

Nematode community shifts in response to experimental warming and canopy conditions are associated with plant community changes in the temperate-boreal forest ecotone

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Abstract Global climate warming is one of the key forces driving plant community shifts, such as range shifts of temperate species into boreal forests. As plant community shifts are slow to observe, ecotones, boundaries between two ecosystems, are target areas for providing early evidence of ecological responses to warming. The role of soil fauna is poorly explored in ecotones, although their positive and negative effects on plant species can influence

plant community structure. We studied nematode communities in response to experimental warming (ambient, +1.7, +3.4 °C) in soils of closed and open canopy forest in the temperate-boreal ecotone of Minnesota, USA and calculated various established nematode indices. We estimated species-specific coverage of understory herbaceous and shrub plant species from the same experimental plots and tested if changes in the nematode community are associated with plant cover and composition. Individual nematode trophic groups did not differ among warming treatments, but the ratio between microbial-feeding and plant-feeding nematodes increased significantly and consistently with warming in both closed and open canopy areas and at both experimental field sites. The increase in this ratio was positively correlated with total cover of understory plant species, perhaps due to increased predation pressure on soil microorganisms causing higher nutrient availability for plants. Multivariate analyses revealed that temperature treatment, canopy conditions and nematode density consistently shaped understory plant communities across experimental sites. Our findings suggest that warming-induced changes in nematode community structure are associated with shifts in plant community composition and productivity in the temperate-boreal forest ecotones.

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Introduction

Global climate warming is a key driving force that can alter floral and faunal community structures in terrestrial ecosystems (Harrington et al. 1999; Tylianakis et al. 2008; Van der Putten et al. 2010). Climate warming can influence abiotic

conditions, such as soil moisture availability, that can influence biotic processes like decomposition (Singh et al. 2010). One key response of ecosystems to climate warming is a shift in floral and faunal community composition, generally following the trend of shifts from lower to higher latitude (Chen et al. 2011). Temperate forests, for instance, have been reported to shift northwards as a result of climate warming into regions formerly dominated by boreal tree species (Leithead et al. 2010; Fisichelli et al. 2013a); this shift has been argued to potentially alter consumer-resource interactions both aboveground and belowground (Frelich et al. 2012). However, such changes are occurring slowly and take a long time to fully observe (Beckage et al. 2008; Fisichelli et al. 2013a).

Ecotone regions—that is boundaries between two ecosystems—can provide early evidence of plant community range shifts and therefore have received particular attention in range-shift studies (Beckage et al. 2008; Fisichelli et al. 2013b). The response of soil fauna to climate warming in such transition zones has been, however, poorly investigated. Soil fauna do not only react sensitively to changes in climate (Bakonyi et al. 2007), they also exert significant feedback effects on plant growth and plant community structure (van der Stoel et al. 2002). For instance, soil fauna can enhance nutrient mineralization and nutrient supply for plants (Bardgett and Chan 1999), but may also act as plant enemies, e.g., by feeding on plant roots and inhibiting plant growth (van Dam 2009). Plant growth in turn influences the dynamics of soil fauna by litter inputs and rhizodeposits, a process referred to as plant-soil feedback (Bever et al. 1997, 2002). Soil fauna can function as plant mutualists or plant enemies depending on the balance between positive and negative effects on plant growth (Klironomos 2002), and such soil effects can provide insights into plant community shifts (Van der Putten 2012).

In addition to warming of soils, soil habitat conditions can also influence soil fauna with distinct shifts in soil food web structure. For instance, higher trophic level soil fauna (such as predatory species) are more sensitive to alterations in habitat conditions than lower trophic level fauna (Komonen et al. 2000; Valladares et al. 2012). Canopy conditions in forests, i.e., closed or open canopy, may differentially affect soil habitat conditions; for instance, soil moisture may fluctuate more in the open canopy than in the closed canopy forests (Gray et al. 2002). In general, soil fauna in the organic layer of forest systems has been found to respond negatively to decreased water availability, possibly due to competition with the plants for moisture (Blankinship et al. 2011). Further, warming effects in open canopy conditions are more pronounced, which may lead to negative effects on soil fauna due to higher exposure to light and higher soil desiccation (Martius et al. 2004).

In this study, we explored the response of nematode communities, a key group of soil fauna encompassing major trophic groups of soil food webs, to experimental warming in closed and open canopy plots in the temperate-boreal forest ecotone of North America. We discuss effects of changes in soil food web structure on plant cover and plant community composition and highlight this approach as useful in understanding community shifts of plant species. Nematodes play key roles in the functioning of soil food webs by encompassing several trophic positions: microbial feeders (bacterial feeders and fungal feeders), plant root feeders, predators (feeding on microbial and plant feeders), and omnivores (feeding on both microorganisms and smaller nematodes) (Neher 2010; Ferris 2010). Trophic-level information of nematodes can provide insights into nematode-plant relations (Neher 2010). For instance, the ratio between the densities of microbial-feeding and plant-feeding nematodes can be used to grossly estimate soil feedback effects on plant community productivity (Wasilewska 1997; Eisenhauer et al. 2011). Such a ratio would indicate the relative increase or decrease in the density of plant-feeding nematodes compared to microbial-feeding nematodes and thus represent the extent of belowground plant antagonistic effects (Wasilewska 1997). The initial colonization success of range-expanding plant species has been shown to be promoted by the lowered presence of plant-feeding nematodes (Engelkes et al. 2008). On the other hand, relative increase in microbial-feeding nematodes stimulates microbial activities through grazing which could increase nutrient availability in soil (Ingham et al. 1985). Further, alterations of predatory or omnivore nematodes highlight functional shifts in soil food webs through reduction in the strength of top-down control, hence indicating a decrease in food web complexity (Neher 2010).

Different trophic groups of nematodes may differ in their responses to environmental stresses (Eisenhauer et al. 2012). Warming-induced water shortage in soil reduced the density of fungal-feeding and omnivore nematodes, but not that of other groups of nematodes in a multi-factor global change experiment in grassland (Kardol et al. 2010). Soil warming additionally enhanced the microbial activities in soil and rate of decomposition processes (Davidson and Janssens 2006), which may promote the performance of microbial-feeding nematodes (Ruess et al. 1999) with potential consequences for nutrient availability for plants. Moreover, open canopy areas are characterized by higher temporal fluctuations in microbial biomass (Sahani and Behera 2001; Nunes et al. 2012), which may accordingly lead to fluctuation in nematode populations feeding on microorganisms.

Soil warming and altered canopy conditions may therefore additively or interactively alter nematode communities, thereby changing soil feedback effects. Population

and community structure of nematodes are often studied in terms of nematode density and diversity, functional diversity indices, and indices based on the ratio of trophic-group densities (Ferris and Bongers 2009). We calculated these well-established indices and expected them to change with experimental warming and canopy conditions in the temperate-boreal ecotone. We hypothesized that the density of different trophic groups of nematodes will be affected by experimental warming and canopy conditions (hypothesis 1). More specifically, we expected higher trophic-level nematodes (i.e., predators and omnivores) to be negatively affected rather than the lower trophic-level nematodes (microbial-feeding and plant-feeding nematodes) by experimental warming and particularly so in open canopy conditions. We further hypothesized that functional indices and the trophic group ratio of nematodes will change with experimental warming and canopy conditions (hypothesis 2). For instance, we expected indices indicating soil food web complexity to decrease with warming, but indices related to plant nutrient availability to increase with warming. We finally hypothesized that warming-induced changes in the nematode community are associated with the cover and composition of understory herbaceous and shrub communities of the temperate-boreal ecotone (hypothesis 3). These hypotheses are not exhaustive due to the complexity of soil food webs and potential responses to warming and canopy conditions. This stresses the urgent need for empirical work on this topic, and we aimed at deriving general patterns by performing the same experiment at two distinct sites (Cloquet and Ely) in Minnesota, USA.

Materials and methods

Experimental design

The study was conducted in the temperate-boreal forest ecotone of Minnesota, USA, in the framework of the project B4WarmED (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 (latitude 46.7, longitude -92.5) and Ely (latitude 47.9, longitude -91.8). The mean annual temperature at Cloquet is 4.6 °C with mean annual precipitation of 807 mm; at Ely the mean annual temperature is 1.4 °C and mean annual precipitation 739 mm (Brzostek et al. 2012). Both experimental sites have six blocks (each with six plots), 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- to 80-year-old aspen overstory (*Populus tremuloides*). Parts

of the aspen overstory were removed to create open canopy plots in 2006 at Cloquet and 2007–2008 at Ely.

In order to investigate warming- and canopy-induced changes in soil food web structure and functioning, we sampled nematodes from 72 circular plots (3-m diameter) in total at the experimental field sites at Cloquet and Ely. For each temperature treatment, six replicates were sampled from closed canopy forest and another six from open canopy forests at each study site. Temperatures were regulated both aboveground and belowground simultaneously through artificial heating using 6–8 ceramic heating elements (model FTE-1000; Mor-Electric, MI) aboveground (Kimball 2005) and heating cables in soil (Devi A/B; Danfoss, Denmark) for belowground warming (Bergh and Linder 1999). At the start of the experiment in 2008, plots were planted with 11 different tree species [as seedlings; see electronic supplementary material (ESM) 2 for further information]. Hence, planted tree communities at the start of experiment were identical across all plots, whereas naturally occurring understory plant communities varied and were not controlled.

In August 2010, we sampled soil nematodes by taking three soil cores (2 cm in diameter, 7 cm deep) from each experimental plot. The soil was pooled per plot, thoroughly but gently mixed and split for soil nematode extraction and soil microbial analyses. Nematodes were extracted from approximately 10 g of fresh soil using a modified Baermann method (Ruess 1995). After an extraction time of 30 h, nematodes were preserved in 4 % formaldehyde, counted and related to grams soil dry weight. Subsequently, 10 % of the individuals (but not less than 100 individuals, if possible) were identified to family level or if necessary to genus level and assigned to the trophic groups as bacterial feeding, fungal feeding, plant feeding, predators and omnivores (Yeates et al. 1993). The list of nematode species is provided in ESM 1.

Nematode indices

First, we calculated the density of total nematodes as well as the density of different trophic groups. For nematode diversity, total nematode richness, the Shannon Wiener index ($H' = -\sum p_i \times \ln p_i$, where p_i is the proportion of the i th taxon) and Pielou's evenness ($J' = \frac{H'}{\ln S}$, where S is the number of taxa) were calculated (Magurran 2004).

Second, we calculated key functional indices for nematodes according to Bongers and Bongers (1998): the maturity index (MI) and plant parasitic index (PPI). MI was calculated after assigning colonizer-persister (c-p) class values for nematode families (Bongers and Bongers 1998). The c-p classes were assigned based on characteristics ranging from colonizers (short life cycle with high reproduction rate, tolerant to disturbance) to persisters (long life cycle with low reproduction rate, sensitive to disturbance) on a scale from 1 to 5, i.e., if a species is a strong colonizer, its

c–p class would be 1, whereas if it is a strong persister, its c–p class would be 5. MI was then calculated for each plot as $\frac{1}{N} \sum (c - p)_i \times n_i$ where N is the total number of individuals for all species in a plot, $(c - p)_i$ is the c–p value for species i and n_i is total number of individuals of species i . PPI was calculated as MI but for only plant-feeding nematode species, i.e., as $\frac{1}{N} \sum (c - p)_i \times n_i$ where $(c - p)_i$ is the c–p value for plant-feeding nematodes and n_i is total number of individuals of plant-feeding nematode i . In addition, the richness of c–p classes for each plot was calculated. The assigned c–p values for nematodes are provided in ESM 1. The MI is a proxy of the environmental disturbance in soil, i.e., low MI indicates a disturbed system or highly enriched soil due to fertilization, whereas high MI reflects stable soil conditions (Bongers and Bongers 1998; Bongers 1999). Therefore, higher MI values indicate higher presence of persister nematode species and lower values indicate presence of colonizer nematode species. Higher PPI values indicate higher presence of persister plant-feeding nematodes. Third, we calculated three trophic group ratios based on the density values of different trophic groups of nematodes as follows:

1. The relative importance of energy channels (bacterial dominated vs. fungal dominated) in soil (Wasilewska 1997; Eisenhauer et al. 2011) is calculated as the ratio between the density of fungal-feeding nematodes (FF) and the sum of fungal-feeding and bacterial-feeding (BF) nematodes $[\text{FF}/(\text{FF} + \text{BF})]$.
2. The ratio between densities of microbial-feeding (FF + BF) and plant-feeding (PF) nematodes $[(\text{FF} + \text{BF})/\text{PF}]$. A $[(\text{FF} + \text{BF})/\text{PF}]$ ratio higher than 1 indicates positive effects of soil nematodes on plant productivity (relative decline in plant-feeding nematodes), whereas values <1 stand for negative effects on the plant biomass production (Wasilewska 1997). However, own meta-analyses indicate that a threshold of 1 may not be representative for all soil and vegetation types (Thakur et al., unpublished data); nevertheless, relative differences in this ratio may still indicate changes in soil feedback effects on plant growth.
3. The ratio between predator nematodes (PR) and plant-feeding nematodes (PR/PF) is an index for food web complexity related to the ability of nematode predator communities to control herbivores, i.e., plant-feeding nematodes (top-down control) (Wasilewska 1997; Eisenhauer et al. 2011). For this ratio, we combined the densities of predators and omnivores.

Vegetation cover

The understory plant community (herbaceous and shrub species) was assessed within two 0.5-m² subplots in each plot. Herbaceous plants and shrubs were identified to

species level and their percent cover was estimated visually. We use total plant cover as proxy for plant productivity for the understory plant community. The list of identified plant species is provided in ESM 2. Two years after the start of the experiment, we estimated diameter and height of planted tree individuals and later used these to estimate the gross volume of tree seedlings by multiplying the square of the mean diameter with seedling height (Stoate 1945). We treated total tree seedling volume and naturally occurring herbaceous and shrub plant community cover separately due to differences in their measured unit. Both measures of plant community productivity were used separately as response variables since we were particularly interested in changes in herbaceous plant community composition and their relationship to nematode communities.

Soil microbial variables

We measured soil microbial parameters to explain changes in nematode communities and potential nematode effects on plant community productivity and composition in response to our treatments. Soil sub-samples were sieved (2 mm) to remove larger plant roots, animals and stones before starting the measurements of soil microbial biomass C and microbial basal respiration. Microbial respiration and biomass C of approximately 5 g soil (fresh weight) were measured by using an O₂-microcompensation apparatus (Scheu 1992). The microbial respiratory response was measured for 24 h at 22 °C at hourly intervals. Substrate induced respiration was then calculated from the measured respiratory response to D-glucose for about 10 h at 22 °C. Glucose was added to saturate the catabolic enzymes of microorganisms (20 mg g⁻¹ dry weight solved in 400 µl deionized water according to preliminary studies). The mean of the lowest three readings within the first 10 h (between the initial peak caused by disturbing the soil and the peak caused by microbial growth) was assessed as the maximum initial respiratory response (MIRR; µl O₂ h⁻¹ g⁻¹ soil dry weight) and microbial biomass (µg C g⁻¹ soil dry weight) was calculated as $38 \times \text{MIRR}$ (Beck et al. 1997).

Statistical analyses

We used experimental warming and canopy conditions as main explanatory variables with two random factors, site (Cloquet and Ely) and block, in a linear mixed-effect model to explain variations in the nematode density and diversity (hypothesis 1) and functional indices of nematodes (hypothesis 2). Some of the nematode indices were log transformed (indicated in Table 1) in order to improve the residual fitting and homoscedasticity of errors. We carried out post hoc Tukey comparisons of means, but only

Table 1 *F*-values of linear mixed models for the effects of experimental warming and canopy conditions and interaction on the nematode community. Sites and block were used as random effects in the mixed model

Nematode indices	Warming (W)	Canopy conditions (C)	W × C
	<i>F</i> -value	<i>F</i> -value	<i>F</i> -value
Density and diversity indices			
Total density (L)	0.72	0.13	0.93
Fungal-feeders density (L)	0.27	0.05	0.85
Bacterial-feeders density (L)	1.25	3.54	0.09
Plant-feeders density (L)	0.03	7.25*	0.18
Predators density (L)	2.39	3.60	0.22
Omnivores density (L)	0.06	6.00*	2.35
Total richness (L)	1.32	0.29	0.14
Shannon Wiener index	0.2	0.07	1.23
Pielou evenness	0.68	0.01	2.08
Functional indices			
c–p1 richness	1.02	1.09	1.12
c–p2 richness	0.55	0.19	1.00
c–p3 richness	1.43	8.77**	1.32
c–p4 richness	<0.01	0.35	0.85
MI	0.69	4.76*	0.18
PPI	6.66**	9.30**	0.41
Trophic group ratio			
FF/FF + BF (L)	1.48	0.19	0.26
FF + BF/PF (L)	5.97**	14.94**	0.03
PR/PF (L)	1.23	12.95**	1.78

L Log₁₀-transformed data

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

when temperature and canopy conditions significantly affected nematode indices, respectively. Data were analyzed using the nlme package (Pinheiro et al. 2013) in R statistical software version 2.15.2 (R Development Core Team 2012). We further used linear mixed-effect models with only significantly affected nematode indices (by both experimental warming and canopy conditions) to explain variations in total understory plant cover using site and block as random factors (hypothesis 3). We also checked statistically if planted tree seedling volume showed any associations with significantly affected nematode indices using the linear mixed-model approach (with sites and blocks as random factors). Redundancy discriminatory analysis (RDA) and an ordination bi-plot were used to visualize the effects of treatments on the plant community composition. We used percent cover of each plant species per plot as response variable and density of nematode trophic groups, microbial biomass, experimental warming, canopy conditions, and sites as explanatory variables in RDA. RDA was only run for understory plant species community as planted tree seedling volume and nematode

indices were not statistically associated (see Results below). Species data were log transformed and significances of nematode trophic groups and microbial biomass on plant community composition were analyzed using Monte Carlo permutation tests (999 permutations). RDA tests were performed in Canoco for Windows version 4.5.

Results

Density and diversity indices

Experimental warming showed no significant effects on population indices of nematodes (Table 1). Canopy conditions, on the other hand, showed some significant effects on the nematode population indices. The densities of plant-feeding nematodes were significantly lower in open canopy areas, whereas densities of omnivores were significantly higher in open canopy sites. However, the density of fungal-feeding, bacterial-feeding and predator nematodes did not significantly vary between canopy conditions (Table 1). Both warming and canopy conditions showed no effects on various measures of nematode diversity, i.e., total richness, Shannon Wiener index and Pielou's evenness. We also did not find any significant interaction effects of warming and canopy conditions on population indices of nematodes. The mean values of nematode indices in response to warming and canopy condition treatments for both Cloquet and Ely are provided in ESM 3.

Functional indices

The taxonomic richness of the different c–p classes were not significantly affected by warming. Only the richness of c–p3 species was significantly higher in open canopy sites (Table 1). The MI was not significantly affected by the warming treatments; however, MI values were significantly higher in open canopy sites. In contrast to MI, PPI decreased significantly with increasing temperature and more so in open canopy plots. The post hoc test showed that the PPI in the ambient treatment was significantly higher than those of the ambient +1.7 °C and ambient +3.4 °C treatments (Fig. 1).

Trophic group ratios

The [FF/(FF + BF)] as well as PR/PF showed no significant variations with experimental warming. The PR/PF ratio however was significantly higher in open canopy sites. The [(FF + BF)/PF] ratio, on the other hand, increased significantly with experimental warming (Table 1), and this effect was consistent in both closed and open canopy areas (Fig. 1). The post hoc test revealed that [(FF + BF)/PF]

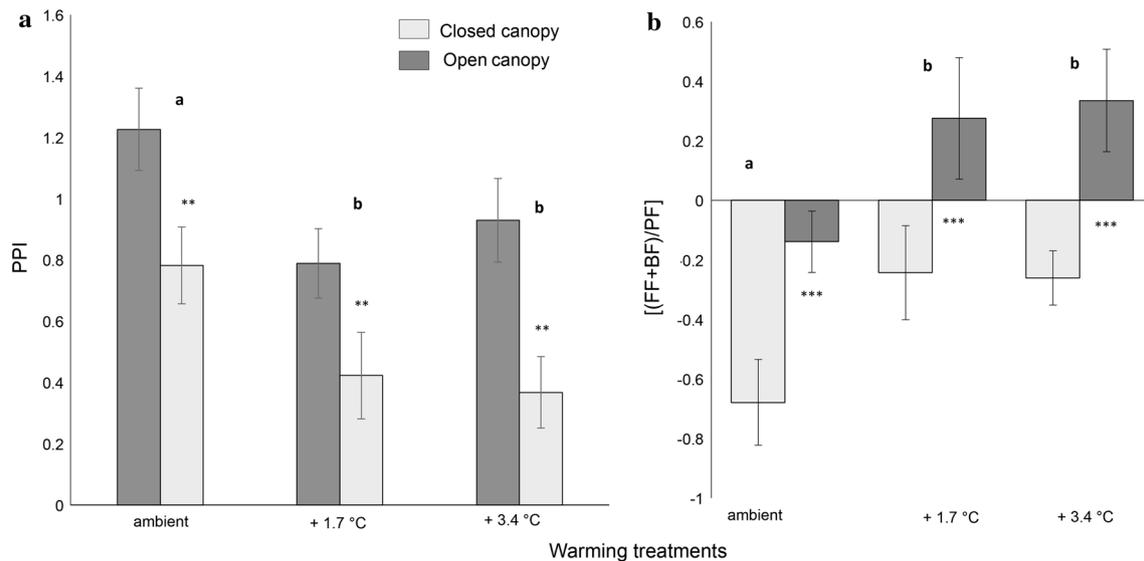


Fig. 1 The mean \pm SE for **a** plant parasitic index (PPI) and **b** trophic group ratios of microbial-feeding and plant-feeding nematodes [(FF+BF)/PF] as affected by experimental warming (ambient, +1.7 and +3.4 °C) and canopy conditions (closed and open canopy). Dif-

ferent letters indicate differences in temperature treatments based on post hoc Tukey test; asterisks indicate significant effects of canopy conditions from the general linear mixed model (LMM) (** $p < 0.01$, *** $p < 0.001$)

ratio was significantly higher in both warmed treatments than in the ambient ones (but did not differ between +1.7 and +3.4 °C).

Nematodes association with plant community cover and composition

Total understory plant cover was positively correlated with the [(FF + BF)/PF] ratio (F -value = 12.94, $p < 0.001$; Fig. 2). On the other hand, PPI showed a negative correlation with the total understory plant cover (F -value = 6.17, $p < 0.05$). However, we did not find any significant relation between [(FF + BF)/PF] ratio (F -value = 2.031, $p > 0.05$) or PPI (F -value = 0.25, $p > 0.05$) and the total tree seedling volume per plot.

The RDA analysis explained 26.8 % of the variation in plant community composition (Fig. 3). The first axis of the RDA bi-plot explained 13.7 % of the variation and the second axis explained an additional 6.2 % of the variation. Among individual trophic groups of nematodes, plant-feeding nematodes significantly correlated with plant community composition (F -value = 2.68, $p < 0.001$). In addition, microbial biomass also explained a significant portion of the variation in total plant community composition (F -value = 2.21, $p < 0.01$). The first axis of RDA bi-plot for total plant community composition showed higher correlation with plant-feeding nematodes (correlation coefficient = -0.45) than the other explanatory variables, whereas the second axis was more correlated with soil microbial biomass (correlation

coefficient = -0.31) and bacterial-feeding nematodes (correlation coefficient = 0.31). Our RDA bi-plot showed that understory plant community composition at the two experimental sites and canopy treatments were affected consistently by warming treatments (Fig. 3).

Discussion

Our results suggest that experimental warming and canopy conditions induce a shift in the functional composition of nematode communities of the temperate-boreal forest ecotone. Although the density and diversity of nematodes were largely unaffected by the treatments, which makes us reject our hypothesis 1, we found a shift in the ratio between microbial-feeding and plant-feeding nematode density [(FF + BF)/PF] in response to experimental warming and canopy conditions, which is in agreement with our hypothesis 2. We further found that the shift in the ratio between microbial-feeding and plant-feeding nematode density showed a significant positive correlation with understory plant cover. Further, the PPI, which declined in response to experimental warming and open canopy conditions, was negatively associated with understory plant cover. Both these results agree with our expectation that changes in the nematode community may associate with changes in the plant community (hypothesis 3). Moreover, plant community composition was consistently affected by experimental warming, canopy conditions and altered

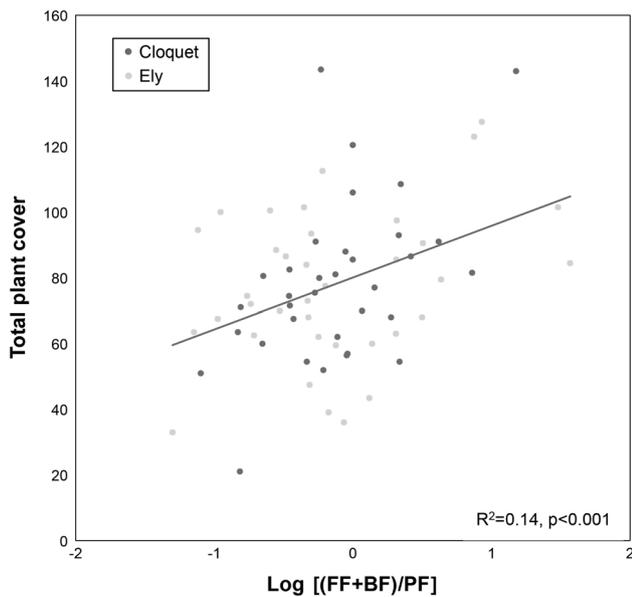


Fig. 2 Positive correlation for trophic group ratio between microbial-feeding and plant-feeding nematodes [(FF + BF)/PF] and total coverage of the understory plant community at both experimental sites (Cloquet and Ely). The R^2 -value is based on the LMM results. For abbreviations, see Fig. 1

nematode community structure at two experimental sites. We highlight that experimental warming and altered canopy conditions can significantly change functional indices of soil nematodes, and that such shifts are associated with variations in plant productivity and community composition. Thus, to understand shifts in plant communities in a changing world, we must also consider the responses and effects of belowground soil organisms.

Shifts in nematode communities (hypotheses 1 and 2)

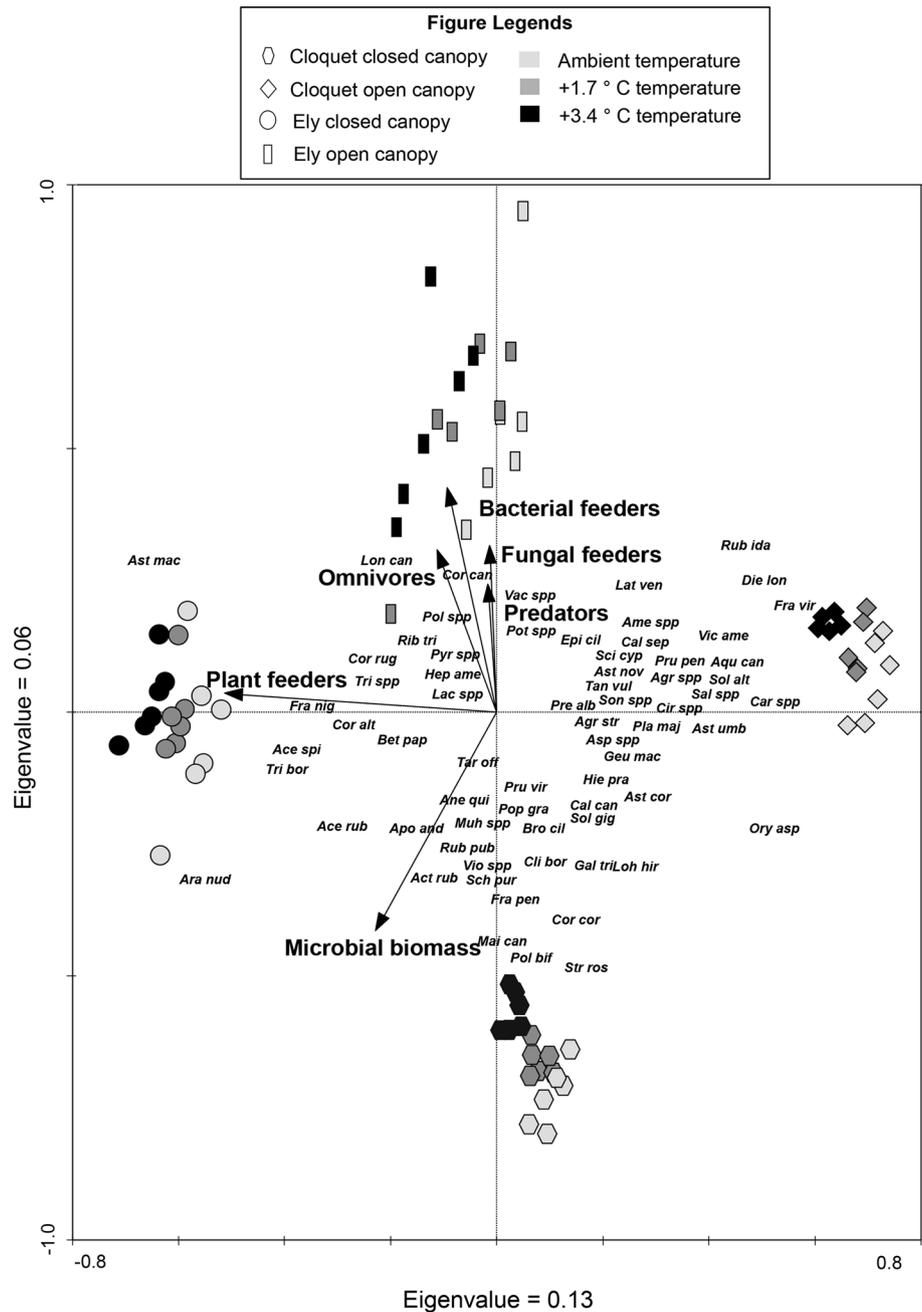
The densities of individual trophic groups of nematodes were not significantly affected by experimental warming; however, we found nematode functional indices to be sensitive to warming effects. Experimental warming significantly increased the ratio between microbial-feeding and plant-feeding nematode density [(FF + BF)/PF] and decreased the PPI. Moreover, the [(FF + BF)/PF] was significantly higher in open canopy than in closed canopy areas (Fig. 1). Notably, this soil feedback index (Eisenhauer et al. 2011) increased significantly and consistently with experimental warming in both open and closed canopy areas. The increase in this index implies a relative decline in the density of plant-feeding nematodes or a relative increase in the density of microbial-feeding nematodes or both. Soil warming can stimulate microbial activities (Davidson and Janssens 2006; Conant et al. 2011), which in turn can benefit nematodes feeding on

these microorganisms. On the other hand, predation pressure of microbial-feeding nematodes on microorganisms would enhance microbial activity, potentially increasing nutrient availability (Irshad et al. 2011). In either case, the density of microbial-feeding nematodes would increase, which would further benefit predator and omnivore nematodes. Such an increase of microbial-feeding nematodes and their predators has been suggested to increase the MI (Bongers and Bongers 1998). However, we only found a significant increase in the MI in open canopy conditions, which could be linked to the post-canopy removal effects, such as increased root decay that may enhance microbial turnover.

The PPI, which is analogous to the MI but only represents plant-feeding nematodes (Bongers et al. 1997), significantly decreased with experimental warming. The PPI often increases with an increase in soil nutrients; however, this occurs only when persister plant-feeding nematodes (c - p values >3) are increased (Bongers et al. 1997; Bongers and Bongers 1998). Persister nematodes are adapted to less disturbed and stable soil conditions (Bongers 1999). In contrast, we did not find any plant-feeding nematodes with c - p values >3 , but only plant-feeding nematodes with c - p values = 2 or 3 were found across the plots (ESM 1). The decrease in the PPI based on only two classes of c - p values (2 and 3) may not be conclusive for its relation with nutrient availability in this study; however, the decline in colonizer plant feeding nematodes could indicate (decreased PPI) conditions favorable for persister nematodes (Bongers 1999). Among persister nematodes (c - p values >3), our results confirm an increase in omnivores (c - p values = 4 in our study; ESM 1) but only at open canopy conditions (Table 1). The trophic group ratio between predators (including omnivores) and plant-feeding nematodes also increased in open canopy conditions. These results support that soil conditions become favorable for persister nematodes at open canopy plots with warming effects only tractable with a decline in colonizer plant-feeding nematodes. Plant-feeding nematodes have been reported to be much more sensitive to changes in plant community composition than other groups of nematodes, such as fungal-feeding nematodes, due to their selective feeding on certain host plants (Yeates 1999; Neher 2010). In open canopy plots, due to removal of tree species, understory plant species with the competitive advantage of light capture would thrive (Rodríguez-García et al. 2011). This may change the plant community composition and negatively influence plant-feeding nematodes associated with the competitively light-inferior herbaceous plant species.

Our results indicate a stronger effect of canopy conditions on various trophic groups of nematodes and also on several functional indices than the effects of experimental

Fig. 3 Ordination diagram of redundancy discriminatory analysis for total cover of plant species constrained by the density of different trophic groups of nematodes and microbial biomass. Eigenvalues for the first and second axes show the variations in total cover of plant species across the plots explained by the density of different trophic groups of nematodes and microbial biomass. *Rectangular blocks* represent sample plots categorized for the experimental sites Cloquet and Ely, and two canopy conditions (closed and open canopy), indicated by the *different symbols*. Warming effects are shown in *different gray shades*. Full names of plant species are provided in ESM 2



warming (Table 1). A recent study highlighted weak effects of warming on the absolute density of different trophic groups of nematode compared to soil N availability (Li et al. 2013). Our results suggest that warming-induced effects on nematode densities could be better reflected in changes in trophic group ratios due to disproportionate responses among trophic groups. In particular, warming-induced changes in the ratio between microbial-feeding and plant-feeding nematodes could be useful to understand associations between nematodes and plant communities in transition zones.

Associations between nematodes and plant communities (hypothesis 3)

We found a significant positive relationship between the ratio of microbial-feeding and plant-feeding nematodes and total understory plant cover. More importantly, this relation was consistent across four environmental contexts: open and closed canopies at both study sites. This finding provides support for our hypothesis that changes in nematode community structure can be associated with alterations in the plant community (Fig. 2). Our RDA results indicate

that plant community composition may be associated with different trophic groups of nematodes as well as with soil microbial biomass. As eigenvalues for RDA axes were not remarkably high, site-specific plant community composition may have been affected by other environmental variables, which were not considered in our study. Nevertheless, RDA analyses showed stronger associations between plant-feeding as well as bacterial-feeding nematodes and plant community composition compared to other trophic groups of nematodes. Remarkably, experimental warming induced a consistent shift of plant community composition across both experimental sites and canopy treatments (Fig. 3).

Our results show that plant-feeding nematodes were closely associated with the plant species *Aster macrophyllus* (*Aster* hereafter) (Fig. 3). Interestingly, *Aster* is a common species in the region, but particularly so at Ely. Our RDA diagram shows that *Aster* was most common in the closed canopy plots of Ely, where absolute densities of plant-feeding nematodes were highest (Fig. 3; ESM 3). The decline in the plant-feeding nematode density with warming was also pronounced in the closed canopy plots at Ely (ESM 3). The increase in the absolute density of bacterial-feeding nematodes with experimental warming was more consistent at Ely in both open and closed canopy conditions (Table 1; ESM 3). Plant species like *Carex* spp. or *Oryzopsis asperifolia* have been found to increase in the North American temperate regions although not specifically in response to climate warming (Wiegmann and Waller 2006). Our results indicate that these species were higher in open canopy plots and, interestingly, negatively related to plant-feeding nematodes (Fig. 3).

Previous studies have shown that changes in soil faunal communities can be associated with changes in plant community properties, such as plant identity, diversity and biomass production (De Deyn et al. 2003; Kardol et al. 2010; Eisenhauer 2012). Essentially, through positive or negative soil feedback effects, soil fauna—including nematodes—may contribute to the survival and growth of plant species (Bever et al. 1997). Over time plant communities shape specific soil faunal communities through litter inputs and rhizodeposits (Bever et al. 1997; De Deyn et al. 2003; Eisenhauer 2012). Greenhouse experiments have revealed that range-expanding plant species face a lower degree of enemy pressure, here from plant-feeding nematodes, compared to native plant species (Engelkes et al. 2008; Morriën et al. 2012). With decrease in belowground enemy pressure, and increase in nutrient availability in response to climate warming, and specifically in open canopy plots, it is likely that opportunistic plant species, such as range-expanding species, can increase in density in transition zones (Van der Putten 2012). Climate warming has already been suggested to increase plant biomass and shape plant communities in the changing world (Lin et al. 2010). Our results from the North

American temperate-boreal ecotone highlight that experimentally induced warming and changes in canopy conditions can influence the functional composition of nematode communities, and that such changes are associated with changes in plant productivity and community composition. Direct effects of climate warming and altered canopy conditions can shape plant community composition and changes in plant composition can then affect the nematode community. However, exploring both directions, i.e., plant effects on nematodes and nematode effects on plants, will be required to mechanistically understand and predict global change effects on plant and soil communities. Our results suggest that the relative decline of plant-feeding nematodes in response to warming may provide better breeding grounds for range-expanding species in the temperate-boreal ecotone, but this hypothesis has to be tested in future studies.

Conclusion

We provide evidence that despite weak effects of experimental warming on the density and diversity of soil nematodes, functional indices of soil nematode communities responded significantly to experimental warming and to altered canopy conditions. The warming-induced increase in the ratio between microbial-feeding nematodes and plant-feeding nematodes indicates a shift in soil feedback effects on plants. Accordingly, the understory plant community cover was positively correlated with the ratio of microbial-feeding to plant-feeding nematodes. Importantly, these findings were consistent in four different environmental contexts and were associated with changes in plant community composition. We highlight that shifts in soil nematode communities due to warming and canopy conditions may cause changes in plant-soil feedback effects, potentially co-determining plant community shifts caused by global change in the temperate-boreal ecotone.

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