



Article

Physical Properties of Soils Altered by Invasive Pheretimoid Earthworms: Does Their Casting Layer Create Thermal Refuges?

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Abstract: Pheretimoid earthworms are invasive in hardwood forests of formerly glaciated regions in the USA. They alter the forest floor structure by creating an extensive, several cm-deep casting layer comprising loose macro-aggregates. Little is known about the physical properties of the casting layer and how they relate to earthworm ecology. Here, thermal and macropore properties of three forest soil textures (clay, silt, and sandy soils, with and without pheretimoids) were measured and compared to explore the possible relationships to their ecology. Thermal properties were significantly different between the casting layer (CAST) and original soil (NOCAST). Results indicate that CAST soils dampen temperature fluctuations occurring at the surface more than NOCAST soil. The increased dampening may be of particular importance to pheretimoid survival in forest fires and during spring when surface fluctuations could expose the hatchlings to fatal temperatures. Macropore volume, an indicator of ease of movement of pheretimoids, was significantly greater in CAST than NOCAST soil. Together, the ease of movement and greater temperature dampening of CAST soils may provide thermal refuges to pheretimoids from temperature variations outside the optimal range. This may improve their chances of survival in newly colonized areas where the climate differs from the original range.

Keywords: *Amyntas agrestis*; *Amyntas tokioensis*; casting layer; inter-aggregate pores; thermal properties; dampening depth; earthworm invasion

1. Introduction

Earthworms are an important part of the soil system and are regarded as ecosystem engineers [1,2]. While their ‘engineering’ activity is to their benefit, they also have effects on other compartments of the soil ecosystem. Their positive effects on soil fertility are well known [3], while their contribution to greenhouse gas emissions has recently been scrutinized [4]. In the last few decades, researchers have highlighted the invasiveness of earthworms in some forested ecosystems [5,6] and the feedbacks on the impacted soil systems [7,8]. Invasive earthworms in northern hardwood forests in North America comprise earthworm species originating from both Europe [7,9] and Asia [10–12]. The Asian species involved in forest invasion belong to a species group called ‘pheretimoids’ in the family of Megascolecidae [13]. There are three pheretimoid species that frequently co-invade North American forested ecosystems [14] and are highly invasive in the eastern USA. These species are *Amyntas tokioensis*, *A. agrestis* (Figure 1), and *Metaphire hilgendorfi*.

One of the ways that invasive earthworms affect northeastern hardwood forests is through the modification of the humus layer [15,16]. The alteration of the forest floor by Lumbricidae and

Megascolecidae results in fundamentally different soil surfaces. The former generate a compacted A-horizon by consuming and/or mixing the O-horizon material with the underlying mineral soil [15]. The latter transform the top soil layers into an extensive layer of loose frass aggregates, or casting [8,12,17] (Figure 1).



Figure 1. *A. agrestis* on its casting layer. Scale reference: Earthworm diameter is 4 mm.

Although the pheretimoid frass layer is very conspicuous and differs considerably from the top soil formed by lumbricids, very little is known about its properties. The castings are coarse and granular, and carpet wide areas in affected forests. The change from an organic forest floor to the coarse aggregates might change the soil's water relations and aeration, and therefore the thermal properties [18]. In turn, one expects changes in the soil's ability to heat and cool in response to temperature fluctuations at the surface.

Could the modified structure of the forest floor provide benefits to the pheretimoid ecosystem engineers? The three pheretimoid species of concern in the New England forests are epi-endogenic earthworms, whose habitat encompasses the leaf litter, casting layer, and the top few centimeters of the native mineral soil [13,19]. For one of the pheretimoid invaders, *Amyntas agrestis*, temperature benchmarks for hatching and survival have been determined in laboratory studies. Hatching occurs after soils warm to 10 °C [20]; embryonic development may require approximately 600 °C degree days when moisture conditions are optimal [20,21]; 100% mortality of adults is expected when they are exposed to temperatures below 5 °C or above 30 °C, but moisture moderates the mortality rate at temperatures greater than 20 °C [22]. There are also upper temperature limits to cocoon viability [23]. Thus, thermal properties may affect pheretimoid phenology.

We compared macroporosity, thermal conductivity, heat capacity, and thermal diffusivity of the casting layer and the original soil to explore the possibility that pheretimoids create thermal refuges to protect them from atmospheric temperatures that deviate from the optimal range. We inspected three soils with different textures for five different tensions ranging from 0 to 100 kPa. Given the aggregated structure of the casting layer, this range of soil tension likely corresponds to soil moistures between saturation and field capacity. Due to the extensive macropore network within the casting layer, we hypothesized that its water release characteristics and its thermal properties differed from unaltered soil.

2. Materials and Methods

2.1. Sites and Soil Sampling

Undisturbed soil cores were taken from three sugar maple (*Acer Saccharum*, Marshall) stands in Shelburne (44.410 N, 73.248 W), South Burlington (44.431 N, 73.200 W) and Huntington (44.347 N, 72.997 W), Vermont, USA. The soils at these locations were mapped (Soil Survey Staff, 2009) as Covington clay loam (very-fine, mixed, active, mesic Mollic Endoaqualfs), Windsor loamy sand (mixed, mesic Typic Udipsamments), and Raynham silt loam (coarse-silty, mixed, active, nonacid, mesic Aeric Epiaquepts). These soils will be referred to as 'clayey', 'sandy', and 'silty' soil, respectively. The casting layers at these sites varied between 5 and 10 cm in depth. We collected 12 undisturbed, cylindrical soil cores (5-cm diam., 5-cm long) from each site for a total of 36 cores. Half of these were taken from patches exclusively occupied by pheretimoid species (six cores at each site, 'CAST'); the other half were taken from pheretimoid-free patches on the same soil series in the same stand (six at each site, 'NOCAST'). The pheretimoid patches were free of lumbricid earthworms. The pheretimoid species present at all sites were *A. agrestis* and *A. tokioensis*. At the sandy site, *M. hilgendorfi* was also present. At the pheretimoid-free patches, several lumbricids species were present. Of these, *Lumbricus terrestris*, *Octolasion cyaneum*, and *L. rubellus* were the most abundant species at all three sites.

2.2. Soil Moisture Release Curve

Soil moisture release curves were measured from saturation to 100 kPa tension. Soil cores were conditioned to the target tension by applying suction at the core bottoms using sand tables (Eijkelkamp, Giesbeek, The Netherlands) [24–26]. In brief, core bottoms were fitted with a fine nylon mesh held in place by a rubber band. The cores were first submerged in a water bath for two days to determine water content at saturation. Then, the cores were placed on a sand table where they were sequentially conditioned to tensions, ψ , of 10, 30, 63, and 100 kPa by adjusting the suction exerted at the surface of the sand table by varying the length of a hanging water column. Core weights were obtained daily until the core weights no longer changed. This occurred between three and four days after the suction was applied.

At the end of this sequence, the cores were dried at 70 °C for 24 h and weighed. At this point, the tare weight (core sleeve, nylon mesh, and rubber band) was determined. The soil mass at each tension point was calculated as the core weight minus the tare. Bulk density was calculated as the ratio of the oven-dried soil mass, m_{OD} , and the core volume. Mass moisture, θ_m , was calculated as

$$\theta_m = \frac{m_\psi - m_{OD}}{m_{OD}} \quad (1)$$

where m_ψ is the tared core weight at tension, ψ , and m_{OD} is the oven dry weight.

The water loss, Δm , was determined as the difference between core mass at consecutive tensions (e.g., between saturation and 10 kPa, or 10 kPa and 30 kPa). Water loss is regarded as the pore volume displaced under the influence of the increased tension. Due to the strong aggregation of CAST, the fraction of large macropores (i.e., the water removed from the cores between saturation and 10 kPa), is of particular interest.

Hydraulic pore diameter, D_H , was calculated from tension as

$$D_H = \frac{300}{\psi} \quad (2)$$

where D_H is in μm and ψ is in kPa.

2.3. Thermal Property Measurements

Specific heat capacity C , thermal conductivity K , and diffusivity, D , were measured at each tension, ψ , and for all cores using the SH1 dual needle probe of a KD2 Pro Thermal Properties Analyzer (Decagon Devices, Pullman, WA, USA).

To translate diffusivity into a biologically meaningful parameter, thermal damping depth at tension ψ , $d(\psi)$, was calculated as

$$d(\psi) = \text{sqr}t\left(2 * \frac{D(\psi)}{\omega}\right) \quad (3)$$

where ω is the angular frequency of a thermal fluctuation, associated with, for example, diurnal or seasonal variation. The damping depth gives the depth where the amplitude of the temperature variation is reduced to 1/e of the amplitude at the soil surface. The greater the dampening depth d , the further the temperature variations occurring at the surface are transmitted to depth.

Soil temperature, T_{zcm} , at soil depth, z , was calculated as

$$T_{zcm} = T_{mean} - 0.5(T_{max} - T_{min})e^{-\frac{d}{z}} \quad (4)$$

where T_{mean} , T_{max} , and T_{min} are the mean daily, maximum daily, and minimum daily temperatures, respectively.

Ambient temperatures were taken from 2014 data measured at a US. National Weather Service station located at Burlington Airport (BTV) 3–30 km from the sites where the soil cores were collected.

Decrease in soil temperature, ΔT , with depth, z , due to fire with temperature T_s at the surface was estimated with

$$\Delta T = T_s e^{-\frac{d}{z}} \quad (5)$$

2.4. Pheretimid Collections and Abundance

Pheretimoids were collected at the three sites from 2011 to 2019 and enumerated at the sandy soil site. Hatchling abundance was estimated from counts in 0.5 by 0.5 cm quadrats [19]. Here, we specifically report the abundance only for mid-May sampling dates in 2011 to 2019. We also used width and length data developed with ImageJ (NIH, Bethesda, Maryland, USA) from specimens collected at the sandy soil site on 30 April and 17 May 2018. Data were sorted and only specimens with body lengths of less than 20 mm were included in the data analysis as these were likely to be new hatchlings.

2.5. Statistical Analysis

Analysis of Variance (ANOVA) was used to evaluate the significance of a general linear model (GLM) with factors “earthworms” (CAST versus NOCAST), “soil tension”, “soil”, and their interaction on each measured variable (K , C , D , θ_m , and Δm). When ANOVA warranted it, Tukey’s HSD was used to estimate whether the means of the variables between CAST and SOIL were significantly different at the 0.05 level. JMP 14 Pro (SAS Institute, Cary, NC, USA) was used to conduct the statistical tests.

3. Results

3.1. Moisture Release

There were significant effects of the factors on soil mass moisture, θ_m (model $r^2 = 0.82$, $p < 0.0001$; Table A1). Mass moisture was strongly affected by tension, earthworm presence (CAST vs. NOCAST), and soil, but not their interaction, except for the interaction between tension and earthworms ($p < 0.0001$). Comparisons between CAST and NOCAST cores (Figure 2) showed that soils modified by pheretimid earthworms always had greater soil moisture than soils that were unmodified (Tukey’s HSD evaluated at $p < 0.05$).

In the overall model, there were significant variations in water loss ($r^2 = 0.88$, $p < 0.0001$; Table A1). All effects including the interactions were significant. Figure 2 shows the variation of water loss for

CAST and NOCAST soils. In all soils, moisture loss between saturation and 0.01 bar was significantly greater for the CAST than for the NOCAST soil (Tukey's HSD $p < 0.05$). However, moisture loss in the other intervals was not always significantly different between CAST and NOCAST.

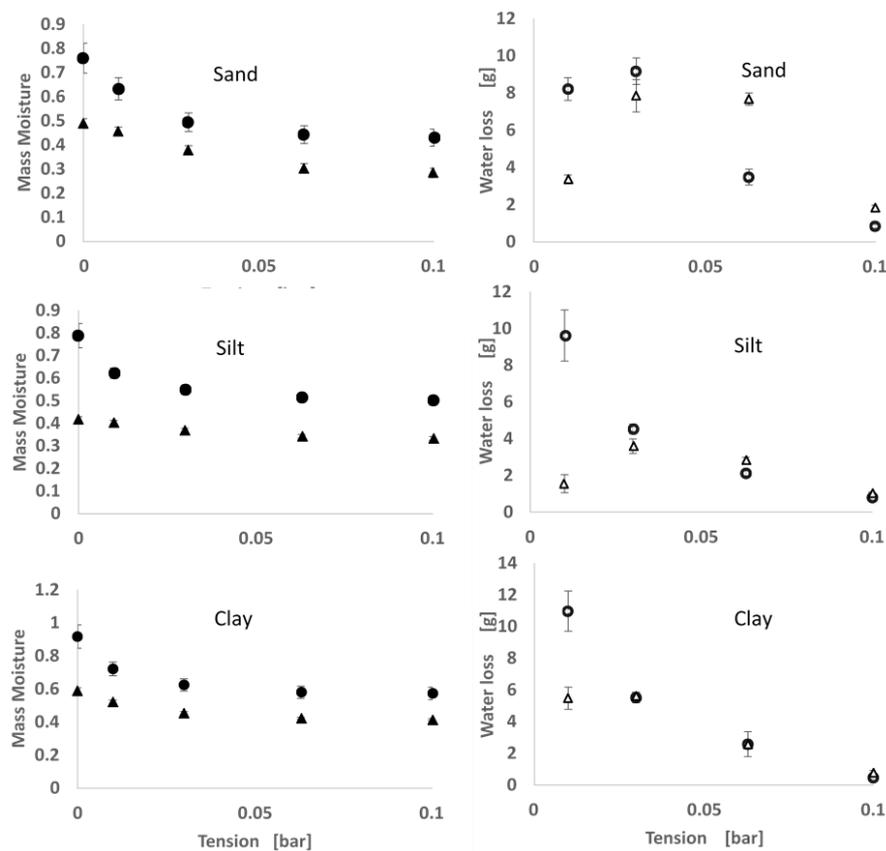


Figure 2. Mass moisture and water loss as a function of tension for CAST (circles) and NOCAST (triangles) soils. Error bars represent 1 standard error.

3.2. Thermal Properties

For each soil texture, the GLM was a good predictor of thermal properties. All factors, earthworm, soil and tension, explained variations in specific heat capacity, C , and thermal conductivity, K ($r^2 = 0.61$ to 0.88 , $p < 0.0001$ to $p = 0.028$; Appendix A Table A1). However, for diffusivity, D , the model was supported by factor earthworm ($p < 0.0001$) and soil ($p < 0.0001$), but not by tension ($p = 0.52$) or the interaction between earthworm and tension and tension and soil ($p = 0.67$ to 0.87). The interaction between earthworm and soil was significant ($p < 0.0001$)

Tukey's HSD (at $p = 0.05$) supported our hypothesis that CAST and NOCAST soil had significantly different thermal properties. CAST cores had greater K and C values than the NOCAST cores at almost all tensions. However, the opposite was found for diffusivity, for which the NOCAST cores were greater than the CAST cores (Figure 3, Appendix A Table A2). Depending on tension, the diffusivity of the CAST and NOCAST cores differed between 11 and 22% for the sand, between 10 and 25% for the silt, and between 4 and 14% for the clay.

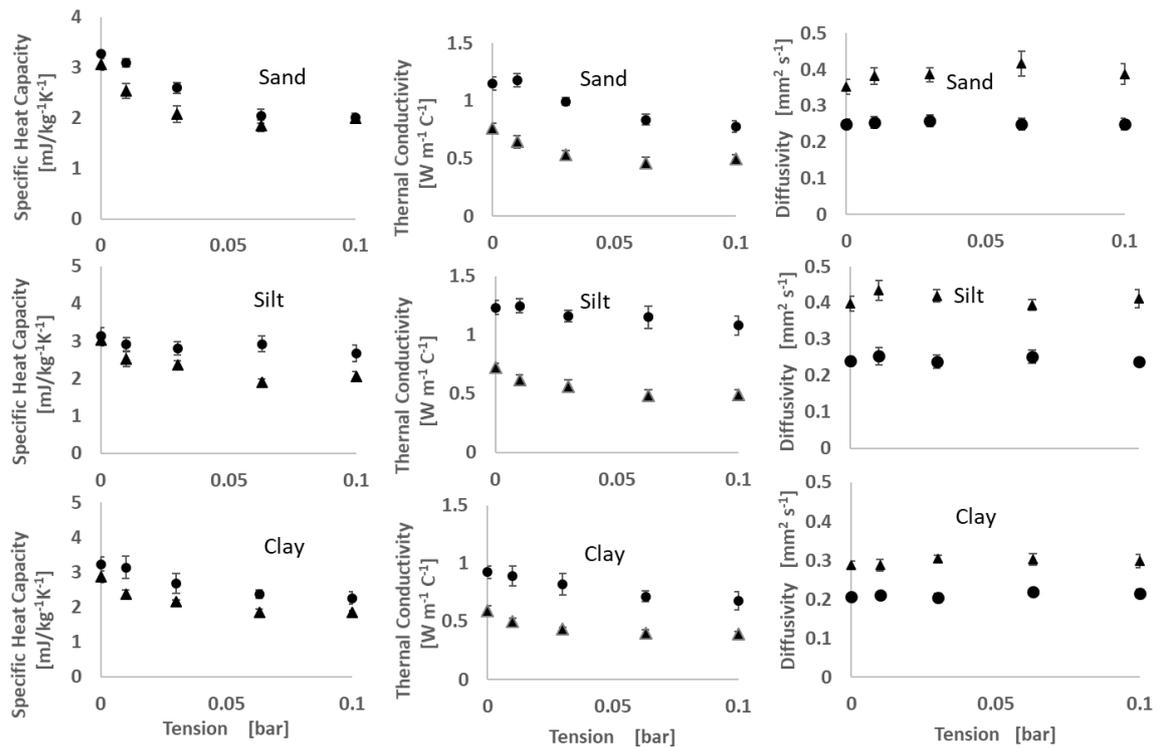


Figure 3. Thermal properties for the three soils for CAST (circles) and NOCAST (triangles) soils. Error bars represent 1 standard error.

3.3. Pheretimid Size

The body diameter of pheretimid hatchlings in the spring of 2018 varied between 0.6 and 1.3 mm. The mode was 0.9 mm (Figure 4). Pheretimid hatchlings were first observed in mid-April of each year and increased to between 26 and 300 m^{-2} by mid-May for 2011 to 2019.

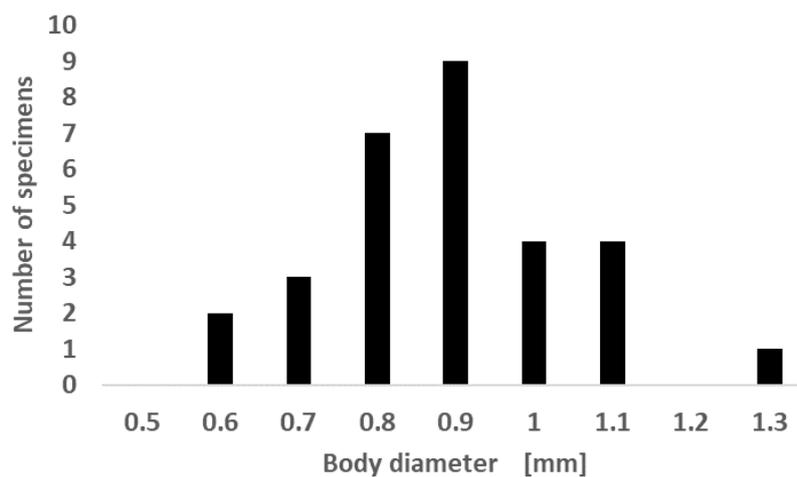


Figure 4. Distribution of body diameters of pheretimid hatchlings collected from April to May 2016.

4. Discussion

4.1. Moisture Release and Thermal Properties

As expected, the large inter-aggregate pores of the CAST soil led to large water losses at low tension levels. Pore neck diameters in the tension interval from saturation to 10 kPa were greater than

300 μm . Therefore, the cast layer was likely to be a good medium for pheretimoid hatchling migration, whose body diameters are of similar size.

Values of K, C, and D found in this study were comparable to those measured for similar soils [27–29]. K and C were linearly related (regressions not shown). Diffusivity (i.e., the ratio of K and C), was therefore relatively constant with tension.

4.2. Ecological Relevance of Thermal Property Differences

The variation of temperature with depth may affect how well some epi-endogeic earthworms respond to temperature fluctuations at the surface. There were large differences between the K, C, and D of the CAST soil and the corresponding NOCAST soil. As such, we expected that there would also be differences in the capacity of the soils to transmit heat and thus propagate to depth any temperature variations occurring at the surface.

Resistance to heat transport can be expressed by the damping depth (Equation (3)). For our soils, the damping depth was shallower for the CAST than for the NOCAST soil (Figure 5). This means that any variations in temperature at the surface do not penetrate as far into the CAST as into the NOCAST soil and thus the cast layer provides some thermal insulation. However, when inspecting the damping depth values, we found only small difference between the CAST and NOCAST soils. For diurnal variation, the damping depths in CAST soil were between 2 mm and 13 mm less in the casting layer than in the NOCAST soil. For short variations that occurred at a scale of about one hour, the damping depth in CAST soil was between 3 and 5 mm shallower than in the NOCAST soil. For variations that occurred at the minute scale (e.g., due to light penetration through gaps in the canopy), damping depths differed only by a fraction of a mm between CAST and NOCAST.

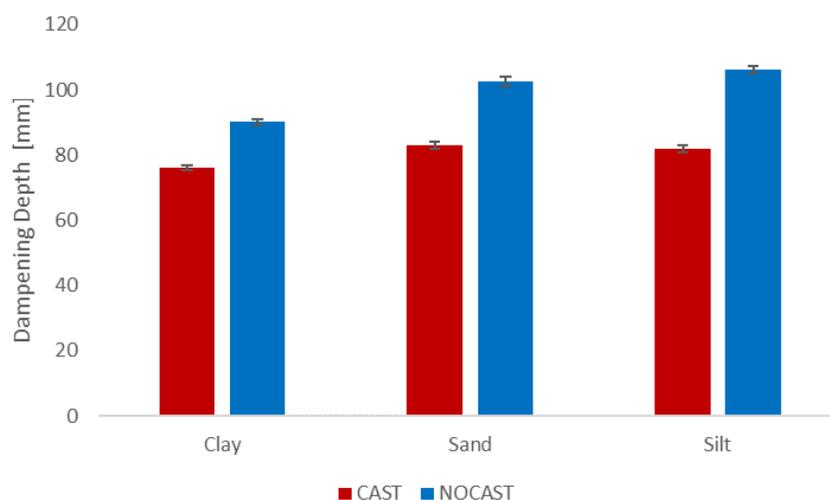


Figure 5. Comparison of dampening depth for CAST and NOCAST soils. Error bars represent 1 standard error.

Differences in damping depth are probably not large enough for the casting layer to provide much of a thermal benefit to adult earthworms under normal circumstances and represent only a fraction of the casting layer depth. However, the damping depth may be important for pheretimoid hatchlings, particularly during the first month after hatching when they are still very small (Figure 5). Between early April and the middle of May, diurnal soil temperature variations are relatively large (Figure 6) and at the surface, dip below the mortality threshold more often than at 5 cm depth. From 9 April (first pheretimoid hatchling recorded that year) to 9 May 2014, the ambient minimum daily temperature at the surface was below the mortality threshold of 5 °C [22] 17 times. The temperature at the dampening depth was below that value only eight times. Furthermore, at the dampening depth, the temperatures stayed above 5 °C after 25 April. At the surface, temperatures were below 5 °C until 8 May 2014.

During this time of the year, the abundance of pheretimoid hatchlings increased (to between 26 and 140 m⁻²), suggesting that the casting layer may be a shelter from conditions colder than the mortality threshold. The greater damping of the temperature variation in the casting layer may shorten the distance that juveniles need to move to reach a safe depth.

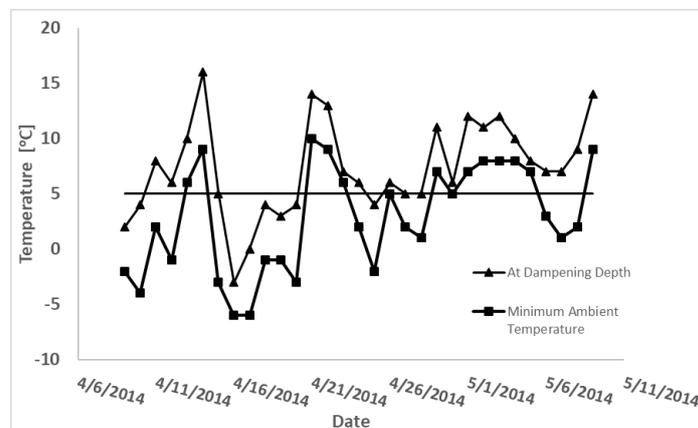


Figure 6. Typical variation of temperature experienced by early hatchlings at the soil surface (ambient) and at the dampening depth.

The loose layer of castings may also facilitate vertical mobility in the soil by eliminating the need to excavate during movement. The water loss data (Figure 2) suggests that a large fraction of the pore volume in the CAST soil resides in pores with necks >300 μm . This is of the same order of magnitude as hatchling diameters (Figure 4).

Under fire conditions, extreme temperatures can occur at the soil surface. However, *A. agrestis* were found to survive controlled fires in a sandy soil [30], with temperatures at a 5 cm depth only increasing by 7 °C above pre-fire conditions. Our calculations (Equation (5)) predicted a 4 °C and 12 °C temperature increase for the cast layer of the sand and 15-min and 30-min fire duration, respectively. For the soil without casts, the increase at 5 cm was calculated as 6 and 25 °C for the 15 and 30 min fire duration, respectively. In the calculations, we assumed that the fire burned at a constant surface temperature of 900 °C. It is difficult to compare our results with those of Blackmon et al. [31] because they did not report the duration of the fire. However, our calculations predicted that the casting layer may represent a better fire refuge for pheretimoids than the equivalent original soil for purely thermal reasons. Survivability of pheretimoids exposed to forest fires may be improved further as the large pores spaces between the loose aggregates of the casting layer allows easier vertical movement to escape the heat. The casting layer may represent protection in newly invaded areas where climate and fire frequencies differ from their original range.

5. Conclusions

1. Significant differences between the thermal properties and macropore structure existed between the CAST and NOCAST soil.
2. Production of CAST soil may improve pheretimoid survival when large daily temperature fluctuations about the mortality threshold occur, or when the soil is exposed to the extreme temperatures that may occur during forest fires.
3. The casting layer may increase the invasiveness of pheretimoids in new environments.

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Appendix A

Table A1. ANOVA parameters for linear model for entire model and individual effects of heat capacity, C, thermal conductivity, K, density (including soil moisture), ρ , mass moisture, θ_m , mass of water loss between soil tensions, Δm , and thermal diffusivity, D.

	ANOVA of Model			Effect Tests											
	r^2			Earthworm		Tension		Soil		Earthworm X Tension		Earthworm X Soil		Tension X Soil	
		F-Ratio	$p > F$	F-Ratio	$p > F$	F-Ratio	$p > F$	F-Ratio	$p > F$	F-Ratio	$p > F$	F-Ratio	$p > F$	F-Ratio	$p > F$
K	0.83	36.56	<0.0001	526.0	<0.0001	23.68	<0.0001	51.27	<0.0001	1.48	0.209	15.53	<0.0001	0.933	<0.491
C	0.61	11.55	<0.0001	57.65	<0.0001	39.60	<0.0001	3.66	<0.0280	1.29	0.275	1.58	<0.210	1.36	<0.219
ρ	0.81	31.14	<0.0001	550.4	<0.0001	0.00	1.00	32.44	<0.0001	0.00	1.00	19.38	<0.0001	0.00	1.00
θ_m	0.82	34.17	<0.0001	324.4	<0.0001	65.03	<0.0001	42.19	<0.0001	8.59	<0.0001	1.95	0.146	1.27	0.261
Δm	0.88	89.28	<0.0001	24.34	<0.0001	188.03	<0.0001	24.55	<0.0001	50.20	<0.0001	4.63	<0.0111	13.10	<0.0001
D	0.77	25.03	<0.0001	392.8	<0.0001	0.798	<0.528	51.95	<0.0001	0.54	0.705	13.43	<0.0001	0.408	0.9150

Table A2. Dependence of mean (1 standard error) heat capacity, C, thermal conductivity, K, density (including soil moisture), ρ , and thermal diffusivity, D on soil tension (from saturation to 0.1 bar) for *Amynthas* cast and unaltered soil material for three soils with different textures. Replication rate = 6 per treatment.

Clay		CAST					NOCAST				
Tension [bar]	0.0	−0.01	−0.03	−0.063	−0.1	0.0	−0.01	−0.03	−0.063	−0.1	
K [$\text{Wm}^{-1}\text{k}^{-1}$]	0.593 (0.042)	0.501 (0.030)	0.438 (0.015)	0.402 (0.025)	0.395 (0.018)	0.925 (0.055)	0.890 (0.085)	0.822 (0.094)	0.716 (0.045)	0.678 (0.076)	
C [$\text{MJm}^{-3}\text{k}^{-1}$]	2.864 (0.173)	2.377 (0.116)	2.149 (0.056)	1.839 (0.101)	1.838 (0.048)	3.233 (0.197)	3.137 (0.324)	2.674 (0.279)	2.369 (0.123)	2.257 (0.187)	
ρ [g/cm^3]	1.057 (0.018)	0.933 (0.020)	0.869 (0.018)	0.841 (0.018)	0.836 (0.017)	1.261 (0.033)	1.201 (0.037)	1.139 (0.035)	1.111 (0.034)	1.103 (0.033)	
D [mm s^{-1}]	0.196 (0.006)	0.226 (0.005)	0.235 (0.009)	0.261 (0.011)	0.257 (0.010)	0.229 (0.012)	0.241 (0.016)	0.269 (0.006)	0.273 (0.012)	0.270 (0.014)	
Sand		CAST					NOCAST				
Tension [bar]	0.0	−0.01	−0.03	−0.063	−0.1	0.0	−0.01	−0.03	−0.063	−0.1	
K [$\text{Wm}^{-1}\text{k}^{-1}$]	0.762 (0.047)	0.645 (0.056)	0.531 (0.037)	0.463 (0.046)	0.499 (0.037)	1.151 (0.059)	1.178 (0.057)	0.996 (0.033)	0.835 (0.047)	0.777 (0.053)	
C [$\text{MJm}^{-3}\text{k}^{-1}$]	3.065 (0.114)	2.540 (0.155)	2.081 (0.158)	1.849 (0.116)	1.994 (0.054)	3.272 (0.065)	3.098 (0.087)	2.597 (0.105)	2.040 (0.140)	2.013 (0.084)	
ρ [g/cm^3]	1.199 (0.027)	1.100 (0.031)	0.991 (0.031)	0.950 (0.030)	0.940 (0.029)	1.423 (0.038)	1.388 (0.038)	1.305 (0.040)	1.223 (0.036)	1.204 (0.036)	
D [mm s^{-1}]	0.208 (0.009)	0.231 (0.012)	0.261 (0.015)	0.262 (0.013)	0.266 (0.014)	0.247 (0.008)	0.275 (0.012)	0.296 (0.010)	0.339 (0.019)	0.321 (0.016)	
Silt		CAST					NOCAST				
Tension [bar]	0.0	0.01	0.03	0.063	0.1	0.0	0.01	0.03	0.063	0.1	
K [$\text{Wm}^{-1}\text{k}^{-1}$]	0.726 (0.035)	0.621 (0.042)	0.565 (0.051)	0.484 (0.048)	0.490 (0.042)	1.235 (0.059)	1.248 (0.061)	1.160 (0.048)	1.150 (0.093)	1.082 (0.080)	
C [$\text{MJm}^{-3}\text{k}^{-1}$]	3.024 (0.154)	2.518 (0.204)	2.366 (0.115)	1.909 (0.079)	2.047 (0.135)	3.144 (0.219)	2.915 (0.180)	2.802 (0.181)	2.920 (0.213)	2.675 (0.223)	
ρ [gcm^{-3}]	1.180 (0.041)	1.055 (0.051)	0.998 (0.052)	0.971 (0.049)	0.961 (0.048)	1.451 (0.032)	1.435 (0.035)	1.396 (0.036)	1.366 (0.037)	1.355 (0.037)	
D [mm s^{-1}]	0.205 (0.009)	0.242 (0.024)	0.243 (0.023)	0.260 (0.017)	0.249 (0.007)	0.276 (0.019)	0.304 (0.024)	0.301 (0.016)	0.289 (0.010)	0.305 (0.024)	

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