

Introduced earthworm species exhibited unique patterns of seasonal activity and vertical distribution, and *Lumbricus terrestris* burrows remained usable for at least 7 years in hardwood and pine stands

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Abstract It is difficult to obtain non-destructive information on the seasonal dynamics of earthworms in northern forest soils. To overcome this, we used a Rhizotron facility to compile 7 years of data on the activity of anecic (*Lumbricus terrestris*) and endogeic (*Aporrectodea caliginosa* complex) earthworms in two contrasting soil/plant community types. We hypothesized that *L. terrestris* burrows would be used for longer than a typical *L. terrestris* lifetime, and that the distribution and activity pattern of the two earthworm species would respond differently to changes in soil moisture and temperature. For 7 years we recorded earthworm distribution and activity state bi-weekly to a depth of 1.5 m, tracked *L. terrestris* burrows using images captured annually, and measured soil temperature and moisture. Activity and vertical distribution of earthworms was closely linked to earthworm species and soil temperature in the fall, winter and spring. *Lumbricus terrestris* typically remained active through the winter, whereas the *A. caliginosa* complex was more likely to enter an aestivation period. Activity of all earthworms decreased substantially in July and August when soil temperature was at its highest and soil moisture at its lowest for the year. Most *L. terrestris* burrows were used continuously and moved very little during the 7-year study, likely creating

spatiotemporally stable hotspots of soil resources. The different patterns of response of these species to soil temperature and moisture suggests that endogeic earthworms are more likely than anecic earthworms to adjust activity states in response to climate change mediated shifts in soil moisture and temperature.

Keywords *Lumbricus terrestris* · *Aporrectodea caliginosa* · Rhizotron · Aestivation · Burrow longevity

Introduction

Earthworms are ecosystem engineers that can alter soil properties and processes in forest ecosystems (Jones et al. 1994; Lavelle et al. 1997; Brown et al. 2000; Jouquet et al. 2006). Northern temperate forests of North America lacked earthworms until 100–150 years ago, and earthworm introduction has had major impacts on the soil biological, physical, and biogeochemical properties (Alban and Berry 1994; James 1995; Hendrix and Bohlen 2002; Hale et al. 2005). These ecological impacts have been studied extensively; however, phenological insights into the activity of earthworms have been elusive due to challenges making in-situ belowground observations. To understand the spatiotemporal impacts on soils and belowground processes in both their native and invasive ranges, as well as their potential responses to climate change, further insight into these phenological patterns is needed.

Earthworms impact belowground processes through their feeding and burrowing behavior (Hale et al. 2005), with distinct differences between functional groups (Bouché 1977; Lavelle et al. 2004). Endogeic earthworms feed on mineral soil near the soil surface, and create horizontal burrows that are repeatedly infilled as they explore for resources (Bouché

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1977; Lee and Foster 1991; Capowiez et al. 2014). Earthworms in the anecic group consume leaf litter and move between the soil surface and deep mineral soil horizons. To facilitate the repeated movement between the surface and mineral soil, these anecic earthworms create deep, open vertical burrows that persist from months to years (Lee and Foster 1991; Jégou et al. 1998; Nuutinen and Butt 2003). When burrows persist in the soil, they function as soil water flow paths (Edwards et al. 1990; Capowiez et al. 2015), zones of carbon and nutrient accumulation, and hotspots of microbial activity (Tiunov and Scheu 1999; Kuzyakov and Blagodatskaya 2015). Although perennial occupation of burrows of anecic earthworms is well-known (Nuutinen 2011; Grigoropoulou and Butt 2012), these are based largely on surface observations, so there is relatively little data on the length of continuous burrow occupancy, and especially the spatial stability of these burrows belowground.

Plant community and soil chemical and physical properties also influence the presence and distribution of earthworm species. Anecic earthworms feed on leaf litter and have the highest growth rates on litter with a high C:N ratio and calcium (Ca) content, which includes sugar maple (*Acer saccharum*), basswood (*Tilia americana*), and red maple (*Acer rubrum*) (Yatso and Lilleskov 2016), and have been found to be negatively correlated with white pine (*Pinus strobus*) (Crumsey et al. 2014). Generally, soils low in pH, Ca content, and organic matter do not support high abundances of earthworms (Lee 1985; Edwards and Bohlen 1996; Reich et al. 2005). Additionally, percent sand and soil water holding capacity and their interactive effects are significantly correlated with earthworm abundance (Crumsey et al. 2014). Given the sensitivity of earthworms to soil and food properties, the burrow longevity of anecic species, and their seasonal activity, the depth distribution of earthworms is expected to vary by soil type and dominant plant community as a result of litter and soil properties. In particular, it seems likely that endogeic earthworm vertical distribution and seasonal activity will be much more sensitive than anecic earthworms to resource availability deeper in the soil, because of their dependence on mineral soils for food. Yet insights into this are constrained by the absence of long-term observation of activity patterns on different soil and/or forest types under similar climates.

We do not fully understand how seasonal variation in temperature and moisture affect earthworm vertical distribution and activity, and how this differs between anecic and endogeic earthworms. Earthworms are typically active during spring, summer, and fall and have differing strategies for surviving extreme cold and dry conditions (Rundgren 1975, Lee 1985). To adapt to cold temperatures, some species are freeze tolerant and some species go in to a period of aestivation (Rundgren 1975; Garnsey 1994; Holmstrup 2003; Nuutinen and Butt 2009). Aestivation is not a true hibernation but a period of

decreased metabolic rate in which the earthworm ties itself into a knot (also described as ‘balled’) to decrease surface area, and excretes mucus to form a barrier around itself (McDaniel et al. 2013). Similar strategies of aestivation are used when soil moisture levels are low in the middle of summer (Lee 1985; Wever et al. 2001). Most endogeic and some anecic species will use aestivation as a drought and cold avoidance behavior; however, it is difficult to determine the activity state of deep burrowing earthworms during these periods of environmental stress. In southern Sweden, most of the population of the anecic *Lumbricus terrestris* burrowed deep in the soil (Rundgren 1975) and became inactive (defined as less ‘excitable’ in response to formaldehyde addition) when soil temperatures went below 0 °C during the winter (Nordström 1975). However, in south-western Finland, where frost depth reaches ~0.4 m, destructive sampling revealed that *L. terrestris* were active, deep in their burrows (Nuutinen and Butt 2009). Beyond the work of Nuutinen and Butt (2009), direct in-situ observations of activity over deep vertical profiles are lacking, and would inform our understanding of the spatiotemporal patterns of activity. This understanding is critical, because how and when a particular species responds to the very cold or very dry conditions will influence their seasonal impact on soil process rates in earthworm burrows (Brown et al. 2000; Bityutskii et al. 2012).

Soil moisture, soil temperature, and seasonality of earthworm activity have been studied extensively, but much of the past research relied on laboratory-based mesocosm studies (Rundgren 1975; Jégou et al. 2000; Wever et al. 2001; Perreault and Whalen 2006) that would not necessarily incorporate effects of intra-annual variation in response to environmental cues. In contrast, most field-based studies of seasonal activity and demography of earthworms primarily rely on destructive approaches, such as soil pits, and measure size and population change (Rundgren 1975; Callaham and Hendrix 1997; Eggleton et al. 2009; Uvarov et al. 2011). These approaches do not address questions relating to location of earthworms in the soil profile and their states of activity, and instead focus on metrics of survival and changes in body mass at a static point (Valle et al. 1997; Wever et al. 2001; Uvarov et al. 2011). With the average life span of most earthworms between 1 and 2 years, and some earthworms living up to 9 years (Lee 1985), there is a need for longer term observation (Brown et al. 2000). The activity and location of earthworms during different times of the year will likely influence soil carbon cycling, the consumption of organic horizons, and interactions with other native and non-native soil organisms.

Rhizotrons and mini-rhizotrons allow for non-destructive, direct observation of belowground soil processes (Klepper and Kaspar 1994). There are limitations with the use of rhizotrons which include the artifacts of construction, modified soil temperature and moisture levels, and altered soil physical properties, but many of these issues can be avoided

with careful engineering and design. While rhizotrons are commonly used for the study of the rhizosphere, the methods are also effective for the study of earthworms (Lussenhop and Fogel 1993). Seasonal changes in earthworm distribution can be observed with rhizotrons by means of long-term in-situ observations without disturbance, and consequently are particularly well-suited to understanding phenological patterns in relation to seasonality and associated environmental factors (soil temperature and moisture).

We used the Houghton Rhizotron facility (described in **Methods** section) to study seasonal earthworm activity, burrow longevity, and distribution by depth. We focus on the dominant species present in the Rhizotron to examine the differences between functional groups, *L. terrestris* L. (anecic) and *Aporrectodea caliginosa* (Savigny) complex (includes *A. caliginosa* and *A. tuberculata*) (endogeic). Our objectives were to quantify the longevity of *L. terrestris* burrows, track the vertical and seasonal activity of *A. caliginosa* complex and *L. terrestris*, and determine how soil moisture and soil temperature influence activity at different times of the year. Based on our understanding of the permanence of anecic burrows, our first hypotheses are (1) *L. terrestris* burrows persist and remain in use in the same location beyond the average lifespan of a single *L. terrestris*; and (2) *L. terrestris* burrows will have greater longevity in soils with higher organic matter content and preferred plant litter present. Next we examine the differences between endogeics (*A. caliginosa* complex) and anecics (*L. terrestris*), and predict that because of *L. terrestris*' ability to migrate vertically in response to adverse environmental conditions (cold and drought), (3) *L. terrestris* will be able to maintain a longer period of activity throughout the year than *A. caliginosa* complex, and (4) seasonal aestivation in *A. caliginosa* complex will be influenced by (a) temperature in the spring and fall, and (b) soil moisture in the late summer.

Methods

Facility description

The Houghton Rhizotron was constructed in 2005 and is located at the USDA Forest Service Northern Research Station, Forestry Sciences Laboratory (<http://www.nrs.fs.fed.us/research/facilities/rhizotron/>) in Houghton, Michigan, USA (N 47° 6' 52.884", W 88° 32' 52.332"). Air temperatures range from an average low of −13.1 °C in January to an average high of 24.3 °C in July. Average yearly precipitation totals 86.6 cm, and average yearly snowfall is 564 cm (NOAA). Due to the snow pack in the region, soils rarely freeze (Fig. 1).

The rhizotron is oriented north to south and has 24 windows measuring 1.5 m tall and 1 m wide. Within each

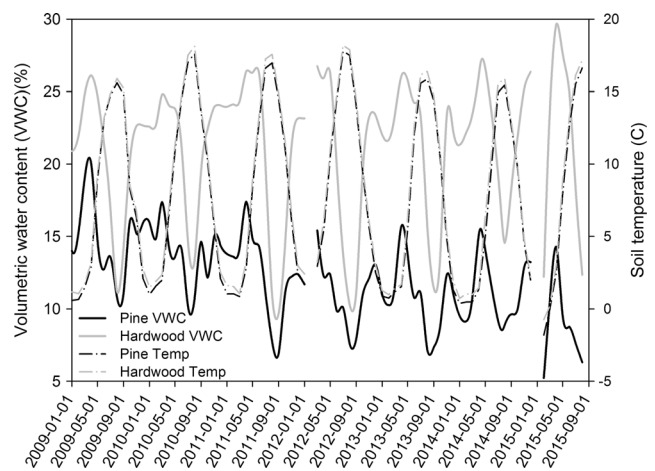


Fig. 1 2009–2015 monthly mean soil temperature and soil moisture (as volumetric water content) at 20 cm depth at the Rhizotron facility, Houghton MI, USA. Date on x-axis as YYYY-MM-DD

window, there are 15 steel reinforced glass panes (29 × 29 cm) sealed using an aquarium sealant (NuFlex Guelph, ON), and each pane can be individually removed for manipulative studies. Insulated panels cover the windows, and are only removed for inventory and imaging. On both sides of the rhizotron, the soil was backfilled in major genetic horizons (A, B, C), and soil was watered as it was placed to adjust bulk density to typical levels for these soils. The two sides of the rhizotron were backfilled with different soil types. The east side (hereafter Hardwood) was constructed adjacent to a mature deciduous northern hardwoods stand. Prior to construction, the native (i.e., local) soil was excavated by genetic horizon. The rhizotron was constructed ~50 cm from the intact vertical soil face, and this gap was backfilled with the native soil (as described above). This sandy loam soil, of the Michigamme-Trimountain soil series (NRCS 2016), is moderately well-drained and very stony with an average depth to bedrock of 170 cm (Table 1). On the west side (hereafter Pine), the soil was excavated 2 m from the edge of the facility, with the first meter from the facility to a depth of 1.8 m and the second meter to a depth of 1 m. This excavated area was then backfilled with soil from a red pine plantation located 15 km from the Rhizotron facility. This imported soil, classified as a Kalkaska soil, is a somewhat excessively drained sand, originating on an outwash plain, and is one of the most common soils in Michigan (Table 1). In the Kalkaska, the O and E horizons were mixed at time of collection to form the A horizon.

The Hardwood side is mature second growth northern hardwoods dominated by *Acer saccharum* (sugar maple), *Fraxinus americana* (white ash), *Quercus rubra* (red oak), and *Tilia americana* (basswood), with sparse shrub and herbaceous cover in the understory (Supplemental Table 1). Maple and basswood support high rates of *L. terrestris* growth (Yatso and Lilleskov 2016). As a result of earthworm activity,

Table 1 Horizon description, soil texture, and pH for Pine and Hardwood side soils

Side	Depth (cm)	Designation	pH in CaCl ₂	(%) organic matter	(%) silt	(%) sand	(%) clay	Texture
Pine	0–20	A _p	4.7	2.7	7.1	87.0	5.9	Loamy sand
Pine	20–25	B _h	4.9	1.6	2.6	93.2	4.2	Sand
Pine	25–75	B _s	4.8	1.4	3.3	94.1	2.6	Sand
Pine	75+	C	4.8	0.5	3.3	92.3	4.3	Sand
Hardwood	0–17	A	5.3	6.4	18.0	76.8	5.2	Sandy loam
Hardwood	17–68	B	5.1	3.5	11.5	83.3	5.3	Loamy sand
Hardwood	68–170	C	5.2	3.5	9.0	90.7	0.3	Sand

there are no O_a or O_c horizons, and fresh litter in the O_i is consumed predominantly by *L. terrestris* throughout the growing season, exposing bare mineral soil for most of the snow-free period. The sole dominant species on the Pine side is *Pinus strobus* (white pine), planted in 2007 as 3 + 0 seedlings, which are now 1.8–3.0 m in height and form a dense overstory with little shrub or herbaceous cover in the understory. A 1–2 cm layer composed of white pine needles plus a small fraction of deciduous litter forms the O_i horizon on the Pine side. There is no O_a or O_c horizon. White pine has litter that is negatively associated with earthworm abundance (Crumsey et al. 2014), and relatively lower litter production than hardwoods because it drops around half of its leaves annually (Gower et al. 1993).

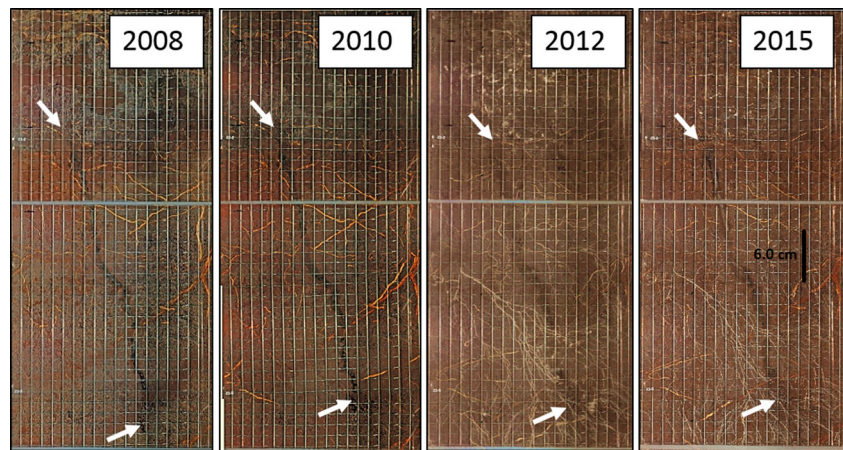
The rhizotron facility was built to follow the natural slope of the forest, with a drain along the foundation to prevent water buildup along the facility wall. The interior environment in the rhizotron tunnel is controlled via a heating/cooling system and a dehumidifier. Interior relative humidity is maintained at <75% to prevent condensation and mold growth inside the facility. A CR10X measurement and control system logs hourly interior and exterior air temperature (107-L), soil temperature (107-L), and soil moisture (CS616) (Campbell Scientific, Logan, UT). Soil temperature and moisture sensors are located at three depths (5, 20, and 50 cm) and two distances (4 and 40 cm) from the rhizotron wall. To minimize temperature gradients, the interior air temperature is adjusted to match the soil temperature at 20 cm depth (± 1 °C); therefore, interior air temperatures range from approximately 4 °C in the winter to >20 °C in the summer. At the 5 cm depth, the soil near the window has a mean volumetric water content 2% less than the soil 40 cm away from the window. At 20 cm depth, the VWC is on average 0.3% drier near the window on the Hardwood side and 3.2% wetter near the window on the Pine side. Field capacity (FC) and permanent wilting point (PWP) were modeled for the two soil types using texture and organic matter content (Saxton and Rawls 2006). On the Pine side, FC averaged 18.8% and PWP was 3.3%, while on the Hardwood side, FC was 24.8% and PWP was 5.6% with the higher values reflecting the higher OM content and water holding capacity of the sandy loam Hardwood soil.

Inventory and imaging

Beginning in 2009, an earthworm inventory was conducted on a bi-weekly basis in the rhizotron. The inventory was divided into seven depth zones, 0–10, 10–20, 20–30, 30–60, 60–90, 90–120, and 120–145 cm and completed for 22 windows, 11 on each side. Panels were removed from windows at the time of the inventory, and an initial quick scan was made for presence of *L. terrestris* which will typically move rapidly away from sources of light, followed by scan for *Aporrectodea*. Earthworm location within a given depth zone, activity state (balled (aestivation) or extended (active)), species (if known), and presumed type of burrow (temporary or permanent) were recorded. A burrow was identified as permanent if it was darker in color than surrounding soil and had visible structure. Temporary burrows are often smaller in diameter with little accumulation of organic matter along the burrow walls. Due to the nature of a rhizotron inventory being 2-dimensional, we were unable to document earthworm mortality or track individuals between sampling periods.

Permanence of adult *L. terrestris* burrows was quantified by identifying burrows in full window images from 2008 and tracking the burrow persistence and location through the remaining 7 years of images (Fig. 2). A burrow was recorded as having disappeared when it was no longer visible in subsequent images, and was determined to be active if it had any of the following indicators: visible burrow outline, lack of infill in shallow depths, and/or earthworm presence in burrow. If these criteria were not met (burrow filled in, in-grown with roots), the burrow was determined to no longer be active/present. This estimate should be considered conservative for two reasons: first, if burrows migrated away from windows, they would be recorded as no longer active; second, burrows were already in existence at the beginning of the study. We can set the upper bound on their age at 2 years longer than the course of the study, as the study was initiated 2 years after construction of the rhizotron. To evaluate spatial stability, burrow initiation and end points were recorded using the window pane grid and burrows were traced in images taken in 2008 and 2015 and analyzed for change over time.

Fig. 2 An example of *L. terrestris* burrows on Hardwood side of rhizotron, photographed over a 7-year period. Arrows on images indicated initiation and termination points of burrow as visible in window. Image illustrates burrow is stable in space and time



Data analysis

Twenty-two windows were inventoried during each bi-weekly sampling, 11 windows on each side (Pine or Hardwood). To analyze and visualize temporal trends by species over time, for a given sampling date, earthworm counts for all windows on each side that were summed (no spatial replication within side for a given sampling date). This summary approach was taken, rather than treating each window as a replicate for a sampling date, due to a lack of independence across windows on a given side. In summaries, seasons were defined as follows: winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November).

The relationship between soil temperature and transitions on the activity state of *Aporrectodea caliginosa* complex in the late fall (active to aestivating) and early spring (aestivating to active) was analyzed using linear regressions, with the soil temperature at the time of the earliest transition date as the independent variable and the Julian date of the transition for a given year as the dependent variable. The activity state transition day was identified through the bi-weekly inventory data, and was the Julian day when a set proportion (at least 40%) of the *A. caliginosa* complex earthworms recorded began aestivating (late fall) or became active (early spring). SigmaPlot 12.5 (Systat Software Inc. San Jose, CA, USA) was used for the linear regressions and chi-square tests to detect differences between soil types in burrow longevity.

Generalized mixed effects models (R version 3.2.5), with the packages “lme4”, “car”, “lsmeans”, and “lattice”, were used to evaluate the differences in the vertical distribution and the influence of environmental variables on summer aestivation. For vertical distribution models, season nested within year was specified as a random (repeated) effect, and species (*A. caliginosa* complex/*L. terrestris*) and side (Pine/Hardwood) were specified as fixed effects, with the continuous response variable vertical distribution (depth). To evaluate the vertical distribution of endogeics by activity, a similar model was created with activity

(aestivation/active) added as a fixed effect, and the vertical distribution of *A. caliginosa* complex as the continuous response variable. Because *L. terrestris* was active during most of the year, we did not model the activity within this species separately. We checked for violations of assumptions by plotting residuals, histograms of residuals, and normal probability plots of residuals. If data was not normally distributed, a negative binomial distribution was used. The influence of environmental variables on the aestivation of *A. caliginosa* complex earthworms during July, August, and September (time period where aestivation was recorded outside of winter months) was analyzed using side, monthly mean soil moisture, and monthly mean soil temperature as fixed effects, month nested within year as the random effect, and the percent of the population in aestivation as the response variable. Hypothesis testing was completed using a Type III chi-square analysis of variance. To examine differences in activity between the two species, we used a generalized mixed-effects model with percent active/month as the response variable. Post-hoc analyses were conducted with lsmeans using the Tukey method and confidence level of 0.95. Significance for all tests was determined at $p \leq 0.05$. Unless noted, all p values in the text reflect the generalized mixed-effects models described above.

Results

Distribution between sides

Location (as defined by side) influenced the abundance of earthworms present (Fig. 3). Within the *A. caliginosa* complex, 63% (2010–2015 annual mean) were inventoried on the Hardwood side while 37% were on the Pine side. Similar trends were present for *L. terrestris*, with 62% of this species on the Hardwood side and 38% on pine. Combining sides, 83% of the earthworms inventoried were *A. caliginosa* complex while the remaining 17% were *L. terrestris*. With both species combined, annual counts were always higher

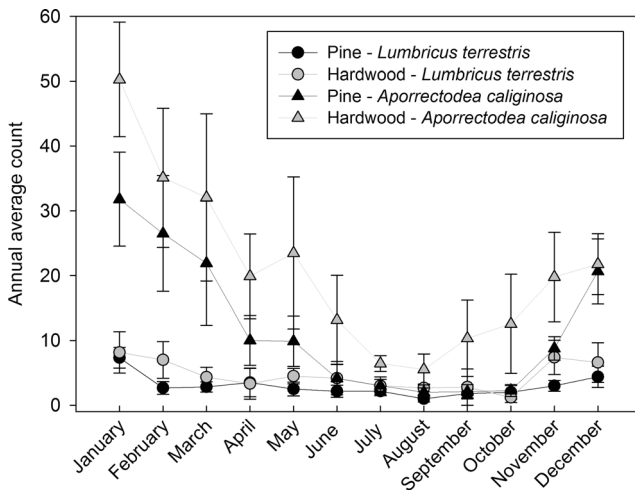


Fig. 3 Average monthly counts of *L. terrestris* and *A. caliginosa* complex earthworms observed at the Pine and Hardwood locations (2010–2015). Error bars are standard error

on the Hardwood side compared to the Pine side (328 ± 58 vs 195 ± 36 , mean \pm SE).

Burrow longevity

Twenty-six *L. terrestris* burrows were identified in whole window photos taken in 2008 (Fig. 2). Of these 26 burrows, 21 were still visible and active in 2015 (Table 2). On the Hardwood side, 94% of the surveyed burrows (Table 2) persisted after 7 years, while on the Pine side 56% of the burrows were still active, although this difference was not significantly different (chi-square test, $p = 0.34$). The burrows that were still present after 7 years were also stable in space, with 67% of burrows occupying the same location in 2008 and 2015 (Table 3). The remaining 33% shifted less than 2 cm with only small sections shifting in most of the burrows surveyed (Table 3). Most of the burrow shifts were located at the basal end of the burrow, where the original burrow terminus

was infilled with soil and gravel (Fig. 2). The spatial stability of the burrows did not differ between the Pine and Hardwoods sides.

Seasonal differences in activity by species

A greater proportion of *A. caliginosa* complex aestivated during the inventory period than *L. terrestris* ($p = 0.0056$). More individuals of the *A. caliginosa* complex population were aestivating during the winter and summer months, with almost 50% of earthworms balled in February and close to 30% balled in August (Fig. 4, Supplemental Table 2). This coincides with the coldest soil temperatures (winter) and warmest and driest soil conditions (summer) (Fig. 4). Observations of *L. terrestris* in aestivation were not common, with the highest percentage ($14.9\% \pm 5.5\%$) of earthworms in aestivation in January (6-year mean \pm standard error). In the summer, a higher percentage of earthworms aestivated on the Hardwood side, but not the Pine side, when the volumetric water content was the lowest (side \times soil moisture interaction, $p = 0.04$). When only the Hardwood side was used in this model, soil moisture predicted earthworms aestivating ($p = 0.002$).

The earliest recorded date over 6 years for the spring transition of earthworms from aestivation to an active state was Julian day 68 (March 9, 2010) when the soil temperature at 20 cm was 2.1 °C. In the fall, the earliest day in which earthworms went in to aestivation was Julian day 324 (November 20, 2014) when the soil temperature at 20 cm was 3.2 °C. Earthworms’ transitioned to inactive states in the late fall/early winter when the average soil temperature was 1.9 ± 0.5 °C (mean \pm standard error) on the Pine side and 2.3 ± 0.4 °C on the Hardwood side. The mean temperature at which earthworms came out of aestivation in the spring was 1.2 ± 0.2 °C on the Pine side and 1.4 ± 0.3 °C on the Hardwood side. Using soil temperature at the earliest date of activity transitions for the 6-year period and the Julian date of

Fig. 4 Monthly mean percent of *L. terrestris* and *A. caliginosa* complex population in aestivation (with standard errors) over a 6-year period. Mean soil temperature and soil moisture (as volumetric water content) at 20 cm also shown

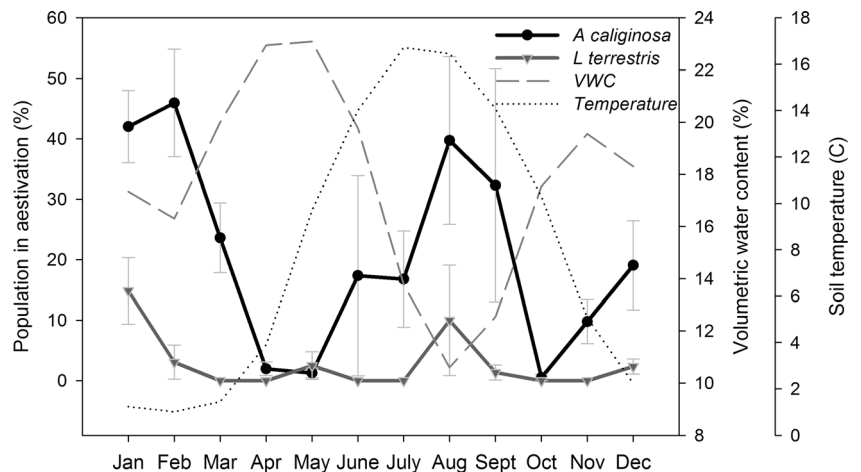


Table 2 *Lumbricus terrestris* minimum burrow longevity tracking from 2008 to 2015 in Rhizotron soils

Side	Original burrow counts	Count of original burrows still present			Burrows remaining after 7 years
	2008	2010	2013	2015	%
Pine	9	9	7	5	56
Hardwood	17	17	16	16	94

the transition for a given year in a linear regression, we found temperature at that date was a strong predictor of the Julian date of activity state transition in the fall ($R^2 = 0.645$, $p = 0.005$) and the spring ($R^2 = 0.683$, $p = 0.003$). Specifically, warmer soil temperatures allowed earthworms to be active later in the fall, and to resume activity earlier in the spring.

Vertical distribution

The vertical distribution of earthworms was affected by species, side, and season (Supplemental Table 3). In winter months when soil temperatures near the surface were near, or at, freezing (Table 4), the majority of earthworms on both Pine and Hardwood sides were distributed ≥ 20 cm below the soil surface (Figs. 5 and 6). For the remaining seasons *L. terrestris* differed from *A. caliginosa* complex, with most *L. terrestris* recorded from 20 to 60 cm (Fig. 6), whereas the majority of *A. caliginosa* complex transitioned to depths near the surface, especially on the Hardwood side, when soil temperatures increased. In the summer on the Hardwood side, the majority of *A. caliginosa* complex species were recorded at 0–10 cm depth, while they were more evenly distributed in the top 20 cm on the Pine side (Fig. 5). *L. terrestris* earthworms were distributed deeper in the soil profile than *A. caliginosa* complex between the Hardwood and Pine sides ($p = 0.026$) and within a given side ($p = 0.021$). Both species were distributed deeper on the Pine side in the spring and on the Hardwood side in the summer (season x side, $p < 0.001$). *L. terrestris* was distributed deeper than *A. caliginosa* complex for all seasons except winter (species x season, $p < 0.001$).

For the activity state (aestivating vs. active) of *A. caliginosa* complex, the interaction of side x activity x season on vertical distribution was significant ($p < 0.0001$) (Fig. 7).

Table 3 Spatial stability of *L. terrestris* burrows on Pine and Hardwood sides after 7 years

Spatial Stability	Percentage of burrows
Stable	67%
<than 50% of burrow; <1 cm shift	19%
>than 50% of burrow; <1 cm shift	5%
<than 50% of burrow; <2 cm shift	9%

Aestivating earthworms were distributed significantly deeper in the winter and spring on the Pine side, and deeper in the spring only on the Hardwood side. During the winter, the Pine side had a 2–4× higher proportion of aestivating earthworms, with over 70% of earthworms at 30–60 cm in aestivation on the Pine compared to 20% on the Hardwood side (Fig. 8). Interactive effects of activity x season were also significant ($p < 0.0001$), with inactive earthworms distributed deeper than active earthworms in the spring and winter, especially on the Pine side.

Discussion

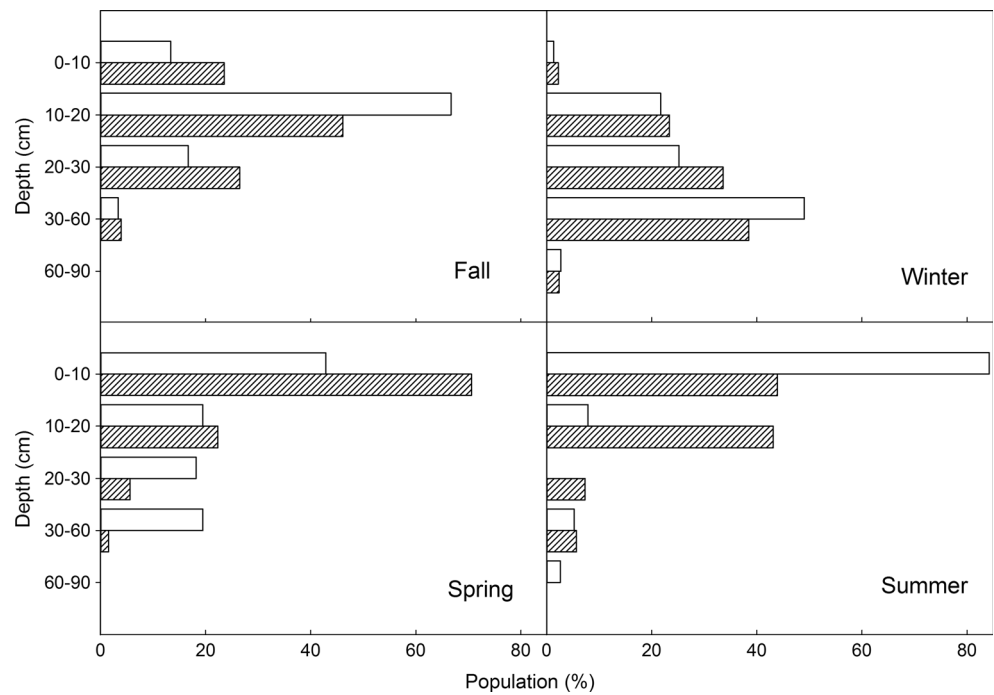
***L. terrestris* burrow spatiotemporal stability**

Our results support hypothesis 1, and are the first to confirm that anecic *L. terrestris* burrows are stable in space for at least 7 years in northern forests, based on non-destructive in-situ measurements. Given the majority (80%) of burrows in the rhizotron that were still in use after 7 years, and *L. terrestris* have an estimated average life span of 1–4 years (Lachani and Satchell 1970, Lee 1985), it is apparent that these permanent burrows consistently outlast the lifetime of a single earthworm. The reuse of *L. terrestris* burrows has been reviewed extensively (Nuutinen 2011), and was demonstrated in a field experiment in the UK; earthworms were removed from the site and during a period of 11 months, around 50% of the burrows were recolonized (Grigoropoulou and Butt 2012). Our test of hypothesis 2 was equivocal, because while the proportion of burrows that survived for 7 years was much higher on the hardwood side, the result was not significant,

Table 4 Mean soil temperature (°C) by season at 5, 20, and 50 cm depth

Side	Depth (cm)	Spring	Summer	Fall	Winter
Pine	5	3.7	14.9	8.8	0.0
Pine	20	3.7	14.7	9.3	0.7
Pine	50	3.7	14.2	9.8	1.4
Hardwood	5	4.3	15.3	9.0	0.5
Hardwood	20	4.4	15.2	9.6	1.1
Hardwood	50	4.0	14.4	10.0	1.7

Fig. 5 Depth distribution of *A. caliginosa* complex by season and side, shown as mean percentage of population by depth class for 2013–2015. Textured bars represent Pine side and open bars represent Hardwood side (N: fall =132, spring =274, summer =161, winter =1192)

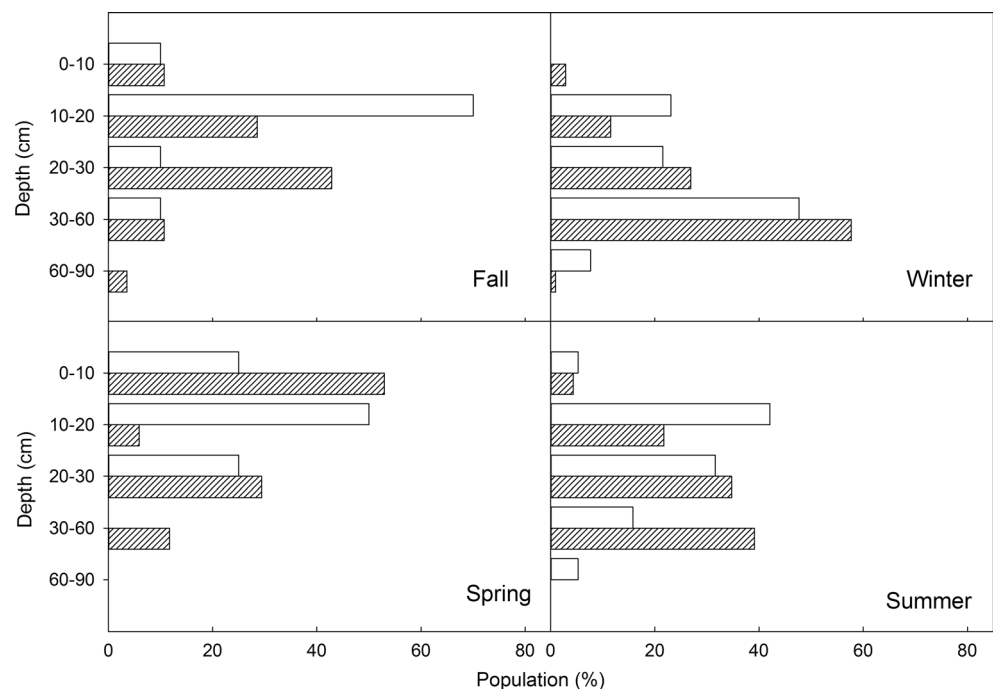


suggesting greater power is needed, which in our case can be achieved by a longer period of observation.

Although the “permanent” nature of anecic burrows has been studied, up until this point the ability to quantitatively measure the longevity and belowground spatial stability of anecic burrows of *L. terrestris* has been limited. The persistence of *L. terrestris* middens (burrow entrances) on the soil surface for 8 years at the soil surface has been documented in previous work (Tiunov and Kuznetsova 2000), but our work

demonstrates the spatial stability of these burrows in the soil column. The observed belowground spatial and temporal stability of anecic earthworm burrows can result in an accumulation of organic matter along burrow walls, with implications for biotic and biogeochemical processes (Brown et al. 2000; Andriuzzi et al. 2013; Nieminen et al. 2015; Uksa et al. 2015; Hoang et al. 2016). Burrow spatial stability also affects the community of organisms that can exploit the burrows (Butt and Lowe 2007), with the potential for mycorrhizal fungi and

Fig. 6 Depth distribution of *L. terrestris* by season and side, shown as mean percentage of population by depth class for 2013–2015. Textured bars represent Pine side and open bars represent Hardwood side (N: fall =38, spring =25, summer =42, winter =169)



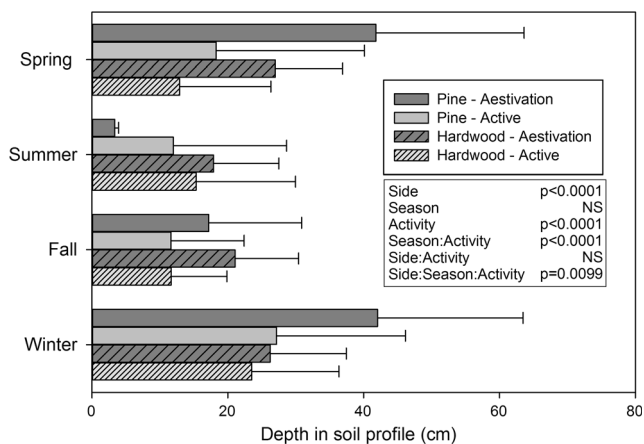


Fig. 7 Mean vertical distribution of active (light gray) and aestivating (dark gray) *Aporectodea caliginosa* complex by season and side. The Hardwood side is shown with textured bars and the Pine is represented by the open bars. Error bars are standard deviation. Results (*p* values) from mixed effects model are also displayed on figure (number of observations =2624)

roots to grow towards these predictable resource patches (Springett and Gray 1997; Han et al. 2015). Our results complement previous literature (Lee and Foster 1991; Nuutinen and Butt 2003), which reported extensive information of the physical characteristics of burrows, their influence on hydrology, and stability of cast material.

Comparisons of *L. terrestris* and *A. caliginosa* complex distribution and activity

Over the duration of the study, we logged 3285 earthworm observations. Total counts of *A. caliginosa* complex were about 5× greater than *L. terrestris* over 6 years, however *L. terrestris* are larger, with maximum per individual biomass approximately 6× higher based on equations of Hale et al.

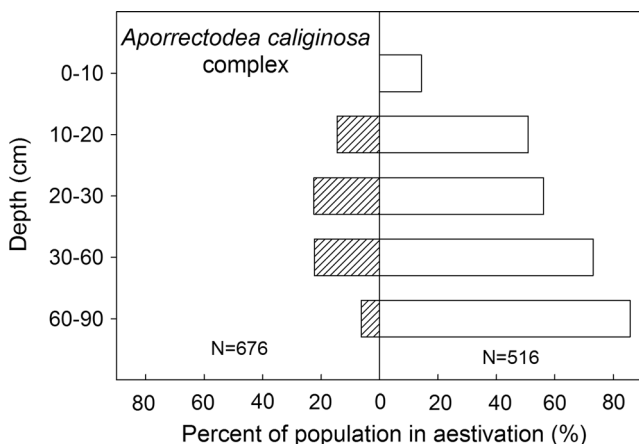


Fig. 8 Winter distribution by depth of *A. caliginosa* complex in aestivation in Hardwood (textured bars) and Pine (open bars) sides. Values represent percent of worms in aestivation out of total worms inventoried at a given depth, averaged over the winter season

(2004). Thus, our observed biomass of the two species were likely quite similar. Combined counts of the two species were higher on the Hardwood side. This is unsurprising due to the litter inputs dominated by white ash, basswood, and sugar maple, all of which are preferred litter types by earthworms (Hendriksen 1990; Yatso and Lilleskov 2016). The Hardwood sandy loam soil also has higher OM content which indicated higher resources availability throughout the soil profile. Both earthworm species have improved growth and survival in organic rich soils with relatively moderate pH (>4.6) and high quality, low-lignin litter (Fisichelli et al. 2013; Yatso and Lilleskov 2016). Interestingly, the proportion of the two species were almost identical for both vegetation/soil types over the 6-year period, indicating niche overlap with similar resource requirements for the two species (Hackenberger and Hackenberger 2014).

Side was also a factor in the depth distribution of aestivating earthworms. This was especially evident for *A. caliginosa* complex during the winter, where on the Pine side with sand soils, 60–80% of the population below 20 cm were aestivating, while on the Hardwood side in sandy loam soils, only 20% of the population went into aestivation. Mean winter temperatures at 50 cm differed by 0.3 °C between the two sides (1.7 °C on Hardwood side vs 1.3 °C on Pine side), so temperature is likely not a causal factor. The percent organic matter on the Hardwood side was 2–3× higher than the Pine side (Table 1). We hypothesize that this difference in aestivation rates can likely be explained by resource availability, which is influenced by plant community. Because quantity and quality of litter is poorer on the pine side, we expect under adverse environmental conditions with limited resources a majority of *A. caliginosa* complex will go into aestivation.

Our findings support hypothesis 4a, as winter soil temperature appeared to be a strong control of vertical distribution and activity. Colder temperatures in the winter resulted in a deeper burrowing of both species, with *L. terrestris* sometimes burrowing over 1 m deep. Perhaps as a result of this avoidance activity, very few *L. terrestris* went into aestivation at cold temperatures while a majority of *A. caliginosa* did enter a resting state during the winter. Although *L. terrestris* does not commonly enter an aestivation state (Nuutinen and Butt 2009), it has been reported in very hot and dry soils (Perreault and Whalen 2006) and we occasionally observed the behavior under both cold and dry conditions. *L. terrestris* has a greater tolerance for frost than *A. caliginosa* (Addison 2008) and can remain active as soil temperatures approach freezing (Nuutinen and Butt 2009). In northern regions, most aestivation is driven by cold soil temperatures (Lavelle 1988); however in areas with heavy snow pack, such as the lake effect snow band of northern Michigan where our study was conducted, the snow forms an insulating layer protecting soils from freezing and permitting continued activity throughout the winter.

Our findings at least partially supported (H4b) that soil moisture would be a strong factor in summer aestivation. To our knowledge, our study is one of the first to observe and record multiple years of activity states and demonstrate soil water content as a significant predictor of summer aestivation in *A. caliginosa* complex earthworms. The significant effect of soil moisture was only detected on the Hardwood side, which is interesting because the soil moisture on the Hardwood side is generally 3–5% higher in the summer when compared to the Pine side. The inability to detect significant soil moisture effects on the Pine side might be attributed to a lower population of earthworms in the summer on this side. Alternatively, this difference might also be attributed to the differences in soil texture, because finer textured soils (as found on the Hardwood side) will have lower water potential at the same water content. Our results are in agreement with field-based destructive sampling studies that have noted inactivity of earthworms in the summer when soil water content is low (Gerard 1967; Garnsey 1994). In a mesocosm experiment, a significant number of *A. caliginosa* went into aestivation after 1 week of water stress, and the number of aestivating earthworms increased as moisture stress increased (McDaniel et al. 2013). Perreault and Whalen (2006) found that high soil temperature and low soil moisture drove the deeper burrowing of earthworms, but did not evaluate activity states.

With increasing winter temperatures driven by global warming, we can expect that in soils with available resources (high OM), earthworm activity will continue to increase during the colder months. This year-round activity will have consequences for soil process rates, water movement, and nutrient cycling. Eggleton et al. (2009) predicted that with warmer winter soils, more earthworm activity would shift to the winter when soil moisture levels are relatively high. The processing of soil organic matter will also be expected to increase as the length of time earthworms are feeding and burrowing expands. However, this increase in soil temperature would only be expected if a persistent snowpack exists in future climatic conditions. In our study area, soil temperatures rarely go below freezing and only right near the surface do we observe a frost layer (top 2–3 cm). A decrease in the seasonal extent and depth of snowpack could enable soils to freeze, which could result in colder than normal soil temperatures and decreased activity (Groffman et al. 2001).

Conclusion

In this paper, we expand the understanding of earthworm functional group burrowing behavior, seasonal activity, and vertical distribution using a unique rhizotron facility. Our ability to observe non-destructively in-situ dynamics permits an in-depth approach to understanding how different species respond to soil temperature and moisture conditions throughout

the year in northern temperate forests. The differential response of the two earthworm species to seasonal temperature dynamics will alter their relative impacts under a changing climate. We hypothesize that the dramatic difference in activity of the endogeic earthworms between the two soils reflects the lower availability of organic matter deeper in the soil on the pine side. Without appropriate temperature, moisture, and food supply, the endogeic earthworms are forced to aestivate to avoid starvation. The ability of *L. terrestris* to migrate through stable vertical burrows permits much greater ability to maintain activity in response to changing environmental conditions. In addition to providing cold and drought avoidance, the *L. terrestris* burrows are used for many years, well beyond the average lifespan of an individual earthworm, and once established appear to shift very little in belowground position. This indicates that these burrows are spatially stable hotspots, with all the implications that has for microbial community development, root foraging, and nutrient cycling. Future work in the rhizotron will build upon burrow dynamics and long-term inventory data and explore the ecological implications of stable anecic burrows as microbial hotspots.

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