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Warming shifts 'worming': effects of experimental warming on invasive earthworms in northern North America

SUBJECT AREAS:

INVASIVE SPECIES

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Climate change causes species range shifts and potentially alters biological invasions. The invasion of European earthworm species across northern North America has severe impacts on native ecosystems. Given the long and cold winters in that region that to date supposedly have slowed earthworm invasion, future warming is hypothesized to accelerate earthworm invasions into yet non-invaded regions. Alternatively, warming-induced reductions in soil water content (SWC) can also decrease earthworm performance. We tested these hypotheses in a field warming experiment at two sites in Minnesota, USA by sampling earthworms in closed and open canopy in three temperature treatments in 2010 and 2012. Structural equation modeling revealed that detrimental warming effects on earthworm densities and biomass could indeed be partly explained by warming-induced reductions in SWC. The direction of warming effects depended on the current average SWC: warming had neutral to positive effects at high SWC, whereas the opposite was true at low SWC. Our results suggest that warming limits the invasion of earthworms in northern North America by causing less favorable soil abiotic conditions, unless warming is accompanied by increased and temporally even distributions of rainfall sufficient to offset greater water losses from higher evapotranspiration.

Future changes in climatic conditions are likely to influence the composition, biodiversity and functioning of ecosystems¹ as well as the spread and success of non-native invasive species². As a consequence, climate change will have direct and indirect effects on ecosystems by altering process rates and changing species interactions, respectively³. For instance, increasing temperatures will change the composition and functioning of ecosystems⁴ and likely shift the competition between native and non-native species by favoring species adapted to higher temperatures and drought.

Rising temperatures can disrupt existing biotic interactions, making ecosystems either less or more susceptible to biological invasions³, and may benefit certain invasive species². Moreover, warming has been shown to cause range shifts in many plant and animal species⁵, with range expansion strongly influenced by biotic interactions^{6,7}. In fact, Lu et al.⁸ recently showed that warming may allow the natural enemy of range-expanding plants to follow their host, changing the enemy release and shifting the geographical gap ('spatial mismatch'⁹) between plants and herbivorous insects.

In that context, several studies have stressed the possibility of warming-induced northward range expansions of some macro-detrivore species^{10,11}. Such range shifts of macro-detrivores may cause increased decomposition rates and changed carbon dynamics in previously macro-detrivore-free areas^{10,12}. Particularly key macro-detrivores, such as those structuring the environment for other organisms ('ecosystem engineers')¹³, may have disproportionately strong effects on the composition and functioning of invaded ecosystems^{10,14,15}.

Large parts of North America are lacking native earthworm species¹⁰, but European species have been invading many forest and grassland ecosystems since European colonization of North America ~400 years ago^{10,14}. Once introduced, exotic earthworms have been shown to change soil physical^{16,17}, chemical^{12,18}, and biological properties of native habitats^{17,19}. Considering the wide distribution of European earthworm species in North America¹⁴ and the dramatic consequences of earthworm invasion for the biodiversity and functioning of ecosystems previously devoid of earthworms¹⁰, better knowledge of distribution patterns and drivers of invasive earthworm populations is crucial to predict and control their further spread²⁰.



Potential interactions between increasing temperature and invasion by exotic earthworm are of considerable interest^{14,21}. Frelich and Reich²² hypothesized that climate warming should accelerate the spread and increase the impacts of invasive earthworms in northern North America. Warming-induced increasing length of growing seasons and enhanced primary productivity at northern latitudes are likely to contribute to more rapid earthworm invasions formerly limited by colder temperatures¹⁰. Indeed, minimal winter soil temperature may be a main determinant of the northern boundary of earthworms' range²³.

Warming may, on the other hand, also reduce biological activity by decreasing soil water content²⁴, and this may be particularly relevant for soil organisms²⁵. Earthworm activity is limited by extreme temperatures and drought stress^{26,27}, and Zaller et al.²⁸ found that moderate experimental warming significantly reduced the density and biomass of epigeic earthworms in a *Carex* fen ecosystem in southern South America. These inconsistent results may be due to the fact that higher soil temperatures are often accompanied by lower soil water content^{29,30}, complicating predictions on how warming will affect earthworm invasion ('worming'³¹). Given the seasonality of relationships between soil temperature and soil water content, repeated assessments of earthworm activity in different contexts may thus be necessary²⁷. In order to do so, we took advantage of a unique field experiment in the temperate-boreal forest ecotone, Minnesota, USA, where invading earthworms pose a major threat to native biodiversity and change the functioning of forest habitats^{32,33}. This experiment consists of two distinct sites and simulates climate warming in closed and open forest canopy (Fig. 1), i.e., in four different environmental contexts altogether (such as indicated by different understory plant community structure)³⁴.

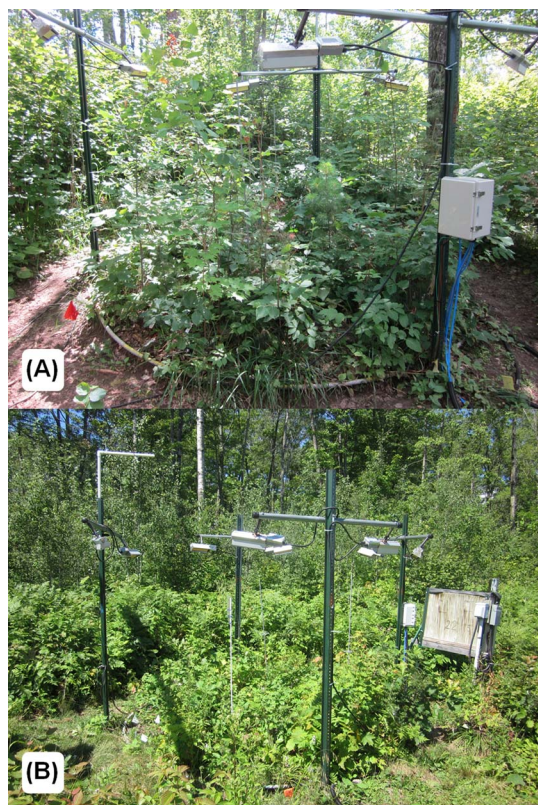


Figure 1 | Exemplary plots of the B4WarmED experiment (Boreal Forest Warming at an Ecotone in Danger) in Minnesota, USA. The upper panel shows one example plot in closed canopy with tree saplings and heat lamps in 2010 (A), the lower panel shows one example plot in open canopy in 2012 (B; photo credit: N. Eisenhauer).

We hypothesized that (1) warming influences exotic earthworm performance positively at high soil water content, but negatively at low soil water content (i.e., warming effects will vary across the growing season). Relatedly, we expected (2) detrimental warming effects to be more pronounced in the open canopy than in the closed canopy treatment due to the fact that open canopy soils may experience a lower average in soil water content and more pronounced fluctuations.

Results

Overall, the results confirmed our hypothesis that temperature effects on invasive earthworms depend on the water content of the soil. We found that warming reduced earthworm densities in three out of eight cases, whereas a significant positive effect was only observed once. Structural equation modeling revealed that detrimental warming effects could indeed be explained in part by warming-induced reductions in soil water content, and the direction of warming effects depended on the current soil water content: experimental warming had neutral to positive effects when average soil water content was high (>21%), whereas the opposite was true at low average soil water content. Warming effects were consistent across experimental sites (Ely and Cloquet) and canopy treatments (open and closed).

General Linear Models showed that canopy effects on earthworm density and biomass were most pronounced in June 2012, with higher density and biomass under closed canopy (Table 1). Further, earthworm density and biomass decreased with increasing temperature in August 2010 and June 2012 in Cloquet and Ely, and in October 2012 in Ely (Fig. 2). In Cloquet, the response of earthworm densities in August 2010 was most pronounced in epigeic earthworms, which decreased from 455 ± 445 ind./m² (ambient temperature) to 325 ± 296 ind./m² (ambient + 1.7°C) to 130 ± 235 ind./m² (ambient + 3.4°C; $F_{1,28} = 5.60$, $P = 0.025$), although endogeic earthworms showed a similar pattern with a decrease from 87 ± 170 ind./m² (ambient temperature) to 71 ± 121 ind./m² (ambient + 1.7°C) to 65 ± 117 ind./m² (ambient + 3.4°C; $F_{1,27} = 1.23$, $P = 0.28$). We could not perform ecological group-specific analyses for Ely (August 2012) due to low earthworm densities.

Earthworm density and biomass increased significantly with increasing temperature in April 2012 in Cloquet, but did not vary significantly in response to warming in April 2012 in Ely and in October 2012 in Cloquet (Fig. 2). Increased earthworm numbers at higher temperatures in April 2012 in Cloquet were due to the significant response of endogeic earthworms ($F_{2,29} = 5.56$, $P = 0.009$), while epigeic earthworms were not significantly affected ($F_{2,29} = 0.52$, $P = 0.60$). In Ely, both earthworm ecological groups did not differ significantly among the temperature treatments (both $P > 0.66$). The decrease in earthworm densities with increasing temperature in June 2012 in Cloquet was mainly due to linearly decreasing numbers of endogeic earthworms ($F_{1,32} = 3.60$, $P = 0.067$), while epigeic earthworms remained largely unaffected ($F_{1,32} = 0.06$, $P = 0.80$). The same pattern was true in June 2012 in Ely: densities of endogeic earthworms decreased significantly with increasing temperature ($F_{1,32} = 11.28$, $P = 0.002$), while epigeic earthworms were not significantly affected ($F_{1,32} = 0.31$, $P = 0.58$). In October 2012, both epigeic ($F_{1,31} = 2.42$, $P = 0.13$) and endogeic ($F_{1,31} = 1.81$, $P = 0.19$) earthworms tended to decrease with increasing temperature in Ely, while densities and numbers of replicates did not allow more detailed analyses for Cloquet. Overall, endogeic and epigeic earthworms did not show opposing responses to temperature, which is why we will focus on the response of total earthworms in the following. Responses to temperature treatment never varied significantly with canopy type (i.e., no significant interactive effects of temperature and canopy on earthworm density and biomass), indicating that warming effects were consistent in the two canopy treatments (Table 1).



Table 1 | GLM table of F and P values of the effects of Canopy (open and closed), Temperature (ambient, ambient + 1.7°C, and ambient + 3.4°C; linear variable), and the interaction between Canopy x Temperature on (a) earthworm abundance and (b) earthworm biomass in Cloquet and Ely in August 2010, April 2012, June 2012, and October 2012

		Canopy		Temperature		Canopy x Temperature	
		F value	P value	F value	P value	F value	P value
(a) Earthworm abundance							
Cloquet							
	August 2010	1.60	0.215	4.99	0.033	0.70	0.408
	April 2012	2.48	0.126	4.63	0.039	1.23	0.276
	June 2012	10.90	0.002	3.42	0.076	0.60	0.445
	October 2012	0.56	0.513	0.42	0.529	0.03	0.863
Ely							
	August 2010	0.23	0.653	4.46	0.046	0.01	0.941
	April 2012	3.28	0.080	0.01	0.906	0.23	0.638
	June 2012	12.46	0.001	9.91	0.004	2.91	0.098
	October 2012	0.67	0.421	3.25	0.081	0.27	0.608
(b) Earthworm biomass							
Cloquet							
	April 2012	1.69	0.203	11.45	0.002	1.07	0.310
	June 2012	11.97	0.002	0.39	0.537	0.27	0.607
	October 2012	1.14	0.307	0.03	0.858	0.19	0.667
Ely							
	April 2012	1.04	0.315	0.21	0.653	0.07	0.799
	June 2012	8.33	0.007	9.90	0.004	1.40	0.247
	October 2012	0.13	0.725	7.14	0.012	0.35	0.557

Degrees of freedom: Canopy = 1, Temperature = 1, Canopy x Temperature = 1, Error = 31 (except Cloquet, October 2012 = 12); significant effects ($P < 0.05$) are given in bold.

Overall, structural equation models supported the results from the GLM analyses and explained 6 to 41% of the variance in earthworm density across samplings (Fig. 3). Indeed, warming-induced reductions in soil water content explained the warming effects in three out of five cases (three significant effects and two trends). The direction of warming effects depended on the current soil water content as experimental warming had neutral to positive effects when average

soil water content was high ($>21\%$), whereas the opposite was true at low average soil water content (Fig. 3). Mean soil water contents were 23 and 14% in August 2010, 25 and 26% in April 2012, 16 and 13% in June 2012, and 5 and 3% in October 2012 in Cloquet and Ely, respectively. In addition, soil water content was significantly higher in the closed than in the open canopy treatment in April 2012 (Ely), June 2012 (Cloquet and Ely), and October 2012 (Ely).

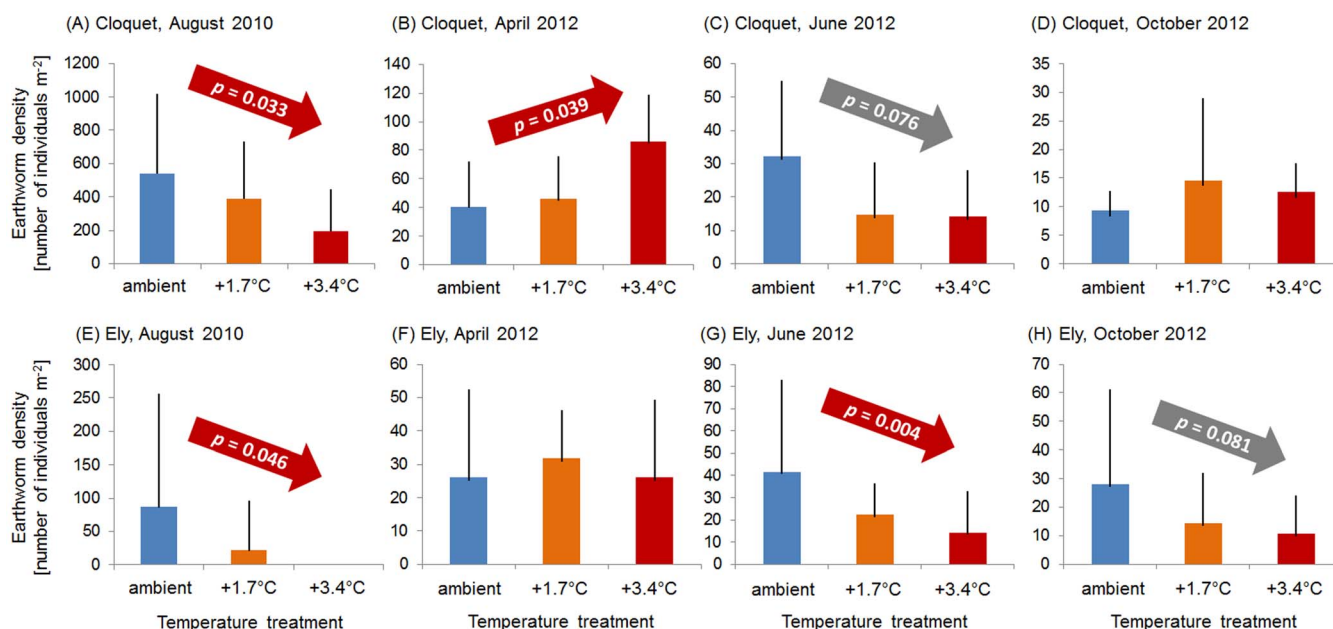


Figure 2 | Earthworm densities as affected by warming (temperature treatments: ambient temperature, ambient + 1.7°C, and ambient + 3.4°C) at the experimental sites at Cloquet (A, B, C, D) and Ely (E, F, G, H) in August 2010 (A, E), April (B, F), June (C, G), and October 2012 (D, H). Red arrows indicate significant warming effects on earthworm densities; grey arrows indicate non-significant trends ($P < 0.1$).

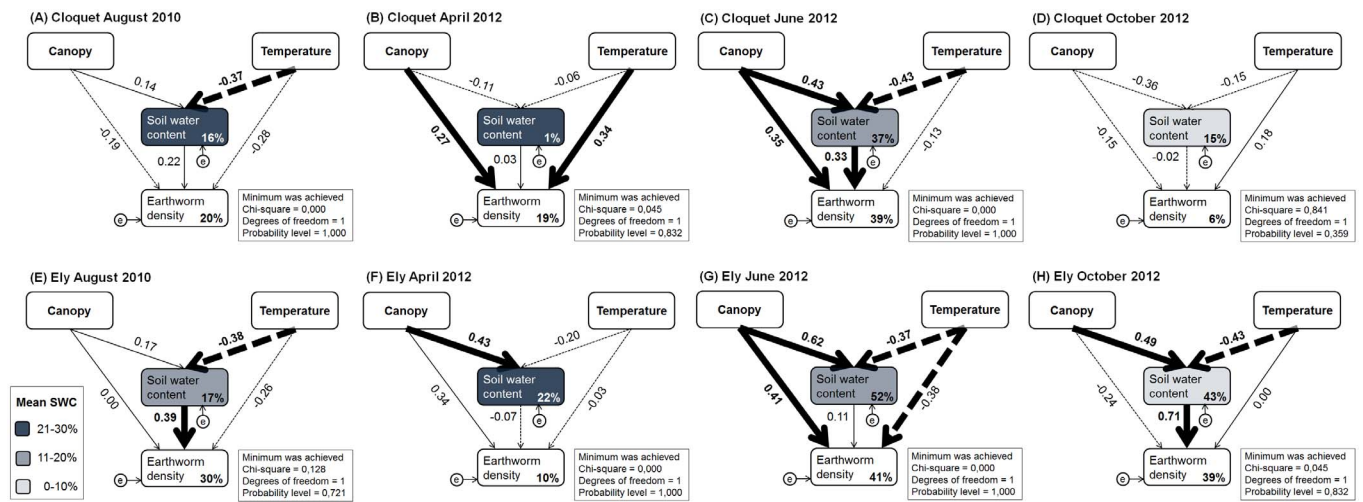


Figure 3 | Structural equation models of causal influences of canopy (open = 0, closed = 1) and temperature (ambient, ambient + 1.7°C, and ambient + 3.4°C) (both exogenous variables) on soil water content and earthworm density (both endogenous variables) in Cloquet in August 2010 (A), April 2012 (B), June 2012 (C), and October 2012 (D), and in Ely in August 2010 (E), April 2012 (F), June 2012 (G), and October 2012 (H). Numbers on arrows are standardized path coefficients (equivalent to correlation coefficients). Average soil water content levels during earthworm extractions are indicated by different shades of blue. Percentages in boxes indicate the proportion of explained variance in endogenous variables. Width of the arrows indicates the strength of the causal influence: bold arrows indicate significant ($P < 0.05$) standardized path coefficients, fine arrows indicate non-significant path coefficients ($P > 0.05$). Solid arrows indicate positive relationships and dashed arrows negative relationships. Circles indicate error terms of endogenous variables.

Discussion

Climate change may alter biotic interactions and the spread and success of exotic invasive species^{2,3,15}. As a consequence, invasive species can have significant impacts on the functioning and biodiversity of native ecosystems³⁵, and particularly so in boreal and northern temperate forest ecosystems, where biological invasions were recently shown to represent the main drivers of biodiversity loss³⁶. The results of the present experimental study confirmed our hypotheses (1): we found pronounced variations in the strength and direction of warming effects on earthworm performance, with detrimental effects occurring under dry soil water conditions and being partly explained through warming-induced reductions of soil water content. On the other hand, neutral and positive effects of warming on earthworm performance occurred at higher soil water contents. However, we found no support for our hypothesis (2) assuming that detrimental warming effects to be more pronounced in the open canopy than in the closed canopy treatment due to different soil water conditions. Although average soil water content was as predicted significantly higher in closed than in open canopy (with differences varying between 1.5 and 14.3%), we only found one non-significant interaction between canopy and warming ($P < 0.1$).

In addition to higher levels of soil water content under closed canopy, we expected greater resource inputs through tree litter material to cause higher earthworm densities and to buffer warming effects. Litter material with high N- and P-contents in aspen forests has been shown to support exceptionally high densities of exotic earthworms^{17,37}. Indeed, we observed higher earthworm densities in the closed than in the open canopy treatment in four out of eight cases, which were only partly explained by higher soil water content. We assume that higher availability and quality of litter material³⁸ under closed canopy increased earthworm densities early in the season (April and June) when litter material from the previous fall was still on the soil surface, but not later in the season (August and October; Fig. 3) when the litter might have been mostly consumed by the decomposer community.

The lack of interactive effects of canopy type and warming treatment on earthworm performance indicate that canopy effects were likely due to resource availability and not through changes in soil

water content: while warming significantly decreased soil water content in five out of eight cases, canopy effects were only partly due to changes in soil water content as indicated by the significant paths from canopy to earthworm densities in April and June (Fig. 3). We therefore conclude that canopy conditions may have little potential to alter warming effects on earthworm invasion success.

Earthworms represent impactful invaders around the globe¹⁴, and – once established in native habitats – it is almost impossible to remove exotic earthworms from native ecosystems²⁰. Therefore, many studies have investigated the main vectors^{32,39} and driving environmental conditions facilitating earthworm invasions⁴⁰. Studying 125 mixed temperate-boreal forest sites across the western Great Lakes region in the US, Fischelli et al.⁴⁰ recently reported that 93% of those sites showed earthworm activity, and that earthworm activity was largely explained by soil pH, precipitation, and litter quality. Although mean summer temperature was not a significant predictor in that study, the authors argued that changes in soil water content due to a warming climate may limit earthworm invasion⁴⁰. Annual precipitation can be regarded as a proxy for site level soil moisture stress, which poses strong limits to earthworm activity²⁶. The boreal-temperate ecotone in Central North America has experienced three decades of relatively wet weather conditions⁴¹, which may have facilitated earthworm invasion⁴⁰. However, climate projections for hotter and drier summers⁴¹ may limit soil water content and thereby reduce the activity of invasive earthworms in the future.

The results of our present study indicate that warming can have positive effects on earthworm performance if soil water content is not decreasing accordingly. However, warming often decreases soil water content due to increased evapotranspiration^{29,30}. In addition, warming may be accompanied by greater variation in precipitation events, which may not yield in strong changes in precipitation amounts, but severe precipitation events could alternate more frequently with droughts¹. Based on our experimental study, we expect future climate warming to decrease the spread of earthworm invasions since earthworm activity is limited by higher temperatures and drought stress^{26,27,28}. Notably, earthworm activity has been shown to intensify warming effects on soil water content³¹, so there may be positive feedbacks of the drying effects of climate warming, and of



earthworms on soil drying, up to the point where the drying reduces earthworm activities. Underlying mechanisms may involve earthworm burrows to function as preferential flow pathways for soil surface water⁴², and the removal of a litter layer, which protects lower soil layers from evaporation⁴³.

Our multiple samplings at two experimental sites and in various seasons indicate that climate warming may also shift activity patterns of earthworms (Fig. 2): warming effects tended to be neutral to positive in spring when soil water content was still high from the melting snow. Later in the growing season, though, warming effects switched to negative at low soil water content. In order to predict warming effects on the future spread of exotic earthworms, one should also consider other indirect drivers of earthworm invasion, which could change with a warming climate. For instance, anglers – which represent the main vectors of current earthworm invasions^{32,39} – may more frequently visit northern and remote lakes, which could create new invasion sites and facilitate jump dispersal. It is therefore vital to inform people about the potential consequences of earthworm invasions as this might be one of the most successful management strategies thus far²⁰.

To adequately assess the consequences of earthworm invasions, experimental manipulations of exotic earthworm densities in the field are urgently needed⁴⁴. Most previous studies observed natural invasion gradients, while the reasons for those gradients are mostly unknown but inferred to represent ongoing invasion patterns^{16,17}. Thus, studying potential interactions between climate change agents, such as warming, and earthworm invasions in long-term field experiments will allow improvements in our predications concerning the future spread of exotic earthworms as well as potential interactive effects on the biodiversity and functioning of native ecosystems.

Our study highlights that climate warming will influence earthworm invasions in the boreal-temperate ecotone of North America with the direction of the effect depending on soil water content. Although we expect climate warming to decelerate earthworm invasions, we highlight the need to consider seasonal shifts in earthworm activity patterns with unprecedented effects on the functioning of native ecosystems.

Methods

Experimental design. The study was conducted in the temperate-boreal forest ecotone of Minnesota, USA, in the framework of the B4WarmED experiment (Boreal Forest Warming at an Ecotone in Danger). This field experiment simulates climate warming in closed and open canopy areas at two different sites: Cloquet (coordinates: 46°31' N, 92°30' W) and Ely (coordinates: 47°55' N, 92°30' W). The mean annual temperature of Cloquet is 4.23°C with mean annual precipitation of 752.31 mm, whereas Ely has 3.57°C and 664.61 mm, respectively (100 y average recorded at Cloquet Forestry Center and Ely airport; years 1912–2012). Both experimental sites consist of six blocks (each with six circular plots 3 m in diameter), three in open and three in closed canopy forest. For each block, three levels of temperature treatments (ambient, ambient + 1.7°C, and ambient + 3.4°C, randomly assigned) were used (n = 2 per temperature treatment per block). Both experimental sites in Cloquet and Ely are forested with 60–80 years old aspen overstory (*Populus tremuloides*). Part of the aspen overstory was removed to create open canopy plots in the year 2006 at Cloquet and in 2007–2008 at Ely. At both sites, the soil is sandy loam, with higher variability in texture at Ely than at Cloquet.

In order to investigate warming and canopy effects on earthworm performance, we sampled earthworms from 72 plots in total at the experimental field sites at Cloquet and Ely in August 2010, and April, June, and October 2012 (see below for details). Elevated temperature treatments were maintained on heated plots both aboveground and belowground simultaneously through artificial heating using 6–8 ceramic heating elements (Mor-Electric, model FTE-1000, MI, USA) aboveground⁴⁵ and heating cables buried in soil (Danfoss GX, Devi A/B, Denmark) for belowground warming⁴⁶ (Fig. 1). The warming treatments represent a continuous (24 h d⁻¹) elevation of plant and soil temperature from ambient plots (which serve as a reference; see electronic supplementary material (ESM) 1 and ESM 2 for more details). Although warming in winter can have important impacts on ecosystem processes⁴⁷, the low levels of biological activity, potential artefacts of warming treatments on snow melt and freeze/thaw cycles, and the high expense of warming year-round led us to decide not to warm in winter. Thus, plots are warmed 24 h d⁻¹ for ~8 months per year (roughly the time of year when ambient temperatures are greater than 1°C, on average).

At the start of the experiment in 2008, plots were planted with a random mix of eleven different tree species (as seedlings; *Abies balsamea*, *Acer rubrum*, *Acer saccharum*, *Betula papyrifera*, *Populus tremuloides*, *Picea glauca*, *Pinus banksiana*, *Pinus*

strobus, *Quercus macrocarpa*, *Quercus rubra*, *Rhamnus cathartica*)³⁴. Thus, tree communities were identical across all plots at the start of experiment, whereas naturally occurring understory plant communities varied and were not controlled³⁴. This experimental setup allowed us to test warming effects on exotic earthworms under field conditions in a standardized way.

Earthworm extraction in 2010. In August 2010, we sampled earthworms by taking one soil core (7 cm in diameter, 7 cm deep) from each experimental plot. Earthworms were extracted by heat as this method has been shown to yield a high number of extracted earthworms⁴⁸. We were not able to take larger soil cores as those would have caused too much damage to the plots, tree root systems, and/or heat cables in the soil. However, since we were interested in relative differences between plots rather than absolute data on earthworm densities and biomass at the sampling locations, this approach did not influence the conclusions of our work. As earthworm densities were rather low (mostly 1–4 individuals per soil core) and as responses in epigeic and endogeic earthworms to warming were similar, we mainly focused on the total density of earthworms in the statistical analyses. Although we performed separate analyses for ecological groups if possible, species-specific analyses were not possible. Dominant earthworm species were *Lumbricus rubellus*, *Aporrectodea caliginosa*, and *Dendrodrilus rubidus*. In 2010, we only recorded the number of extracted earthworms, not biomass.

Earthworm extractions in 2012. In order to cover potential seasonality in warming effects on the density of earthworms, we performed three extractions in 2012: in April (early spring), June (early summer), and October (fall). Since repeated destructive measurements, such as in the case of heat extraction (see above), were not feasible in the experimental plots, we performed mustard extractions⁴⁹. Therefore, mustard solutions were prepared by shaking 60 g of dry mustard powder (Frontier Natural products Co-op, Frontier Bulk Mustard Seed Yellow Powder, Organic, 1 lb. package) with 6 l of water; the solution was mixed intensively before application. We applied 6 l of mustard solution to the sample frame of 0.4 × 0.4 m split into three sub-applications every 5 min using ~2 l of the solution each time (applications after 0, 5, and 10 min). Sampling was terminated after 15 min per plot with a very low number of earthworms emerging afterwards. Earthworms were identified as detailed above.

We lost part of the samples from the extraction at the Cloquet site in October 2012 during shipping, which is why we were restricted to data from 16 plots from that extraction campaign. In order to allow comparisons with other studies, we report earthworm density and biomass data as number of individuals and earthworm fresh weight with gut content per m², respectively. In the other sections of this paper, we will mostly refer to 'earthworm performance' implying both earthworm density and biomass (which showed very similar patterns; Table 1).

The reader should note that a low number of extracted earthworms could also indicate that earthworms entered dormancy, which means that they are not necessarily dead and may outlast detrimental environmental conditions in deeper soil layers. However, we believe that earthworm activity (and thus extractability) is one important component of earthworm performance⁵⁰ and is likely to be the basic requirement and determinant of further spread of exotic earthworms. Given that the efficiency of mustard extraction can vary depending on soil conditions⁵¹, we verified the earthworm density and biomass data by determining the weight of surface cast material⁵⁰ in April 2012, which is an indicator of earthworm activity integrating information over longer time intervals (from weeks to months depending on the frequency and severity of precipitation events). Therefore, we sampled earthworm casts from 0.16 m² (0.4 × 0.4 m) of the experimental plots and dried the cast material in an oven (70°C, 48 h). Then, we plotted earthworm density and biomass data against the dry weight of earthworm casts. Both the density and biomass of earthworms were significantly positively correlated with the dry weight of earthworm casts in Ely (density: R² = 0.41, P < 0.001; biomass: R² = 0.33, P < 0.001) and Cloquet (density: R² = 0.37, P < 0.001; biomass: R² = 0.38, P < 0.001), indicating that the earthworm density and biomass data well predicted earthworm activity at both sites.

Dominant earthworm species were *Lumbricus rubellus*, *Aporrectodea caliginosa*, *Dendrobaena octaedra*, and *Dendrodrilus rubidus*, with very low numbers of *Aporrectodea rosea*, *Allolobophora chlorotica*, and *Octolasion lacteum*. We only used the density and biomass of epigeic or endogeic earthworms to explore functional responses of earthworm communities and to account for low densities of several species.

We measured gravimetric soil water content as a potential explanatory variable for detrimental warming effects. Therefore, three soil cores were taken from the upper 7 cm (2 cm in diameter) per plot, pooled, and stored in plastic bags. On the same day we determined soil fresh weight of ~5 g soil and dried the soil afterwards (drying oven, at 70°C for 48 h). After reaching constant weight, we measured soil dry weight and calculated the ratio between soil dry weight and fresh weight to get gravimetric soil water content (%).

Statistical analyses. General Linear Models (GLMs) were performed to analyze the effects of Canopy (open and closed), Temperature (ambient, ambient + 1.7°C, and ambient + 3.4°C; linear variable), and the interaction between Canopy × Temperature on (a) earthworm abundance and (b) earthworm biomass in Cloquet and Ely in August 2010, April 2012, June 2012, and October 2012 using the statistical software SAS 9.3 (SAS Institute, Cary, USA). Fitting temperature as categorical variable did rarely yield more significant results than testing as a linear variable (only results of the best models are presented).



In addition to GLM, we used structural equation modeling (SEM) to investigate how canopy and temperature directly and indirectly affect earthworm densities by using gravimetric soil water content as explanatory variable. SEM allows testing direct and indirect relationships between variables in a multivariate approach⁵². Treatment factors (canopy and temperature) were treated as exogenous variables, whereas soil water content and earthworm density were endogenous variables. Due to the complete factorial design we expected exogenous variables to be independent from each other. The adequacy of the model was determined via χ^2 tests, AIC, and RMSEA, but we always kept the full model in order to discuss the strength of all paths in all analyses. Standardized path coefficients are given in Fig. 3. SEM was performed using Amos 21 (Amos Development Corporation, Crawfordville, FL, USA).

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Author contributions

N.E. and P.B.R. conceived the ideas. A.S., K.R. and R.R. ran the field experiment. N.E., A.S., N.F., K.R. and R.R. collected the data. N.E. analyzed the data and wrote the first draft of the manuscript. A.S., N.F., K.R., R.R. and P.B.R. contributed to the manuscript text and reviewed the manuscript.

Additional information

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