Effects of earthworms and warming on tree seedling growth: A small-scale microcosm experiment

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Effects of earthworms and warming on tree seedling growth: A small-scale microcosm experiment

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Abstract

Warming global temperatures are expected to strongly influence plant communities, yet there is limited knowledge of how these changes will interact with stressors such as the invasion of exotic earthworms. We conducted a small-scale microcosm experiment to assess the individual and interactive effects of warming and exotic anecic earthworms (*Lumbricus terrestris* L.) on the growth of sugar maple seedlings (*Acer saccharum* Marsh.). After fifty days, the elevated temperature chamber created warmer and drier soil conditions and increased several measures of plant growth, including stem width, stem width : stem height ratio, stem biomass, and fine root biomass. Earthworms did not have any clear impacts on plant growth either on their own or in interactions with the temperature chambers. However, earthworms did reduce surface leaf litter cover and exposed soils, which could exacerbate evaporative losses and moisture stress in field soils resulting from a warming climate under different growing conditions. Future studies should consider long-term earthworm-temperature interactions on sugar maple growth as well as diurnal and seasonal changes in temperature.

Keywords

*Lumbricus terrestris, Acer saccharum, earthworm density, climate change, invasive species*
**Introduction**

Anthropogenic greenhouse gas emissions are contributing to rapid and unprecedented change in global ecosystems, including rises in temperature that have been observed since the 1950s (IPCC 2014). This warming is expected to have complex impacts on plant communities (Kandeler et al. 1998; Rustad et al. 2001). An additional stressor that could further influence these warming impacts is the invasion of exotic earthworms, taxa widely regarded as the most influential soil macrofauna (Jouquet et al. 2006; Lavelle et al. 2006). Exotic earthworms are continuing to spread globally including into Canadian forests (Addison 2009). Earthworms can positively and negatively influence plant growth through changes in the physical, chemical, and biological properties of soil and interactions with plant roots and seeds (Edwards and Bohlen 1996; Lee 1985; Scheu 2003). To date, however, there have been few studies considering the potential interactive effects of warming and earthworms on plant growth.

The few existing laboratory studies have documented interactive effects of different earthworm species and elevated temperatures on soil properties as well as herbaceous plants (Eisenhauer et al. 2012) and birch tree, *Betula* spp. L. (Betulaceae), seedlings (Makoto et al. 2016). Researchers hypothesize that these interactive effects are related to changes in soil fertility and soil moisture. For example, elevated temperatures and earthworms may increase rates of decomposition and nutrient availability for plants (Coûteaux and Bolger 2000; Curry and Schmidt 2007; Edwards and Bohlen 1996; Johnston et al. 2018; Makoto et al. 2016; Sánchez-de León et al. 2018). Moreover, water stress under warmer temperatures may be exacerbated by the removal of surface litter and soil compaction by earthworms (Blouin et al. 2007; Eisenhauer et al. 2012; Milcu et al. 2006) or mitigated by organic matter incorporation and increased surface infiltration (Eisenhauer et al. 2012; Zaller et al. 2011). These effects may include complex
feedbacks of soil temperature, soil moisture, and plant growth on earthworm activity (Andriuzzi et al. 2015; Coûteaux and Bolger 2000; Curry and Schmidt 2007; Eisenhauer et al. 2012, 2014; Johnston et al. 2018).

Earthworm interactions with roots could include both positive and negative impacts that influence the ability of plants to acquire soil nutrients and moisture, though these interactions are generally poorly understood (Scheu 2003). Roots may respond to temperature effects based on trade-offs between nutrient storage and resource capture (Kandeler et al. 1998; Rustad et al. 2001; Way and Oren 2010) and may be affected by earthworms through preferential growth via earthworm burrows (Pitkänen and Nuutinen 1997; Springett and Gray 1997), changes in resource availability and spatial heterogeneity (Eisenhauer et al. 2009), and root abrasion and feeding (Arnone and Zaller 2014; Cortez and Bouche 1992; Curry and Schmidt 2007; Jana et al. 2010).

However, it remains unclear whether earthworm damage to tree roots occurs through intentional feeding or accidental abrasion, or if no significant direct damage occurs.

The purpose of this study was to determine whether earthworms and elevated temperature have an interactive effect on tree seedling growth. We studied the responses of sugar maple, *Acer saccharum* Marsh. (Sapindaceae), an economically-important species in northern hardwood forests in North America (Hassegawa et al. 2018) already thought to be negatively impacted by exotic earthworm invasion in the midwestern United States (Hale 2006; Corio et al. 2009). For our focal earthworm species, we chose to use the common, ecologically-influential, and deep-burrowing anecic earthworm *Lumbricus terrestris* L. (Lumbricidae) (Addison 2009; Bouché 1997). To complement the existing literature (Eisenhauer et al. 2012; Makoto et al. 2016), we focused particularly on whether surface foraging and burrowing by earthworms would affect soil moisture (and subsequent seedling growth) by increasing water stress and disrupting plant roots.
Materials and methods

This experiment was designed as a two-way factorial microcosm experiment in which we assessed the effects of earthworm density (none, ‘low’, ‘high’) and temperature (‘normal’, elevated) on soil properties, earthworms, and tree seedling growth in temperature chambers (n = 11 microcosms per unique treatment combination).

We purchased 66 dormant sugar maple seedlings as 15-cm root plugs (Connon Nurseries NVK, Ontario, Canada) and planted them dormant in 2-gallon nursery pots filled 16 cm-deep with lightly-compacted Agro Mix G2 potting mix. To simulate the forest floor and provide food for earthworms, we covered the soil surface of each pot with 3 g of dried leaf litter [mixed sugar maple and Norway maple, *Acer platanoides* L. (Sapindaceae)] collected from the field (Monarch Woods Park, Kitchener, Ontario, Canada). Pots were topped with a paper ring to prevent earthworm escape. Adult *L. terrestris* were purchased commercially (Big Bear Foodmart, Ontario, Canada), weighed fresh, and added to pots at addition rates of 0 earthworms (0 m$^{-2}$), 1 earthworm (50 m$^{-2}$), or 3 earthworms (150 m$^{-2}$), simulating a plausible range of earthworm densities found in Canadian forests (Edwards and Bohlen 1996; Fründ et al. 2010).

We chose to simulate early spring, a crucial period for sugar maple seedling growth and planting (Dumbroff and Webb 1978; Morrow 1950). We estimated ‘normal’ ambient conditions based on a five year average (2014-2018) of mean daily temperature (15.1 °C) and precipitation (2.1 mm) for May in Toronto, Ontario, Canada using historical climate data (http://climate.weather.gc.ca/). For our elevated temperature treatment, we used + 4 °C based on the end of century predictions for the global increase in mean surface temperature (Eisenhauer et al. 2012; Pachauri et al. 2014). We randomly assigned and placed microcosms into one of two
temperature chambers (Econair Model GC20) programmed at either 15 °C (‘normal’) or 19 °C (elevated), and set to a 16 : 8 h day : night cycle. We randomized pot placements weekly to minimize chamber edge effects. Because we only had access to two growth chambers (i.e., one for each temperature treatment), we adopted an approach used by Davies and Bodart (2015) and cautiously interpret our results in terms of differences between temperature chambers rather than the effects of temperature per se.

The duration of the experiment was 50 days with an additional 7 days for seedlings and earthworms to adapt to experimental conditions before taking any measurements (Fründ et al. 2010). Pots were watered twice per week to receive an average of 80 mL of water per day, approximating local spring conditions (http://climate.weather.gc.ca/), plus 25 % extra to account for dry conditions within the temperature chambers. At the end of the experiment, three soil temperature and three soil moisture measurements were taken using a thermometer and SM150T moisture sensor (Delta-T Devices, Cambridge, UK) and averaged. Leaf litter remaining on the soil surface was then collected, air-dried, weighed, and expressed as a percentage of the initial litter. Pots were deconstructed and living earthworms were counted and weighed fresh. We also measured stem height to the nearest 0.1 cm and stem width (as an average of three measurements at the base, middle, and tip of each stem) to the nearest 0.01 cm, and the number of leaves per seedling. Plants were divided into stems, leaves, fine roots (< 2 mm), and coarse roots (> 2 mm). Leaves were weighed fresh, and then all components were oven-dried (60 °C for 48 h) and weighed.

Of the original 66 microcosms, analyses excluded any replicates in which the final earthworm density exceeded the initial density (indicating one or more earthworms transferred from other microcosms) or an initial non-zero earthworm density was reduced to zero (indicating
total earthworm escape or mortality). This left a total of 57 microcosms in our experiment, with
27 microcosms in the ‘normal’ temperature condition and 30 microcosms in the elevated
temperature condition. The mean number of earthworms (± 1 SD) in the no earthworm, ‘low’
earthworm density, and ‘high’ earthworm density replicates at the end of experiment were 0 ± 0
(n = 9), 1.1 ± 0.3 (n = 10), and 2.5 ± 0.8 (n = 8) earthworms respectively in the ‘normal’
temperature chamber, and 0 ± 0 (n = 10), 1 ± 0 (n = 9), and 2.6 ± 0.5 (n = 11) earthworms
respectively in the elevated temperature chamber.

We analyzed the effects of temperature chamber and earthworm density on each of the
responses for soils (moisture, temperature, remaining leaf litter cover), earthworms (change in
biomass), and seedlings (stem height, stem width, stem width : stem height ratio, number of
leaves, leaf moisture, fine root biomass, coarse root biomass, aboveground : belowground
biomass ratio, stem biomass) using two-way ANOVAs with Type II sums of squares to account
for unbalanced data (Langsrud 2003) and Tukey’s HSD post-hoc tests. For statistically-
significant model terms, we also report partial omega-squared (ω_p²) as an effect size to show the
magnitude of the temperature chamber and earthworm treatments (Lakens 2013; Maxwell and
Delaney 2004) (Table 1). We tested assumptions of normality and equal variance using graphical
examination (Henderson 2006) and Levene’s test respectively (Gastwirth et al. 2009). We
conducted all statistical tests at α = 0.05 using R, version 3.6.1 (R Core Development Team
2019). We used functions in the package ‘car’ to fit models and assess their assumptions, and
functions in the package ‘agricolae’ for post-hoc tests.

Results and discussion
Several measures of soil properties and plant growth differed between temperature chambers (Table 1). Relative to the ‘normal’ temperature treatment, we found that the chamber with the elevated temperature had decreased moisture content of soils (-23%) and number of leaves (-4%), and increased soil temperature (+11%), stem width (+10%), stem width : stem height ratio (+14%), stem biomass (+27%), fine root biomass (+98%), and litter remaining on the soil surface (+13%). Earthworms affected only the amount of leaf litter remaining at the soil surface and did not detectably affect any of the other responses or interact with the temperature treatment (Table 1). The amount of leaf litter remaining at the soil surface was 87% in the absence of earthworms, decreasing in the presence of ‘low’ (-38%) or ‘high’ densities of earthworms (-54%) (Fig. 1).

Overall, the temperature chambers were responsible for most of the significant responses in this experiment, and we hypothesize that this is due to the different temperature treatments applied to the chambers. These changes suggest that the temperature treatment created warmer, drier growing conditions (increased soil temperature, decreased soil and leaf moisture) that resulted in overall greater plant growth (stem width, stem width : stem height ratio, stem biomass, fine root biomass) consistent with the known impacts of warming on plants (Rustad et al. 2001; Way and Oren 2010). More surprisingly, the temperature chamber with the higher temperature was also associated with more leaf litter mass; this may have indicated decreased microbial decomposition at higher temperatures because of lower soil moisture, though this seems unlikely over the relatively short duration of the experiment. Alternatively, the apparent temperature effect may have been due to reduced earthworm foraging under warmer, drier conditions (Andriuzzi et al. 2015; Coûteaux and Bolger 2000; Eisenhauer et al. 2012). For example, Makoto et al. (2014) found that macrofauna (millipedes) decrease their feeding under
warming temperature within as little as three months. Examining the temperature effect by
earthworm density, the additional litter remaining at the elevated temperature was low when
earthworms were absent (±1%) but higher at ‘low’ or ‘high’ earthworm densities (±28-33%),
even though our models indicated no statistically significant earthworm-temperature chamber
interaction (Table 1).

In contrast with the effects of the temperature chambers, neither earthworms nor their
interactions with temperature chamber had any substantial net impact on soil properties or sugar
maple seedling size or biomass. Importantly, this may be the result of trade-offs that produce no
net effect (Arnone and Zaller 2014), such as a simultaneous increase in nutrient availability and
root abrasion (Jana et al. 2010; Scheu 2003). Future studies can monitor additional variables
(e.g., soil nutrients or organic matter content) to explore these potential effects. One earthworm
effect that we observed but that did not seem to have a strong impact on plant growth in this
study was the removal of surface leaf litter (38-54% decrease) (Fig. 1), though we suspect this
could affect soil moisture and plant growth under different environmental conditions, including
field-collected soil with a lower water-holding capacity. Litter removal is one of the most
pronounced impacts of earthworms on forest ecosystems (Gundale 2002; Suárez et al. 2006).
Long-term litter removal could increase water stress in exposed soils, though this may be
partially mitigated by organic enrichment of the soil (Eisenhauer et al. 2012). Warming impacts
exacerbated by leaf litter removal could be particularly strong for plant communities adapted to
thicker forest floors already threatened by exotic earthworms and litter disappearance (Nuzzo et
al. 2009).

Several logistical constraints should be considered when interpreting these results. First,
because we had access to only two temperature chambers, we were not able to statistically
separate the effects of temperature and the chambers themselves and must be cautious in
discussing the effects of the temperature treatments. However, we believe it is a reasonable
hypothesis that the differences seen between the chambers were a result of the temperature
treatment as we made every effort to keep the chambers otherwise identical and the observed
responses were ecologically-consistent with a temperature effect (e.g., drier soils and increased
plant growth). Second, although this experiment was logistically constrained to examine
relatively short-term effects (i.e., over 50 days), it would be useful for future studies to consider
longer-term earthworm-temperature interactions for a long-lived species such as sugar maple.
Future studies might also wish to consider diurnal and seasonal changes rather than conditions
that remain constant over time, especially as we expect increases in extreme weather events
(Meehl et al. 2000; Rosenzweig et al. 2001). Last, the use of a commercial potting mix (needed
because we were unable to collect frozen field soil at the time of the experiment) and a relatively
generous watering regime (used because the temperature chambers were dry and we were
concerned about seedling survival) likely reduced the risks of moisture or nutrient stress
compared to conditions than might be encountered in the field. We suspect that certain
earthworm-temperature effects, particularly exacerbated moisture stress and growth impacts,
may become more apparent in soils with lower fertility and moisture-holding capacity.

Overall, though we did not observe any earthworm-temperature interactions in this short-
term study, we did document earthworm removal of leaf litter that could plausibly interact with
temperature under certain conditions and be an important driver of soil and plant community
change. We recommend continued study, particularly of the effects of earthworm litter removal
on plants and soil moisture and the impacts of earthworms on plant roots. Other future research
priorities might include investigating the interactions of different soils, plant species, and
earthworm species (Eisenhauer et al. 2012; Makoto et al. 2016); additional responses, including mycorrhizal colonization and root morphology (Kim et al. 2015; Zaller et al. 2011); and the importance of other environmental variables associated with a changing climate, including changes in precipitation and CO₂ (IPCC 2014).

**Author contributions**

JMG and MJM conceived and designed the experiment and analyzed the data; JMG, MJM, SS, EHUS, and SMMA conducted the experiment; and all authors contributed to writing the manuscript.

**Compliance with ethical standards**

**Conflict of interest**

The authors declare that they have no conflict of interest.

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Tables and Figures

Table 1 Summary of two-way ANOVA (Type II sums of squares) output and effect sizes ($\omega_p^2$) assessing the effects of earthworm density (none, ‘low’, ‘high’) and temperature chamber (‘normal’, elevated) on different measures of soil properties, earthworms, and sugar maple seedling growth. The number of replicates varied for each unique treatment combination (none, ‘normal’ n = 9; none, elevated n = 10; ‘low’, ‘normal’ n = 10; ‘low’, elevated n = 9; ‘high’, ‘normal’ n = 8; ‘high’, elevated n = 11).

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Figure captions

**Fig. 1** Boxplot illustrating the effect of earthworm density on the amount of leaf litter remaining at the soil surface (%) (n = 19 replicate pots per density). Group means are depicted as crosses (X). Lowercase letters denote Tukey groupings comparing earthworm densities within each temperature chamber; means that do not share a letter are significantly different.
Fig. 1

![Box plot showing the effect of earthworm density on surface leaf litter remaining.](image-url)