

# Effects of soil warming history on the performances of congeneric temperate and boreal herbaceous plant species and their associations with soil biota

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## Abstract

### Aims

Climate warming raises the probability of range expansions of warm-adapted temperate species into areas currently dominated by cold-adapted boreal species. Warming-induced plant range expansions could partly depend on how warming modifies relationships with soil biota that promote plant growth, such as by mineralizing nutrients. Here, we grew two pairs of congeneric herbaceous plants species together in soil with a 5-year warming history (ambient, +1.7°C, +3.4°C) and related their performances to plant-beneficial soil biota.

### Methods

Each plant pair belonged to either the mid-latitude temperate climate or the higher latitude southern boreal climate. Warmed soils were extracted from a chamberless heating experiment at two field sites in the temperate-boreal ecotone of North America. To isolate potential effects of different soil warming histories, air temperature for the greenhouse experiment was identical across soils. We hypothesized that soil with a 5-year warming history in the field would enhance the performance of temperate plant species more than boreal plant species and expected improved plant performances to have positive

associations with plant growth-promoting soil biota (microbial-feeding nematodes and arbuscular mycorrhizal fungi).

### Important Findings

Our main hypothesis was partly confirmed as only one temperate species performed better in soil with warming history than in soil with history of ambient temperature. Further, this effect was restricted to the site with higher soil water content in the growing season of the sampling year (prior to soil collection). One of the boreal species performed consistently worse in previously warmed soil, whereas the other species showed neutral responses to soil warming history. We found a positive correlation between the density of microbial-feeding nematodes and the performance of one of the temperate species in previously wetter soils, but this correlation was negative at the site with previously drier soil. We found no significant correlations between the performance of the other temperate species as well as the two boreal species and any of the studied soil biota. Our results indicate that soil warming can modify the relation between certain plant species and microbial-feeding nematodes in given soil edaphic conditions, which might be important for plant performance in the temperate-boreal ecotone.

**Keywords:** seedling emergence, soil biota, range shift, ecotone, *Aster cordifolius*

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## INTRODUCTION

The geographic ranges of many plant species are changing as a consequence of climate warming (Chen *et al.* 2011; le Roux and McGeoch 2008). For example, temperate plant species appear to be expanding northward into boreal regions (Fisichelli *et al.* 2013a; Leithead *et al.* 2010). Understanding the complex suite of local ecological conditions that underpin successful plant performance is a key challenge in predicting future range shifts (Fisichelli *et al.* 2013b; Sexton *et al.* 2009). Climate change affects the ecological success of a plant species not only by influencing the plant carbon balance (Reich *et al.* 2015), but also by altering the plants' belowground interactions with enemies, such as soil-borne pathogens (van der Putten 2012), and with soil biota that promote plant growth, such as microbial-feeding nematodes (Morriën *et al.* 2012; Thakur *et al.* 2014a) and mycorrhizal fungi (Pringle *et al.* 2009). Although previous studies have confirmed the important roles of plant enemies in soil in determining the warming-induced range expansion of plants (Engelkes *et al.* 2008; Morriën and van der Putten 2013), the relevance of plant growth-promoting soil biota remains largely unexplored in this context (Kivlin *et al.* 2013).

Successful invasion by non-native plant species is often attributed to the 'enemy release hypothesis', whereby aboveground and belowground herbivory or pathogen attacks are reduced at new locations (Keane and Crawley 2002). Reduced enemy pressure is also proposed as an explanation for successful range expansion of plant species at new locations (Engelkes *et al.* 2008). Additionally, recent studies have indicated that the acquisition of novel soil biota that promote plant growth could enhance the success of plant invasion and range expansions (Callaway *et al.* 2011; Sun and He 2010; Traveset and Richardson 2014; van der Putten 2012). Enhanced beneficial plant-soil biota interactions at new locations is proposed as an additional mechanism ('enhanced mutualism hypothesis') to the enemy release hypothesis (Reinhart and Callaway 2006). The key premise of the enhanced mutualism hypothesis is that soil biota beneficial to plants associate with the plant species expanding into the new range and, in turn, enhance their competitive advantage over resident plant species (Kulmatiski and Kardol 2008). Warming might also promote the enhanced mutualism mechanism if warming increases the population of beneficial soil biota in the new range of expanding plant species.

There is a large diversity of belowground soil organisms that promote plant growth (Eisenhauer 2012; Wagg *et al.*

2014; Wall and Moore 1999). For example, microbial-feeding nematodes promote plant growth by contributing to nutrient mineralization (Bonkowski *et al.* 2000). Symbiotic soil microorganisms, such as mycorrhizal fungi, benefit plants by acquiring nutrients in forms that are largely inaccessible to plants (van der Heijden *et al.* 2006). These beneficial interactions between soil biota and plants may change as a result of climate warming (Kiers *et al.* 2010). Climate warming may accelerate feeding rates of microbial-feeding soil biota (Carrera *et al.* 2009) and thus increase nutrient availability (Natali *et al.* 2012), with a possibility of surpassing the pre-existing nutrient demand of the resident plant species. These effects of soil biota can generate relative advantages for range-expanding plants over resident plant species (van Grunsven *et al.* 2010). Surplus nutrients in soil would provide selective advantages for those range-expanding plant species with a greater soil nutrient demand as reported for invasive plant species (Davis *et al.* 2001).

The effect of climate warming on soil biota is partly mediated by the extent to which warming reduces the availability of soil water (Kardol *et al.* 2011; Ruess *et al.* 1999). Soil drying induced by warming could diminish microbial growth and biomass (Liu *et al.* 2009), which may have detrimental effects on higher trophic groups of soil biota, such as microbial-feeding nematodes (Bakonyi and Nagy 2000). Similarly, the effect of warming on microbe-mediated nutrient mineralization in soil may be weakened as water in the soil becomes scarce (Suseela *et al.* 2012). In contrast, warming-induced increases in nutrient mineralization are commonly observed in soils with high water content (Davidson and Janssens 2006; Rustad *et al.* 2001). Warming was also shown to enhance mycorrhizal colonization of roots as warming boosted nutrient turnover in soil with sufficient moisture (Rillig *et al.* 2002). Taken together, these observations suggest that the influence of climate warming on the relationship between plants and soil biota is likely to be mediated by the water content of the soil (Compant *et al.* 2010).

Here, we report the effects of soil warming history, defined by soil preconditioned with warming for several years (see Meisner *et al.* 2013; Thakur *et al.* 2014b) on the performance of four herbaceous plant species representing an ecotonal climatic zone (northern temperate and southern boreal). We relate plant performances in soils with different warming histories to two important groups of soil biota that promote plant growth: free-living microbial-feeding nematodes and arbuscular mycorrhizal fungi (AMF) to elucidate the potential role of the enhanced mutualism hypothesis. We hypothesized that soils with a history of experimentally

increased temperature should favor plant species that occur in temperate regions (modestly warm-adapted) over plant species of boreal regions (modestly cold-adapted) because warm-adapted plant species might be superior at acquiring greater amounts of nutrients in warmed soils (Fisichelli et al. 2015). Secondly, we hypothesized that warmed soils support higher densities of microbial-feeding nematodes and promote AMF colonization (as long as soils maintain sufficiently high water content) due to increased nutrient turnover rates caused by warming. Finally, we hypothesized that such changes in soil biota (density of microbial-feeding nematodes and AMF colonization) would be associated with plant responses to soil warming. We expected that the above-hypothesized relations would partially depend on soil water content.

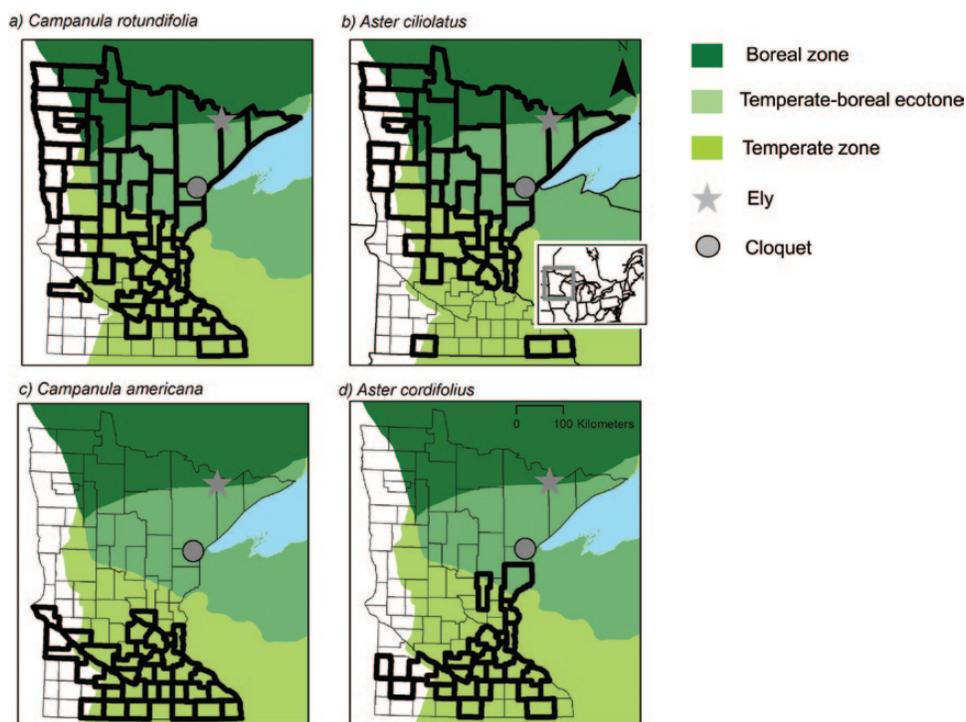
## METHODS

### Field experiment

The soil used in the experiment came from the open-heating/chamberless experiment called ‘Boreal Forest Warming at an Ecotone in Danger’ (B4WarmED; Rich et al. 2015). The B4WarmED experiment started in 2008 with a simultaneous heating above (infrared canopy heating) and below the ground (resistance heating cables) in the temperate-boreal forest ecotone, i.e. a boundary region between temperate and boreal forests in Northern Minnesota, USA. This

experiment consists of three warming treatments (ambient, ambient +1.7°C and ambient +3.4°C) replicated six times and grouped into three blocks in open (clear-cut) and forested habitats at two sites, Cloquet and Ely in Northern Minnesota, USA (Fig. 1). Technical details of the B4WarmED experiment can be found in Rich et al. (2015). Briefly, temperatures were manipulated aboveground and belowground using 6–8 ceramic heating elements (Mor-Electric, model FTE-1000, MI, USA) aboveground (Kimball 2005) and heating cables in soil (Danfoss GX, Devi A/B, Denmark) for belowground warming (Bergh and Linder 1999). All plots were planted with 11 different tree species to establish identical tree communities across plots (see online supplementary Table S1 for tree species list), whereas naturally occurring understory plant communities varied and were not controlled. The soils in this study were taken only from the closed forest canopy treatments in 2013, i.e. 5 years after the start of the field experiments (details below in Greenhouse experiment). Two of the species used in the greenhouse experiment (*Campanula rotundifolia* and *Aster ciliolatus*) also occur at the field sites (Fig. 1)

The Cloquet study site is situated at the Cloquet Forestry Center (46°40′45″N, 92°31′12″W). The Ely study site is situated at Hubachek Wilderness Research Center (47°56′46″N, 91°45′29″W). Although Cloquet and Ely are >150 km apart (Fig. 1), both sites are located in the temperate-boreal forest ecotone. Cloquet has a historically higher mean annual



**Figure 1:** geographic distribution of four plant species in Minnesota (USA) used in this study. The thick black lines indicate the presence of the species in the particular region (county) of Minnesota. B4WarmED study sites are located in the temperate-boreal transition zone. Information on plant species distributions can be found online at [http://www.dnr.state.mn.us/eco/mcbs/plant\\_lists.html](http://www.dnr.state.mn.us/eco/mcbs/plant_lists.html) (29 March 2015, date last accessed).

temperature (4.5°C) than Ely (3.0°C), but during the B4WarmED experiment, growing season temperatures at the two sites have been very similar (Rich *et al.* 2015). The growing season mean air temperature of Cloquet in 2013 was 12°C ( $\pm 0.22$ ), whereas that of Ely was 13°C ( $\pm 0.20$ ) for the closed canopy ambient warming treatments (see Rich *et al.* 2015 for details about temperature measurement). The soil at Cloquet is a Typic Dystrudept composed of ~61% sand, ~9% clay and ~30% silt, whereas the soil at Ely is a Lithic Udorthent composed of ~60% sand, ~12% clay and ~28% silt in the surface soils (upper 5 cm).

### Greenhouse experiment

Four herbaceous plant species were selected based on their occurrence in temperate and boreal forests in North America. The two species pairs belonged to two genera: *Aster* and *Campanula*. *Aster cordifolius* (common name: Heart-leaved aster) and *Campanula americana* (common name: American bellflower) are both temperate plant species with a mid-temperate and north temperate range (Fig. 1). *Aster ciliolatus* (common name: Lindley's aster) and *Campanula rotundifolia* (common name: Harebell) are boreal species, which occur predominantly in southern boreal climate in central North America, extending slightly into the north temperate climate zone (Fig. 1; see online supplementary Fig. S1). All seeds were purchased from Prairie Moon Nursery (Winona, MN, USA). Based on their geographic ranges, we assumed that the two selected northern temperate species have adapted to a warmer climate compared to the southern boreal species.

In total, we set up 36 microcosms in the greenhouse: two sites  $\times$  three soil warming history  $\times$  six replicates. We took 150 g of soils from 36 B4WarmED field plots (18 plots from each of the two sites) using a 10-cm-deep soil core with a diameter of 5 cm. These soils were sampled in mid-November of 2013 and cooled at 4°C until used for germinating plants. Large stones and roots were gently removed before adding the soil to microcosms (diameter = 11 cm, height = 9.4 cm). Each microcosm received 150 g of sand on top of which B4WarmED soils were added. For each of the four plant species, 10 seeds were randomly added on the soil subsurface (2–3 mm deep) of each microcosm (40 seeds per microcosm) for the 36 B4WarmED plots. Each microcosms received 60 ml of sterilized water every second day. Water was carefully added to prevent flooding in the microcosm but in sufficient amounts to avoid water limitation. Plants were grown at 20°C for 16 h in daylight and at 16°C for 8 h in the dark.

### Plant and soil biota measurements

We recorded seedling emergence weekly for 14 weeks in total. Seedling emergence is the epicotyl breaking the soil surface from seeds beneath the soil. Plants were harvested in the 14th week after the start of the experiment because no new plants emerged after week 10 (see online supplementary Fig. S2; Eisenhauer *et al.* 2012). Plant species-specific shoot biomass was measured after drying at 70°C for 72 h. Roots

were gently and thoroughly cleaned with tap water before drying. A part of the fresh root material was separated during the harvest to determine AMF colonization and kept in 50% ethanol until further processing (details below). A soil core of 2 cm diameter and 5 cm depth was taken for nematode extraction (20 g of fresh soil) using a modified Baermann technique (Ruess 1995).

The roots for the determination of AMF colonization were cleared in 10% KOH solution at 70°C for ~40 min, followed by staining in a 5% pen ink–vinegar solution for 5 min at 70°C (Vierheilig *et al.* 1998). Roots were subsequently mounted on glass slides with 50% glycerine and scored for the presence of AMF structures (intraradical hyphae, arbuscules and vesicles/intraradical spores) following the transect-intersect method as described in McGonigle *et al.* (1990). Nematodes were counted and identified to genus levels and then grouped into trophic groups following Yeates *et al.* (1993) as bacterial-feeding, fungal-feeding and plant root-feeding. Total and trophic group densities of nematodes are expressed as individuals 20 g<sup>-1</sup> of fresh soil.

Volumetric soil water content was measured at the experimental field sites throughout the year before the start of the emergence experiment using a Campbell Scientific method for user-specific calibration of water reflectometers (Rich *et al.* 2015). In total, we report soil water content based on the measurement for 212 days at Cloquet and 198 days at Ely in 2013. The soils that were used for germinating plants were also used to prepare extracts for ammonium and nitrate measurements immediately after the field sampling. Ten grams of sieved soils (2 mm) were added to 50 ml 2 M potassium chloride solution to prepare extracts, which were used to estimate ammonium and nitrate concentrations using microplate colorimetric methods (Hood-Nowotny *et al.* 2010).

### Statistical analyses

First, prior to the greenhouse experiment, the effect of soil warming treatments on soil water content and nutrient availability at each experimental site was investigated. Soil water content was analyzed for the two sites using mixed-effects repeated measures analysis of variance (ANOVA) (Gaussian error) due to repeated measurements of soil water content throughout the year. Experimental blocks were used as a random effect term in all the mixed-effects models. Post hoc Tukey's honestly significant difference (HSD) tests were performed on the models with significant warming effects to compare the means of the three warming treatments. Post hoc Tukey tests were only performed when warming treatments were used as a categorical variable.

Second, after the greenhouse experiment, seedling emergence, plant shoot biomass and plant shoot biomass per individual (total shoot biomass/number of emerged seedlings) in response to soil warming history were analyzed for each species using linear mixed-effects models (with Gaussian error, experiment blocks as a random effect) by pooling data from the two sites as they have the same design.

Linear model assumptions of the residual normality were assessed using the Shapiro–Wilk normality test, while homogeneity of variance was visually inspected using fitted versus residuals graphs. We used permutation tests to calculate *P*-values when models failed to meet linear model assumptions, even after data transformation. Permutation test generates *P*-values by randomly generating reference distributions from the original data and are suitable for lower sample sizes and data with non-normal distribution (Legendre and Legendre 2012).

Third, warming history effects on nematode densities were assessed using generalized linear mixed-effects model ANOVA with negative binomial errors due to overdispersion in the count data, i.e. residual deviance  $\gg$  degrees of freedom (Zuur et al. 2009). We analyzed the density of bacterial-feeding nematodes, fungal-feeding nematodes and the sum of both (i.e. microbial-feeding nematodes) as affected by warming history. For warming history effects on AMF colonization (expressed as a fraction) and AM arbuscules (the functional part of the nutrient exchange site in roots), we again used linear mixed-effects models with Gaussian errors. Linear model assumptions of variance homogeneity and normality of residual errors were met.

Fourth, plant species-specific responses (plant shoot biomass per individual) were associated with soil biota responses using Spearman rank correlation tests. These correlation tests were informed by our full models for plant performance and soil biota responses. Based on those results (e.g. warming and site interaction effects), we ran site-specific correlation analyses to associate plant performance with soil biota responses in soil with warming history. Plant shoot biomass per individual was used as a proxy for plant performance. We used Spearman rank correlation tests because most of the response variables were not normally distributed. The correlation tests were run between the response of each plant species and soil biota responses. We also used partial Spearman rank correlation analysis between each plant species and soil biota responses by controlling the responses of the other three plant species. Partial correlation test is applicable to situations, where the relationship between two variables is influenced by their relationship with other variables (details in Legendre and Legendre 2012). We used non-parametric partial correlations to test whether the association between the performance of each plant species and soil biota was influenced by the performance of the other three neighboring plant species.

All statistical analyses were carried out in R statistical software version 3.1.0 (R Development Core Team 2014). Mixed-effects models were run with the ‘lme4’ package (Bates et al. 2015). Using the ‘car’ package, we obtained chi-square values for mixed-effects model objects using Type II Wald chi-square tests (Fox and Weisberg, 2011). Tukey HSD tests were performed using the ‘multcomp’ package (Hothorn et al. 2008). Permutation tests were carried out using the ‘minque’ package (Wu 2014). Spearman rank correlation tests were performed using the ‘Hmisc’ package (Harrell 2016). Partial Spearman

rank correlation tests were carried out in the ‘ppcor’ package (Seongho 2012).

## RESULTS

### Warming effects on soil edaphic conditions

Volumetric soil water content during the 2013 growing season was significantly lower in warmed plots at both sites as compared to soil from ambient plots (Cloquet:  $\chi^2 = 18.67$ ,  $P < 0.001$  and Ely:  $\chi^2 = 32.02$ ,  $P < 0.001$ ), with a ~14% decline in soil water in the +3.4°C treatment (see online supplementary Fig. S3). Ely had higher mean soil water content compared to Cloquet at ambient temperature in 2013 (27% at Ely versus 21% at Cloquet). Soil ammonium concentrations did not change with warming at either of the sites (Cloquet:  $\chi^2 = 3.76$ ,  $P = 0.15$  and Ely:  $\chi^2 = 0.78$ ,  $P = 0.67$ ). The same was also true for the soil nitrate concentrations (Cloquet:  $\chi^2 = 1.93$ ,  $P = 0.38$  and Ely:  $\chi^2 = 1.18$ ,  $P = 0.55$ ).

### Warming history effects on plant performance

Warming history effects on seedling emergence and shoot biomass in the greenhouse experiment varied among plant species and between the two sites (Table 1; all means and respective standard errors are provided in online supplementary Table S2). We found a significant interaction effect of warming history and site on the performance of *A. cordifolius*—a temperate species. This was true for seedling emergence, total shoot biomass and shoot biomass per individual (Table 1). The significant interaction between warming history and site was due to opposing warming effects on the performance of *A. cordifolius* between the two sites (Fig. 2). At Cloquet, the performance of *A. cordifolius* decreased with warming (Fig. 2a and c), whereas warming enhanced its performance at Ely (Fig. 2b and d). We did not find significant effects of warming, site and as well as the interaction of warming and site effects on the performance of the other temperate species (*C. americana*) (Table 1; Fig. 2). The seedling emergence of *A. ciliolatus*—one of the two boreal plant species—decreased with soil warming history at both sites (Table 1; Fig. 2a and b). However, the shoot biomass and shoot biomass per individual of this plant species were not significantly affected by warming history (Fig. 2c and d). We also found no significant effect of warming history on the other boreal plant species (*C. rotundifolia*) (Table 1; Fig. 2).

### Warming history effects on soil biota

Among the tested soil biota, we only found a significant increase in the density of fungal-feeding nematodes with warming history (Table 2; Fig. 3c and d; all means and respective standard errors are provided in online supplementary Table S3). The density of fungal-feeding nematodes was also significantly higher in Ely soils than Cloquet soils (Fig. 3c and d; note different scales on y-axes). The density of bacterial-feeding nematode was not significantly affected by soil warming history (Table 2). Further, we found a marginally significant increase ( $P = 0.05$ ) in the density of microbial-feeding

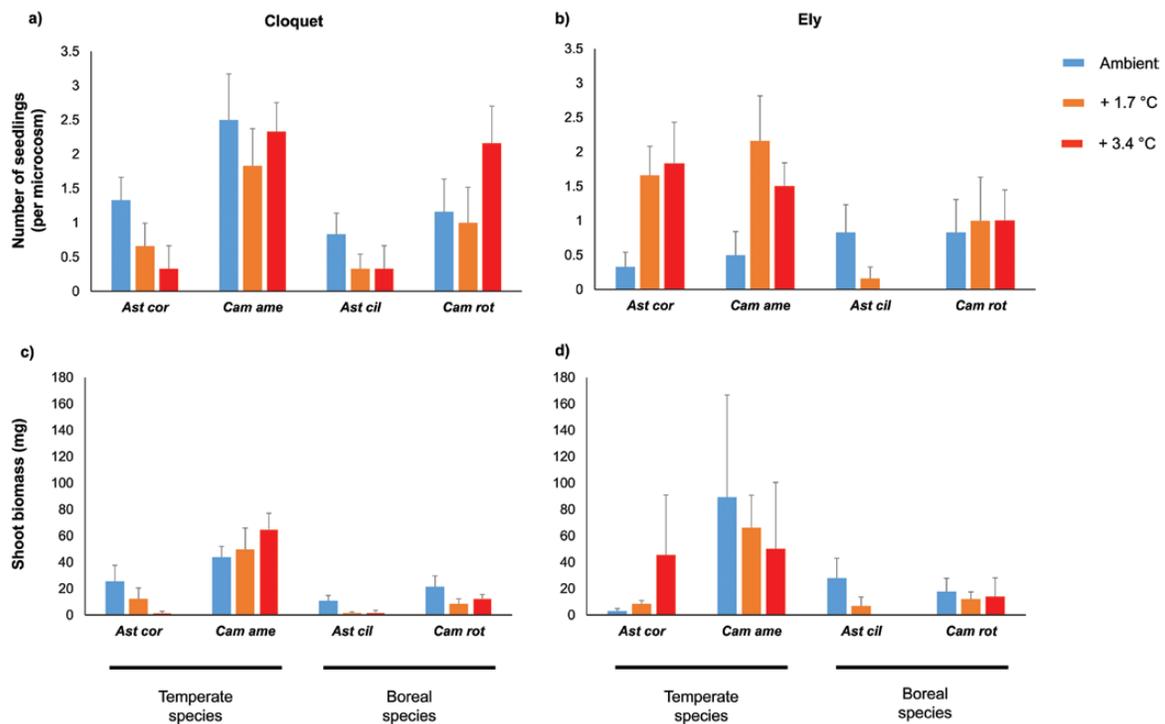
**Table 1:** effects of soil warming history (ambient, ambient +1.7°C, and ambient +3.4°C) on plant species-specific performances (seedling emergence, total shoot biomass and shoot biomass per individuals) at two different sites (Cloquet and Ely; both in MN, USA)

Plant performance	Warming			Site			Warming × Site		
	Slope estimates	$\chi^2$ -value	<i>P</i> -value	Slope estimates	$\chi^2$ -value	<i>P</i> -value	Slope estimates	$\chi^2$ -value	<i>P</i> -value
Emergence <sup>a</sup>									
<i>Aster cordifolius</i> (T)	-0.50	0.46	0.49	-0.75	1.37	0.24	<b>1.25</b>	<b>11.71</b>	<b>&lt;0.001</b>
<i>Campanula americana</i> (T) (log-transformed)	0.02	2.38	0.12	-0.58	1.84	0.17	0.24	1.65	0.19
<i>Aster ciliolatus</i> (B) (log-transformed)	<b>-0.17</b>	<b>7.99</b>	<b>&lt;0.01</b>	-0.04	0.46	0.49	-0.06	0.21	0.64
<i>Campanula rotundifolia</i> (B)	0.5	1.55	0.21	-0.08	0.66	0.41	-0.41	0.79	0.37
Total shoot biomass (mg)									
<i>Aster cordifolius</i> (T)	<b>-0.06</b>	—	<b>0.02</b>	-0.03	—	0.12	<b>0.05</b>	—	<b>&lt;0.001</b>
<i>Campanula americana</i> (T)	0.02	—	0.30	<0.01	—	0.47	-0.01	—	0.35
<i>Aster ciliolatus</i> (B)	0.01	—	0.2	<b>0.02</b>	—	<b>&lt;0.01</b>	<b>-0.01</b>	—	<b>0.03</b>
<i>Campanula rotundifolia</i> (B)	-0.01	—	0.43	-0.01	—	0.42	<0.01	—	0.42
Shoot biomass per individual (mg)									
<i>Aster cordifolius</i> (T)	<b>-0.04</b>	—	<b>&lt;0.01</b>	<b>-0.02</b>	—	<b>0.01</b>	<b>0.03</b>	—	<b>&lt;0.01</b>
<i>Campanula americana</i> (T)	0.04	—	0.30	0.04	—	0.11	-0.03	—	0.24
<i>Aster ciliolatus</i> (B)	<0.01	—	0.41	0.01	—	0.07	<-0.01	—	0.11
<i>Campanula rotundifolia</i> (B)	<-0.01	—	0.24	<-0.01	—	0.39	<-0.01	—	0.31

Statistical significances are based on Wald and permutation (when  $\chi^2$  values are missing) tests in mixed-effects models. Bold slope coefficients,  $\chi^2$  and *P*-values are statistically significant (*P* < 0.05). Shoot biomass refers to dry weight (see Methods).

<sup>a</sup>Number of seedlings.

Abbreviations: B = boreal species, T = temperate species.



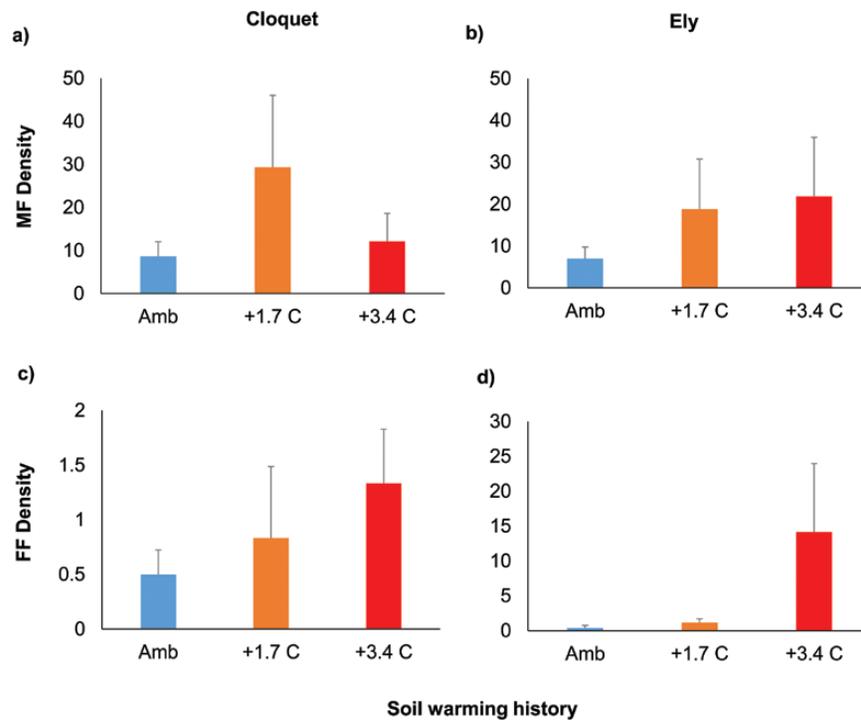
**Figure 2:** species-specific means (+standard error) of seedling emergence (a, b), and shoot biomass per individual per microcosm (c, d) of four plant species in three soil warming history treatments of Cloquet (a, c) and Ely (b, d). Abbreviations: *Ast cil* = *Aster ciliolatus* (boreal), *Ast cor* = *Aster cordifolius* (temperate), *Cam ame* = *Campanula americana* (temperate), *Cam rot* = *Campanula rotundifolia* (boreal).

**Table 2:** soil warming history (ambient, ambient +1.7°C and ambient +3.4°C) effects on the plant-beneficial soil biota at two different sites (Cloquet and Ely; both in MN, USA)

	Warming			Site			Warming × Site		
	Slope estimates	$\chi^2$ -value	<i>P</i> -value	Slope estimates	$\chi^2$ -value	<i>P</i> -value	Slope estimates	$\chi^2$ -value	<i>P</i> -value
<b>Nematodes</b>									
MF density	<i>0.15</i>	3.74	0.05	-0.66	<0.01	0.98	0.65	1.43	0.23
BF density	0.08	0.88	0.34	-0.64	0.11	0.73	0.35	0.36	0.54
FF density	<b>0.48</b>	<b>10.45</b>	<b>&lt;0.01</b>	<b>-0.63</b>	<b>4.07</b>	<b>0.04</b>	<i>1.43</i>	3.79	0.05
<b>AMF</b>									
Colonization	-0.06	0.09	0.75	-0.22	2.13	0.14	0.10	1.26	0.26
AM arbuscules	-0.03	0.11	0.73	-0.04	<0.01	0.99	0.04	1.24	0.26

Bold slope coefficients,  $\chi^2$  and *P*-values are statistically significant ( $P < 0.05$ ), and values in italics are marginally significant ( $P < 0.1$ ). Nematode density is expressed as per 20 g fresh soil.

Abbreviations: BF= bacterial-feeding nematodes, FF= fungal-feeding nematodes, MF= microbial-feeding nematodes.



**Figure 3:** means (+standard error) of nematode density (# 20g<sup>-1</sup> fresh soil) for microbial-feeding (a, b) and fungal-feeding nematodes (feeding guild within the microbial-feeding nematodes) (c, d) in three warming history treatments of Cloquet (a, c) and Ely (b, d). Abbreviations: FF = fungal-feeding nematodes, MF = microbial-feeding nematodes.

nematodes (sum of fungal- and bacterial-feeding nematodes) with soil warming history (Table 2; Fig. 3a and b). Warming history had no significant effect on plant-feeding nematodes ( $P > 0.05$ ). We also found no significant effect of soil warming history on AMF colonization and AM arbuscules (Table 2).

### Relationships between plant performance and soil biota

In Ely soils, the greater growth of individual of *A. cordifolius* in the warmed than ambient treatment soils was

associated with higher densities of microbial-feeding nematodes ( $\rho = 0.49$ ,  $P = 0.04$ ) and fungal-feeding nematodes (marginally significant;  $\rho = 0.48$ ,  $P = 0.05$ ; see online supplementary Table S4). Partial correlation tests revealed that the significant positive association between the density of microbial-feeding nematodes and *A. cordifolius* became insignificant in Ely soils ( $\rho = 0.40$ ,  $P = 0.12$ ) when we statistically controlled for the performance of the other plants in the microcosms. In Cloquet soils, the performance of *A. cordifolius* was negatively correlated with the density of

microbial-feeding nematodes, but only so in the partial correlation tests ( $\rho = -0.49$ ,  $P = 0.03$ ). We did not find any significant correlation between the performance of the other three plant species and nematode densities in either Cloquet or Ely (see online [supplementary Table S4](#)) or between plant performance and AMF colonization (see online [supplementary Table S4](#)).

## DISCUSSION

In this study, we found some evidence in support of our overarching hypothesis that warming enhances the performance of temperate herbaceous species relative to boreal species and that this could be associated with warming-induced changes in soil biota. However, there were also some results inconsistent with this hypothesis and many neutral responses. Support for the overarching hypothesis was restricted to one of the two boreal species (one responded negatively to warming, the other neutrally) and for one of the temperate species (one responded positively to warming, the other neutrally). Further, the positive response of one of the temperate species was also restricted to the site with higher soil water content in the growing season just prior to soil collection (Ely), whereas a decrease in performance in response to warming history was observed at the site with lower soil water content (Cloquet). The density of total microbial-feeding nematodes and that of fungal-feeding nematodes in particular increased in the warmed soils. In support of our hypothesis, we found that the enhanced performance of *A. cordifolius* and the density of microbial-feeding nematodes in soils with warming history were positively correlated at Ely, but we found no such associations between plant performances and AMF colonization of roots. Recent studies have proposed that, in order to successfully expand their geographical range, plant species should experience a reduction in enemy pressure and/or a greater association with beneficial soil biota in the new range (Callaway *et al.* 2011; van der Putten 2012). We only found partial support for the enhanced mutualism hypothesis in one of the two temperate plant species and also only at one of the sites.

The improved performance of *A. cordifolius* (both in terms of seedling emergence and shoot biomass) in warmed soils of Ely is in line with previous studies reporting higher plant production in warmer temperatures (Baldwin *et al.* 2014; Lin *et al.* 2010; Natali *et al.* 2012; Thakur *et al.* 2014b). Improved plant performance could partly be related to a plant's ability to exploit soil nutrients made available by warming-induced increases in nutrient mineralization in the soil (de Frenne *et al.* 2014; Natali *et al.* 2012). In addition, the physiology of these plants may also be sufficiently adjustable to take advantage of the warmed soil environment, which could be related to the geographic range of the species (Lin *et al.* 2010; Reich *et al.* 2015; Walker *et al.* 2006). For instance, species performing better in the warmed soil can increase their root length

and diameter to acquire greater amounts of resources (Kaspar and Bland 1992).

Differences in site-level soil water content during the growing season of the sampling year provide a potential explanation why the same temperate plant species responded inconsistently to warmed soil. Warming generally increases the activity of soil organisms and the nutrient turnover in soil at sufficiently high water content (Allison and Treseder 2008; Carrera *et al.* 2009). Our results suggest that a greater adaptability to warmer conditions related to geographic ranges alone does not guarantee the success of plant species in the warmed soil of the new locations, but also other soil edaphic conditions such as water content could play a crucial role. However, further direct experimental manipulations of soil water content together with soil warming are required to test the generality of such assumptions. The decreased performance of *A. ciliolatus* (boreal species) in warmed soil of both sites indicates that warming-induced alterations in soil biotic and abiotic conditions can detrimentally affect the performance of cold-adapted plant species. Further, the poor performance of *A. ciliolatus* could also be due to negative plant–soil feedback effects (such as via soil pathogens) as this species occurs at both field sites.

Our results also revealed that the association between the density of plant-beneficial nematodes (i.e. microbial-feeding) and *A. cordifolius* performance depended on the soil water content: a positive relationship occurred at Ely, whereas a negative trend was observed in Cloquet soils. In particular, higher density of fungal-feeding nematodes in the warmed soil (Fig. 3) may have increased nutrient availability by enhancing turnover rates of soil fungi (Chen and Ferris 1999). Surprisingly, soils warmed prior to the greenhouse experiment did not show significantly higher nutrient concentrations (ammonium and nitrate); however, this may have changed as the experiment progressed. Although the correlation between *A. cordifolius* performance and the density of fungal-feeding nematodes was only marginally significant in warmed Ely soils (see online [supplementary Table S4](#)), these results indicate that warming may reinforce plant–soil biota relations by potentially benefitting nutrient-demanding plants (de Deyn *et al.* 2004; Morriën *et al.* 2012). In addition to nutrient effects, fungal-feeding soil fauna could also indirectly support seedling emergence by reducing fungi that may decay seeds and decrease the persistence of seeds in the soil (Mitschunas *et al.* 2006). Why such an advantage was true only for one of the two temperate species at one of the sites merits further exploration.

Some of the observed associations between the nematode density and plant performance, such as the negative correlation between *A. cordifolius* performance and the density of microbial-feeding nematodes in Cloquet soils, were also influenced by the performance of other plant species in warmed soils as shown by the partial correlation tests (see online [supplementary Table S4](#)). This indicates that a given plant–soil

biota relation may also depend on the competitive interactions among the co-occurring plant species. Such plant–plant interactions and plant–soil biota relations could play a crucial role in determining the success of range-expanding plants (de Frenne et al. 2014). Moreover, the finding of both positive and negative correlations between microbial-feeding nematodes and *A. cordifolius* performance depending on soil water conditions calls for experiments to directly test the causal effects of nematodes on the performance of range-expanding plant species in different soil water availability treatments, such as by manipulating densities of nematodes with pesticides (Eisenhauer et al. 2010).

In summary, our study provides some evidence that soil warming history can alter the performance of plant species in the temperate-boreal ecotone. Soil warming history resulted in increased performance of one of the two temperate herbaceous plant species (*A. cordifolius*) in the soil of one of the two study sites (Ely), whereas the opposite pattern was true at the other site (Cloquet). Soil water content in the sampling year was higher in Ely than Cloquet, which may have caused inconsistent performance of the temperate species *A. cordifolius* in the warmed soil of the two sites. Notably, the increased performance of *A. cordifolius* at the wetter site was associated to elevated densities of microbial-feeding nematodes. We also observed that the same plant species in the drier site of the sampling year negatively associated with the density of microbial-feeding nematodes. We caution, however, that differences in soil water content are not consistently different in these two sites during all years of warming (Rich et al. 2015) and thus legacy effects related with variations in soil water content together with the legacy effects of soil warming require further assessment. Our conclusions are based on only two congeneric plant species pairs, and more experiments are needed to test the generality of our results. Nevertheless, our study highlights that warming can modify the relation between certain plant species and soil biota, such as microbial-feeding nematodes, which could play an important role for the successful establishment of plants beyond their present range limits.

## SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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## REFERENCES

- Allison SD, Treseder KK (2008) Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Glob Chang Biol* **14**:2898–909.
- Bakonyi G, Nagy P (2000) Temperature- and moisture-induced changes in the structure of the nematode fauna of a semiarid grassland-patterns and mechanisms. *Glob Chang Biol* **6**:697–707.
- Baldwin AH, Jensen K, Schönfeldt M (2014) Warming increases plant biomass and reduces diversity across continents, latitudes, and species migration scenarios in experimental wetland communities. *Glob Chang Biol* **20**:835–50.
- Bates D, Maechler M, Bolker B, et al. (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* **67**:1–48.
- Bergh J, Linder S (1999) Effects of soil warming during spring on photosynthetic recovery in boreal Norway spruce stands. *Glob Chang Biol* **5**:245–53.
- Bonkowski M, Cheng W, Griffiths BS, et al. (2000) Microbial-faunal interactions in the rhizosphere and effects on plant growth. *Eur J Soil Biol* **36**:135–47.
- Callaway RM, Bedmar EJ, Reinhart KO, et al. (2011) Effects of soil biota from different ranges on Robinia invasion: acquiring mutualists and escaping pathogens. *Ecology* **92**:1027–35.
- Carrera N, Barreal ME, Gallego PP, et al. (2009) Soil invertebrates control peatland C fluxes in response to warming. *Funct Ecol* **23**:637–48.
- Chen J, Ferris H (1999) The effects of nematode grazing on nitrogen mineralization during fungal decomposition of organic matter. *Soil Biol Biochem* **31**:1265–79.
- Chen I-C, Hill JK, Ohlemüller R, et al. (2011) Rapid range shifts of species associated with high levels of climate warming. *Science* **333**:1024–6.
- Compant S, van der Heijden MG, Sessitsch A (2010) Climate change effects on beneficial plant-microorganism interactions. *FEMS Microbiol Ecol* **73**:197–214.
- Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**:165–73.
- Davis M, Grime J, Thompson K (2001) Fluctuating resources in plant communities: a general theory of invasibility. *J Ecol* **88**:528–34.
- de Deyn GB, Raaijmakers CE, van Der Putten WH (2004) Plant community development is affected by nutrients and soil biota. *J Ecol* **92**:824–34.

- de Frenne P, Coomes DA, de Schrijver A, *et al.* (2014) Plant movements and climate warming: intraspecific variation in growth responses to nonlocal soils. *New Phytol* **202**:431–441.
- Eisenhauer N (2012) Aboveground–belowground interactions as a source of complementarity effects in biodiversity experiments. *Plant Soil* **351**:1–22.
- Eisenhauer N, Ackermann M, Gass S, *et al.* (2010) Nematicide impacts on nematodes and feedbacks on plant productivity in a plant diversity gradient. *Acta Oecologica* **36**:477–83.
- Eisenhauer N, Fisichelli NA, Frelich LE, *et al.* (2012) Interactive effects of global warming and “global worming” on the initial establishment of native and exotic herbaceous plant species. *Oikos* **121**:1121–33.
- Engelkes T, Morriën E, Verhoeven KJ, *et al.* (2008) Successful range-expanding plants experience less above-ground and below-ground enemy impact. *Nature* **456**:946–8.
- Fisichelli NA, Frelich LE, Reich PB (2013a) Temperate tree expansion into adjacent boreal forest patches facilitated by warmer temperatures. *Ecography* **37**:152–61.
- Fisichelli NA, Frelich LE, Reich PB (2013b) Climate and interrelated tree regeneration drivers in mixed temperate–boreal forests. *Landscape Ecol* **28**:149–59.
- Fisichelli NA, Stefanski A, Frelich LE, *et al.* (2015) Temperature and leaf nitrogen affect performance of plant species at range overlap. *Ecosphere* **6**:1–8.
- Fox J, Weisberg S (2011) *An R Companion to Applied Regression*, 2nd edn. Thousand Oaks, CA: Sage.
- Harrell F Jr (2015) *hmisc: Harrell Miscellaneous*. R package version 3.17-3. <https://CRAN.R-project.org/package=Hmisc> (20 May 2016, date last accessed).
- Hood-Nowotny R, Umana NH-N, Inselbacher E, *et al.* (2010) Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. *Soil Sci Soc Am J* **74**:1018.
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* **50**:346–63.
- Kardol P, Reynolds WN, Norby RJ, *et al.* (2011) Climate change effects on soil microarthropod abundance and community structure. *Appl Soil Ecol* **47**:37–44.
- Kaspar TC, Bland WL (1992) Soil temperature and root growth. *Soil Sci* **154**:290–9.
- Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. *Trends Ecol Evol* **17**:164–70.
- Kiers TE, Palmer TM, Ives AR, *et al.* (2010) Mutualisms in a changing world: an evolutionary perspective. *Ecol Lett* **13**:1459–74.
- Kimball BA (2005) Theory and performance of an infrared heater for ecosystem. *Glob Chang Biol* **11**:2041–56.
- Kivlin SN, Emery SM, Rudgers JA (2013) Fungal symbionts alter plant responses to global change. *Am J Bot* **100**:1445–57.
- Kulmatiski A, Kardol P (2008) Getting plant–soil feedbacks out of the greenhouse: experimental and conceptual approaches. *Prog Bot* **69**:449–72.
- le Roux PC, McGeoch MA (2008) Rapid range expansion and community reorganization in response to warming. *Glob Chang Biol* **14**:2950–62.
- Legendre P, Legendre L (2012) *Numerical Ecology*, 3rd edn. Amsterdam, Netherlands: Elsevier.
- Leithead MD, Anand M, Silva LC (2010) Northward migrating trees establish in treefall gaps at the northern limit of the temperate-boreal ecotone, Ontario, Canada. *Oecologia* **164**:1095–106.
- Lin D, Xia J, Wan S (2010) Climate warming and biomass accumulation of terrestrial plants: a meta-analysis. *New Phytol* **188**:187–98.
- Liu W, Zhang Z, Wan S (2009) Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. *Glob Chang Biol* **15**:184–95.
- McGonigle TP, Miller MH, Evans DG, *et al.* (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol* **115**:495–501.
- Meisner A, de Deyn GB, de Boer W, *et al.* (2013) Soil biotic legacy effects of extreme weather events influence plant invasiveness. *Proc Natl Acad Sci USA* **110**:4–7.
- Mitschunas N, Wagner M, Filser J (2006) Evidence for a positive influence of fungivorous soil invertebrates on the seed bank persistence of grassland species. *J Ecol* **94**:791–800.
- Morriën E, Duyts H, van der Putten WH (2012) Effects of native and exotic range-expanding plant species on taxonomic and functional composition of nematodes in the soil food web. *Oikos* **121**:181–90.
- Morriën E, van der Putten WH (2013) Soil microbial community structure of range-expanding plant species differs from co-occurring natives. *J Ecol* **101**:1093–102.
- Natali SM, Schuur EAG, Rubin RL (2012) Increased plant productivity in Alaskan tundra as a result of experimental warming of soil and permafrost. *J Ecol* **100**:488–98.
- Pringle A, Bever JD, Gardes M, *et al.* (2009) Mycorrhizal symbioses and plant invasions. *Annu Rev Ecol Evol Syst* **40**:699–715.
- R Development Core Team (2014) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Reich PB, Sendall KM, Rice K, *et al.* (2015) Geographic range predicts photosynthetic and growth response to warming in co-occurring tree species. *Nat Clim Change* **5**:148–52.
- Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. *New Phytol* **170**:445–57.
- Rich RL, Stefanski A, Montgomery RA, *et al.* (2015) Design and performance of combined infrared canopy and belowground warming in the B4WarmED (Boreal Forest Warming at an Ecotone in Danger) experiment. *Glob Chang Biol* **21**:2334–48.
- Rillig MC, Wright SE, Shaw MR, *et al.* (2002) Artificial climate warming positively affects arbuscular mycorrhizae but decreases soil aggregate water stability in an annual grassland. *Oikos* **97**:52–8.
- Ruess L (1995) Studies on the nematode fauna of an acid forest soil: spatial distribution and extraction. *Nematologica* **41**:229–39.
- Ruess L, Michelsen A, Schmidt I, *et al.* (1999) Simulated climate change affecting microorganisms, nematode density and biodiversity in subarctic soils. *Plant Soil* **212**:63–73.
- Rustad LE, Campbell JL, Marion GM, *et al.* (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **126**:543–62.
- Seongho K (2012) *ppcor: Partial and Semi-Partial (Part) Correlation*. R package version 1.1. <https://CRAN.R-project.org/package=ppcor> (20 May 2016, date last accessed).

- Sexton JP, McIntyre PJ, Angert AL, et al. (2009) Evolution and ecology of species range limits. *Annu Rev Ecol Evol Syst* **40**:415–36.
- Sun ZK, He WM (2010) Evidence for enhanced mutualism hypothesis: *Solidago canadensis* plants from regular soils perform better. *PLoS One* **5**:e15418.
- Suseela V, Conant RT, Wallenstein MD, et al. (2012) Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Glob Chang Biol* **18**:336–48.
- Thakur M, Reich P, Fisichelli N, et al. (2014a) Nematode community shifts in response to experimental warming and canopy conditions are associated with plant community changes in the temperate-boreal forest. *Oecologia* **175**:713–23.
- Thakur MP, Reich PB, Eddy WC, et al. (2014b) Some plants like it warmer: increased growth of three selected invasive plant species in soils with a history of experimental warming. *Pedobiologia* **57**:10–3.
- Traveset A, Richardson DM (2014) Mutualistic interactions and biological invasions. *Annu Rev Ecol Evol Syst* **45**:89–113.
- van der Heijden MG, Streitwolf-Engel R, Riedl R, et al. (2006) The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. *New Phytol* **172**:739–52.
- van der Putten WH (2012) Climate change, aboveground-belowground interactions, and species' range shifts. *Annu Rev Ecol Evol Syst* **43**:365–83.
- van Grunsven RHA, van Der Putten WH, Martijn Bezemer T, et al. (2010) Plant-soil interactions in the expansion and native range of a poleward shifting plant species. *Glob Chang Biol* **16**:380–5.
- Vierheilig H, Coughlan AP, Wyss U, et al. (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl Environ Microbiol* **64**:5004–7.
- Wagg C, Bender SF, Widmer F, et al. (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proc Natl Acad Sci USA* **111**:5266–70.
- Walker MD, Wahren CH, Hollister RD, et al. (2006) Plant community responses to experimental warming across the tundra biome. *Proc Natl Acad Sci USA* **103**:1342–6.
- Wall DH, Moore JC (1999) Interactions underground: soil biodiversity, mutualism, and ecosystem processes. *BioScience* **49**:109–17.
- Wu J (2014) *minque: An R Package for Linear Mixed Model Analyses*. R Package Version 1.1. <http://CRAN.R-project.org/package=minque> (20 May 2016, date last accessed).
- Yeates GW, Bongers T, de Goede RG, et al. (1993) Feeding habits in soil nematode families and genera—an outline for soil ecologists. *J Nematol* **25**:315–31.
- Zuur A, Ieno E, Walker N, et al. (2009) *Mixed Effects Models and Extensions in Ecology with R*. New York, NY: Springer.