results

3.3.1 Abundance

A total of 13,798 microarthropods were extracted from the samples and classified as Astigmata/Prostigmata (28.4%), Collembola (14.9%), Mesostigmata (9.4%), or Oribatida (47.3%). Some macroarthropods, including spiders, thrips, aphids, staphylinid beetles, and dipterans, were also extracted but were not included in analyses. Significant differences in abundance among treatments were observed for all microarthropods together (Figure 3.1a; $F_{3,36} = 5.49$; $P = 0.0036$), oribatids (Figure 3.1b; $F_{3,36} = 3.04$; $P = 0.0412$), and Astigmata/Prostigmata (Figure 3.1c; $F_{3,36} = 2.98$; $P = 0.0443$). Post-hoc comparisons indicated abundance differences were greatest between the control and both species treatments ($P = 0.007$ for microarthropods; $P = 0.116$ for Oribatida; $P = 0.100$ for Astigmata/Prostigmata) and the *L. terrestris* and both species treatments ($P = 0.034$ for microarthropods; $P = 0.111$ for oribatids; $P = 0.190$ for astigmatids/prostigmatids). In each case, abundance was lowest in the treatment with both earthworm species and highest in the control treatment, although differences were significant only for all microarthopods together. No significant differences were observed between the both species treatment and the *D. octaedra* treatment for any taxon. Although they followed the same trends, abundances of Mesostigmata (Figure 3.1d; $F_{3,36} = 2.84$; $P = 0.0512$) and Collembola (Figure 3.le; $F_{3,36} = 2.45$; $P = 0.0793$) did not differ significantly among treatments. Survival rates of earthworms also did not differ significantly across treatments and earthworms were alive in all replicates at the end of the experiment, except for one *D. octaedra* replicate (Cameron & Bayne 2011). Despite this lower survival of *D. octaedra*, we observed large numbers of cocoons (egg cases) of *D. octaedra* in all replicates, suggesting that most individuals survived until near the end of the experiment (Cameron & Bayne 2011).

3.3.2 Oribatid taxa

Oribatid taxon richness did not differ between treatments (Figure 3.1f; $F_{3,36} = 0.49$; P = 0.6899). However, oribatid assemblage structure differed significantly according to the permutational MANOVA (Table 3.1; $F_{3,36} = 2.35$; P = 0.001). Examination of the NMDS graphs suggests this significance is largely due to the control and *L. terrestris* treatments differing from the *D. octaedra* and both-species treatments, although separation of replicates in these treatment categories was partial and only visible in a graph of the first two axes (Figure 2). In the indicator species analysis, *Suctobelbella* (indicator value (IV) = 0.43; P = 0.027) and *Tectocepheus* (IV = 0.50; P = 0.009) had high indicator values in the control treatment and lowest values in the treatment containing both earthworm species. *Phthiracarus* (IV = 0.46; P = 0.030) and *Quadroppia* (IV = 0.60; P = 0.002) were strong indicators of the *D. octaedra* and *L. terrestris* treatments, respectively.

3.4 Discussion

Microarthropod abundance and oribatid assemblage structure were strongly affected by non-native earthworms in our boreal forest soil mesocosms. Total microarthropod abundance was lowest in the treatment that included both *L. terrestris* and *D. octaedra*, as predicted, and it was significantly lower than in both the no earthworm treatment and the *L. terrestris* only treatment. Therefore, the effects of the two earthworm species did not appear to be synergistic, as would be expected in an invasional meltdown. Nor was the effect of both species combined lower than the additive expectation. *Lumbricus terrestris* can negatively affect *D. octaedra* reproduction (Cameron & Bayne 2011; Chapter 2), but such an effect might be more likely to influence microarthropod abundances on a time scale greater than the length of this experiment. No significant differences between pairs of treatments were observed for individual microarthropod taxa (Oribatida, Mesostigmata, Astigmata/ Prostigmata, Collembola). In the single earthworm species treatments, *D. octaedra* appeared to have a larger, but not significant, effect on microarthropod abundance than did *L. terrestris*, even though the *D. octaedra* treatment had only half the earthworm biomass of the *L. terrestris* treatment on average. Structure of oribatid assemblages in the *D. octaedra* and both-species treatments were also the most distinct from those of the control treatment.

Although no other studies have investigated the effects of epigeic versus anecic earthworms on microarthropods, previous research has suggested several mechanisms by which epigeic earthworms may affect microarthropods (McLean & Parkinson 1998, 2000). Changes to the physical structure of the organic layers of the soil, particularly the conversion of litter materials into earthworm casts, is suggested to be the major mechanism involved (McLean & Parkinson 2000; Frelich et al. 2006; Eisenhauer 2010). In our study system, *D. octaedra* had limited effects on soil structure while *L. terrestris* caused larger decreases in leaf litter depth and increases in bulk density (Chapter 2; Cameron & Bayne 2011). More detailed measurements of soil texture or chemistry might have provided a better indication of changes in the organic layers caused by *D. octaedra*. Alternatively, factors other than changes to soil structure could be involved, such as competition for microbial food resources or predation (McLean & Parkinson 1998, 2000; Eisenhauer 2010). However, predation of microarthropods by earthworms appears to be very rare (Gutiérrez López et al. 2006). A small survey of field-collected *D. octaedra* from Alberta yielded no recognizable microarthropod material in the gut contents of nine individuals (K. M. Knysh, personal observation).

Unlike epigeic species, anecic earthworms live in deep burrows, although both groups feed on leaf litter. As a result, the potential for direct interactions of anecic earthworms with litter-dwelling microarthropods may be reduced as compared to epigeic species which live only within the organic horizons. The spatial extent of foraging by the two *L. terrestris* per replicate in our study could also be expected to be less than that by the thirty *D. octaedra*. The limited effects of *L. terrestris* in our experiment have also been observed in several other studies (Migge 2001; Eisenhauer et al. 2007; Straube et al. 2009), although not in all (Migge 2001; Burke et al. 2011). The effects of *L. terrestris* appear to differ depending on the scale of examination, with positive impacts on microarthropods being observed at the microhabitat level (within burrows and middens versus in soil without burrows/middens) (Eisenhauer 2010). Anecic species have also been suggested to have positive effects on microarthropods in mineral soil due to incorporation of organic material or stimulation of soil microbial activity (Eisenhauer 2010). As we did not examine microarthropod abundance at either the microhabitat scale or within the mineral soil, it is unclear whether such positive impacts occurred in our study.

Oribatida and Astigmata/Prostigmata experienced the greatest reductions in abundance due to earthworms, while Collembola and Mesostigmata did not decrease significantly in our experiment. As predators, Mesostigmata may be less affected than detritivores and microbivores by competition or loss of microbial food resources due to earthworms (Salmon et al. 2005; Eisenhauer 2010). It is also possible that effects might later cascade to the predatory groups if examined over a longer time period. However, members of each of the other microarthropod taxa vary widely in their diets and many presumed detritivores have been shown to avidly consume nematodes in the laboratory (Oliveira et al. 2007). Therefore, examination at a finer level of taxonomic resolution, together with more research on microarthropod diets, is needed to more clearly assess whether earthworms have a stronger effect on detritivores than predators.

Abiotic and biotic disturbances appear to be the main forces structuring oribatid assemblages, and the low reproductive rates of most oribatid species may result in slow recovery from such disturbances (Maraun & Scheu 2000). Consequently, as ecosystem engineers, earthworms may have strong effects on oribatid assemblage structure. However, our treatments had varying effects on individual oribatid taxa. *Suctobelbella* and *Tectocepheus* were the genera most negatively affected by the presence of earthworms. This is similar to previous research in which abundance of *Suctobelbella* was negatively correlated with *D. octaedra* biomass in a pine forest (McLean & Parkinson 2000) and in which lower abundance of *Tectocepheus* occurred in hardwood forest stands with earthworms (Burke et al. 2011). In Alberta, species of both genera are mostly very small-bodied, parthenogenetic mites that show no clear gut contents when slide-mounted, which suggests that they feed on bacteria and/or yeast (D. E. Walter, personal communication). *Phthiracarus* was also negatively affected by epi-endogeic, endogeic, and anecic earthworms in the Burke et al. (2011) study, but in our experiment *Phthiracarus* individuals were more abundant in the presence of the epigeic *D. octaedra*. Juvenile *Phthiracarus* are often found burrowing inside conifer needles and small twigs, and adult gut contents include hyphae (Walter et al. 2011; D.E. Walter, personal communication). Although it has been proposed that smaller oribatid species are more likely to be negatively impacted by earthworms (McLean & Parkinson 2000), our data do not show a clear pattern with respect to size. The species of *Phthiracarus* and *Tectocepheus* commonly found in

Alberta are larger than *Quadroppia*, which had a positive association with *L. terrestris*, and are also larger than most species of *Suctobelbella*, which had a higher abundance in our control treatment (Walter 2011; Walter et al. 2011). It is difficult to propose a biological rationale for this sensitivity, as the biology of *Suctobelbella* species in Alberta is not known (D.E. Walter, personal communication).

Our study demonstrates that earthworms can cause large decreases in microarthropod abundance, as well as shifts in oribatid community composition. Although endogeic and anecic earthworms often have stronger effects on both the physical environment and on other organisms than smaller epigeic species (Gundale 2002; Hale et al. 2008), in our experiment the anecic *L. terrestris* did not have a larger impact on microarthropods than the epigeic *D. octaedra*. This suggests that epigeic species should not be overlooked in assessments of the impacts of earthworm invasions. Further research should investigate whether earthworm effects on microarthropods result in cascading changes to litter decomposition, nutrient cycling, or plant growth. Additional information on the combined effects of earthworm species is also needed to assess whether different earthworm assemblages have antagonistic, additive, or synergistic impacts on microarthropods.

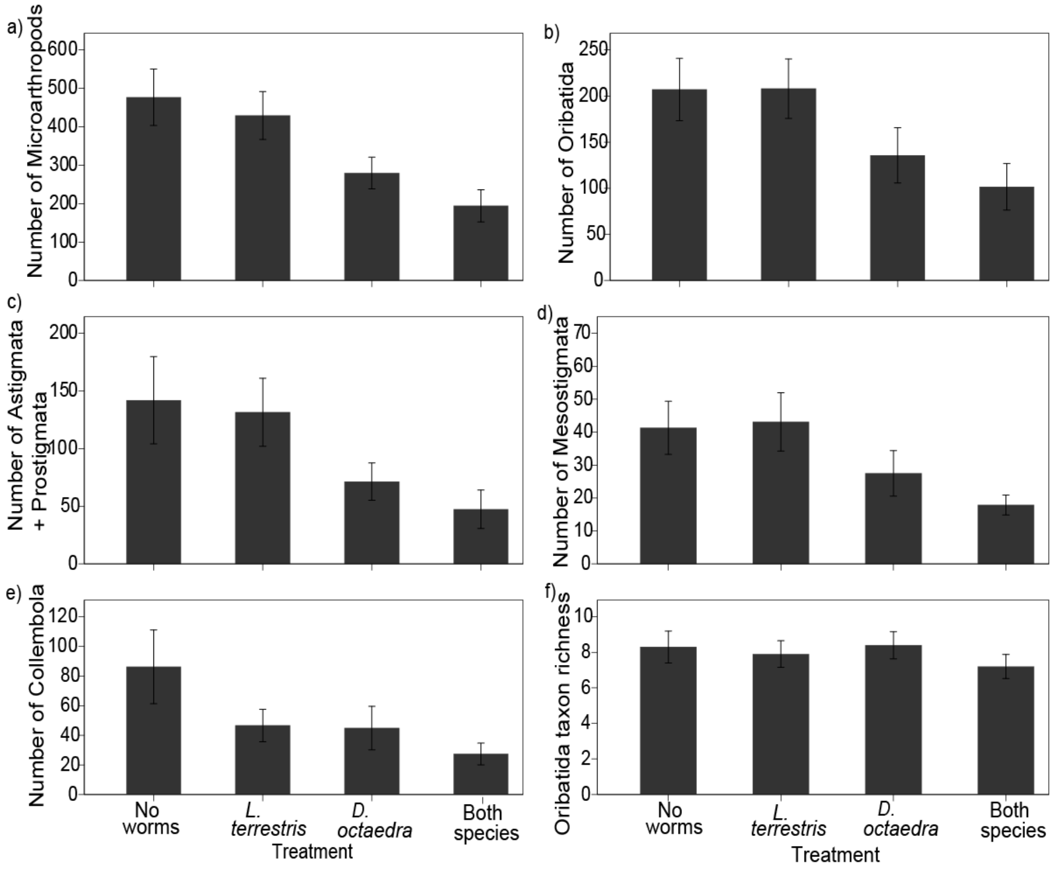

Figure 3.1. a) Mean abundance of microarthropods; b) Oribatida mean abundance; c) Astigmata+Prostigmata mean abundance; d) Mesostigmata mean abundance; e) Collembola mean abundance; and f) Oribatida taxon richness in the four treatments (no earthworms, *Lumbricus terrestris*, *Dendrobaena octaedra*, both earthworm species together). N = 10 replicates per treatment. Error bars represent ± 1 SE.

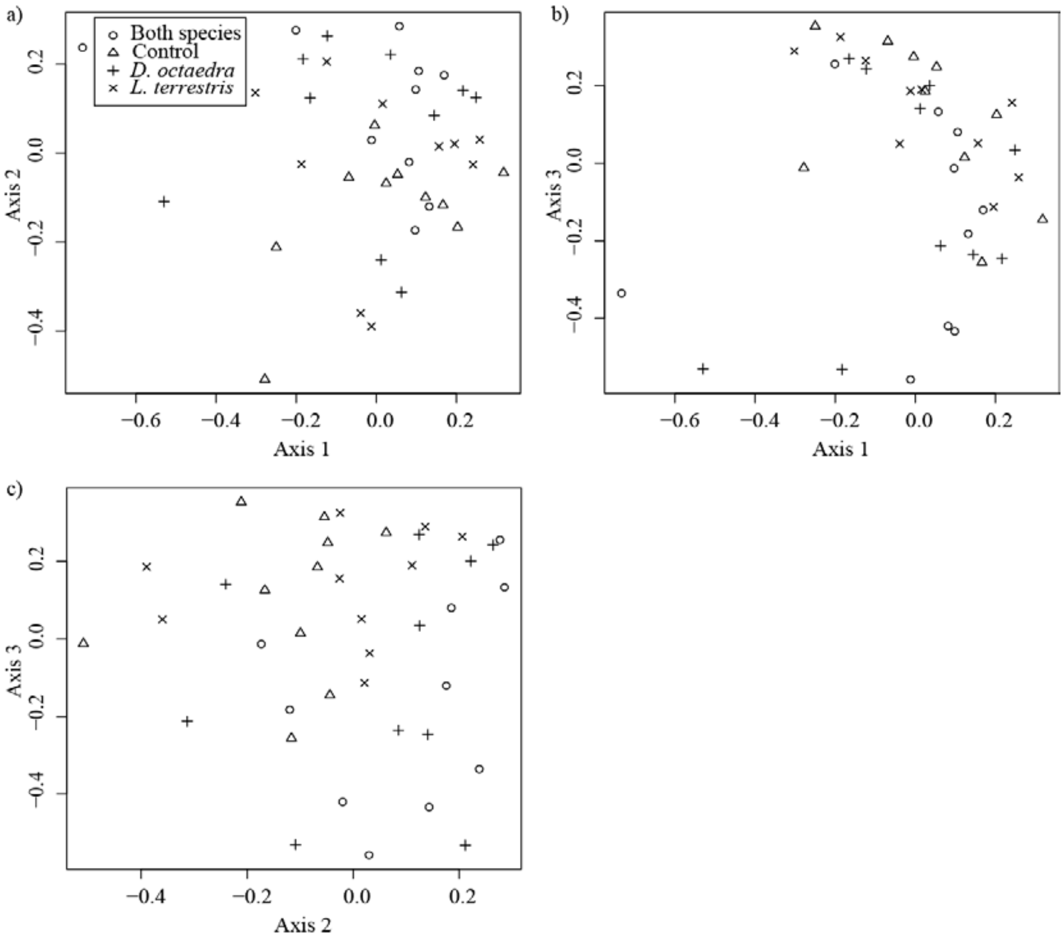

Figure 3.2. Nonmetric multidimensional scaling (NMDS) plots of replicates based on oribatid taxa. Symbols represent the four treatments: ○ = both species, Δ = control, + = *Dendrobaena octaedra* only, and x = *Lumbricus terrestris* only. a) Plot of ordination axes 1 and 2; b) plot of ordination axes 1 and 3; and c) plot of ordination axes 2 and 3.

Table 3.1. Mean abundances (±1 SE) of Oribatida taxa in the four treatments (no earthworms, *Lumbricus terrestris* alone, *Dendrobaena octaedra* alone, and both earthworm species together). N = 10 replicates/treatment.

Family	Genus	No earthworms	*L. terrestris*	*D. octaedra*	Both species
Achipteriidae	*Achipteria*	0 (0)	0.5 (0.40)	0.1 (0.10)	0.1 (0.10)
Brachythoniidae	-	60.3 (22.68)	48.2 (14.43)	19.8 (8.33)	10.6 (4.19)
Camisiidae	*Platynothrus*	0 (0)	0.1 (0.10)	0 (0)	0.1 (0.10)
Cepheidae	*Oribatodes*	0 (0)	0 (0)	0.1 (0.10)	0 (0)
Ceratozetidae	*Ceratozetes*	4.6 (1.70)	2.9 (0.67)	1.6 (0.56)	3.3 (0.96)
Chamobatidae	*Chamobates*	1.2 (0.61)	0.7 (0.33)	0.8 (0.29)	1.5 (0.43)
Damaeidae	*Epidamaeus*	0.1 (0.10)	0 (0)	0 (0)	0.1 (0.10)
Euphthiracaridae	*Eupthiracarus*	0.8 (0.33)	0.8 (0.39)	0.6 (0.34)	1.0 (0.45)
Galumnidae	*Pergalumna*	0.1 (0.10)	0.1 (0.10)	0 (0)	0 (0)
Galumnidae	*Pilogalumna*	0.6 (0.27)	0.1 (0.10)	1.0 (0.68)	0.2 (0.13)
Gymnodamaeidae	*Gymnodamaeus*	0.1 (0.10)	0.4 (0.27)	0.4 (0.22)	0 (0)
Haplozetidae	*Peloribates*	0 (0)	0 (0)	0.1 (0.10)	0 (0)
Hermanniellidae	*Hermanniella*	0 (0)	0 (0)	0.1 (0.1)	0 (0)
Mycobatidae	*Mycobates*	0 (0)	0 (0)	0 (0)	0.1 (0.1)
Nanhermannidae	*Nanhermannia*	0 (0)	0 (0)	0.3 (0.21)	0 (0)
Oppiidae	*Oppiella*	82.5 (14.68)	116.7 (28.17)	82.5 (24.42)	69.0 (22.44)
Oribatulidae	*Oribatula*	0.4 (0.27)	0 (0)	0 (0)	0 (0)
Oribatulidae	*Zygoribatula*	0 (0)	0 (0)	0.2 (0.13)	0 (0)
Parakalummidae	*Neoribates*	0 (0)	0.1 (0.10)	0 (0)	0 (0)
Phenopelopidae	*Propelops*	1.0 (0.37)	0.7 (0.26)	1.5 (0.70)	0.5 (0.22)
Phthiracaridae	*Phthiracarus*	3.2 (1.71)	1.0 (0.52)	6.2 (1.97)	1.8 (0.81)
Quadroppidae	*Quadroppia*	0.3 (0.15)	3.5 (1.92)	0.3 (0.30)	0.6 (0.34)
Scheloribatidae	*Scheloribates*	0.3 (0.21)	0 (0)	0.3 (0.21)	0 (0)
Suctobelbidae	*Suctobelbella*	45.1 (13.54)	29.4 (4.51)	18.5 (4.34)	11.9 (3.01)
Tectocepheidae	*Tectocepheus*	6.6 (2.45)	3.0 (1.17)	1.4 (0.40)	0.8 (0.33)

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CHAPTER 4. EARTHWORM EFFECTS ON BELOW-GROUND MOVEMENT OF MICROARTHROPODS[4]

4.1 Introduction

Changes in habitat structure due to ecosystem engineering can influence resource availability, species' abundances, and community composition (Jones et al. 1994, 1997; Badano et al. 2006). Movement of organisms is another key factor that may be affected by engineers, although it has been poorly studied. For example, post-larval dispersal of some macrofauna is greater in beds of the mussel *Mytilus edulis* L. as compared to in bare sediment patches, where turbulence and sediment flux are lower (Commito et al. 2005). Modeling suggests that ecosystem engineers can even cause increases in their own rate of spread because of their ability to alter habitat structure (Cuddington & Hastings 2004). They may also decrease movement, as in the case of shrubs in Mediterranean woodland that act as a physical obstacle to seed dispersal and thereby affect herbaceous plant communities (Gabay et al. 2012).

The effects of ecosystem engineering on movement may be particularly important below-ground, where permeability of the soil matrix limits the active movement of many groups (Dighton et al. 1997; Ettema & Wardle 2002). Below-ground movement is estimated to be approximately four times slower than aboveground locomotion for some microarthropods (Kaczmarek 1978). However, burrowing by soil macrofauna can increase porosity and create openings that might facilitate the movement of other organisms (Dighton et al. 1997).

Earthworms are frequently cited examples of below-ground ecosystem engineers because of their large impacts on soil structure, which can lead to cascading effects on other species. For example, earthworms and their burrowing activity can facilitate the movement and germination of giant ragweed (*Ambrosia trifida* L.) seeds (Regnier et al. 2008). In their native and introduced ranges, earthworms have been observed to significantly affect soil microarthropods (Chapter 3; Cameron et al. 2013; McLean & Parkinson 1998; Migge-Kleian et al. 2006; Eisenhauer 2010). Both positive and negative effects on microarthropod

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abundance and species richness have been reported. Impacts seem to depend on the density of earthworms, their ecological group, and the microarthropod taxa being examined. For example, microarthropods that are mainly detritivorous or microbivorous (Collembola, Astigmata, Oribatida) have been suggested to be more negatively affected than the predatory Mesostigmata due to competition or to greater disruption of their food resources by earthworms (Salmon et al. 2005; Eisenhauer 2010). Earthworms can also have variable effects on microarthropod distributions due to their effects on physicochemical properties of soil, with microarthropods being attracted to earthworms and their excreta in some cases (Salmon & Ponge 1999; Salmon 2001; Gutiérrez López et al. 2011) but not in others (Salmon 2001; Gutiérrez López et al. 2009; Gutiérrez López et al. 2011). These responses appear to vary depending on the earthworm species and microarthropod species involved (Gutiérrez López et al. 2011).

One of the ways in which earthworms are thought to affect microarthropods is by altering their rate of movement (Salmon 2004; Salmon et al. 2005; Eisenhauer 2010). Some microarthropods can disperse above-ground via wind, water, phoresy, or walking (Mitchell 1970), but their movement below-ground has been less well studied. Earthworms may influence microarthropod movement via a number of mechanisms. Firstly, earthworm burrows have been suggested to act as corridors for movement of microarthropods by forming an interconnected network of pores within the soil that are easier to move through (Salmon 2004; Salmon et al. 2005; Eisenhauer 2010). Secondly, microarthropods may be attracted to burrows by the earthworm secretions and excreta (mucus, castings, and urine) that line burrow walls (Salmon & Ponge 1999; Salmon 2004) and may subsequently move through the burrows in the pursuit of microbial food resources present there (Salmon & Ponge 1999). Thirdly, earthworms themselves may transport microarthropods via phoresy, as appears to occur with nematodes (Shapiro et al. 1995; MacMillan et al. 2009). Finally, earthworms could consume some microarthropod taxa or negatively affect them via disturbance of the soil or competition for microbial food resources, thereby causing a reduction in movement and abundance (McLean & Parkinson 1998, 2000; Eisenhauer 2010).

Here we examine the effects of an epi-endogeic earthworm (*Lumbricus rubellus* Hoffmeister, 1843) on movement of microarthropods in boreal forest soil using a microcosm experiment. *Lumbricus rubellus* lives and feeds in the upper soil layers and is a common invader in Canada and many other countries (Reynolds 1994; Hendrix et al. 2008). Our experiment tested whether movement of springtails and mites is affected by the presence of tunnels in the soil, of tunnels lined with excreta, or of earthworms themselves. We predicted microarthropod movement would be greater when there were tunnels in the soil, particularly for Mesostigmata and Collembola, which typically have a larger body size than other microarthropod taxa and hence may be more limited by soil porosity than smaller-bodied taxa (Edwards 1967; Vreeken-Buijs et al. 1998). Variation in the impacts of earthworms on movement of microarthropod taxa is one factor that could explain changes in community composition of microarthropods in response to earthworms reported in previous studies.

4.2 Methods

4.2.1 Experimental design

We used a short-term microcosm experiment to investigate the effects of non-native earthworms on microarthropod movement. Each microcosm consisted of an open-ended 10 cm x 4 cm x 4 cm box with one 750 mL (~12.5 cm height x 9 cm diameter) opaque plastic container and one 120 mL (~5 cm height x 5.5 cm diameter) translucent plastic container attached to either end (Figure 4.1a). The 750 mL container acted as a “source” of microarthropods and the 120 mL container as a “destination”. The sides of the plastic containers were cut where the box was attached to allow movement of microarthropods and earthworms across the microcosm. The bottom and sides of each box were constructed from 0.6 cm thick plywood, while the top was 0.3 cm thick acrylic plastic. We drilled two 0.5 cm diameter screw holes in the bottom and top of each box and bolted the top down after the box was filled with soil to limit movement of microarthropods on the soil surface.

Soil was collected from the organic horizon (H layer) of a trembling aspen (*Populus tremuloides* Michx.)/balsam poplar (*Populus balsamifera* L.) forest in the North Saskatchewan River valley in Edmonton, Alberta (53°32’ N 113°33’ W). Earthworms were also collected from this forested area. The soil used to fill the boxes and 120 mL destination containers was sterilized by autoclaving for 1 hour at 121°C. We mixed unsterilized soil and distilled water and sieved the slurry through a 36 µm sieve to obtain water containing microbes but no microarthropods.

After allowing the autoclaved soil to cool, the sieved liquid was added to it as a food source for the earthworms and microarthropods. The 750 mL containers were filled with unsterilized soil and acted as the microarthropod source.

Two days before the start of the experiment, we filled the unattached boxes with sterilized soil and set up the treatments within them (Figure 4.1b). Our experiment included four treatments: 1) a “control” treatment with no earthworms and no tunnels; 2) an “artificial burrow” treatment with no earthworms but two tunnels running the length of the box made by a 0.6 cm thick wooden dowel; 3) an “abandoned burrow” treatment in which two tunnels were made using the dowel and one earthworm was introduced into each tunnel for the 30 hour period immediately prior to the experiment to lay down excreta, and then removed; and 4) an “earthworms present” treatment in which earthworms were introduced into two dowel-made tunnels 30 hours before the experiment began and remained in the microcosms during the experiment. The width of the dowel was approximately equal to the body width of the *L. rubellus* individuals used in the experiment. Although our dowel-made tunnels likely did not mimic the sinuosity of real earthworm burrows, we chose to create the tunnels in this way for all treatments in order to ensure that they were similar lengths across all replicates. For all treatments, the ends of the boxes were capped with aluminum foil that was secured with elastic bands until the microcosms were assembled. Earthworms were washed with distilled water before being added to the boxes. For the abandoned burrow and earthworms present treatments, the earthworms were removed from the boxes after 30 hours by shining a bright light at one end of the box and placing a dark cloth over the other end or by using electric shock as needed. The electric shock was administered to one end of the box using wall current. In the earthworms present treatment, the earthworms were then placed into the source container that was attached to the same box they had been removed from. This was done to test whether microarthropods might travel from the source container to the 120 mL destination container using the earthworms as phoretic hosts. Nothing was used to attract earthworms to the destination containers but, when we removed the containers for sampling, an earthworm was present in the destination container in nine of the twenty-four microcosms in the earthworm treatment.

The experiment was carried out over eight days in October 2011 in a growth chamber located at the University of Alberta with settings of 18°C, relative humidity of 40%, and day length of 14 hours. The eight day time period was chosen to reduce the possibility that reproduction of microarthropods would occur in the destination containers and affect abundances, as we were interested primarily in movement. We randomly assigned the source containers to the treatment boxes and placed the microcosms randomly within the growth chamber. Pin holes were made in the lids of the plastic containers for ventilation, as the lids were closed to prevent escape of microarthropods and earthworms. All microcosms were misted daily to maintain moist conditions.

4.2.2 Microarthropod sampling

We examined both the total number of microarthropods that dispersed by the end of the experiment and the cumulative movement of microarthropods over time. Each of the four treatments had 24 replicate microcosms, with 12 replicates sampled only at the end of the experiment and 12 replicates sampled at multiple times to investigate movement over time. At each sampling time, we extracted microarthropods from the soil in the destination containers (extraction method described below). For the replicates in which the 120 mL destination container was not removed until the end of the experiment (192 hours), there were a total of 48 extractions (4 treatments x 12 replicates). This provided cumulative numbers of dispersers over 8 days. For the replicates sampled at multiple times, the destination container was removed from the source container at 4 h, 12 h, 24 h, 48 h, 96 h, and 192 h after the start of the experiment and immediately replaced with a new destination container filled with autoclaved soil (except following removal of the 192 h container at the end of the experiment). Thus, for example, the destination containers removed at 192 h would have had 96 h between the time of their attachment and removal to accumulate dispersing microarthropods, since the previous samples were removed at 96 h. A total of 288 extractions (4 treatments x 12 replicates x 6 time steps) were performed for this analysis and these repeated samples were used to determine whether there was an effect of treatment on rates of movement over time.

At each time step, we also extracted microarthropods from an additional 120 mL sample of autoclaved soil that was not attached to a box or source container to verify that the autoclaving had indeed sterilized the soil of microarthropods. At the end of the experiment, we extracted a 120 ml sample from each of the 96 750 mL source containers.

Microarthropods were live-extracted at the Royal Alberta Museum in Edmonton using Tullgren funnels (Macfadyen 1961). All extractions ran for 7 days and microarthropods were preserved in 80% ethanol. Microarthropods were sorted into non-astigmatan Oribatida, Astigmata, Prostigmata, Mesostigmata, and Collembola. Because there were low numbers of similarly sized (very small) Prostigmata and Astigmata, they were grouped together for statistical analyses. The mites were not identified to finer taxonomic levels because there was inconsistent taxonomic representation among the samples. The majority of Collembola belonged to the Onychiuridae, although representatives of Entomobryidae, Isotomidae, and Hypogastruridae were present as well.

4.2.3 Statistical analysis

We assessed the effects of our treatments on the total number of microarthropods dispersing into the destination container using Poisson or negative binomial regression in Stata 9.1 (Stata-Corp, College Station, Texas) depending on the distribution that best fit the data for each group. Negative binomial regression was used when data were overdispersed. Analyses were conducted for Collembola, Mesostigmata, Oribatida, Prostigmata+Astigmata, and all microarthropods together. There were 24 replicates per treatment in all of the models examined, for a total sample size of 96. In 12 of the replicates in each treatment, microarthropods were extracted at six time steps (4 h, 12 h, 24 h, 48 h, 96 h, 192 h) and the total number of microarthropods across all time steps was used in the analysis. Because microarthropod extraction occurred only at the end of the experiment in the remaining 12 replicates per treatment, all models included a variable to account for whether multiple or single extractions were performed for a given replicate microcosm. AIC_c (Akaike's information criterion, corrected for small sample sizes) was used to assess support for three competing models explaining microarthropod abundance in the destination container. AIC_c estimates the relative distance between a model and the mechanism that generated the observed data (Burnham & Anderson 2002). It relies on information theory and the principle of parsimony to provide a weight of evidence for different hypotheses. We considered models with $\Delta AIC_c<2$ to be plausible (Burnham & Anderson 2002).

The models examined were the presence of tunnels (earthworms present, abandoned burrow, and artificial burrow treatments versus the control treatment), the presence of earthworms during the experiment (earthworms present treatment versus abandoned burrow, artificial burrow, and control treatments), and the presence of earthworm castings/mucus (earthworms present and abandoned burrow treatments versus artificial burrow and control treatments). Negative binomial regression was also used to test whether abundances of taxa differed among the source containers.

We also investigated whether our treatments influenced the cumulative total number of microarthropods dispersing over time using Poisson and negative binomial regression. This separate analysis had a sample size of 48 (12 replicates per treatment) for all models because time series data were only available for the replicates from which multiple samples were taken. A random effect was included in all models to account for the lack of independence between multiple samples taken from the same replicate over time. AIC_c was again used to rank three competing models (presence of tunnels, presence of earthworms during the experiment, and presence of earthworm castings/mucus). We examined support for these models with time and treatment as predictor variables, as well as with an interaction between time and treatment to assess whether rates of movement varied between treatments.

4.3 Results

4.3.1 Abundance

A total of 2482 microarthropods were extracted from the destination containers, including 2186 Collembola, 126 Mesostigmata, 139 Oribatida, 27 Prostigmata, and 4 Astigmata. No microarthropods were recovered from the autoclaved soil samples that were not attached to source containers, confirming that the destination containers were indeed free of microarthropods at the beginning of the experiment. Oribatida abundance fit a Poisson distribution, while all other taxa showed evidence of overdispersion and therefore negative binomial regression was used in those cases. According to the ΔAIC_c scores, the tunnel model was the best supported and most parsimonious model for explaining the abundances of Collembola, Mesostigmata and all microarthropods together, although the global model was also well supported for all microarthropods and Collembola (Figure

4.2a-e; Table 4.1). For Oribatida abundance, the earthworm presence (ΔAIC_c = 0.00), global (ΔAIC_c = 0.20), and null models (ΔAIC_c = 1.79) received the most support. The null model was the best supported model for Prostigmata+Astigmata, but the worm, castings, and tunnels models were also well supported with ΔAIC_c scores less than 2. In the source container samples, the abundance of each taxon did not differ significantly across treatments (Figure 4.2f; Collembola χ^2 = 1.85, P = 0.604; Mesostigmata χ^2 = 1.87, P = 0.599; Oribatida χ^2 = 0.970, P = 0.809; Prostigmata+Astigmata χ^2 = 2.11, P = 0.551).

4.3.2 Cumulative movement over time

Some individuals crossed the 10 cm treatment box and reached the destination containers within the first time step (4 hours) in the artificial burrow and earthworm present treatments for Mesostigmata and in all treatments for Oribatida and Collembola (Figure 4.3). Therefore, the maximum movement rate for these taxa was 2.5 cm/hour. The first Prostigmata+Astigmata individuals dispersed within 12 hours of the start of the experiment in the artificial burrow treatment, resulting in a maximum movement rate of 0.83 cm/hour. Movement was slower in the control treatment for Mesostigmata and Prostigmata+Astigmata, with the first individuals arriving within 48 hours and 24 hours, respectively. Oribatida and Mesostigmata abundances were analyzed using Poisson regression and negative binomial regression was used for the remaining taxa. For all microarthropods together, the global model that included interactions between time and each of the other variables (tunnel, earthworm, and excreta) was the best supported model explaining cumulative movement over time (Table 4.2). The tunnel model with no interaction received the most support for Collembola. For Mesostigmata, the tunnel (ΔAIC_c = 0.00), tunnel interaction (ΔAIC_c = 1.10), and global (ΔAIC_c = 1.92) models were best supported. The tunnel (ΔAIC_c = 0.00), global (ΔAIC_c = 0.052), tunnel interaction (ΔAIC_c = 0.34), and earthworm models (ΔAIC_c = 0.42) were the best models for Oribatida. There were too few Prostigmata+Astigmata to allow analysis of their movement over time. Assuming there was no reproduction in the source containers for any of the microarthropod groups, a disproportionately smaller number of Oribatida moved to the destination containers (about 1/10th of the totals per treatment) than for the other groups (about 1/3-1/2 of the totals) (Figure 4.2f versus Figure 4.2b-e).

4.4 Discussion

Our results suggest that activity of *L. rubellus* earthworms can facilitate microarthropod movement. Artificial tunnels in the soil increased the total number of individuals dispersing for several groups including Collembola, Mesostigmata and all microarthropods together, while the presence of earthworm excreta and earthworms themselves resulted in little additional increase. Cumulative movement over time for all microarthropods together was affected by the presence of tunnels and excreta in addition to *L. rubellus*. Abundances of Oribatida and Prostigmata/Astigmata in the destination containers were not well explained by any of the variables considered, with the null models receiving the most support. However, the rate of accumulation of Oribatida over time was influenced by both tunnels and earthworms. Movement of oribatid mites was greater in the presence of tunnels, while presence of earthworms was associated with reduced movement.

Although little is known about below-ground dispersal of microarthropods, corridors have been demonstrated to facilitate above-ground dispersal in moss microcosms (Gilbert et al. 1998; Gonzalez et al. 1998). Increased movement of microarthropods occurred when moss patches were connected by thin corridors of moss, particularly when the rate of emigration to the hostile surrounding matrix is low (i.e., when movement is biased to occur along the moss corridors) (Hoyle 2007). Our results are also consistent with several studies in which microarthropod abundance increased with soil pore volume (Vreeken-Buijs et al. 1998; Ducarme et al. 2004; Nielsen et al. 2008). Increased access to resources and reduced predation or competition due to greater availability of refuges have been suggested as mechanisms by which soil pore volume could lead to increased microarthropod abundances (Nielsen et al. 2008). The greater soil heterogeneity created by the tunnels in our experiment might additionally contribute to increases in diversity (Wardle 2002).

The maximum rates of movement observed in our study, even within the control treatment, are much higher than previously recorded for microarthropods within soil in the field (Sjogren 1997; Ojala & Huhta 2001). The highest potential rate of movement recorded previously was approximately 20 cm per week for some genera of Oribatida and Collembola (Ojala & Huhta 2001), in contrast to our maximum estimated rates of 2.5 cm per hour (= 420 cm/week) for Collembola, Mesostigmata, and Oribatida and 0.83 cm per hour for Prostigmata/Astigmata (=

210 cm/week). Converting from hours to weeks undoubtedly leads to overestimation of spread rates as it is unlikely that microarthropods would continue to move in the same direction for a week. The fact that movement could only occur along the box also may have resulted in greater estimates of movement speed than would be the case in a natural system, where movement could occur in any direction. The straightness and artificial nature of the tunnels might also have resulted in greater movement rates than would be observed along burrows. Additionally, soil type can have large impacts on movement rates in nematodes (MacMillan et al. 2009). If soil type has similar effects on microarthropod movement, it could account for some variation among studies. Nonetheless, the substantially greater rates of movement in our experiment suggest maximum movement rates may be much larger than previously estimated when movement is highly directional.

Microarthropod taxa can exhibit differing responses to soil pores or corridors (Nielsen et al. 2008). As predicted, artificial tunnels had a stronger effect on movement of Mesostigmata and Collembola which tend to have larger body sizes than Oribatida, Prostigmata, and Astigmata (Edwards 1967; Vreeken-Buijs et al. 1998). Large soil pores may be particularly beneficial for taxa that are larger and cannot move as easily through the soil matrix (Vreeken-Buijs et al. 1998). These varying effects on movement of different microarthropod groups may result in altered predator-prey dynamics or competition among microarthropods and could ultimately lead to shifts in community assembly. We observed that a disproportionately smaller number of Oribatida moved to the destination containers than did the other groups (Fig. 4.2f versus 4.2b-e), resulting in a different composition of taxa in the destination containers than in the source containers; however, the treatments themselves did not seem to have a strong influence on the relative proportions of the different taxa remaining in the source containers. A more detailed analysis would be necessary to assess whether other traits besides body size (e.g., diet) can influence the responses of microarthropod taxa to tunnels.

Our artificial and abandoned burrow treatments were designed to mimic the physical changes that occur in the structure of the soil due to earthworm burrowing but not the ongoing disturbance of the soil caused by earthworms burrowing continually. Continual disturbances can be a key factor structuring microarthropod communities, and oribatid mite populations are thought to be particularly sensitive

(Maraun & Scheu 2000; Maraun et al. 2003). For example, repeated sieving and mixing of litter and soil resulted in declines in densities of most groups of oribatid mites, as well as Collembola, in a beech forest (Maraun et al. 2003). It was therefore suggested that high densities of microarthropods in some soils could be related to less mechanical disturbance by earthworms (Maraun et al. 2003). Although we found a lower cumulative number of Oribatida dispersing over time when *L. rubellus* were present in the microcosms, the presence of earthworms did not have a strong effect on the abundance of any taxa in the destination containers. However, the short duration of our experiment and the disruption of the soil during transport and construction of our microcosms may have reduced our ability to detect negative effects of earthworms per se. How earthworm effects on movement rates influence microarthropod communities over the long-term warrants further investigation.

Our study suggests that the effects of ecosystem engineers on habitat structure may strongly affect movement of other species. Movement of microarthropods was greatest when artificial tunnels were present in the soil indicating that changes in soil structure, rather than phoresy or changes in nutrient distribution, may influence microarthropod movement belowground. The stronger influence of *L. rubellus* on movement of Collembola and Mesostigmata, as compared to Oribatida, Prostigmata, and Astigmata, suggests that alteration of movement routes is a possible mechanism driving the variable responses of different microarthropod taxa to earthworms. Little is known about movement below-ground and the effects of edges and corridors on soil organisms, despite the extensive amount of research in this area in above-ground systems. Consequently, future research should further examine effects of soil structure on soil organisms, including the impacts of other earthworm species on microarthropods.

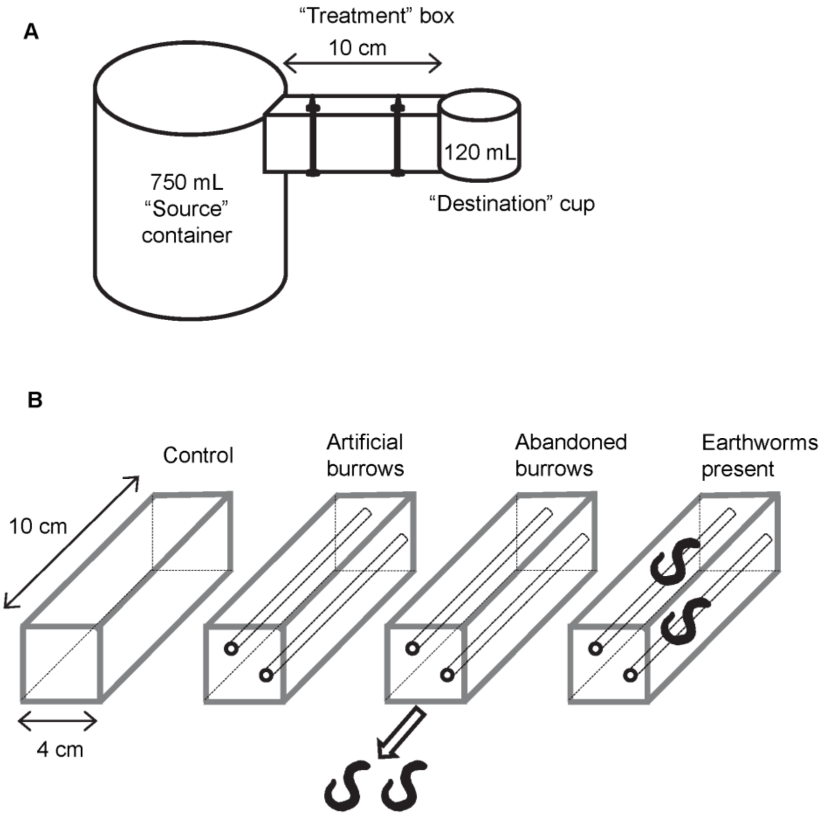

Figure 4.1. Experimental design. a) A microcosm consisting of a 750 mL "source" container, a 10 cm long "treatment" box in which the treatments were implemented, and a 120 mL "destination" container; b) The four treatments within the boxes, including the control treatment with no earthworms, the artificial burrows treatment with two tunnels made by a dowel, the abandoned burrows treatment in which earthworms were removed before the experiment, and the earthworms present treatment in which earthworms were present throughout the experiment.

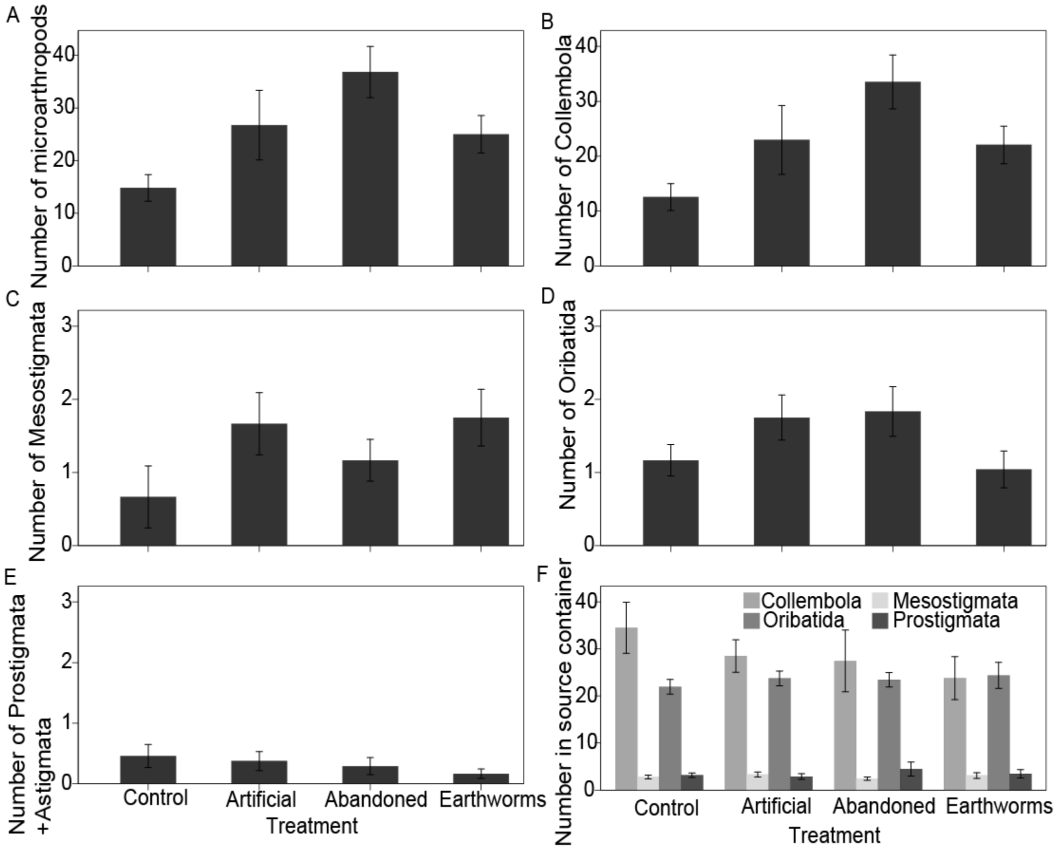

Figure 4.2. Total numbers of microarthropods in destination containers at the end of the experiment in the control, artificial, abandoned, and earthworm treatments. a) All microarthropods together (± 1 SE) in the destination containers; b) Collembola in the destination containers; c) Mesostigmata in the destination containers; d) Oribatida in the destination containers; e) Prostigmata+Astigmata in the destination containers; and f) Collembola, Mesostigmata, Oribatida, and Prostigmata+Astigmata in a 120 mL sample from the source containers. N = 24 replicates per treatment.

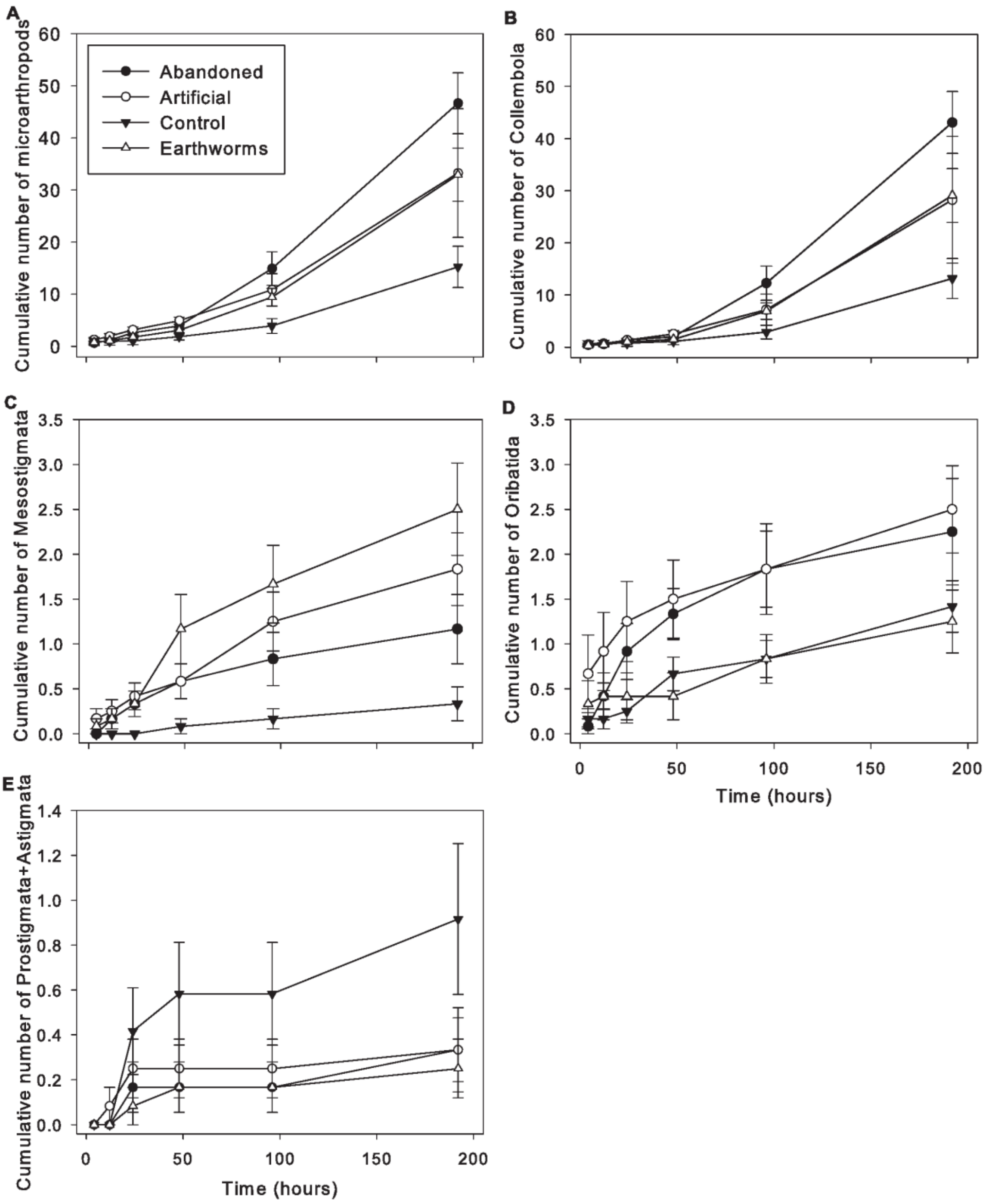

Figure 4.3. Cumulative number of microarthropods dispersing over time (hours) in the abandoned, artificial, control, and earthworm treatments. a) All microarthopods together (± 1 SE); b) Collembola; c) Mesostigmata; d) Oribatida; and e) Prostigmata+Astigmata. N = 12 replicates per treatment.

Table 4.1. Regression fit statistics for models predicting microarthropod abundance.

Taxa	**Model**	***K***	**LL**	**ΔAIC$_c$**	***w*AIC$_c$**
Microarthropods	**Tunnels**	**4**	**-393.80**	**0.00**	**0.52**
	Global	**6**	**-391.68**	**0.26**	**0.45**
	Castings	4	-396.85	6.09	0.02
	Null	3	-399.67	9.55	0.00
	Worms	4	-399.59	11.59	0.00
Collembola	**Tunnels**	**4**	**-387.89**	**0.00**	**0.53**
	Global	**6**	**-385.94**	**0.59**	**0.40**
	Castings	4	-390.15	4.51	0.06
	Null	3	-392.91	7.84	0.01
	Worms	4	-392.85	9.92	0.00
Mesostigmata	**Tunnels**	**4**	**-148.57**	**0.00**	**0.53**
	Null	3	-150.71	2.11	0.18
	Worms	4	-150.09	3.05	0.12
	Global	6	-148.04	3.43	0.09
	Castings	4	-150.50	3.85	0.08
Oribatida	**Worms**	**3**	**-147.68**	**0.00**	**0.36**
	Global	**5**	**-145.58**	**0.20**	**0.32**
	Null	**2**	**-149.64**	**1.79**	**0.15**
	Tunnels	3	-148.72	2.09	0.13
	Castings	3	-149.64	3.91	0.05
Prostigmata+Astigmata	**Null**	**3**	**-68.34**	**0.00**	**0.31**
	Worms	**4**	**-67.33**	**0.16**	**0.28**
	Castings	**4**	**-67.51**	**0.52**	**0.24**
	Tunnels	**4**	**-68.02**	**1.56**	**0.14**
	Global	6	-67.14	4.29	0.04

Predictors included presence of tunnels (*Tunnels*), earthworm excreta (*Castings*), and earthworms (*Worms*). All models included a variable to account for whether microarthropods were extracted from a replicate at multiple times versus only once at the end of the experiment. The best model has a ΔAIC$_c$ of zero and the highest *w*AIC$_c$ value. Models with ΔAIC$_c$<2 are also considered to be plausible and are shown in bold. With *k*, number of parameters; LL, log likelihood; ΔAIC$_c$, difference in the Akaike’s information criterion (corrected for small sample size) value between model and the most strongly supported model; *w*AIC$_c$, weight given by the AIC (i.e., relative strength of support for model).

Table 4.2. Regression fit statistics for models of microarthropod abundance over time.

Taxa	**Model**	***K***	**LL**	**ΔAIC_c**	**$wAIC_c$**
Microarthropods	**Global*Time**	**1**	**-682.19**	**0**	**0.68**
	Tunnels*Time	6	-690.02	2.91	0.16
	Tunnels	5	-690.30	3.48	0.12
	Global	7	-689.62	6.31	0.03
	Castings*Time	6	-690.48	8.03	0.01
	Castings	5	-696.92	16.73	0.00
	Worms	5	-701.08	25.05	0.00
	Worms*Time	6	-700.02	27.09	0.00
	Null	3	-869.92	358.60	0.00
Collembola	**Tunnels**	**5**	**-579.95**	**0**	**0.49**
	Tunnels*Time	6	-579.95	2.08	0.17
	Global*Time	1	-575.72	2.12	0.17
	Global	7	-578.97	2.22	0.16
	Castings	5	-587.58	15.26	0.00
	Worms	5	-592.86	25.81	0.00
	Castings*Time	6	-584.65	11.49	0.00
	Worms*Time	6	-592.13	26.45	0.00
	Null	3	-748.20	332.37	0.00
Mesostigmata	**Tunnels**	**4**	**-224.48**	**0**	**0.50**
	Tunnels*Time	**5**	**-224.00**	**1.10**	**0.29**
	Global	**6**	**-223.36**	**1.92**	**0.19**
	Global*Time	9	-222.71	6.96	0.02
	Worms	4	-229.88	10.80	0.00
	Castings	4	-230.55	12.14	0.00
	Worms*Time	5	-229.81	12.73	0.00
	Castings*Time	5	-230.55	14.20	0.00
	Null	2	-280.15	107.24	0.00
Oribatida	**Tunnels**	**4**	**-303.43**	**0**	**0.22**
	Global	**6**	**-301.37**	**0.052**	**0.21**
	Tunnels*Time	**5**	**-302.56**	**0.34**	**0.18**
	Worms	**4**	**-303.63**	**0.42**	**0.18**
	Castings	4	-304.55	2.26	0.07
	Worms*Time	5	-303.63	2.48	0.06
	Global*Time	9	-299.79	3.23	0.04
	Castings*Time	5	-304.42	4.05	0.03
	Null	2	-339.18	67.42	0.00

Predictors included presence of openings (*Tunnels*), excreta (*Castings*), and earthworms (*Worms*). Time was included in all models, either on its own or in interaction with the other predictor variables. The best model has a ΔAIC_c of zero and the highest $wAIC_c$ value. Models with $\Delta AIC_c<2$ are also considered to be plausible and are shown in bold. With *k*, number of parameters; LL, log likelihood;

ΔAIC$_c$, difference in the Akaike's information criterion (corrected for small sample size) value between model and the most strongly supported model; *w*AIC$_c$, weight given by the AIC (i.e., relative strength of support for model).

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CHAPTER 5. EARTHWORM INFLUENCES ON ECTOMYCORRHIZAL COLONIZATION AND GROWTH OF WHITE SPRUCE[5]

5.1 Introduction

Exotic earthworms can cause dramatic changes to forest plant communities, but there is limited understanding of their impacts in many forest types (Bohlen et al. 2004; Frelich et al. 2006; Eisenhauer et al. 2007; Addison 2009). In heavily invaded areas, tree seedling numbers have significantly decreased while diverse understory herb communities have shifted to simpler communities dominated by only a few species (Frelich et al. 2006; Nuzzo et al. 2009). Mycorrhizal dependent species tend also to decline following earthworm invasion, leaving these simplified communities dominated by non-mycorrhizal plants (Gundale 2002; Frelich et al. 2006; Nuzzo et al. 2009). Consequently, a change in the mycorrhizal community is one of the mechanisms suggested to be driving the shifts in plant communities that occur when earthworms invade (Frelich et al. 2006).

Mycorrhizas occur in the vast majority of plant families and play important roles in the provision of nutrients for, and the balance of carbon in, trees (Lilleskov et al. 2002; Lambers et al. 2008; McLean et al. 2006). The limited amount of research available on the interactions between exotic earthworms and mycorrhizal fungi focuses almost exclusively on arbuscular mycorrhizas (Lawrence et al. 2003; McLean et al. 2006). This research indicates that the presence of exotic earthworms decreases colonization rates, probably as a result of physical disruption of fungal mycelium by the earthworms (Lawrence et al. 2003; McLean et al. 2006). Unlike arbuscular mycorrhizal fungi, ectomycorrhizal fungi form a sheath around root tips and have a complex extraradical mycelium system (Smith & Read 2008). Consequently, the effects of exotic earthworms may differ considerably between these two groups. Ectomycorrhizal fungi also mainly colonize the root systems of conifers, as compared to arbuscular mycorrhizas, which are common in most plant families (Smith & Read 2008; Wagg et al. 2008). Despite the importance of ectomycorrhizas in some ecosystems due to their colonization of the dominant tree species (Smith & Read 2008), the response of ectomycorrhizal communities to

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exotic earthworms is effectively unknown (Baxter et al. 1999; McLean et al. 2006; Addison 2009).

Because ectomycorrhizal colonization and fungal species composition are important determinants of tree seedling growth and survival in some species (Miller et al. 1998; Purdy et al. 2002; Nuñez et al. 2009), any effects of earthworms on ectomycorrhizas may impact seedling performance. A clear understanding of the impact of exotic earthworms on native tree seedlings is necessary, as changes in seedling success may have long-term implications for the trajectory of ecological succession (Frelich et al. 2006). However, conflicting results exist regarding the response of tree seedlings to earthworm invasion (Welke & Parkinson 2003; Frelich et al. 2006; Hale et al. 2008), and nothing is known about their response to exotic earthworms in boreal soils that are dominated by mineral horizons with high clay content.

We examined how exotic earthworms impact ectomycorrhizal colonization, community composition of ectomycorrhizal fungi, and growth of tree seedlings through a short-term greenhouse mesocosm experiment. We observed both the combined and individual effects of the anecic earthworm species *Lumbricus terrestris* and the epigeic earthworm species *Dendrobaena octaedra* on the growth of *Picea glauca* (white spruce) seedlings and their associated ectomycorrhizal fungi.

Mycorrhizal and seedling growth responses may depend upon which species of earthworms are introduced, as the degree to which soil horizons are mixed and altered varies depending on the life history strategy of the species of earthworm invading (McLean et al. 2006). Ectomycorrhizal colonization levels and diversity are predicted to decrease in the presence of earthworms, with a greater impact expected from the addition of *L. terrestris* relative to *D. octaedra*, based on previous studies of other fungal types (McLean & Parkinson 2000; Lawrence et al. 2003; McLean et al. 2006). If earthworm introduction simplifies ectomycorrhizal communities and/or reduces their contact with roots, it is expected that white spruce seedlings will exhibit reduced growth and poor health in the presence of earthworms. However, white spruce seedlings might also benefit from earthworm invasion, particularly by *L. terrestris*, because substrates with high amounts of available mineral soil and thin organic horizons are favourable for white spruce establishment and growth (Purdy et al. 2002). Given the relatively high nutrient demands of white spruce (Neinstadt & Zasada 1990), the increases in nutrient availability often observed during the early stages of earthworm invasion (Bohlen et al. 2004; Frelich et al. 2006) could also result in a positive effect of earthworms on this species.

5.2 Methods

5.2.1 Experimental design

Our experiment consisted of five treatments, as described in greater detail in Chapter 2: (1) no earthworms; (2) thirty *D. octaedra*; (3) two *L. terrestris*; (4) thirty *D. octaedra* combined with two *L. terrestris*; and (5) three *L. terrestris*. Twenty replicate mesocosms were set up for each treatment in plastic pails (30 cm diameter by 38 cm tall) (Hale et al. 2008; Belote & Jones 2009). Mesocosms consisted of a 3 cm layer of surface leaf litter, a 4-6 cm intact organic soil core, and a base 18 cm layer of mineral soil. The study was conducted over one growing season, from 9 May 2009 to 20 October 2009. This amount of time is similar to the average length of experiments (21 weeks, range of 8-104 weeks) used to test the effects of ectomycorrhizal inoculation on seedling growth (Karst et al. 2008), and earthworm impacts on plants are also often observed over similar or shorter time periods (Hale et al. 2008; Belote & Jones 2009).

One-year old white spruce seedlings (mean height 26.96 cm ± 0.56 cm, SE) were purchased from Treetime Services Inc. (http://treetime.ca/). These seedlings were grown in earthworm free peat soil and were not subject to mycorrhizal inoculation, but colonization of fungi from spores present in the greenhouse or our soil cores was possible. One seedling was planted in the center of each bucket, with the top of the root plug roughly one centimetre below the organic soil surface. No supplemental lighting was used, as the experiment occurred during the growing season.

5.2.2 Morphotyping and assessing mycorrhizal colonization

Replicates were processed through destructive sampling between in October 2009. Forbs and grasses were cut and spruce seedling roots were separated from shoots. The root systems with adhering soil intact were stored at -20°C until further processing. At that time, roots were thawed in a lukewarm water bath, and thoroughly rinsed clean over a 1.4 mm sized sieve. Washed root systems were cut into approximately 1 cm fragments, which were randomly selected for observation under a dissecting microscope, similar to previously described methods (Welke & Parkinson 2003). All root tips on each 1 cm root fragment were recorded as mycorrhizal or non-mycorrhizal until 100 tips were counted, excluding dead tips. Only those root tips with a distinct and visible mantle were considered mycorrhizal, and representative mycorrhizal tips were observed under a compound microscope to confirm mantle presence. Ectomycorrhizal colonization was calculated as the number of ectomycorrhizal root tips divided by the sum of living mycorrhizal and non-mycorrhizal tips. Ectomycorrhizas were morphotyped based on differences in tip colour, texture, thickness, shape, and the presence of hyphae (Jones et al. 1997). Following mycorrhizal characterization, root systems were dried for a minimum of 48 hours at 60°C and weighed to obtain below-ground biomass measurements.

5.2.3 Molecular confirmation of ectomycorrhizal morphotypes

DNA from three root tips representing each morphotype was extracted using Sigma Extract-N-Amp Tissue Kit following the manufacturer’s protocol (Sigma, Dorset, UK). Polymerase chain reaction (PCR) amplification was carried out using the fungal-specific primer combination NSI1 and NLB4 (Martin & Rygiewicz 2005). An aliquot of 0.5 μL of extracted DNA was combined with 4 μL of Extract-N-Amp PCR solution in an 8 μL reaction. Amplifications were performed with an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of 94°C for 45 seconds, 58 °C for 45 seconds and 72°C for 72 seconds, with a final extension of 72°C for 10 minutes. Successful PCR products were purified using ExoSAP-IT (USB, Cleveland, OH, USA). Cycle sequencing was conducted using BIGDYE v3.1 (Applied Biosystems, Foster City, CA, USA) with the ITS1F primer and the resulting products were precipitated following the manufacturer’s instructions for EDTA/ethanol. Uni-directional sequences were analysed on an ABI Prism 3730 Genetic Analyser (Applied Biosystems) and edited with Sequencher (GeneCodes, Ann Arbor, MI, USA). DNA sequences were checked against the GenBank sequence database to assign a taxonomic name to each morphotype.

5.2.4 Seedling growth measurements

Above-ground spruce seedling measurements, including seedling height, stem diameter, primary branch number, and branch length, were made at the beginning and end of the experiment. Primary branches were defined as those branches growing off of the main shoot and included new branch buds without needles. For branch length, we marked the first branch from the top of the seedling that exceeded 1 cm in length and measured it.

After separation from the roots, the shoots were qualitatively ranked for dead needles and then dried for a minimum of 48 hours at 60°C before being weighed. To assess dead needle profiles, each seedling was separated into three distinct regions: the lower half of the seedling, the upper half of the seedling, and new apical stem growth. Division of the seedlings into these regions provided a more precise estimate of dead needle occurrence than simply treating each tree as a whole. Each segment was then visually ranked for the presence of dead needles on the following scale: 0 = no dead needles; 1 = <1% of needles dead; 2 = 1-50% of needles dead; and 3 = 51-100% needles dead. Finally, we also determined N concentration in the organic horizon, where most of the spruce roots were located, using a CEC (Control Equipment Corporation) Model 440 Elemental Analyzer.

5.2.5 Statistical analysis

To test for significant differences in proportion of root tips colonized by ectomycorrhizal fungi between earthworm treatments, we used a Kruskal-Wallis test because this variable had a non-normal residual distribution according to the Shapiro-Wilks test. Nonmetric multidimensional scaling (NMDS) was used to qualitatively determine the effects of each treatment on ectomycorrhizal morphotype diversity and abundance, as this method is compatible with datasets containing multiple zeroes (McCune & Grace 2002). Replicates were colour-coded by treatment and positioned on three axes, which provided a less stressed configuration than two axes. Replicates with 0% colonization were excluded from the ordination matrix, as these replicates were unable to be spatially plotted. Multi-response permutation procedure (MRPP), a non-parametric form of MANOVA, was used to assess if morphotype composition differed between earthworm treatments (McCune & Grace 2002).

To examine differences in seedling growth between earthworm treatments, we performed ANOVAs on above- and below-ground measurements of seedling biomass. A Kruskal-Wallis test was used to analyze change in branch length, which had a non-normal residual distribution. Seedling diameter, height, and branch number were tested using ANCOVAs, in order to control for initial size of the seedlings. An ordered logistic regression was applied to dead needle ranks to determine if significant differences were present in seedling needle profiles between treatments. Needle ranks were clustered by replicate ID, and each treatment was compared to the no worms control group. Exact logistic regression was used to test whether the proportion of seedlings dying differed among treatments. Exact logistic regression can produce more reliable results than logistic regression for small sample sizes or unbalanced data by using the conditional distribution of the parameter sufficient statistics to estimate the logistic model parameters (Cox & Snell 1989; Hilbe 2009). Such estimations use exact probability statements which are valid for any sample size, whereas logistic regression using maximum likelihood is based on asymptotic theory and thus is more likely to be unreliable at small sample sizes.

For all other statistical analyses, replicates where the seedling died or earthworm contamination occurred (see discussion) were excluded. This resulted in final sample sizes of 12, 18, 17, 19, and 17 for treatments one to five, respectively. As well, for mycorrhizal colonization and composition and spruce biomass (i.e., those variables measured only at the end of the experiment), we randomly selected fifty replicates for measurement in order to maintain a balanced sample size in each treatment. We also assessed differences in above-ground forb biomass and species composition, above-ground grass biomass, soil nitrogen concentrations, and earthworm survival, as these factors could influence spruce growth and mycorrhizal colonization (Appendix 5.1; Chapter 2; Chapter 3; Cameron & Bayne unpublished data). Analyses were conducted using Stata 11 (StataCorp, College Station, TX, USA) and R version 2.8.1 (R Foundation for Statistical Computing, Vienna, Austria) and significance was set to $P < 0.05$.

5.3 Results

5.3.1 Ectomycorrhizal fungal community composition and colonization

Ectomycorrhizas were categorized into eight identifiable groups (Table 5.1). We were able to amplify DNA and obtain sequences for only three of these morphotypes. Query matches to Genbank accessions did not provide species-level information. All three amplified morphotypes were described as uncultured ectomycorrhizas from different families. Specifically, morphotypes were identified from the clades Pyronemataceae (gb│GU452518.1; 703/704; 99%; accession number, identities / length, %), Thelophora (gb│EF218819.1; 771/787; 98%) and Tomentellopsis (emb│AJ893354.1; 679/682; 99%).

Proportion of root tips colonized did not differ significantly across treatment groups for our eight morphotypes, as indicated by a Kruskal-Wallis ranks test (χ^2_4 = 1.164, P = 0.8840; Figure 5.1). Similarly, multi-response permutation procedures (MRPPs) revealed no significant differences were present in morphotype composition among treatments (Distance measure = Sorensen, A = -0.0278, P = 0.951). Congruent with MRPP results, no clear patterns were detectable within the nonmetric multidimensional scaling ordination plots (Figure 5.2). A 3-dimensional solution provided the lowest value for stress (stress = 0.129). Analysis of the three sequenced morphotypes alone produced similar results in all cases.

5.3.2 Impact of earthworms on white spruce growth

Earthworms had a limited impact on indicators of spruce seedling growth (Figure 5.3). One-way ANOVAs for both above-ground ($F_{4, 45}$ = 1.14, P = 0.352) and below-ground biomass ($F_{4, 45}$ = 0.99, P = 0.424) revealed no significant differences among treatments. No significant differences were observed in change in branch length (H_4 = 1.505, P = 0.837). After controlling for initial size, no significant differences were found for primary branch number ($F_{4, 76}$ = 1.90, P = 0.120), height ($F_{4, 76}$ = 1.95, P = 0.111), and diameter ($F_{4, 76}$ = 0.37, P = 0.829). Needle death was unaffected by earthworm treatment (*D. octaedra* only: odds ratio = 1.005, P = 0.978; *L. terrestris* only: odds ratio = 0.909, P = 0.320; three *L. terrestris*: odds ratio = 1.109, P = 0.429; Both species: odds ratio = 0.855, P = 0.269). The proportion of dead seedlings did not differ significantly between treatments (model score = 8.348, P = 0.0733).

Above-ground grass biomass (mean of 9.8 g ± 0.44 1 SE), above-ground forb biomass (mean of 15.6 g ± 0.40 1 SE), and forb species composition did not differ significantly between treatments, and heights of these species were similar to those typically observed in the understory in this region at the end of the growing season (Appendix 5.1; Cameron & Bayne unpublished data). Leaf litter depth decreased significantly during the experiment, and the survival rates for each earthworm species were insensitive to treatments (Chapter 2; Cameron & Bayne 2011). Survival of *D. octaedra* was lower than that of *L. terrestris*, although we observed high reproductive success of *D. octaedra* and continued earthworm activity in an extra replicate throughout the experiment, suggesting that most survived until near the end of the experiment. Nitrogen concentration in the organic horizon differed significantly among treatments, according to an ANOVA (Fig. 4, $F_{4, 45} = 3.08$, $P = 0.0250$); nitrogen was significantly higher in the *L. terrestris* treatment than the treatment containing both species ($P = 0.034$).

5.4 Discussion

No major differences were observed among any of the earthworm treatments across all measured variables for both ectomycorrhizas and seedlings. This suggests that increased earthworm biomass or multiple species invasions do not have an added impact on mycorrhizas or spruce seedlings relative to single species invasions, at least over a single growing season. In our study, no significant effects were seen on colonization and community structure of ectomycorrhizal fungi of white spruce. In contrast, previous studies suggest that exotic earthworms can reduce abundance of arbuscular mycorrhizal fungi (Lawrence et al. 2003; McLean et al. 2006). These decreases in arbuscular mycorrhizal colonization following earthworm invasion are suggested to be the result of mechanical disruption of hyphal networks as worms move through the soil (Lawrence et al. 2003; Frelich et al. 2006). However, we did not examine soil hyphal networks, and the effects of the disruption of these networks on colonization of roots by ectomycorrhizal fungi are unclear.

Community structure of ectomycorrhizal fungi did not vary among treatments. The observed taxa of ectomycorrhizal fungi appear insensitive to earthworm modifications of soil, however, only a subset of the mycorrhizal community was present in our study. Given that we severed all hyphal connections with mature trees when we collected the soils, only those fungal species able to tolerate and recover from such a disturbance would be included in our field collection. Those species of fungi unable to withstand severed connections from a host may be those that are sensitive to earthworm activity. Mycorrhizal colonization of individual root tips in our study was low across all treatments (mean = 15.5% ± 0.02 1 SE), although 90% of the seedlings we examined had at least some mycorrhizal colonization. It is not uncommon to observe low levels of colonization and numbers of fungal morphotypes in young seedlings grown in the greenhouse or the field following disturbance (Jones et al. 1997; Teste et al. 2004; Cline et al. 2005; Karst et al. 2011).

Greater soil nutrient availability as a result of non-native earthworm activity has also been suggested as a possible mechanism contributing to reduced arbuscular mycorrhizal colonization (Lawrence et al. 2003). Increased soil nitrogen input is strongly and negatively correlated with ectomycorrhizal abundance (Lilleskov et al. 2002). Nitrogen concentration in the organic horizon did not differ among most treatments in our study but was significantly higher in the *L. terrestris* treatment than in the treatment containing both species. Such increases in nutrient flux as a result of earthworm invasion may occur mainly in the early stages of invasion (Bohlen et al. 2004; Frelich et al. 2006) and therefore changes in availability may differ in longer term studies. In our experiment, ectomycorrhizal abundance was not correspondingly lower in the *L. terrestris* treatment, suggesting that the difference in nitrogen concentration was not large enough to result in a response by ectomycorrhizal fungi.

Spruce seedling performance was unaffected by earthworms suggesting that this species may be resistant to effects of earthworm invasion over the short-term. Previous work has shown earthworm species can increase tree seedling growth. For example, the addition of the endogeic earthworm, *Aporrectodea caliginosa tuberculata* (now known as *Aporrectodea tuberculata* (Pérez-Losada et al. 2009)) resulted in increased height and biomass of birch seedlings (Haimi & Einbork 1992). Similarly, Douglas-fir seedlings had significantly greater root biomasses in endogeic worm-worked soils relative to controls (Welke & Parkinson 2003). Our results show no effect on seedling mortality in the presence of exotic earthworms, unlike previous studies in sugar maple stands of northern Minnesota (Frelich et al. 2006). This suggests that the responses of tree seedlings to earthworm invasions may be species-specific, and consequently further research examining effects on multiple tree species by multiple earthworm species could be informative.

Seedling mortality was relatively high in this experiment (ranging from 0% in the *L. terrestris* treatment to 30% in the control). There were no significant differences in mortality among treatments, although this reduced our sample size and resulted in an unbalanced design. Most of the seedling deaths occurred in the second month of the experiment and were in pots that became water logged. This suggests a better approach would have been to water in an amount that ensured soil moisture was kept constant across all replicates rather than using a set amount. Also, as is common in many earthworm mesocosm experiments (Fründ et al. 2010), we confirmed the presence of *D. octaedra* (mean abundance of 2.1 ± 0.74 1 SE) in several no earthworm control and *L. terrestris* replicates. It is unlikely that contamination of these replicates was a result of undetected earthworms initially present in collected soil or root plugs of white spruce seedlings. Instead, some earthworms appear to have been able to overcome our sand strip barriers and escape into adjacent replicates. To maintain the validity of our treatments, contaminated replicates were excluded from analysis.

In our greenhouse experiment, we determined the exotic earthworm species *L. terrestris* and *D. octaedra* have no direct short-term impact on ectomycorrhizal colonization or spruce seedling growth. As earthworms continue to spread in North American forests, an increased understanding of the impact of earthworm invasion on both mycorrhizal colonization and diversity and seedling growth will be necessary in order to assess the impacts of earthworm invasion on forests.

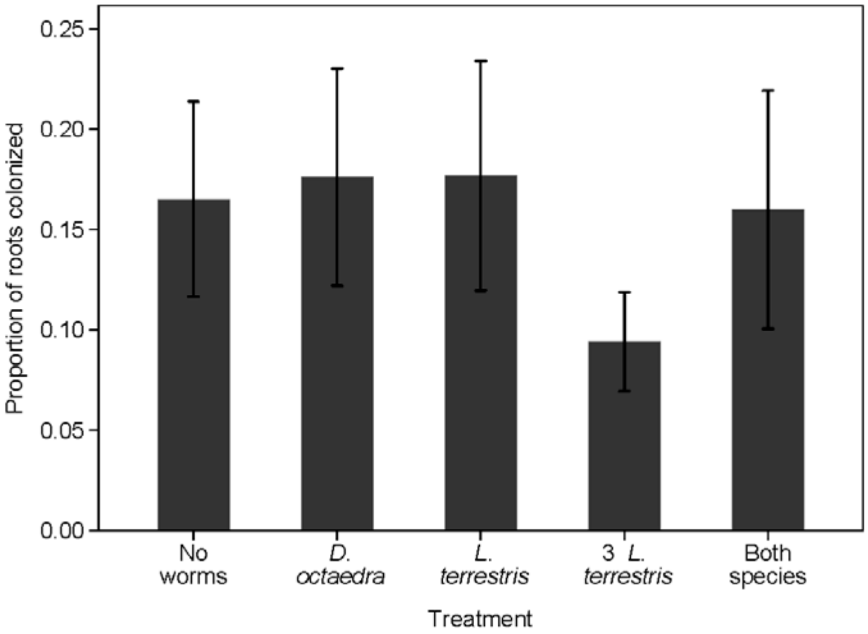


Figure 5.1. Means (± 1 SE) for proportion of root tips of *Picea glauca* colonized by ectomycorrhizal fungi within each treatment (no worms, *D. octaedra*, *L. terrestris*, 3 *L. terrestris*, both species) for all eight morphotypes.

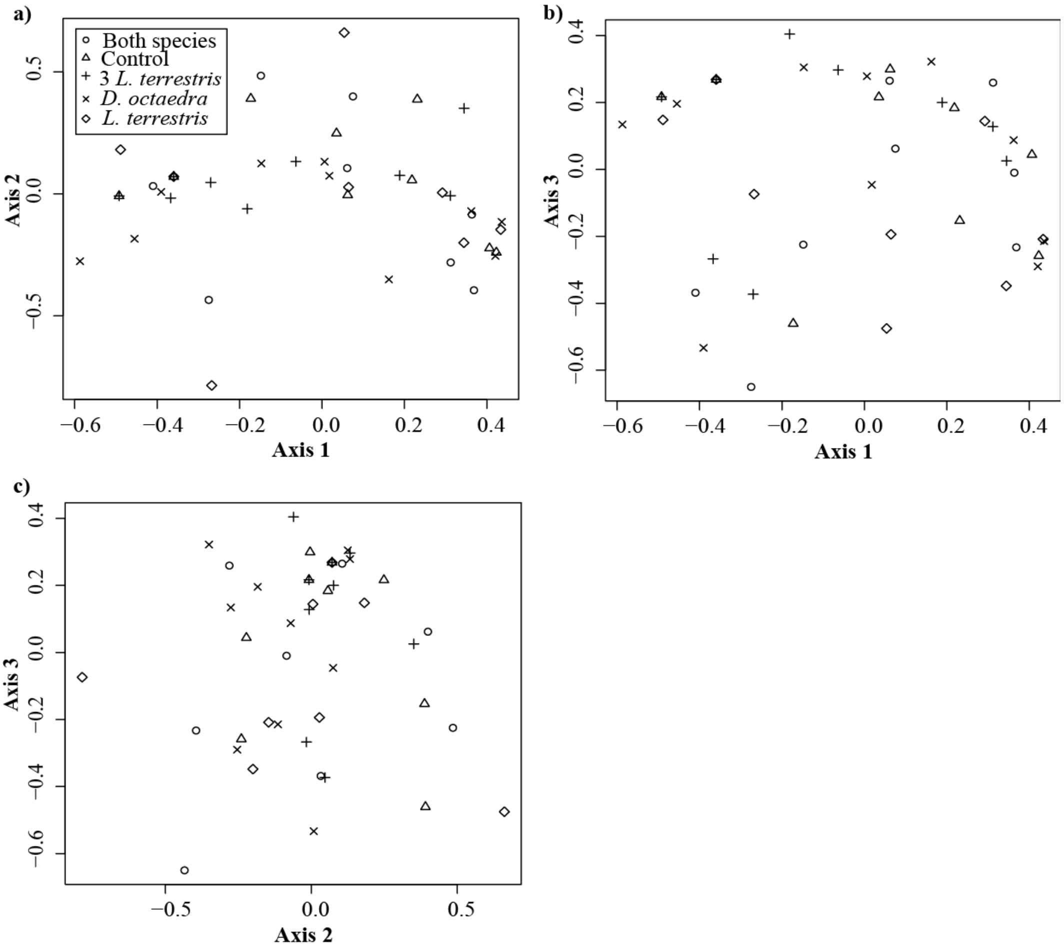


Figure 5.2. Nonmetric multidimensional scaling (NMDS) plots of replicates rank-ordered and arranged by ectomycorrhizal morphotype composition for all eight morphotypes. Symbols represent the five treatments: ○ = both species, Δ = no worms, + = 3 *L. terrestris*, x = *D. octaedra* only, and ◊ = *L. terrestris* only. a) Plot of ordination axes 1 and 2; b) plot of ordination axes 1 and 3; and c) plot of ordination axes 2 and 3.

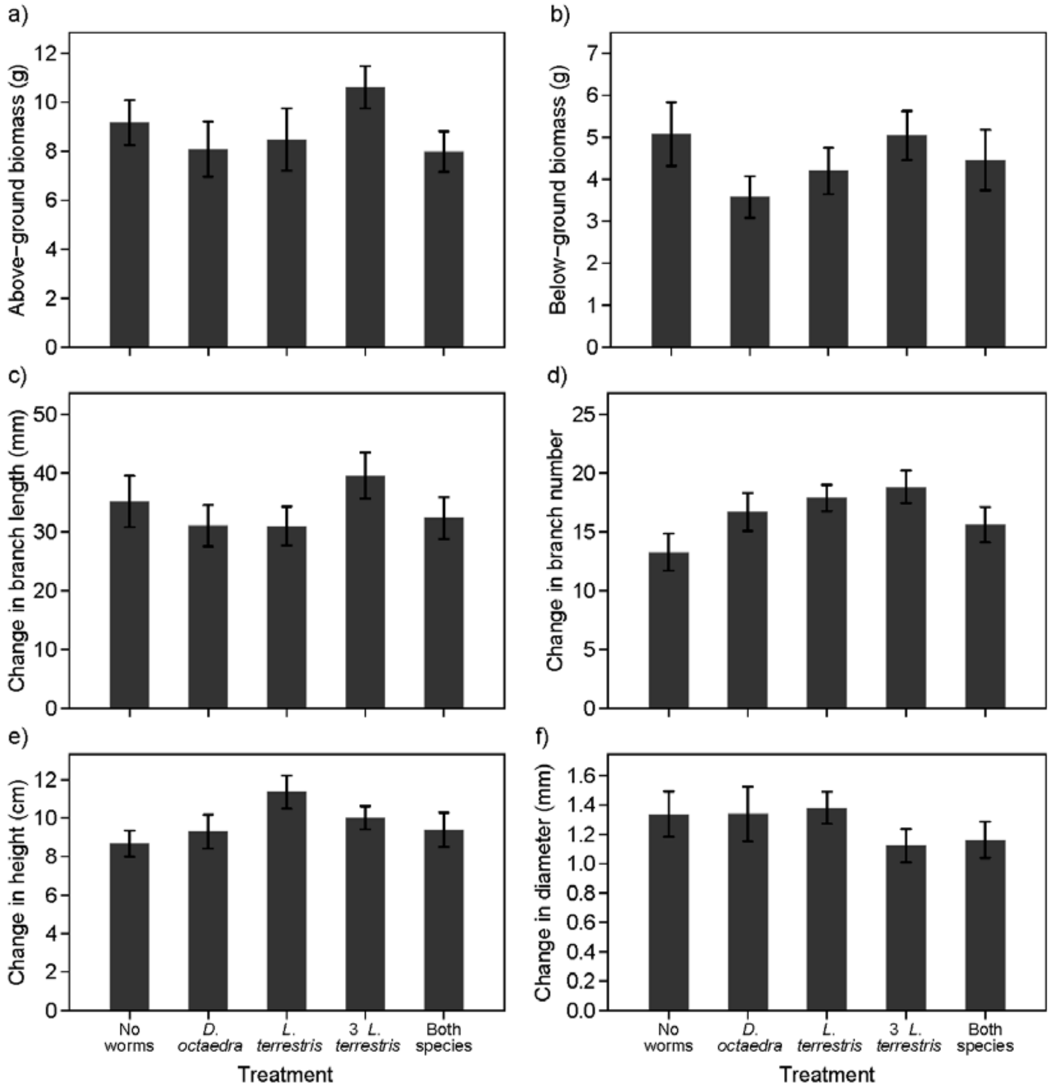


Figure 5.3. Means (± 1 SE) for indicators of white spruce (*Picea glauca*) seedling growth across the five treatments (no worms, *D. octaedra*, *L. terrestris*, 3 *L. terrestris,* both species): a) above-ground biomass (g); b) below-ground biomass (g); c) change in branch length (mm); d) change in primary branch number; e) change in height (cm); and f) change in stem diameter (mm).

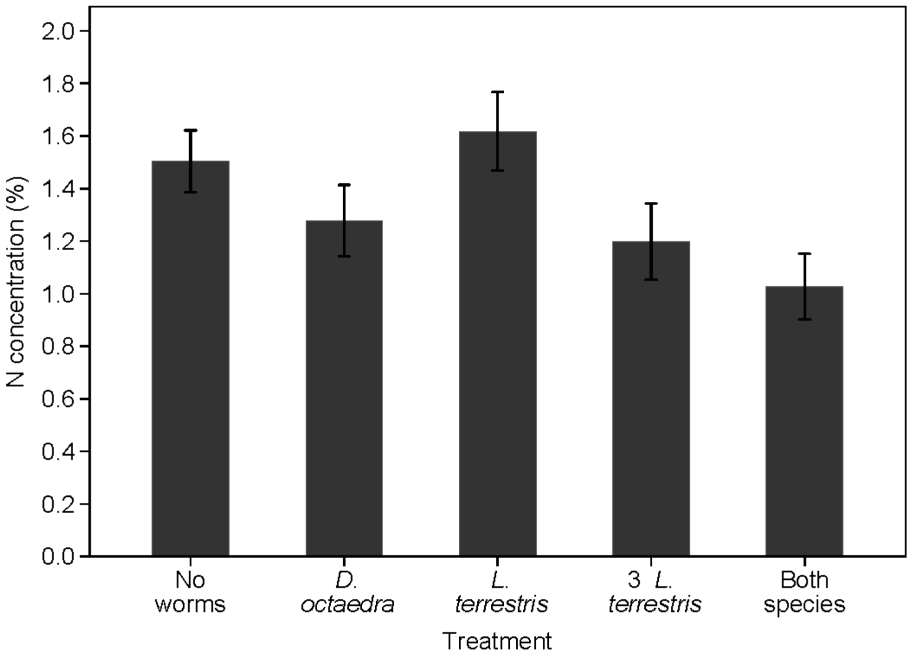
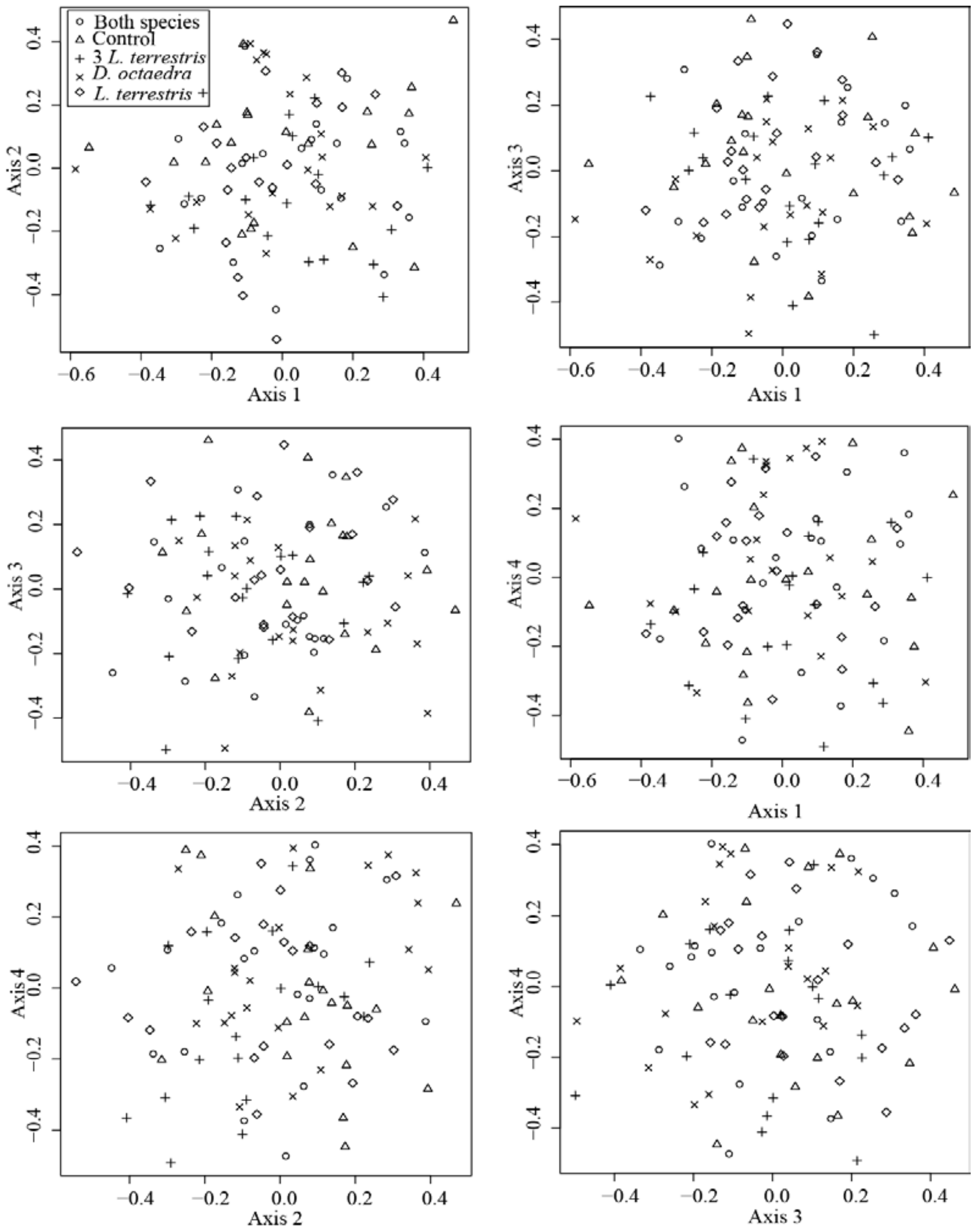


Figure 5.4. Mean (± 1 SE) nitrogen concentration (%) in the organic horizon for the five treatments (no worms, *D. octaedra*, *L. terrestris*, 3 *L. terrestris*, and both species).



Appendix 5.1. Nonmetric multidimensional scaling plots of replicates rank-ordered and arranged by forb species composition. Symbols represent the five treatments: ○ = both species, Δ = control, + = 3 *Lumbricus terrestris*, x = *Dendrobaena octaedra* only, and ◊ = *Lumbricus terrestris* only. Plot of ordination axes: a) 1 and 2; b) 1 and 3; c) 2 and 3; d) 1 and 4; e) 2 and 4; and f) 3 and 4. Stress was 0.1629. A multi-response permutation procedure indicated no significant differences between groups (A=0.0029; P=0.316).

Table 5.1. Mean (± 1 SE) number of root tips colonized for each ectomycorrhizal morphotype across the five treatments. 100 root tips were examined per *Picea glauca* seedling.

	Morphotype							
Treatment	Tomentellopsis sp.	Pyronemataceae sp.	Thelophora sp.	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E
No worms	11.6 ± 4.57	0.7 ± 0.70	0.0 ± 0.0	0.2 ± 0.20	3.3 ± 2.06	0.4 ± 0.40	0.0 ± 0.0	0.3 ± 0.30
D. octaedra	14.3 ± 5.51	1.3 ± 0.94	0.0 ± 0.0	0.3 ± 0.21	0.3 ± 0.30	1.4 ± 1.29	0.0 ± 0.0	0.0 ± 0.00
L. terrestris	13.4 ± 5.43	1.3 ± 0.90	0.0 ± 0.0	0.2 ± 0.20	2.1 ± 1.67	0.0 ± 0.00	0.7 ± 0.7	0.0 ± 0.00
3 *L. terrestris*	6.0 ± 1.59	1.7 ± 1.19	1.0 ± 1.0	0.1 ± 0.10	0.5 ± 0.50	0.2 ± 0.20	0.0 ± 0.0	0.0 ± 0.00
Both species	9.5 ± 3.98	1.1 ± 0.69	0.0 ± 0.0	0.0 ± 0.00	1.8 ± 1.14	1.5 ± 1.39	0.0 ± 0.0	2.1 ± 2.10

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CHAPTER 6. EFFECTS OF EARTHWORM BURROWING ON PLANT ROOT GROWTH

6.1 Introduction

Although it is broadly recognized that belowground interactions can affect aboveground community structure (Wardle et al. 2004; van der Putten et al. 2009), the mechanisms through which detritivores influence plants are not well understood (Scheu 2003). Earthworm invasions in North American forests are causing dramatic shifts in plant community composition (Gundale 2002; Hale et al. 2006; Holdsworth et al. 2007; Nuzzo et al. 2009). Native herbaceous plants have declined in richness and cover (Gundale 2002; Hale et al. 2006; Holdsworth et al. 2007), while some groups, such as sedges and non-native plants, have increased following earthworm invasions in historically earthworm-free northern hardwood and boreal forests (Hale et al. 2006; Nuzzo et al. 2009). Multiple possibilities have been proposed to explain these changes (Frelich et al. 2006) including decreases in leaf litter thickness (Gundale 2002), changes in nutrient availability (Frelich et al. 2006), disruption of mycorrhizal fungi associations (Lawrence et al. 2003; Chapter 5), and consumption or movement of seeds (Eisenhauer et al. 2009). Research has focused on indirect effects mediated through reductions in leaf litter thickness (Gundale 2002, Hale et al. 2006, Holdsworth et al. 2007). In contrast, direct effects of earthworm burrowing have been largely overlooked as a factor influencing plant communities.

Earthworm burrowing could affect plant roots in several ways: 1) indirectly via burrows acting as pathways for root elongation (Edwards & Lofty 1980; Ehlers et al. 1983); 2) indirectly via altered nutrient distributions; or 3) directly via consumption or abrasion (Cortez & Bouche 1992). Burrows are often lined with earthworm excreta which have higher concentrations of nutrients than surrounding soil, thereby creating nutrient patches (Lunt & Jacobson 1944; Tiunov & Scheu 1999). Although Darwin (1881) stated that earthworms “greatly facilitate the downward passage of roots of moderate size; and these will be nourished by the humus with which the burrows are lined”, surprisingly little attention has since been given to potential effects of earthworms on plant root growth and foraging behaviour.

Root growth of plants is highly dynamic and species vary in their ability to adjust foraging in response to variation in the environment (Cahill & McNickle 2011), such as the presence of competitors (Cahill et al. 2010) and herbivores (Stevens & Jones 2006a, 2006b). Thus, differences in root foraging strategies in response to earthworm activity may account for some of the variability observed among plant species following earthworm invasion (Scheu 2003). Here we tested whether earthworms exhibit direct and/or indirect effects on root growth and whether these effects vary with a plant's ability to forage for mineral resources. Species that are high precision foragers (i.e., able to concentrate roots in nutrient patches) should benefit from increased nutrient availability in earthworm burrows. Conversely, such plants should experience greater costs if earthworms consume roots in burrows. We conducted a greenhouse experiment using two herbaceous perennial species native to the Canadian boreal forest: *Achillea millefolium* (Linnaeus) and *Campanula rotundifolia* (Linnaeus). Prior research indicates *A. millefolium* is a more precise root forager than *C. rotundifolia* (Johnson & Biondini 2001).

6.2 Methods

6.2.1 Experimental design

To examine the effects of earthworm burrowing on root foraging, we grew *A. millefolium* and *C. rotundifolia* individually in pots with and without earthworms (Figure 1). Treatments were randomly assigned to pots, with 15 replicate pots per treatment and a total of 60 pots in the experiment. Pots were arranged in three blocks of 20, within which treatments were randomly assigned to the pots. Pots were 27 cm x 11 cm x 26 cm deep wooden boxes filled with a mixture of mineral soil from the boreal forest of northeastern Alberta (54°36'N, 110° 59'W) and sand at a ratio of two parts soil to one part sand, with a uniform vertical soil structure. A transparent acrylic tube (5.7 cm in diameter) ran lengthwise through each pot approximately 5 cm below the surface of the soil to allow images to be taken with a mini-rhizotron camera (Bartz Technology). Each tube ran through a line of five pots.

Three adult *Lumbricus terrestris* (Linnaeus) individuals were added to each pot in the earthworm treatment. *Lumbricus terrestris* feeds on surface leaf litter but lives in permanent burrows that are typically vertical. Trembling aspen (*Populus* *tremuloides*) and balsam poplar (*Populus balsamifera*) leaves were supplied *ad libitum* as food, such that a thin layer of leaves (~5 g) was continually present in pots from all treatments. Earthworms were added to the pots one week prior to the start of the experiment to allow burrow establishment before plants were added (Felten & Emmerling 2009). The rims of the pots were covered with a 1.5 cm thick strip of Velcro to prevent earthworms from leaving the pots. After we found one earthworm had escaped into an *A. millefolium* control pot, we also placed a 10 cm tall strip of 1 mm plastic mesh around the top edge of the pots.

Seeds for *A. millefolium* and *C. rotundifolia* were obtained from Bedrock Seeds and Wild About Flowers, who collect and propagate seeds from local populations in Alberta. Following cold stratification, the seeds were planted in starter trays in late May and late June 2010, respectively. Seeds germinated in early to mid-July and were transplanted into experimental pots on 1 September 2010. We took images monthly starting on 1 September, for a total of four times over 14 weeks. The camera was positioned horizontally facing one side of the tube and images were taken along a belt transect in each tube. After 14 weeks, plants were harvested and shoots were dried at 60°C and weighed. Roots were stored at -20°C, washed in a 2 mm sieve, dried at 60°C, and weighed.

The experiment was conducted in a greenhouse in the Biological Sciences building at the University of Alberta. Room temperature was maintained between 14.5 to 19°C and supplemental lighting was used to achieve a 16:8 L:D light cycle. Plants were watered regularly to field capacity.

6.2.2 Analysis

We digitized the locations of roots, burrows, and cracks in images obtained at four time steps (01 September 2010, 29 September 2010, 03 November 2010, 08 December 2010) in ArcGIS (v 10, Esri). The cracks formed naturally in the soil during the filling of the pots. They changed very little in shape during the experiment and were thus distinguishable from burrows, which shifted slightly over time due to earthworm movements and were often lined with earthworm castings. Images from each pot (18 mm x 222 mm) were divided into 6 mm x 6 mm grid cells, with 111 cells per pot. We then determined occurrence of roots, burrows, and cracks within each cell at each time step.

Mixed effects logistic regression was used to examine root occurrence over time within grid cells containing burrows and cracks for each species separately. These models included void type (burrow, crack, or none), date, and the interaction of void type and date as fixed effects. The first date was not included in the analysis as planting had occurred just prior to imaging and there were no roots present at the depth of the mini-rhizotron tube. Pot identity and grid cell were used as random effects to account for correlations among grid cells within pots and within grid cells over time. Root detectability may have differed in the soil versus in openings (burrows and cracks) because roots should only be observable within the soil matrix if they were at the surface of the soil/mini-rhizotron tube interface. In contrast, roots could be visible farther from the mini-rhizotron tube in openings. Therefore, we conducted post-hoc pairwise comparisons at each time step to compare root occurrence in cracks versus burrows only, where detectability should be similar. We used a Bonferroni correction for multiple testing. Analyses were also performed using a random effect for tube identity (there were five pots along each tube), but they produced similar results and thus are not shown.

A similar mixed effects logistic regression analysis was used to examine differences in root mortality within grid cells containing burrows, cracks, and soil. We included a random effect to account for pot identity and a fixed effect to control for the date of initial colonization of cells by roots. In this analysis, we examined only grid cells with roots present during the experiment. Roots were considered to have died when they were no longer visible in the cell at subsequent time steps.

To assess effects of earthworms on root and shoot biomass, we used mixed effects linear regression with earthworm presence as a fixed effect and tube as a random effect. Species were analyzed separately. Normality was assessed by visual inspection of residuals and data were log transformed if non-normal. All analyses were conducted in Stata (v 12, StataCorp).

6.3 Results and Discussion

Both plant species responded to the physical changes in soil structure caused by earthworm burrowing. Root distributions were strongly affected by openings (cracks or burrows) in the soil, consistent with the idea that foraging by roots is non-random. Roots of *A. millefolium* (Figure 2; $\chi^2_2 = 85.84$, $P < 0.0001$;

generalized linear model) and *C. rotundifolia* ($\chi^2_2 = 10.37$, $P = 0.0056$; generalized linear model) occurred more frequently in openings than in the soil matrix, where mechanical resistance to root growth is presumably higher. This suggests that burrows and cracks act as pathways for root elongation. However, differences in detectability of roots in the soil versus in openings could also lead to observations of higher occurrence in openings where they are likely easier to see. Thus, we focus on relative occurrence in burrows versus cracks in the subsequent discussion.

Our results also support the idea that earthworms influence plant root foraging via redistribution of soil nutrients. Only *A. millefolium*, the higher precision forager, exhibited more extensive root growth in burrows, where nutrient concentrations tend to be higher due to earthworm excreta (Tiunov & Scheu 1999). There was a significant interaction effect of void type (crack, burrow, or none) and time for *A. millefolium* ($\chi^2_4 = 29.80$, $P < 0.0001$; generalized linear model). This species initially was more likely to place its roots in burrows than cracks ($\chi^2_1 = 19.38$, $P < 0.0001$; generalized linear model, monthly contrasts adjusted for multiple testing), but root occurrence in cracks then increased and was similar to occurrence in burrows after two ($\chi^2_1 = 3.90$, $P = 0.14$) and three months ($\chi^2_1 = 0.30$, $P = 1.00$). In contrast, *C. rotundifolia* did not concentrate roots in burrows versus cracks at any point during the experiment, although the distribution of its roots was also significantly affected by an interaction between treatment and time ($\chi^2_4 = 31.36$, $P < 0.0001$; generalized linear model). Occurrence of *C. rotundifolia* roots was initially similar within cracks and burrows ($\chi^2_1 = 0.22$, $P = 1.000$; generalized linear model, monthly contrasts adjusted for multiple testing). After two months, roots were less likely to be present in burrows than cracks ($\chi^2_1 = 5.91$, $P = 0.0452$), and this difference became more pronounced by the end of the experiment with roots occurring 40% less frequently in burrows ($\chi^2_1 = 32.54$, $P < 0.0001$).

Consistent with a general understanding of the mechanisms that can cause altered degrees of root foraging precision (Gross et al. 1993), the patterns of root occurrence observed in burrows, cracks, and soil may be driven by differences in both root production and mortality rates. Lower occurrence of *C. rotundifolia* roots in burrows relative to cracks could occur if *C. rotundifolia* reduced root production in burrows, or if roots in burrows were more likely to die than those in cracks. We found that initial root production of *C. rotundifolia* did not differ as a function of cracks versus burrows, but the proportion of roots dying in burrows was almost twice that in cracks and the soil matrix ($\chi^2_2 = 9.75$, $P = 0.0076$; generalized linear model). Thus, we suggest this species did not alter root production but instead suffered higher root mortality in burrows possibly because of grazing or abrasion by the earthworms. In contrast, in the first month of growth, we observed more new roots of *A. millefolium* in burrows than cracks. Additionally, there was increased mortality of *A. millefolium* roots in soil than in cracks or in burrows (Fig. 3; $\chi^2_2 = 20.56$, $P < 0.0001$; generalized linear model). These results suggest *A. millefolium* altered both aspects of root demography in response to the soil environment.

Despite the initial preference of *A. millefolium* roots for burrows, we did not observe a growth response due to the presence of *L. terrestris*. Earthworms did not affect *A. millefolium* shoot biomass (Fig. 4; $F_{1, 28} = 1.32$, $P = 0.25$; general linear model) or root biomass ($F_{1, 28} = 0.31$, $P = 0.58$). However, consistent with our finding that *C. rotundifolia* roots occurred less frequently in burrows than soil or cracks at the end of the experiment, its root and shoot biomasses were reduced by 25% and 33% respectively when earthworms were present (Root: $F_{1, 28} = 6.04$, $P = 0.014$; log-transformed data; Shoot: $F_{1, 28} = 23.43$, $P < 0.0001$; general linear model).

Our study provides evidence that the behavioural activity of earthworms can interact with the root foraging strategies of plants in soil to impact root distributions and plant growth. Previous research indicates precisely foraging plant species can experience greater risks of root herbivory than less precise foragers due to higher herbivore abundances in nutrient patches (Stevens & Jones 2006a, 2006b). However, we did not observe a negative effect of earthworms on *A. millefolium* or greater mortality of its roots in burrows as would be expected under high herbivory pressure. It is possible that *A. millefolium* was subject to a similar amount of consumption or abrasion as *C. rotundifolia,* but the greater proliferation of *A. millefolium* roots in burrows prevented a noticeable decline in root occurrence. Thus, the benefits derived from increased nutrient concentrations in burrows may balance the costs associated with increased herbivory. Although our data do not allow us to assess this possibility, our results do suggest earthworms can directly affect root foraging via consumption or abrasion and lead to detrimental effects on some plant species.

Here we provide the first evidence of a decline in plant growth due to the interaction between detritivore activity and root foraging strategies. Our results suggest that plants with more responsive foraging strategies may be less negatively impacted by the activity associated with earthworms. Thus, earthworm effects on plant roots may be one factor involved in shifts in North American forest plant communities following earthworm invasions. Little is known about the foraging strategies of most species in these forests. However, one key group increasing in areas that have been invaded by earthworms is non-native plants (Nuzzo et al. 2009), and evidence indicates invasive perennial forbs and grasses may have higher foraging precision than similar native species (Drenovsky et al. 2008; James et al. 2010). Further investigation of the mechanisms involved in invasive earthworm effects on plant communities, and how effects vary depending on plant traits, is needed. Nonetheless, our study demonstrates that soil animals can influence root foraging via both direct and indirect pathways and lead to variable, although substantial, effects on plants.

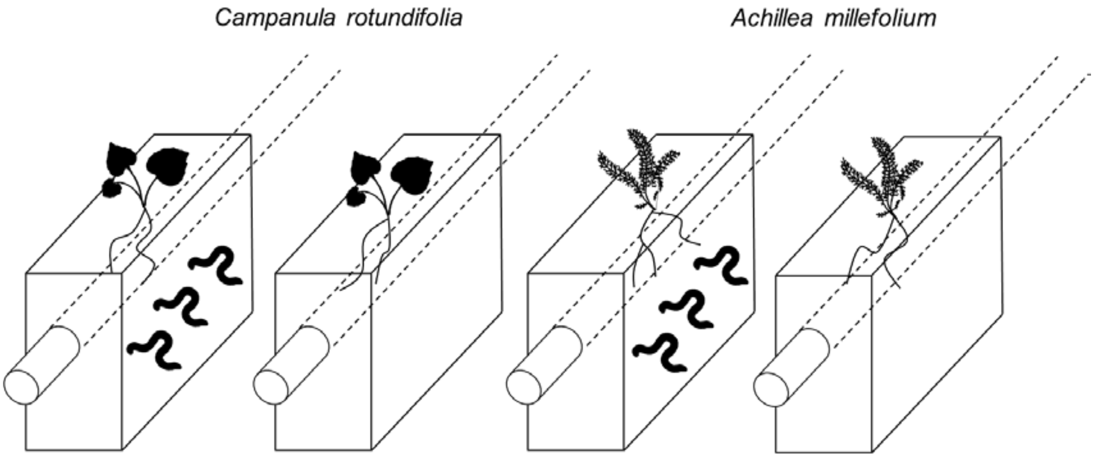
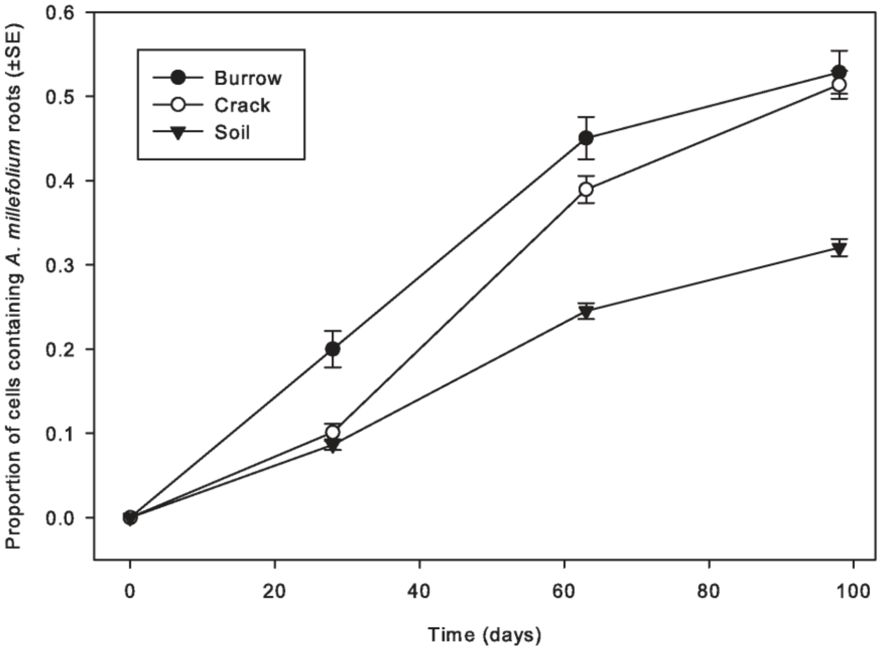


Figure 6.1. Schematic of experimental treatments. *Achillea millefolium* and *Campanula rotundifolia* were grown individually in 15 replicate pots (27 cm x 11 cm x 26 cm) with and without earthworms. Three *Lumbricus terrestris* earthworms were added to each of the earthworm treatment pots. A transparent mini-rhizotron tube (5.7 cm in diameter) ran lengthwise approximately 5 cm below the soil surface of each pot to allow mini-rhizotron images to be obtained. Each mini-rhizotron tube ran through five adjacent pots.

a)



b)

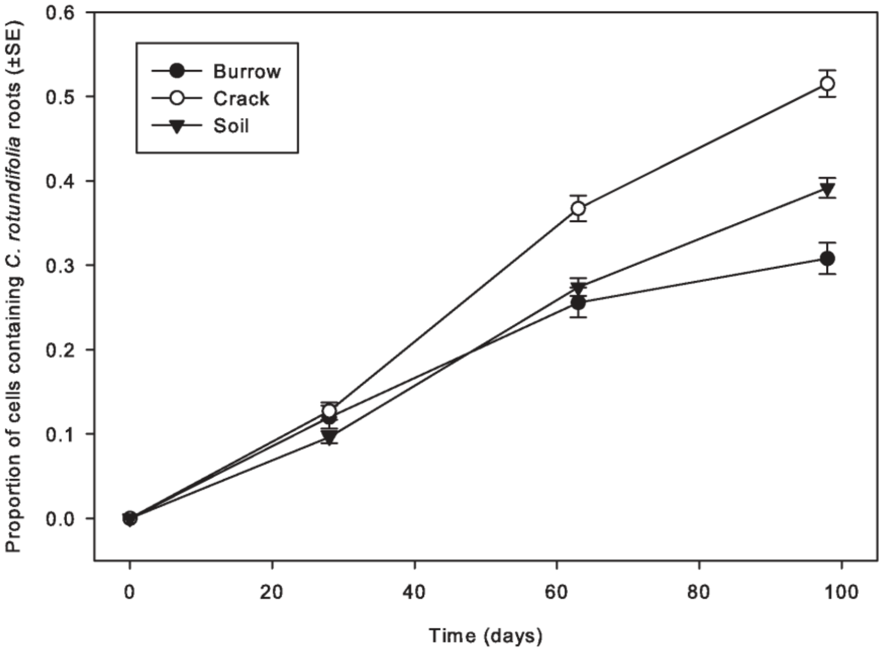


Figure 6.2. Occurrence of roots (± 1 SE) in 6 mm x 6 mm grid cells containing burrows (●), cracks (○), and soil (▼) for a) *Achillea millefolium* and b) *Campanula rotundifolia*.

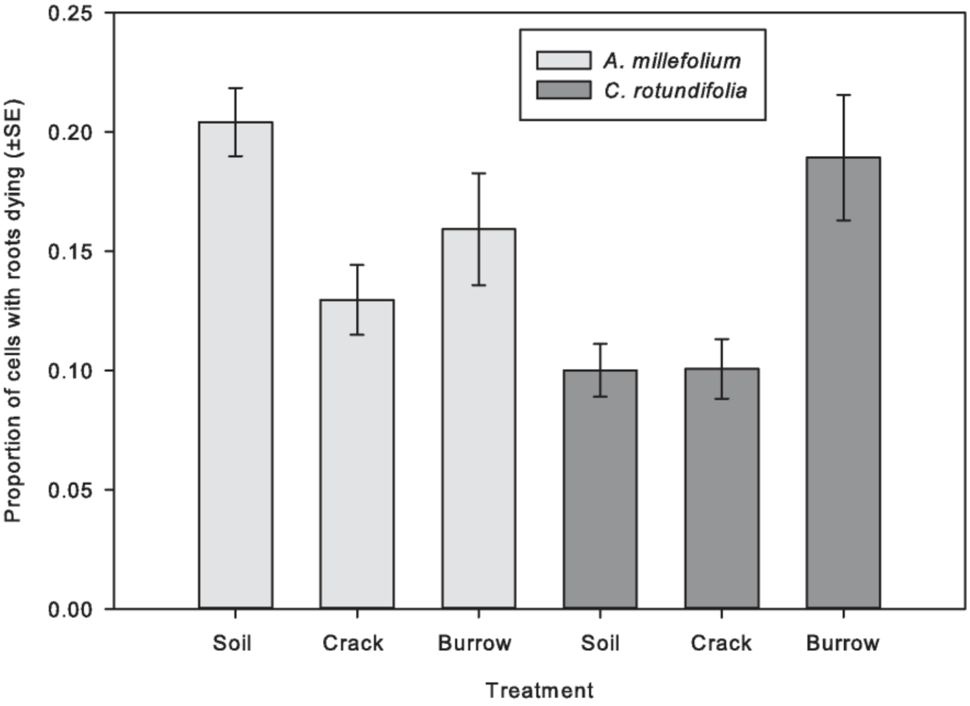


Figure 6.3. Proportion of 6 mm x 6 mm grid cells with roots dying (± 1 SE), out of all cells occupied by roots during the experiment, in soil, cracks, and burrows for *Achillea millefolium* and *Campanula rotundifolia*.

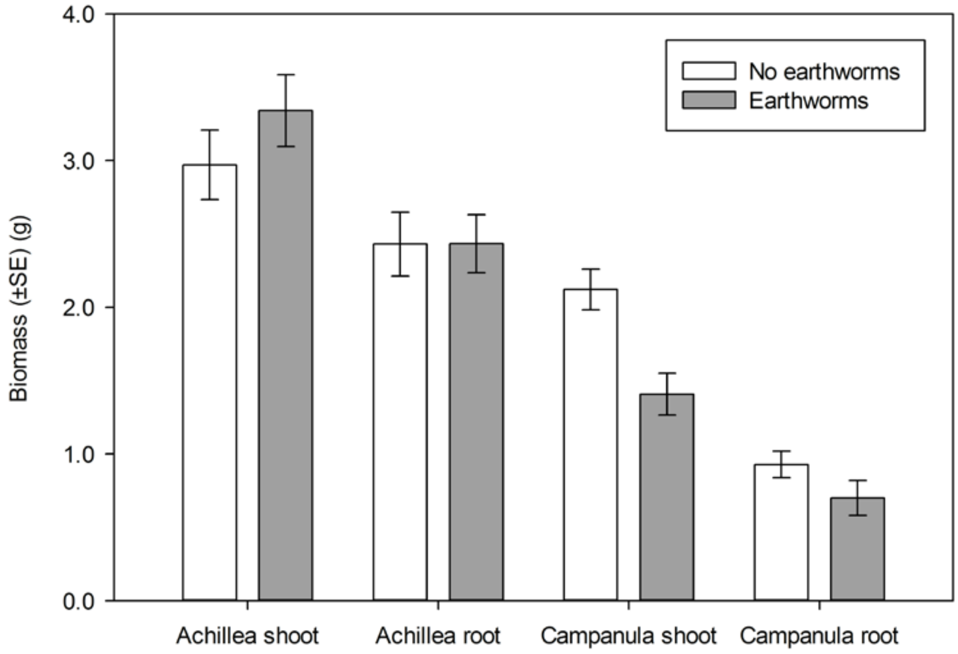


Figure 6.4. Shoot and root biomass in grams (± 1 SE) for *Achillea millefolium* and *Campanula rotundifolia*.

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CHAPTER 7. EARTHWORM EFFECTS ON DISTRIBUTION OF A NATIVE PREDATOR, THE AMERICAN ROBIN[6]

7.1 Introduction

Predator-prey interactions are a key factor influencing the success and impacts of invasive species (Colautti et al. 2004; Salo et al. 2007). Given the large population sizes that can be achieved by invasive species, non-native prey may act as an important food source for native predators (Barber et al. 2008). Such trophic subsidies may lead to changes in native predators' diets (Maerz et al. 2005; King et al. 2006), abundances (Tablado et al. 2010), or distributions (Roemer et al. 2002; Barber et al. 2008). However, the effects of exotic prey on native predators have not been well-examined (Rodriguez 2006; Carlsson et al. 2009).

Anthropogenic modification of habitats can also cause shifts in distributions of both native and non-native species (Didham et al. 2007). Habitat fragmentation and loss can result in population declines of native species (Murcia 1995), while non-native species often benefit due to a greater ability to disperse or survive in the altered environments (With 2002). However, human disturbances benefit some native species, such as habitat generalists that have broad niches and thus can exploit habitats altered by anthropogenic activities (Devictor et al. 2008). Native generalists may be better able to exploit non-native species as food resources due to the fact that they have more flexible diets than specialist native species (Maerz et al. 2005; Carvalheiro et al. 2010).

One generalist species that benefits from anthropogenic activity and consumes non-native food resources is the American robin (*Turdus migratorius* L., 1766) (Hawrot & Niemi 1996; Lafleur et al. 2007). Robins are habitat generalists that can thrive in both urban and forested environments (Howell 1942). Forest fragmentation is believed to have had a positive effect on robin populations due to their preference for both natural and anthropogenically-created edge habitats (Howell 1942; Hawrot & Niemi 1996). Robins are omnivorous, with diets that vary seasonally and include both invertebrates and fruit (Wheelwright 1986). This dietary flexibility appears to allow inclusion of novel items as major components of

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[6] A version of this chapter has been published. Cameron, E. K., and E. M. Bayne. 2012. Diversity and Distributions 18:1190-1198.

their diet, such as non-native fruits (Lafleur et al. 2007) or earthworms (Howell 1942).

Consequently, earthworm expansion into previously earthworm free areas might provide an additional food source that could influence robin distributions. Increased forest dissection and fragmentation by roads, pipelines, seismic lines (8-10 m wide linear features used for oil and gas exploration), campgrounds, and well pads may benefit robin populations both by providing increased edge habitat for nesting and by facilitating spread of earthworms due to vehicle traffic along these features (Cameron et al. 2007; Cameron & Bayne 2009).

In addition to benefiting from earthworms as a novel prey source, robins themselves could act as important vectors of earthworm spread. Transport and release of live earthworms by robins probably occurs only over short distances, when an earthworm is dropped prior to consumption. Similarly, earthworm cocoons are not likely to become attached to robins' plumages or feet. These cocoons are oval and have a minimum diameter of approximately 2 mm (Sims & Gerard 2005), whereas the propagules most likely to adhere to birds for significant periods of time are very small or have a hooked shape (Green & Figuerola 2005). However, earthworm cocoons could be moved over larger distances if they are consumed and survive digestion by robins (Langmaid 1964). This possibility has been investigated with aquatic invertebrates, which can be transported internally by waterfowl (Green & Figuerola 2005).

We examined whether American robin and earthworm distributions are correlated at landscape- and local-levels in the boreal forest of northern Alberta. To address this question, we first assessed whether robin occurrence varies depending on anthropogenic edge type in this region. Because robins are often closely associated with humans and forest edges (Hawrot & Niemi 1996), we predicted that the probability of robin occurrence would increase as the intensity of human activity increased. Specifically, we expected robins would most often be present at well pads and campgrounds, followed by roads, pipelines, seismic lines, and forest interiors. Secondly, we sampled earthworms to assess whether earthworm and robin distributions are correlated at the landscape-level. Because anthropogenic activity is a key vector of earthworm spread, we expected that the probability of earthworm occurrence would also increase as intensity of human activity increased in our larger dataset. Thirdly, we investigated whether non-native earthworm and robin distributions are correlated at the local-level by conducting surveys of earthworms at paired edge locations where robins were present versus absent and which experienced similar amounts of human activity. Such a correlation would suggest robins are either exploiting earthworms as prey or acting as a dispersal vector for earthworms, rather than the two groups' distributions being separately correlated with anthropogenic activity. Fourthly, by conducting a captive feeding experiment, we tested whether robins could act as a dispersal vector for earthworms via internal transport of their cocoons.

7.2 Methods

7.2.1 American robin surveys

Our research was conducted in an 80 888 km^2 area in the boreal forest of northern Alberta, Canada, between 54.4°N and 58.8°N latitude and 110.1°W and 119.3°W longitude (Figure 7.1). To assess American robin distributions in relation to forest edges, we conducted point count and playback surveys at six location types, including campgrounds, well pads, roads, pipelines, seismic lines, and in forest interiors. Sampling took place from 8 May to 27 May 2007 and 12 May to 9 June 2009 between 04:35 and 09:50 MST. In 2007, surveys were conducted along road edges, with twelve points spaced 800 m apart on each 8 km long road transect (Figure 7.1). In 2009, we sampled in the centre of a well pad or campground ("nodes"), at four points spaced 600 m apart along a linear feature leading away from this node, and at four forest interior points located 250-300 m into the forest and halfway (300 m) between the points on the feature (Figure 7.1). Thus, each of these transects was 2.4 km in length. The high levels of anthropogenic activity within our study area prevented us from finding forest interior points that were further than 300 m from a linear feature. In some cases, there were no nodes along the linear feature and an additional survey point was instead sampled on the feature, or the node was shared between two features (e.g., a campground with a road and a seismic line extending from it). Sites were selected using the Alberta Base Features Layer and the Alberta Ground Cover Classification (AGCC) obtained from Alberta-Pacific Forest Industries in ArcGIS 9.3 (ESRI Inc., Redlands, California). We first identified campground locations in ArcGIS and then selected the nearest well pad and linear features.

Sampling points were visited once, by one of three randomly assigned observers. At each point, we performed a five minute point count, followed by a one minute playback of a male robin song (Titus & Haas 1990), and an additional three minute observation period. Use of playbacks increases the likelihood of detecting individuals that are present but silent (Yahner & Ross 1995), although this method still assumes there are no systematic differences in detectability between locations. If a singing male was heard or a pair of robins was observed during the count, the observer stopped the survey, identified the location of the robin, and moved to the next point. The habitat type of each point count location was classified as deciduous, coniferous, black spruce bog, other wetland, or mixedwood. A total of 591 points were sampled, including 14 campgrounds, 21 well pads, 198 roads, 64 pipelines, 73 seismic lines, and 221 forest interiors.

7.2.2 Earthworm surveys

To test whether earthworm distributions show a similar relationship to anthropogenic activity as robins at the landscape-level, we combined data collected in this study with previously published data (Cameron et al. 2007). In the surveys from Cameron et al. (2007), earthworms were sampled in six 50 cm by 50 cm quadrats along a 50 m transect running parallel to the forest edge. A total of 92 surveys were conducted at campgrounds, roads, seismic lines, and forest interiors. In the present study, we added 97 surveys at campgrounds, roads, seismic lines, pipelines, and well pads, as described below. Thus, with both years together, the analysis included 23 campgrounds, 6 well pads, 45 forest interiors, 15 pipelines, 71 roads, and 29 seismic lines. From mid-May to mid-June in both 2007 and 2009, we sampled earthworms at forest edge points where a singing male robin or a robin pair were present and at edge points where robins were absent. Because we were interested in assessing earthworm occurrence in robin territories, rather than in areas where robins were migrating or passing through, points where a single silent robin was sighted were excluded from these surveys. As well, we conducted an additional robin playback when we returned to sample earthworms in order to verify that robins were not actually present in areas we had previously identified as having no robins. We sampled at two randomly selected points with robins present and two points where robins were absent per transect in 2007 and at one point of each type on the shorter transects in 2009. In both years, after randomly selecting a point where robins were present along a transect, we restricted the selection of its paired point with no robins to stations in similar habitat types. On transects where robins were present or absent at all points, only one randomly selected point was sampled.

Earthworms were sampled in a 25 cm by 25 cm quadrat at the point where the robin was first observed. At points where robins were absent, we sampled 1 m into the forest from the point count station because most robin observations occurred at or close to the forest edge and the point count station. We sampled up to eight additional quadrats located 25 m and 50 m from that point, in a cross running perpendicular and parallel to the linear feature. If earthworms or cocoons were found in a quadrat, we stopped sampling at that point count station. We removed the O_i, O_e, and O_a horizons from the quadrats and hand-sorted this material to determine whether earthworms or cocoons were present. Hand-sorting of leaf litter is the most accurate method for estimating abundance for most earthworm species (Callaham & Hendrix 1997), particularly litter-dwelling species such as *D. octaedra*. Mineral soil layers were not sampled, as previous research in northern Alberta indicated sampling these layers did not significantly increase detection rates (Cameron et al. 2007).

7.2.3 Feeding experiment

To assess whether earthworm cocoons could survive digestion, we captured eight adult robins using mist-nets near Edmonton and Slave Lake, Alberta in May 2010. We force-fed them each five *Dendrobaena octaedra* Savigny, 1826 cocoons and held them individually in 1 m x 1 m x 2 m cages. Food and water were supplied ad libitum. Droppings were collected and examined for cocoons after six hours. This time length is substantially greater than the mean digestion time of approximately 60 minutes reported for robins fed fruit or invertebrates (Levey & Karasov 1992). Robins were released at their capture sites.

7.2.4 Data analysis

We assessed the effects of edges on robin distribution using mixed-effects logistic regression in Stata 9.1 (Stata-Corp, College Station, Texas). A random effect was included to account for the spatial clustering of sites on the landscape (clusters included point count stations less than 8 km apart). There were 591 point count locations and 21 clusters in this analysis. An analysis using transects as a random effect produced similar results, but we report only results for clusters because model fit was better in that case. We used Akaike's information criterion corrected for small sample sizes (AIC_c; Burnham & Anderson 2002) and the dimension-consistent Bayesian information criterion (BIC; an approximation of the Bayes factor when no prior information on relative model support is available; Burnham & Anderson 2002) to assess support for three competing models explaining robin occurrence. The models included location type (i.e. anthropogenic edge type), spatial gradient (east-west and north-south), and habitat composition (proportion of forest and proportion of conifer). Julian date and year were included in all models to account for temporal sampling effects. The spatial gradient model was included because of east-west and north-south gradients of increasing intensity of anthropogenic activity and earthworm occurrence in northern Alberta (Cameron & Bayne 2009). Forest cover at the landscape-level has previously been shown to affect robin occurrence (Drolet et al. 1999), and therefore proportion of forest and proportion of coniferous forest within a 200 m radius area of each point count station were calculated in ArcGIS 9.3 (ESRI Inc., Redlands, California). A radius of 200 m was chosen because it is the approximate size of a robin territory (Knupp et al. 1977). Location type was coded as a categorical variable with six categories (campground, well pad, road, pipeline, seismic line, forest interior). No strong correlations ($r^2 > 0.6$) were present between variables.

We also examined the effects of location type and spatial gradients on earthworm occurrence using mixed-effects logistic regression. A random effect was included to account for spatial clustering of sites and support for competing models was evaluated using AIC_c and BIC. Year was included in all models to account for temporal sampling effects. Finally, we used a mixed-effects logistic regression model to assess the effects of earthworm occurrence on robin occurrence in our paired surveys at 97 points. Because of the paired design used in this survey, the only independent variable in this model was earthworm occurrence, but a random effect was used to account for spatial clustering of sites. The Hosmer-Lemeshow test for goodness of fit was used to assess model fit in all cases (Hosmer & Lemeshow 1980).

7.3 Results

Location type was present in all of the best supported models, suggesting it had a strong effect on robin occurrence (Table 7.1). The global model was the best supported model according to AIC_c, while the model containing only location type had the strongest support according to BIC. The model that included location type and habitat composition had both ΔAIC_c and ΔBIC values of less than two. Therefore, we consider the location type and habitat composition model to have the strongest support overall, and we report odds ratios and significance levels for variables in that model only. In this model, location type had a significant effect on robin occurrence (Figure 7.2a, $\chi^2 = 29.71$, $P < 0.0001$). As compared to roads, robins were significantly more likely to occur at campgrounds (odds ratio = 6.63, $P = 0.009$) and significantly less likely to occur on seismic lines (odds ratio = 0.41, $P = 0.03$) and in forest interiors (odds ratio = 0.33, $P < 0.001$). Occurrence at well pads (odds ratio = 1.50, $P = 0.45$) and pipelines (odds ratio = 0.55, $P = 0.15$) did not differ from occurrence at roads. Robin occurrence was also significantly affected by proportion of conifer forest within 200 m (odds ratio = 0.24, $P = 0.008$). The remaining variables were not significant. The Hosmer-Lemeshow goodness of fit test indicated that fit was adequate ($\chi^2 = 9.93$, $P > 0.25$).

Location type also had a strong effect on earthworm occurrence (Figure 7.2b, $\chi^2 = 35.61$, $P < 0.0001$) and was the only variable present in the model with the most support (Table 7.2). As compared to roads, earthworm occurrence did not differ significantly at campgrounds (odds ratio = 2.92, $P = 0.21$), pipelines (odds ratio = 0.40, $P = 0.27$), and well pads (odds ratio = 1.20, $P = 0.89$). Earthworms were less likely to occur on seismic lines (odds ratio = 0.066, $P < 0.001$) and in forest interiors (odds ratio = 0.019, $P < 0.001$) than roads. The global model had the next strongest support, with the spatial model receiving little support. The Hosmer-Lemeshow goodness of fit test was not significant, suggesting model fit was adequate ($\chi^2 = 10.48$, $P > 0.20$).

Robins were significantly more likely to occur at locations where earthworms were present than at those where they were absent (Figure 7.3, odds ratio = 4.41, $P = 0.002$). Model fit was adequate according to the Hosmer-Lemeshow test ($\chi^2 = 5.78$, $P > 0.25$). Most earthworms sampled were the epigeic (litter-dwelling) species *D. octaedra* (98%), while the remaining earthworms were the epigeic species *Dendrodrilus rubidus* Savigny, 1826 (1%) and the endogeic (mineral soil-dwelling) species *Aporrectodea tuberculata* Eisen, 1874 (1%).

Inspection of droppings from eight robins experimentally fed cocoons did not yield any whole cocoons or cocoon fragments.

7.4 Discussion

Distribution of American robins in northern Alberta was strongly associated with both anthropogenic habitat modification and presence of non-native earthworms which may act as a novel prey source. Robin occurrence was highest in areas with the greatest amount of human activity and a higher probability of earthworm occurrence in our landscape surveys. Thus, robins were most likely to occur at campgrounds, followed by well pads, roads, pipelines, seismic lines, and forest interiors. As in previous studies (Howell 1942; Hawrot & Niemi 1996), robins preferred areas with decreased forest cover. Robin occurrence was also positively correlated with earthworm occurrence at the local-level within linear features, providing stronger evidence that the two species' concordant distributions across the landscape were not simply due to separate correlations of robins and earthworms with human activity or habitat type.

We also tested whether robins could internally transport earthworm cocoons in order to exclude the possibility that the observed correlation between earthworm and robin distributions was due to robins acting as a dispersal vector. No earthworm cocoons survived digestion, suggesting that robins are unlikely to be key vectors of earthworm spread. As spread of earthworms via songbirds (i.e. zoochory) would be difficult to control via management actions, it may be fortunate that robins do not appear to be major vectors of spread. Avian frugivores, particularly American robins, are important in the dispersal of a variety of non-native plants (Bartuszevige & Gorchov 2006) but there is no comparable evidence for internal transport of invertebrate propagules without shells by terrestrial songbirds.

To our knowledge, the present investigation is the first study to show an association of exotic prey with the local-level distribution (i.e. habitat selection) of a native predator. As soft-bodied invertebrates, earthworms may represent a more valuable food resource for native predators than hard-bodied species, because of reduced handling and digestion costs (Maerz et al. 2005). In addition, earthworms can often reach high densities and are relatively large in size, which could reduce the foraging effort required to locate them, as compared to native prey. Two previous studies have demonstrated broad-scale effects on native predator distributions owing to non-native prey (Roemer et al. 2002; Barber et al. 2008). Black-billed (*Coccyzus erythropthalmus*) and yellow-billed cuckoos (*C. americanus*) shifted their distributions within their current range in the northeastern United States to areas experiencing gypsy moth outbreaks (Barber et al. 2008). Similarly, within the Channel Islands of California, native golden eagles (*Aquila chrysaetos* L.) colonized new islands in response to the introduction of feral pigs (*Sus scrofa* L.) (Roemer et al. 2002). However, it should be noted that the golden eagle has also been described as invasive on these islands, rather than native (Collins et al. 2009).

As with shifts in occurrence, there are few examples of changes in native predator abundance due to exotic prey (Rodriguez 2006; Maerz et al. 2009; Tablado et al. 2010). In the boreal taiga plains of western Canada, robins have increased significantly in abundance at a rate of 1.1% per year since 1973 (Environment Canada 2010). Both earthworm occurrence and forest dissection have been increasing in northern Alberta during this time period (Schneider et al. 2003; Cameron & Bayne 2009), suggesting that this population increase could be related to either of these factors. However, our results do not allow the causal mechanism leading to this increase to be identified. Further increases are projected in both the density of human-origin edges (Schneider et al. 2003) and earthworm occurrence (Cameron & Bayne 2009) over the next 50 years in northeastern Alberta, suggesting that robin populations may continue to grow in this region if there is a causal link between earthworms and robins.

Distributional shifts or population growth of robins due to human activity and earthworm availability may also affect competing songbird species. Robins have similar habitat requirements to Swainson’s thrushes (*Catharus ustulatus*) and hermit thrushes (*Catharus guttatus*), and all three are ground- and shrub-foraging insectivores and frugivores (Dilger 1956; Paszkowski 1982). However, research on hermit thrushes and American robins when sympatric suggests that habitat partitioning occurs between the two species possibly because of competitive interactions (Dellinger et al. 2007). Changes in predator abundance or distribution as a result of increases in one exotic prey species can also affect other prey through apparent competition (Carlsson et al. 2009), but it is unclear whether this possibility is likely to apply to robin and earthworm populations.

Inclusion of non-native earthworms in the diets of robins may cause them to experience greater temporal variability in prey availability. Earthworm abundances and activity levels are strongly dependent on soil moisture levels and temperatures (Curry 1998). As a result, earthworm availability may fluctuate more over time than other invertebrate prey species and could consequently cause greater variability in robin population dynamics (Maerz et al. 2005). Research on red-backed salamanders (*Plethodon cinereus*) found that non-native earthworms created more dynamic resource levels than native prey, with large increases in prey volume on cool rainy nights compared to warm or dry nights when earthworm availability was reduced (Maerz et al. 2005). Because we did not examine the diet composition or fitness parameters of robins, it is not known whether such fluctuations could have a strong influence on robin populations, nor whether robins consuming more earthworms or occurring in areas with higher earthworm availability have increased fitness.

Our research suggests that robins, as a native predator, are responding to a trophic subsidy provided by non-native prey. This type of positive interaction between native and invasive species can complicate management efforts, particularly if the native species that benefits is of conservation concern (Barber et al. 2008). In the case of earthworm invasions in northern forests, negative impacts on native species including fungi, microarthropods, and plants (Gundale 2002; Frelich et al. 2006; Eisenhauer et al. 2007) appear to be more common than positive effects. Vertebrates, including woodland salamanders and ground-nesting songbirds, also appear to be negatively affected by earthworms, likely due to decreases in forest floor thickness (Maerz et al. 2005; Maerz et al. 2009; Loss & Blair 2011). These examples provide clear evidence of negative effects of earthworm invasions via their actions as ecosystem engineers, while our study indicates that non-native earthworms can also result in effects that are propagated upwards via trophic interactions.

There is no effective method known for controlling earthworm invasions in forests after introduction has occurred and management efforts have consequently focused on prevention of introductions (Callaham et al. 2006). As predators, robins might help to control earthworm populations since native predators can suppress populations of some non-native species (deRivera et al. 2005; Carlsson et al. 2009). However, few species appear capable of exerting substantial predation pressure on earthworm populations and it therefore seems unlikely that robins could limit their invasions. For example, predation by large populations of ring-billed gulls (*Larus delawarensis*) in ploughed fields concluded they had little impact on earthworm population size and persistence (Tomlin & Miller, 1988).

Generalist species often appear to be positively affected by global changes due to their greater flexibility in habitat preferences or diets than specialist species (Clavel et al. 2010). This can lead to biotic homogenization, in which species similarity between systems increases over time (McKinney & Lockwood, 1999; Olden et al. 2004). Our results indicate that generalist American robins may benefit from anthropogenic habitat modification and spread of non-native earthworms in the boreal forest. Further investigation using earthworm removals, earthworm additions, or paired plots with high and low earthworm abundances could provide greater insight into the effects of earthworms on robin densities and fitness. Previous research has emphasized that inclusion of non-native prey in the diets of native predators can result in greater variability in prey availability over time due to population cycling (Maerz et al. 2005; Barber et al. 2008), while our study further suggests that variability in space at local scales can have an effect on native predators.

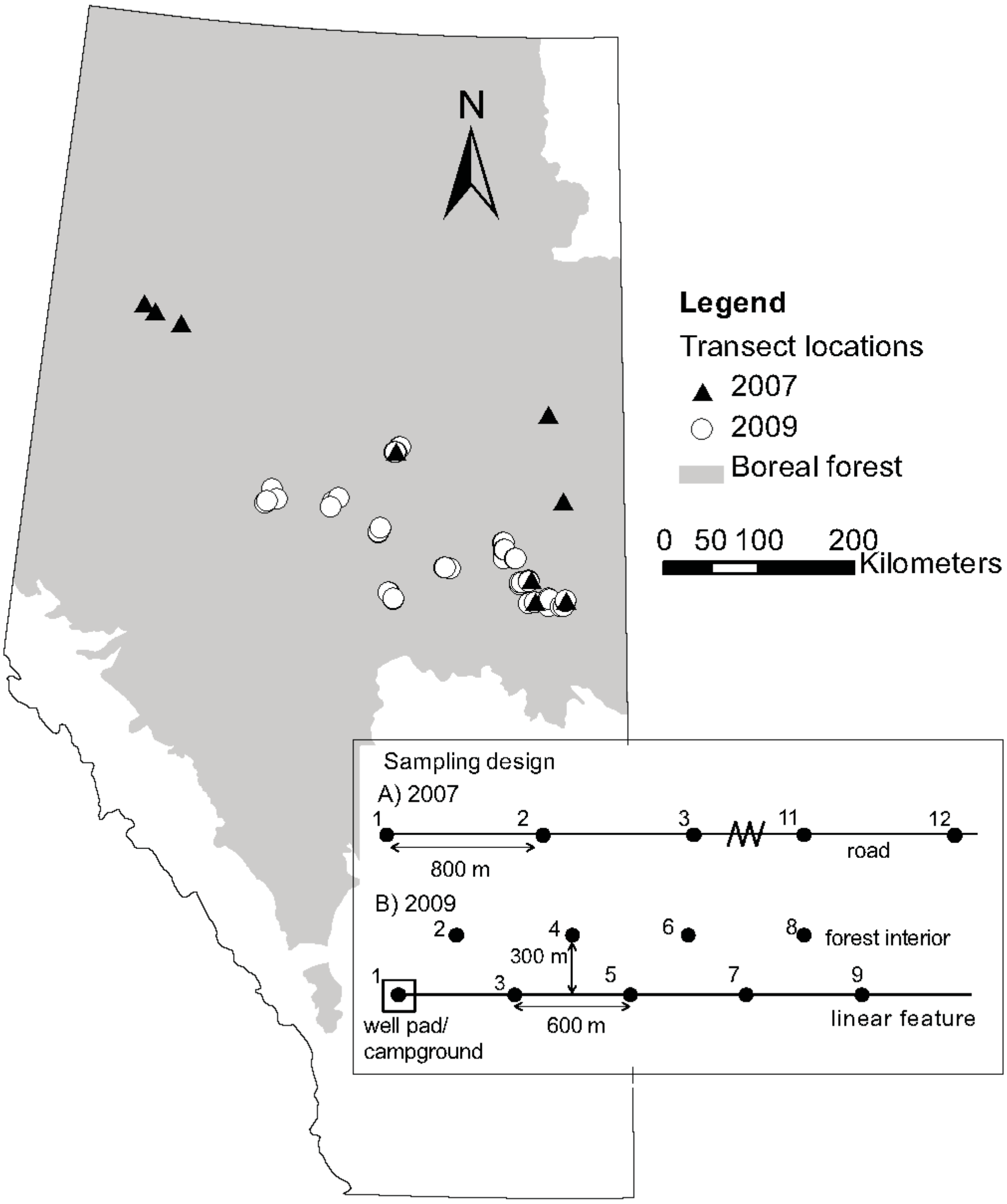

Figure 7.1. Locations of point count transects (Δ = 2007, ○ = 2009) within the boreal forest of northern Alberta (shaded in gray). Inset shows: a) Sampling design in 2007, with 12 points surveyed per road; b) sampling design in 2009, with 9 points surveyed per pipeline, road, and seismic line.

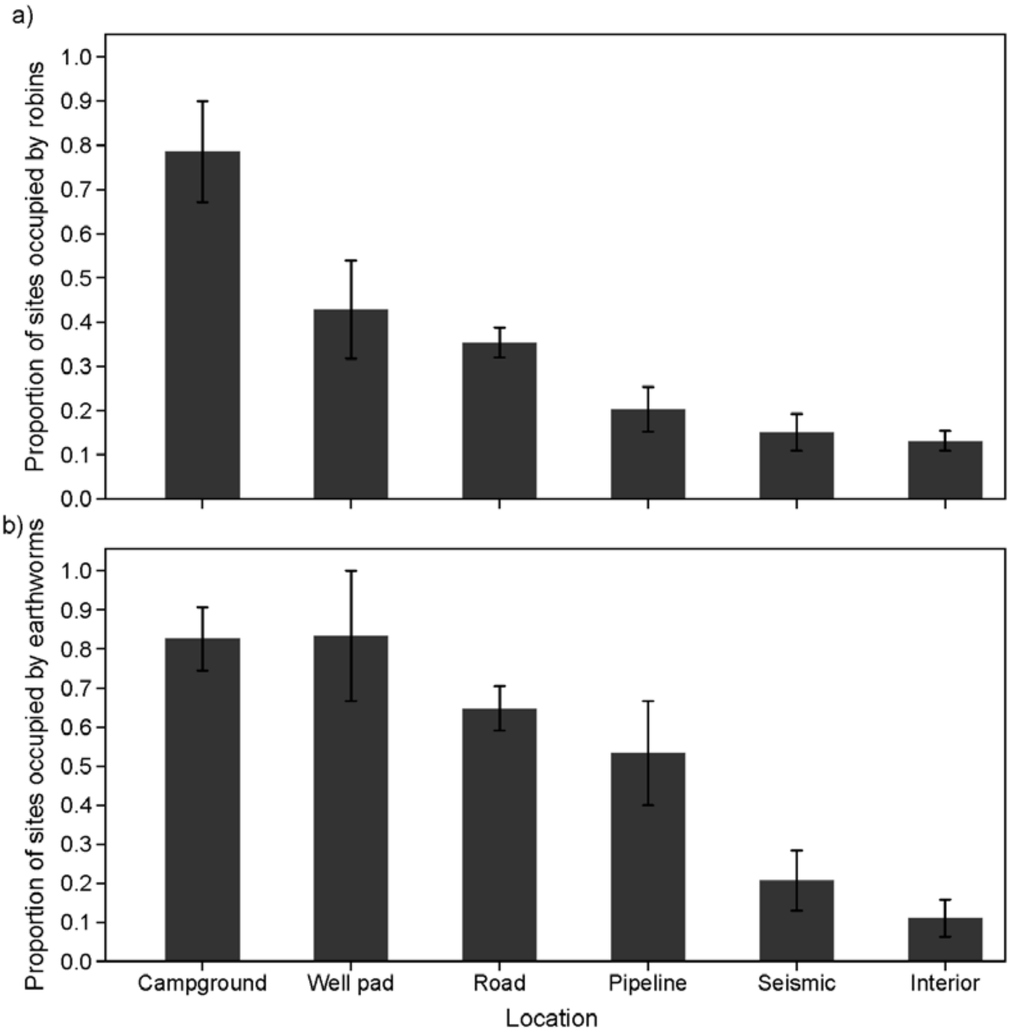

Figure 7.2. a) Proportion of point count stations (n= 591) (± 1 SE) with American robins present in relation to location type (campground, well pad, road, pipeline, seismic line, and forest interior); b) proportion of sites (n = 189) (± 1 SE) with earthworms present in relation to location type (with data from the present study and Cameron et al. 2007).

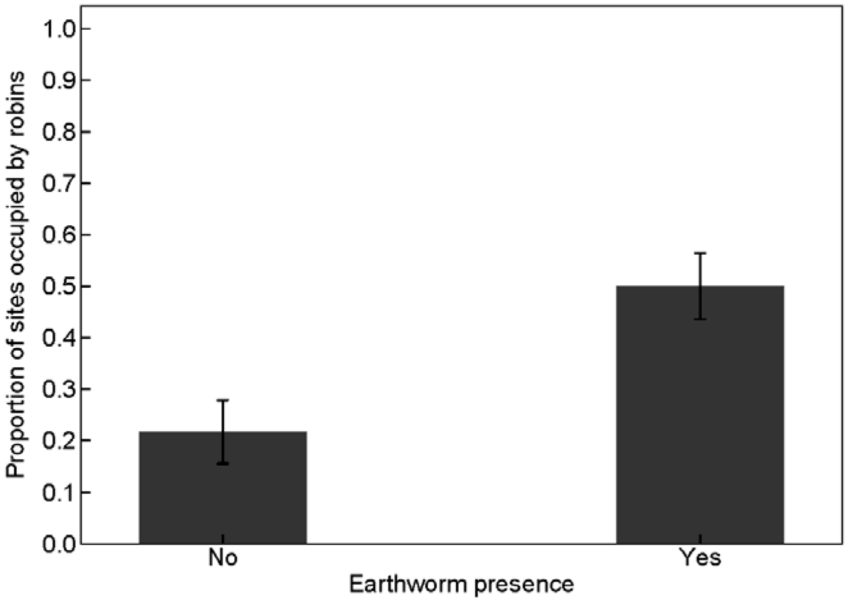

Figure 7.3. Proportion of locations (n = 97) (± 1 SE) with robins present in relation to earthworm occurrence.

Table 7.1. Logistic regression fit statistics for models predicting the occurrence of American robins. Model predictors include location type (*Edge*), forest composition (*Composition*), and spatial gradients (*Spatial*).

Model	K	LL	ΔAIC_c	ΔBIC	$w\text{AIC}_c$	wBIC
Null	2	-322.42	49.57	11.84	0.00	0.00
Edge	9	-294.16	7.35	0.00	0.01	0.64
Composition	6	-305.54	23.95	3.62	0.00	0.10
Spatial	6	-314.76	42.37	22.05	0.00	0.00
Edge + Composition	11	-288.75	0.66	1.94	0.41	0.24
Edge + Spatial	11	-292.02	7.22	8.49	0.02	0.01
Composition + Spatial	8	-302.41	21.78	10.12	0.00	0.00
Global	13	-286.33	0.00	9.86	0.57	0.00

With k, number of parameters; LL, log likelihood; ΔAIC_c, difference in the Akaike's information criterion (corrected for small sample size) value between model and the most strongly supported model; ΔBIC, difference in the Bayesian information criterion value between model and the most strongly supported model; $w\text{AIC}_c$, weight given by the AIC (i.e., relative strength of support for model); wBIC, weight given by the BIC.

Table 7.2. Logistic regression fit statistics for models predicting the occurrence of earthworms. Model predictors include location type (*Edge*) and spatial gradients (*Spatial*) (with data from the present study and Cameron et al. 2007).

Model	*K*	LL	ΔAIC_c	ΔBIC	$wAIC_c$	*w*BIC
Null	2	-125.46	53.49	31.74	0.00	0.00
Edge	9	-91.24	0.00	0.00	0.83	0.99
Spatial	6	-120.90	52.78	43.60	0.00	0.00
Global	11	-90.56	3.13	9.13	0.17	0.01

With *k*, number of parameters; LL, log likelihood; ΔAIC_c, difference in the Akaike's information criterion (corrected for small sample size) value between model and the most strongly supported model; ΔBIC, difference in the Bayesian information criterion value between model and the most strongly supported model; $wAIC_c$, weight given by the AIC (i.e., relative strength of support for model); *w*BIC, weight given by the BIC.

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CHAPTER 8. EVALUATION OF AN INVASIVE EARTHWORM EDUCATION PROGRAM[7]

8.1 Introduction

Anthropogenic activities are causing unprecedented changes to the environment worldwide, leading to calls for ecologists to devote a greater amount of their time to communicating research findings with the public and policy makers (Lubchenco 1998; Holdren 2008). Many ecologists now consider such communication to be important part of their careers (Pace et al. 2010). Public communication, and even advocacy, is viewed by some as a social responsibility of scientists (Lovejoy 1989; Noss 2007; Nelson & Vucetich 2009). This sense of duty may be a particularly important source of motivation for senior researchers, while junior scientists appear to be driven by enjoyment and personal satisfaction as well (Martin-Sempere et al. 2008). Unfortunately, regardless of their motivation for participation in public outreach, many scientists' academic training provides them with little opportunity to learn effective methods for communicating with the public. Further, scientists often do not view public communication as a core aspect of their work and may believe it has neutral or negative impacts on promotion because of the time it takes away from research (Gascoigne and Metcalfe 1997, Martin-Sempere et al. 2008).

Professional organizations such as the American Association for the Advancement of Science and the International Council for Science include enhancing communication among scientists and the public and improving science education as key goals for their organizations (AAAS 2012, ICSU 2012). Funding agencies including the National Science Foundation in the United States, and the National Science and Engineering Research Council in Canada have also attempted to promote public communication activities by including criteria relating to outreach efforts in their grant evaluation processes (Holbrook 2005; NSERC 2012). These types of initiatives should encourage public communication and education to be viewed as an integral component of scientific research. However, the high rates of failure (40-50%) estimated for public education campaigns (Ostergaard 2002)

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[7] A version of this chapter has been submitted for publication as Cameron, E. K., S. J. Yuckin, and E. M. Bayne. Communicating research with the public: evaluation of an invasive earthworm education program. Neobiota.

suggest that evaluation of the success of public communication efforts should be a critical part of outreach activities carried out by ecologists. Researchers' assumptions about a program's success are not sufficient.

Ideally, evaluation should include both formative evaluation, which is used to improve the program while it is being designed or run, and summative evaluation, which is used to determine if the program has achieved its objectives once it is complete (Carleton-Hug & Hug 2010). Yet, assessment of the success of public outreach efforts, whether conducted by academic scientists or others, is often overlooked. In a review of articles published from 1993 to 2008, only 20 evaluations of environmental education programs were found in the three leading environmental education journals (Carleton-Hug & Hug 2010). Lack of funding or time, inexperience with survey design, and fear of negative consequences if the program was unsuccessful are key reasons evaluations are not included in programs (Bitgood 1996; Carleton-Hug & Hug 2010).

Invasive species are often the focus of public education programs because humans can act as a key vector for invasive spread (Jordan et al. 2011). Our research on earthworm spread in Alberta, Canada (Cameron et al. 2007, 2008) led us to work with various agencies to develop a public education program about the effects of earthworms in Alberta. Such non-charismatic species may pose particular challenges for education and management, as their invasions may receive little public attention or initially proceed undetected. In part due to the limited availability of information on earthworm invasions, “vegetation change facilitated by earthworms in North American forests” was recently identified by leading scientists as one of the top 15 global conservation issues (Sutherland et al. 2011).

Management of invasive earthworms in northern forests has focused on trying to prevent introductions since there is no known method of control once populations are established (Callaham et al. 2006). Education programs about the ecological effects of earthworms have attempted to reduce abandonment of live earthworm bait by anglers in areas where earthworms may establish new populations, although most efforts are on a small scale (Keller et al. 2007). The largest program in the United States, the Great Lakes Worm Watch (developed by the Natural Resources Research Institute), has done this, in part, through the distribution of more than 1500 posters and fact sheets to bait shops, nature centers, and park visitor centers (Callaham et al. 2006). Callaham et al. (2006) describe public response to this educational campaign as favourable and state that the message to stop dumping bait has been well received. However, there does not appear to have been any formal published evaluation of the success of this program, or other earthworm-related programs, at informing the general public and altering behavior. If scientists are to effectively raise public awareness, more critical and objective evaluations of the success of public education projects are required.

We examined the effectiveness of our education program by testing whether changes occurred in knowledge or behavior after program implementation. Using pre- and post- surveys, we evaluated whether anglers had encountered program materials, their level of knowledge about earthworm invasions, and their attitudes towards changing their method of disposing of earthworm bait. An increase in awareness of the fact that earthworms are not historically found in Alberta and a decrease in the number of anglers discarding bait on land or in water would suggest that our program was effective.

8.2 Methods

8.2.1 Education program

The Alberta Worm Invasion Project was designed to increase public awareness of earthworm invasions in forests. The key messages of the program were that earthworms are invasive and can harm plants and animals in forests, and the public can help prevent earthworm introduction by not dumping bait. The program’s overall goals were thus to increase knowledge about earthworm invasions but also to change the behavior of anglers who release live earthworm bait into forests and lakes and convince them instead to save their bait or dispose of it in the garbage. It included five types of media: posters placed in bait shops, a website, two radio interviews, two televised informational clips, and two magazine articles (Table 8.1).

Posters were sent to 250 bait stores throughout northern Alberta, Edmonton, and Calgary by a bait distributor in 2010 (Figure 8.1). Stores included large specialized shops as well as gas stations that sell bait and approximately 100 of the stores accepted the posters and displayed them. The posters included a link to our website (http://worms.biology.ualberta.ca), which was also developed in 2010. This site can also be found if the phrase “worm invasion” or “Alberta worms” is searched in Google. On the website, five pages provide detailed information on earthworm spread, impacts on forests, ecological groups, how to avoid introduction, and an overview of our current research.

Let's Go Outdoors Radio aired two interviews about the spread of earthworms and also created two television clips in partnership with the Alberta Conservation Association (ACA). In the spring of 2010 and 2011, the radio interviews ran during a weekend show in 16 communities across Alberta with an expected audience of 100,000 in each case. One television clip was played in 2010 and the other in 2011. Each was played 48 times over the course of two weeks in early summer, and was estimated to have an audience of 600,000 viewers. A magazine article was published in the ACA's *Conservation Magazine* (Cameron 2010) and in the University of Alberta's alumni magazine *New Trail* (Habib 2011). These magazines have readerships of approximately 40,000 and 140,000, respectively. *Conservation Magazine* is targeted to anglers and hunters, while *New Trail* reaches a larger number of Albertans but is not targeted to anglers. All interviews, television clips, and articles included the information that earthworms are not native to Alberta's boreal forest and can be spread by anglers dumping bait, as well as some discussion of the effects of earthworms in forests.

8.2.2 Program evaluation

In-person pre- and post-interviews were used to evaluate our invasive earthworm education program. All individuals surveyed were a minimum of eighteen years old. The pre-surveys were carried out in the summer of 2009, with the ACA conducting 213 surveys and Alberta Sustainable Resource Development (ASRD) conducting 2018 surveys as part of their larger creel surveys of anglers. The post-surveys took place during the summers of 2011 and 2012. The ACA conducted 15 surveys and ASRD conducted 150 surveys in 2011, while we carried out a further 245 surveys in 2012. All surveys were performed at lakes across Alberta where participants were engaged in fishing activities. ACA conducted surveys in northern Alberta and ASRD conducted surveys in south-central Alberta, while our surveys in 2012 were carried out at previously sampled lakes across Alberta (Figure 8.2). In addition to these before-after surveys, we carried out a further 346 surveys in March 2011 at the Edmonton Boat and Sportsmen Show to obtain additional information on program effectiveness. Because the anglers at the show may have represented a different population of anglers and these surveys were conducted prior to the airing of the second television and radio clips, this data was not compared directly to the 2009 surveys but instead was examined qualitatively.

The initial 2009 survey contained three questions (Table 8.2), which addressed participants' use of earthworm bait, how they dispose of bait, and their awareness that earthworms are not native to Alberta's forests. The surveys in 2011/12 included the same three questions and one to three additional questions (Table 8.2). The additional questions examined whether participants had seen material from our education program and whether they had changed their bait disposal behavior or would be willing to change their behavior in the future. If the participant was not using earthworm bait, only the questions examining if they knew earthworms were historically found in Alberta and if they had seen any information from our program were asked (questions 1 and 4 in Table 8.2). If they did use earthworm bait and had seen the information, they were also asked if the information caused them to change their bait use (question 6 in Table 8.2). If they had not seen the information but used earthworm bait, they were asked if they would change their bait use (question 7 in Table 8.2). In the 2012 surveys and the Edmonton Boat and Sportsmen Show surveys, additional time was available as our questions were not part of a lengthy creel survey. Therefore, we asked anglers if they would change their bait use if they ever use bait, rather than asking question 7 only to people using bait that day.

8.2.3 Data analysis

Logistic regression was used to determine whether earthworm bait use (proportion of anglers who used earthworm bait), the location where bait was acquired (proportion bringing bait from home versus digging it up at the lake) and bait disposal (proportion who saved bait/discarded it in the trash versus discarded it on land/water) changed after implementation of the program. We also used logistic regression to examine whether awareness of earthworm invasion (i.e., the proportion of anglers who knew earthworms were not native to Alberta) changed after the program was implemented. Analyses were performed in Stata version 11 and were considered statistically significant at $P < 0.05$.

8.3 Results

8.3.1 Before-after surveys

A similar proportion of anglers reported using earthworm bait on the day they were surveyed in 2009 (9.2% SE 0.61) and 2011/12 (7.6% SE 1.3) according to a logistic regression (odds ratio =0.81, $P = 0.29$). In both years, most people brought their bait from home rather than digging it up at their fishing location. However, the proportion of people bringing bait from home was significantly higher in 2009 at 99% (SE 0.69) than in 2011/12 at 83.9% (SE 6.7) (odds ratio = 0.05; $P = 0.001$).

We used logistic regression to compare methods of disposing of earthworms that could contribute to their spread (releasing them in the lake or on land) versus disposal of earthworms that could prevent anglers from contributing to their spread (disposing of them in the trash or saving them for the next trip). There was a significant difference between 2009 and 2011/12 (odds ratio = 2.59, $P = 0.015$), with 39.2% (SE 3.4) of participants in 2009 and 62.5% (SE 8.7) in 2011/12 disposing of earthworm bait in lakes or on land (Figure 8.3).

Before implementation of our education program, 15.8% (SE 0.77) of anglers were aware that earthworms were not historically found in Alberta, compared to 15.1% (SE 1.8) of anglers in 2011/12. This difference was not significant (odds ratio = 0.95, $P = 0.72$).

8.3.1 Qualitative results (boat show and 2011/12 lake surveys)

When the 2011/12 survey respondents (including both those at the boat show and lakes; n = 756) were asked if they had seen any information about earthworm invasions in Alberta during the past year, only 31 people, or 4.1%, indicated that they had. Most of these participants saw an article on earthworm invasions (35.5%) or a TV clip (22.6%). The remainder received information from the website (16.1%), a poster (12.9%), another person (6.5%), the radio (3.2% - 1 person), or could not remember where they saw the information (1 person).

Only eight of the participants who had seen information from our program were users of earthworm bait. Of these eight, only three indicated they had changed their behavior by reducing their use of earthworm bait or putting leftover bait in the garbage. A total of 276 participants used earthworm bait at least occasionally and had not seen the education program material. When they were asked if they would change their use of earthworms as bait knowing that they are invasive and can negatively affect plants and animals, 46.7% responded that they would not.

8.4 Discussion

8.4.1 Program's level of success

Despite using a variety of forms of media, including print, television, radio, and internet, our program appeared to reach only a small number of anglers and to have a limited effect on knowledge and behavior. Only 4.1% of the surveyed anglers reported seeing information from the program. This low proportion suggests that our program might have benefitted from a more detailed preliminary examination of whether the media employed were likely to successfully reach our target audience. No increase in awareness of earthworm invasions was observed, with 15.8% and 15.1% of respondents before and after our program, respectively, stating correctly that earthworms were not historically found in Alberta. This level of awareness is similar to that encountered in surveys in New York, where 17% of people were aware that earthworms were exotic invasive species (Seidl & Klepeis 2011). Overall, our results emphasize the importance of conducting evaluations to assess the effectiveness of public awareness programs, rather than assuming programs have been successful based on the amount of program literature distributed or the expected audience size for various media.

It has been suggested scientists should devote one tenth of their professional time to outreach efforts and other activities intended to increase the societal benefits of science and technology research (Holdren 2008). We estimate that our program took approximately 140 hours for one of us to initiate over the course of three years, which is considerably less than this suggested amount but still represents a substantial time commitment. Given the limited change in awareness, it could be questioned whether these types of activities are a worthwhile investment for ecologists. However, it is likely that the amount of time and money (~$5000, although we were not charged for television and radio clips) required to set up this program was less than would have been required by a conservation or government group. Although we lacked experience with public education programs, we were already familiar with research on earthworm invasions and had resources (e.g., pictures used on the website and magazine articles) available from previous work.

Rather than viewing the program’s limited success as an indication that academic scientists should not attempt this type of work, it could instead be seen as providing evidence of the importance of evaluating programs while carrying them out. Conservation education programs which include some type of formative evaluation are more likely to be successful (Jacobson & McDuff 1997; Norris & Jacobson 1998).

8.4.2 Effectiveness of media types

Some types of media were more effective than others at reaching our target audience. The articles (35.5%) and television clips (22.6%) were the forms of information most often encountered. Use of mass media such as television and radio has been identified as a factor contributing to the success of conservation programs (Jacobson & McDuff 1997). Fewer participants reported seeing our poster or website and only one mentioned the radio interview. Previous research on bait use recommended that bait stores would be an ideal location for placing signs or other sources of information about earthworm invasions (Keller et al. 2007). Our survey at the Edmonton Boat and Sportsmen Show also confirmed that many anglers do purchase their bait and thus targeting bait stores with program materials does seem to be a reasonable approach. However, signs had similarly limited effects on behavior in a study on bear-proof garbage containers (Baruch-Mordo et al. 2011). Although we consulted with other biologists and environmental program coordinators when designing our program, a more interdisciplinary approach involving greater initial consultation with other disciplines (e.g., psychology, education, business) might have improved our program’s success but would also have required a substantially greater investment of time.

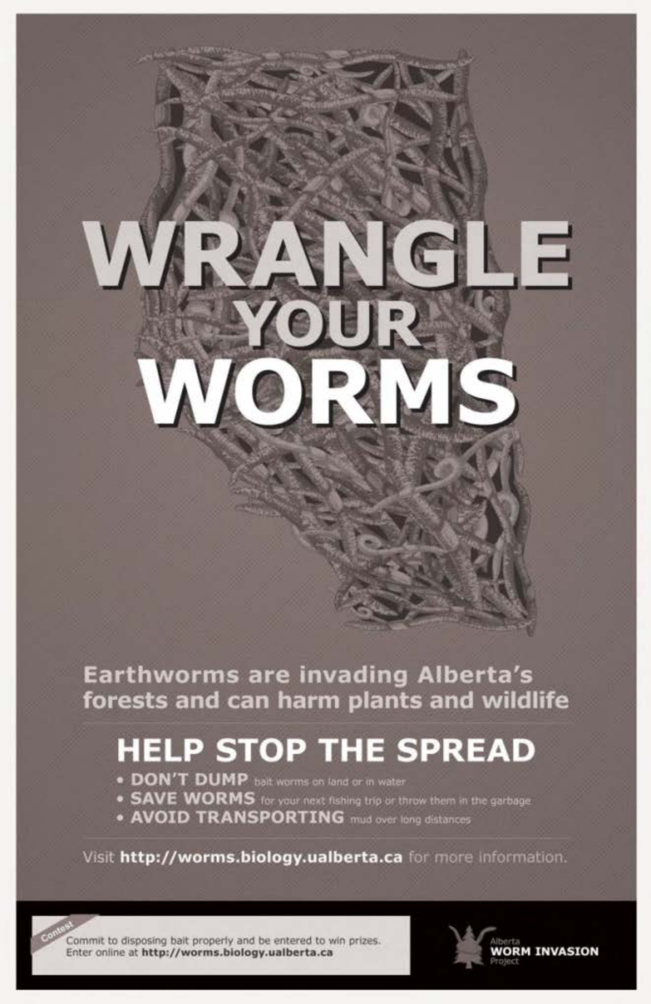
Several alternative methods of delivering program material, which we have been developing through consultation with researchers in sociology and education, may be more effective than the strategies used in our program thus far. First, warning labels could be placed on bait container lids to reach anglers in bait stores. Because anglers should see these labels whenever they use their bait, stickers are less likely to be overlooked than posters on bait fridges. We conducted a small pilot study to test this idea, in which we placed stickers on 5000 containers in 2012. Because we wanted to understand whether bait disposal behavior changed when stickers were present, we enlisted the assistance of store cashiers to collect contact information of bait purchasers. We then surveyed these anglers two weeks after their purchase and 75% stated they saved their bait or put it in the trash, compared to 61% and 37% in our before-after surveys. Unfortunately, the response rate was extremely low with only 12 people responding to the survey, although a greater number left their email addresses. Nonetheless, these results suggest this strategy deserves further examination. Secondly, involvement in citizen science programs can lead to increased knowledge among the public (Jordan et al. 2011). Thus, we have begun collaborating with researchers in the Faculty of Education at the University of Alberta to develop a smartphone application that allows students and the public to participate in data collection on earthworm distributions in Alberta. Thirdly, two participants in our surveys indicated they heard about our program from other anglers, rather than by directly encountering program material. Research on behavioral choices indicates that knowing others are behaving in a particular way can strongly encourage people to conform to the same behavior, regardless of their own level of environmental awareness (Michel-Guillou & Moser 2006; Clayton & Myers 2009). Consequently, encouraging anglers who are active in anglers' associations or work in fishing stores to pass information on to other anglers might lead to greater changes in behavior.

8.4.3 Behavioral changes

Only three out of the eight people who used earthworm bait and also saw our program material stated that they had changed their approach to disposing of bait as a result. Furthermore, many people (46.7%) who did not see our material indicated they would not change their earthworm bait use or disposal after being told that earthworms were not native to the area and could harm plants and animals. Although we did not ask participants for comments on why they would or would not change their bait disposal, 30% of the people at the Edmonton Boat and Sportsmen Show who stated that they would not change commented that they did not believe earthworms could be a problem. Similar to this, almost 85% of residents surveyed in a study in New York moderately or strongly agreed that earthworms have a positive impact on plants (Seidl & Klepeis 2011). Many adults have likely heard or been taught about the benefits of earthworms for soil in gardens and agricultural systems. The reputation of earthworms as beneficial therefore appears to present an additional challenge for management efforts. On the other hand, even when people are aware that species are invasive, they may be unwilling to change their behavior. In a recent survey on attitudes towards invasive species, almost 30% of individuals were willing to introduce non-native species to an area if they would personally benefit (Garcia-Llorente et al. 2008).

8.4.4 Conclusions

The limited impact of our invasive earthworm education program highlights the importance of evaluating conservation programs. Formative evaluation is particularly essential as it allows for the improvement of programs while they are being carried out. A more interdisciplinary approach to program design may also lead to greater program success. Our survey confirmed that anglers are a significant source of earthworm introduction in Alberta, and therefore efforts to target anglers are needed if a reduction in spread of earthworms is desired. Increased access to artificial lures or proper disposal methods, such as labeled trash cans at boat launches, could make it easier for anglers to behave responsibly (Seidl & Klepeis 2011). However, evaluations of other education campaigns have concluded that regulations or regulations combined with education are more effective than education alone at bringing about behavioral changes (Baruch-Mordo et al. 2011). The non-charismatic nature of earthworms and the disbelief many respondents expressed upon being told earthworms could be harmful in forests suggest that regulations restricting bait dumping or bait sales, as have been implemented in other jurisdictions (Callaham et al. 2006; Kilian et al. 2012), are likely needed for a significant reduction to occur in earthworm introductions.



Earthworms are invading Alberta's forests and can harm plants and wildlife

HELP STOP THE SPREAD

- DON'T DUMP bait worms on land or in water
- SAVE WORMS for your next fishing trip or throw them in the garbage
- AVOID TRANSPORTING mud over long distances

Visit **http://worms.biology.ualberta.ca** for more information.

Contest

Commit to disposing bait properly and be entered to win prizes.
Enter online at **http://worms.biology.ualberta.ca**

Figure 8.1. Poster distributed to bait stores as part of our earthworm education program.

Figure 8.2. Locations of surveys across Alberta, with ● representing lakes surveyed in 2009 and 2012, ○ = lakes surveyed in 2011, □ = Edmonton Boat and Sportsman Show in 2011, and ▲ = lakes surveyed in 2011 and 2012.

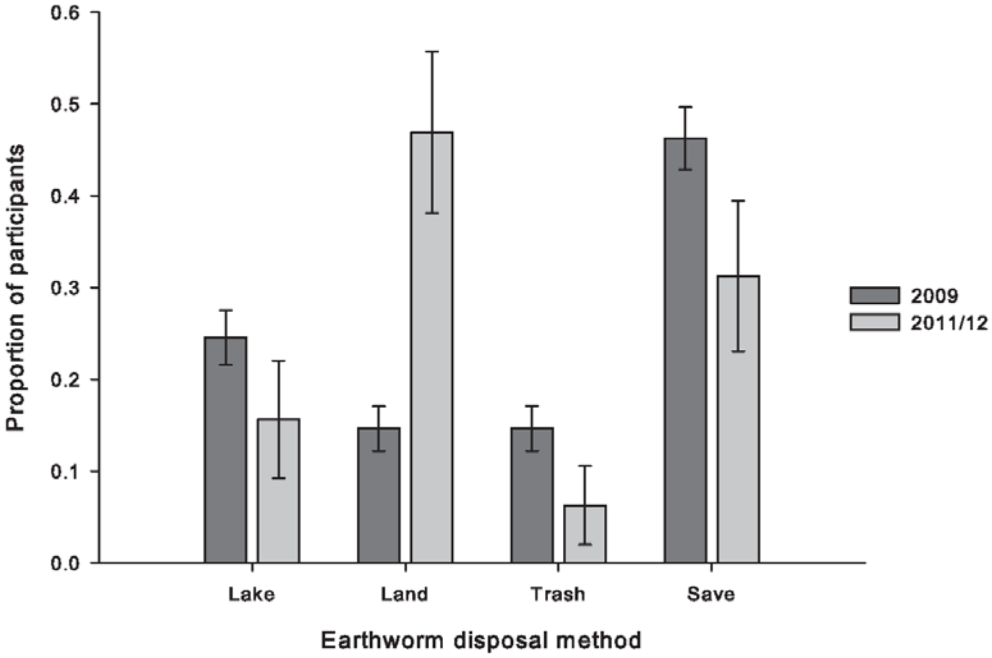


Figure 8.3. Proportion of participants (± 1 SE) using different earthworm bait disposal methods (release in lake, release on land, put in trash, save for next trip) in 2009 (n=212) and 2011/12 (n=32).

Table 8.1. Educational program media and estimated audience sizes.

Media	Audience
Conservation magazine article	40,000
New Trail magazine article	140,000
Posters	~100 stores, number of people unknown
Radio interviews	100,000 (in each of 2010 and 2011)
TV clips	600,000 (in each of 2010 and 2011)
Website	Not available

Table 8.2. Angler survey questions. Questions 1 to 3 were asked in 2009 and 2011/12, while questions 4 to 7 were only asked in 2011/12.

Question	Closed answer options
1. If you use earthworm bait, where do you get it from?	a) did not use b) caught near fishing location c) brought from home
2. At the end of the day, what do you do with your leftover earthworm bait?	a) release in lake, b) release on land, c) dispose of in trash, d) save for next trip
3. Do you think earthworms were historically found in this region?	a) yes, b) no, c) do not know
4. In the past year, have you seen any information about earthworm invasions in Alberta?	a) yes, b) no
5. Where was this information from? If other, provide source.	a) posters, b) TV commercial, c) website, d) article, e) other
6. If you have heard about earthworm invasions, has it changed your use of bait?	a) yes, b) no
7. Knowing that earthworms are invasive to boreal forests in Alberta and can negatively affect plants and animals, will you change your use of earthworm bait?	a) yes, b) no

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CHAPTER 9. SYNTHESIS.

9.1 Invasional meltdowns and ecosystem engineering

In this dissertation, I investigated above- and below-ground effects of exotic earthworms in Alberta's boreal forest. Although it has been suggested that facilitation between non-native earthworm species may lead to synergistic effects on other species or invaded ecosystems (Hale et al. 2005, Suarez et al. 2006, Hendrix et al. 2008), my results provide no support for the suggestion that earthworm invasions could constitute an invasional meltdown. The non-native earthworm species *L. terrestris* and *D. octaedra* did not facilitate each other's survival, growth, or reproduction (Chapter 2). Rather, *L. terrestris* had a competitive effect on *D. octaedra*, with *D. octaedra* producing significantly fewer cocoons when *L. terrestris* was present. Similarly, these two species did not have synergistic or over-additive effects on leaf litter depth, bulk densities, soil nitrogen concentrations, microarthropod abundance or composition, white spruce biomass, or colonization of spruce roots by ectomycorrhizal fungi (Chapters 2, 3, 5). In some cases, the impacts of both earthworm species combined were larger than either species by itself, but effects were only additive. Although these results do not exclude the possibility of an invasional meltdown involving different earthworm species or forest types, they emphasize the need for experimental tests rather than relying on observational data alone when assessing occurrence of invasional meltdowns.

As ecosystem engineers, earthworms can cause population and community level changes similar to non-engineering species, but they can also directly affect ecosystem functioning. These ecosystem level changes can then feedback to influence populations and communities. However, it is not clear whether many of the effects caused by non-native earthworms are the result of ecosystem engineering or direct interactions (e.g., predation, herbivory). In my experiments, changes in soil structure associated with ecosystem engineering had strong effects on both movement of microarthropods and growth of plant roots. More individuals of Collembola and Mesostigmata dispersed when artificial burrows were present in the soil, although Oribatida, Prostigmata, and Astigmata movements were not well explained by any of the models tested (Chapter 4). *Achillea millefolium* roots grew preferentially in earthworm burrows before occupying cracks in the soil (Chapter

6). In contrast, *C. rotundifolia* roots occurred less frequently in burrows than cracks, possibly due to consumption of roots by earthworms. Distributions of American robins and earthworms also appeared to be linked trophically via predation of earthworms by robins (Chapter 7). Thus, both ecosystem engineering and direct trophic effects should be considered when examining the mechanisms driving changes following earthworm invasions.

9.2 Future research questions

This dissertation examined only a subset of the interactions that could occur between non-native earthworms and other species or ecosystem functions in the boreal forest. Studies conducted in temperate and montane forests indicate earthworm invasions can significantly impact microbial communities (McLean and Parkinson 1997), ground-nesting songbirds (Loss and Blair 2011), amphibians (Maerz et al. 2005, 2009), and other invertebrates (Snyder et al. 2011). Further investigation of earthworm effects on a wider range of boreal forest taxa is therefore needed. Earthworm invasions in temperate hardwood forests and montane forests have also led to changes in carbon and nitrogen concentrations in soil (Scheu and Parkinson 1994, Alban and Berry 1994, Bohlen et al. 2004a, 2004b, Wironen and Moore 2006). However, results have been inconsistent across studies and little is known about how earthworms affect nutrient cycling in the boreal forest. Research should also examine impacts of earthworms on other ecosystem functions including hydrology (Shipitalo et al. 2004, Eisenhauer et al. 2012) and decomposition of coarse woody debris (Hendrix 1996).

In general, there is a lack of understanding of the mechanisms driving changes in northern hardwood and boreal forests following earthworm invasions. Most research has focused on describing patterns rather than on understanding processes. Earthworms can affect other species both via ecosystem engineering and direct trophic pathways as demonstrated in Chapters 5, 7, and 8. Thus, experiments that simultaneously test multiple hypotheses for effects, rather than examining single mechanisms, would be especially useful. For example, research is needed to investigate the relative importance of mechanisms proposed to explain earthworm-caused changes in plant communities, which include decreases in leaf litter thickness, consumption and movement of seeds, disruption of mycorrhizal fungi, and changes in nutrient dynamics (Frelich et al. 2006). Structural equation modeling could be used to assess whether these various mechanisms have direct or indirect effects on plants. Long term studies, in which changes in ecosystems are tracked over multiple years following earthworm introductions, are also critical to examine whether effects vary nonlinearly over time (e.g., Straube et al. 2009).

A key unanswered question is whether climate change will influence the spread and effects of non-native earthworms in forests. Earthworm expansion in the boreal forest is likely limited by cold temperatures, although the six species present in northern Alberta have all been found north of the -20°C isotherm for mean January air temperature, which appears to restrict earthworm distributions in Europe (Tiunov et al. 2006, Cameron et al. 2007, Addison 2008). Warmer temperatures may allow increased population growth or greater spread by increasing the size of the area that earthworms are capable of invading. Eight additional species currently occur in Alberta but are not yet present in the boreal forest (Addison 2008). Climate change and earthworm invasions may also have interacting effects on other species. For example, warming and non-native earthworms interactively affected soil water content and plant community composition in a greenhouse experiment (Eisenhauer et al. 2012).

Although earthworm invasions in the boreal forest are not well understood, there is less information available on earthworm distributions and effects in most other forest types in Canada (Addison 2008). Thus, examination of earthworm invasions in a range of other forested ecosystems should be a key priority for future research. Greater coordination of research efforts across regions and forest types would allow a more comprehensive understanding of non-native earthworms in forests. Such research would permit an assessment of whether the effects of earthworm invasions in the boreal forest are similar to those found in other forest types in Canada.

9.3 Management implications

It is unclear whether boreal forest stands invaded by earthworms will follow similar trajectories of change as observed in invaded temperate forests. For example, although I observed significant effects of earthworms on plants in my mini-rhizotron experiment (Chapter 6), non-native plant occurrence and native forb cover across northern Alberta do not appear to be strongly influenced by earthworms (Cameron and Bayne unpublished data). In contrast**,** plant communities have been heavily impacted by earthworms in northern hardwood forests (Frelich et al. 2006, Hale et al. 2006, Holdsworth et al. 2007). These differing effects may be related to the lower abundances of epi-endogeic, endogeic, and anecic earthworms, which typically have the largest impacts in hardwood forests. Alternatively, the different soils, climate, and vegetation types found in northern boreal forests might increase the resistance of this system to earthworm invasions. However, my research does indicate earthworms can have substantial impacts on soil structure, some species of plants, songbirds, and microarthropods. Thus, regardless of whether the changes caused by earthworm invasions are as large as those observed in other areas, earthworms are common in Alberta's boreal forest (Cameron et al. 2007, Cameron and Bayne 2009) and are impacting other species.

Because earthworms are difficult to eradicate once they invade, prevention of invasions via awareness programs is believed to be the most effective method of control (Callaham et al. 2006). In Alberta, only 15% of anglers surveyed were aware that earthworms are not native to the province (Chapter 8). This percentage did not increase after implementation of an education program. Many anglers were doubtful that earthworms could have negative impacts, with almost half stating they would not change their bait disposal behaviour after being told about the effects of earthworm invasions in forests. These results highlight the need to evaluate education programs, rather than assuming they have been successful.

Our understanding of the effects of species invasions on above- and below-ground interactions is still limited (Bardgett and Wardle 2010). In particular, little is known about how invasive species may interact with other types of global change, such as climate change, to influence above- and below-ground linkages (Bardgett and Wardle 2010, Eisenhauer et al. 2012). Because below-ground invasions often proceed undetected initially, it can be difficult to accurately examine impacts, and society may be less likely to recognize the negative effects of such invaders. Thus, a combination of increased research, public education, and regulations are likely required for effective management of below-ground invaders such as earthworms.

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