

Long-term nitrogen addition shifts the soil nematode community to bacterivore-dominated and reduces its ecological maturity in a subalpine forest



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ABSTRACT

Nitrogen deposition from anthropogenic sources is a global problem that reaches even the most remote ecosystems. Responses belowground vary by ecosystem, and have feedbacks to geochemical processes, including carbon storage. A long-term nitrogen addition study in a subalpine forest has shown carbon loss over time, atypical for a forest ecosystem. Loss of microbial biomass is likely linked to lower soil carbon, but the mechanism behind this is still unknown. One possible explanation is through increased turnover due to grazing by soil fauna. Because nematodes occupy many trophic levels and are sensitive to trophic and environmental changes, assessing their communities helps reveal belowground responses. In this study, we tested the hypothesis that long-term nitrogen fertilization affects nematode community structure and maturity beneath coniferous forests in the Rocky Mountains, indicating a faster cycling, bacterial-driven system. We identified and enumerated nematodes by trophic group and family from experimental plots. Total nematode abundance was 40–96% greater in fertilized plots compared to the control, but richness, diversity, and ecological maturity were lower. The differences in abundance were driven by opportunistic bacterivores (e.g., Rhabditidae) and plant parasites (e.g., Tylenchidae), which made up 23 and 13% of the community in fertilized compared to 7 and 5% in control plots, respectively. Nematode maturity indices showed that the nematode food web was enriched (indicating high nutrient/resource status) and structured (all trophic levels present, including long-lived predators) in both treatments, but significantly more enriched in the fertilized treatment. Nonmetric multidimensional scaling of the relative abundance of nematode families demonstrated that nematode community composition differed between treatments, driven largely by opportunistic bacterivores (e.g., Rhabditidae) in the fertilized plots. The mechanism of the aboveground–belowground link between nitrogen deposition and nematode community composition is likely through increased microbial turnover, and sustained high-quality food for microbial grazing nematodes.

1. Introduction

Anthropogenic nitrogen (N) deposition has increased more than an order of magnitude over the last century and is far greater than N deposition from natural sources (Galloway et al., 2004, 2008). Largely from food and energy production and use, N deposition can have extensive effects on greenhouse gases, above- and belowground biodiversity, and soil biogeochemical cycles (Tilman, 1986; Vitousek et al., 1997; Sala et al., 2000; Gough et al., 2012; Ramirez et al., 2012). These

effects are not isolated to human-managed systems, and reach even very secluded places (Fenn et al., 2003; Pardo et al., 2011). While the impacts of N deposition aboveground generally include increased primary production, which stores carbon (Quinn et al., 2009), belowground responses differ by ecosystem (Liu and Greaver, 2010; Lu et al., 2011; Zhou et al., 2014). These responses vary from net soil carbon storage (e.g., forests, Janssens et al., 2010) to net loss (e.g., arctic tundra, Mack et al., 2004).

A long-term nitrogen addition study in the subalpine forest of the

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Colorado Rocky Mountains found that soil organic horizon carbon decreased by 11% in fertilized plots compared to control plots (Boot et al., 2016), which contrasts findings in other forests undergoing N amendments (e.g., Frey et al., 2014). Additional results from this study included reduced soil pH and microbial biomass; these results are more typical of N addition studies (Boot et al., 2016). Reduced microbial biomass after N addition is often attributed to lower plant C flux through reduced litter, root growth, and exudate input (Liu and Greaver, 2010), but could also be a result of increased aluminum toxicity caused by lower soil pH (Vitousek et al., 1997). Although food web dynamics are often left out of N addition studies, elevated grazing by soil fauna resulting in increased turnover could explain the loss of microbial biomass (Lokupitiya et al., 2000; Parfitt et al., 2010).

Nematoda is an incredibly diverse phylum, ubiquitous in soil. Nematodes span multiple trophic groups, with different taxa feeding on fungi, bacteria, cyanobacteria, algae, protozoans, roots, and other soil fauna (Yeates et al., 1993; Wardle et al., 1995; Bongers and Bongers, 1998). Because they occupy many trophic levels and are sensitive to environmental changes, their communities reveal the soil's condition and are useful as environmental and food web indicators (Bongers, 1990; Ruess et al., 1999; Ferris et al., 2001; Yeates, 2003). Furthermore, evidence for trophic cascades has been identified in nematode communities, where both top-down and bottom-up effects exist between bacterial and fungal components of soil food webs and their nematode consumers (Wardle and Yeates, 1993). Specifically, microbivore nematodes are most likely limited by resource quality and respond to microbial biomass growth, while abundance of predaceous nematode groups are also related to microbial biomass via their microbivore prey; thus, a high ratio of bacterivore nematodes to bacterial biomass would indicate quick turnover rates and productivity for bacteria (Wardle and Yeates, 1993; Wardle et al., 1995).

To better understand the nematode community and how it relates to ecosystem status, the maturity index (MI; Bongers, 1990) was developed to assess successional maturity. The MI is a weighted mean of the frequency distribution of colonizer-persister (*c-p*) values of nematode taxa in the community. This *c-p* scaling ranges from 1 to 5 and corresponds to *r* and *K* life strategies, with *c-p* values of 1 associated with *r* strategists and 5 with *K* strategists. In short, undisturbed soil communities with sufficient resources have greater MI values than those from disturbed systems. These mature nematode communities have low abundance of bacterivore opportunists (i.e., colonizers; *c-p* 1) and all trophic levels are present including long-lived omnivore and predator nematodes (i.e., persisters, *c-p* 3 to 5). Soil communities with lower MI values are either associated with: 1) disturbances (chemical and physical), which decrease abundance of persister groups (*c-p* 4 to 5), typically omnivore and predators; or 2) enrichment of resources, which triggers population growth of colonizers (*c-p* 1) (Bongers et al., 1997). The extensions of the MI – including the enrichment index (EI) and structural index (SI) – are useful for assessing specific changes to soil food web structure (Ferris et al., 2001). Higher EI values indicate greater availability and turnover of resources, and are characterized by opportunistic groups; while greater SI values indicate low stress, high stability, and are systems where greater diversity, number of trophic links, and long-lived omnivore-predators are present (Ferris et al., 2001).

Commonly, opportunistic nematode groups (particularly *r*-selected bacterivores) increase in abundance with N addition (Ettema et al., 1999; Lokupitiya et al., 2000; Ruess et al., 2002) while long-lived omnivore-predators decrease (Todd, 1996; Sarathchandra et al., 2001), reflecting an increased abundance of resource availability along with stress caused by N addition. Plant parasitic taxa also often increase with N addition due to higher quality input from plants (Bongers et al., 1997). However, the effects of elevated N on nematode community vary by ecosystem. For example, lower nematode abundance and diversity with N addition has been found in grasslands (Wei et al., 2012) and temperate forests (Sun et al., 2013). Meanwhile, Zhao et al. (2014)

showed that nematode diversity and trophic composition were unaffected by N addition for tropical forests. Nematodes likely track the ecosystem response to N addition – through effects on their plant and microbial resources – rather than responding directly to elevated N.

The objective of our study was to use the MI and similar nematode community indices, to test the hypothesis that nematode community maturity and diversity are correlated negatively with long-term fertilization. We hypothesized a switch to a bacterial-driven, faster cycling soil food web with long-term nitrogen fertilization, which would be reflected by a lower MI, higher EI, lower SI, and overall significant increase in the total abundance of nematodes. We expected that nematode communities in control plots would have greater MI and SI, lower EI, greater richness and diversity values, and a distinct composition compared to fertilized plots.

2. Methods

2.1. Study site description

Loch Vale watershed (LVWS) is located on the east side of the continental divide in Rocky Mountain National Park, Colorado, USA, where soils are shallow and coarse entisols (Baron et al., 1992). Located at about 3200 m ASL in elevation, the mean annual temperature is 1.2 °C and mean annual precipitation is 105 cm (Mast et al., 2014). This area receives background N of approximately 3–4 kg N ha⁻¹ yr⁻¹ as wet deposition (Baron et al., 2000). A National Atmospheric Deposition Program monitoring site is present in LVWS and precipitation amount and chemistry are assessed on a weekly basis (<http://nadp.slh.wisc.edu/data/ntn/>). In 1996, a nitrogen fertilization experiment was established in LVWS in a split-plot design (for experiment details see: Rueth et al., 2003). Briefly, three pairs of experimental plots (30 m × 30 m) are located on undisturbed, closed-canopy, old growth Engelmann spruce and subalpine fir stands on northeast facing slopes. Each pair of plots includes one control and one fertilized plot. For fertilized plots, dry ammonium nitrate (NH₄NO₃) was applied throughout the year to mimic natural atmospheric deposition. Specifically, NH₄NO₃ pellets were applied at a rate of 2.5 kg N ha⁻¹ month⁻¹ from April to October and 7.5 kg N ha⁻¹ after the first snow in October. In total 25 kg N ha⁻¹ yr⁻¹ was applied. The rate chosen was similar to natural atmospheric N deposition rates in other parts of the United States during the setup of the experiment in 1996.

2.2. Soil collection and analyses

In July 2014 and July 2015, 24 soil cores per year (5.5 cm diameter × 10 cm depth) were collected in the LVWS experimental plots (4 samples × 2 treatments × 3 plots × 2 years = 48 total samples). Cores were taken approximately 3 m apart in the innermost 15 m² area of experimental plots to minimize any edge effects. Samples were stored at 4 °C at Colorado State University until processing. Soil nematodes were extracted from 100 g subsamples within 48 h of collection by the sugar centrifugation floatation method (Jenkins, 1964; Freckman and Virginia, 1993). Five nematode trophic groups (bacterivore, fungivore, plant parasite, omnivore, and predator) were identified according to Yeates et al. (1993) using an inverted microscope (Olympus CKX41¹, 200× magnification); all nematodes extracted from the 100 g soil sample were identified and counted. After trophic group identification, the microscope lens was moved to a random location over the counting dish and the first 50 nematodes viewed were identified to family level. Additional fauna groups present in the extracts were enumerated and included Rotifera, Tardigrada, and enchytraeids (Annelida). Mass loss of soils dried at 105 °C for 48 h was assessed to determine gravimetric

¹ Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

soil moisture (water mass per unit soil mass). Soil fauna absolute abundance was then expressed on an oven dry weight basis as the number of individuals kg dry soil⁻¹. Bulk density of each sample was calculated by using its volume (cm³) measured during sample collection, and its dry weight (g). In addition, 5 organic horizon 6.5 cm diameter cores per plot (n = 15 per treatment) were taken in July 2015, homogenized, roots and soil removed, and then dried at 60 °C. Next soils were ground and analyzed for C and N analysis (LECO Tru-Spec CN analyzer, Leco Corp.¹, St. Joseph, MI, USA).

2.3. Data analysis

2.3.1. Nematode community indices

We measured nematode family richness, family diversity (Shannon), and ecological maturity indices of nematode communities (plant parasitic and free-living). Two nematode maturity indices were used: 1) the maturity index for free-living nematodes (MI) and 2) the plant-parasitic nematodes index (PPI). To calculate these indices, nematodes are first assigned a colonizer–persister value (*c-p*) ranging from enrichment colonizers (*c-p* 1) and disturbance colonizers (*c-p* 2) to persisters (*c-p* 5). This *c-p* scaling is generally an *r-K* strategy continuum where life cycle, body size, and sensitivity to disturbance increase, while metabolism and fecundity decrease with increasing *c-p* value. Nematodes were assigned to these *c-p* groups based on Bongers (1990). Briefly (summarized from Bongers and Bongers, 1998): *c-p* 1 nematodes are bacterivore taxa that have a short life cycle, high metabolic rate, high fecundity, and are only active during pulses of bacterial growth. The *c-p* 2 nematodes also have a short life cycle and high fecundity, but do not form the dauerlarvae (a resting state) that is found in *c-p* 1 groups; the *c-p* 2 group includes fungivore, plant parasitic, and bacterivore taxa. The *c-p* 1 and *c-p* 2 groups are generally tolerant of disturbance, while *c-p* 1 responds most rapidly to resource addition. The *c-p* 3 and 4 groups have increased life cycle lengths and are more sensitive to disturbance. These groups include few bacterivore groups, along with some fungivores, plant parasites, omnivores, and predators. The *c-p* 5 group contains large nematodes with a long life cycle and low reproduction. This group includes omnivore, predator, and plant parasitic taxa that are the most sensitive to disturbance. The MI is the weighted mean for the frequency distribution of collective *c-p* values for all taxa excluding plant parasites, while the PPI is for plant parasitic taxa only. These indices are calculated separately because plant parasitic nematode response could mask changes in the MI if plant parasites (*c-p* 2 to 5) and colonizer bacterivores (*c-p* 1) both respond to increased nutrients. Specific details for MI and PPI calculations can be found in Bongers (1990). Additionally, two maturity index extensions - the enrichment index (EI) and structural index (SI) - were calculated according to Ferris et al. (2001) to identify nematode food web properties. The EI and SI differ from the MI because they are based on nematode functional guild, which adds feeding group to life history characterization (i.e., *c-p* scaling). The guilds are weighted according to their responses to disturbance and enrichment (Ferris et al., 2001). The EI and the SI were then plotted as a structure-enrichment plot (Fig. 2), which reflects an increase in trophic linkages along the x-axis (SI) and increased presence of opportunistic groups along the y-axis (EI). In this sense, the x-axis corresponds to disturbance (either physical or chemical), and the y-axis corresponds to resource availability (Ferris et al., 2001).

2.3.2. Analysis of treatment effects

We assessed the effects of fertilization treatment and sampling year on nematode total absolute abundance, trophic group absolute abundance, family relative abundance, family diversity (Shannon), and maturity indices with mixed effect models. Treatment and year were fixed effects and plot was random. We also tested these effects on rotifer, tardigrade and enchytraeid absolute abundances. We used Tukey's HSD for post-hoc comparisons. Distributions were assessed for

normality and data were transformed ($\log x + 1$) when necessary to meet assumptions. For all analyses $n = 12$. Statistical significance was accepted at $\alpha < 0.05$. These analyses were performed in R using the nlme and lsmeans packages (Oksanen et al., 2013; R-Core-Team, 2014).

2.3.3. Community ordinations (NMDS)

Non-metric multidimensional scaling (NMDS) was performed to show soil nematode community position in ordination space and to investigate if fertilization was a significant driver of community structure. NMDS is a robust unconstrained ordination method often used for community ecology (Minchin, 1987). Unlike other ordination techniques that rely on distances, such as Euclidean distances, NMDS relies on the rank order of dissimilarity in a community (Kruskal, 1964). Using relative abundances of nematode families, we defined the original position of the community in multidimensional space using the Bray-Curtis distance coefficient. We then ran the NMDS with 1 through 6 dimensions and chose 6 dimensions as the best, rerunning the NMDS ordinations multiple times with several random starting configurations and then choosing the best configuration. This returned a stress value of 4.5%. Permutational multivariate analysis of variance (PERMANOVA) was used to test if communities significantly grouped by treatment and year. These analyses were performed in R using the vegan package (Oksanen et al., 2013; R-Core-Team, 2014).

3. Results

Gravimetric soil moisture (% g/g) did not differ between treatments, but did differ by year. Soil moisture in 2014 was $48.25 \pm 3.27\%$. In 2015, soils were saturated and gravimetric soil moisture was $132.18 \pm 15.44\%$. In July 2015, soil C was $30.95 \pm 2.33\%$ for fertilized and $31.75 \pm 2.56\%$ for control plots. Soil organic horizon N % was 1.33 ± 0.10 for fertilized and 1.45 ± 0.09 for control plots. Fertilized plots had C:N of 23.26 ± 0.91 and control plots had C:N of 22.15 ± 1.08 . Overall, bulk density was 37.00 ± 4.63 kg soil m⁻² and 42.99 ± 5.01 kg soil m⁻² for control and fertilized plots, respectively (mean \pm standard error).

There were significant main effects of treatment on total nematode abundance, nematode family richness, Shannon diversity, MI, PPI, and EI. Overall, the total nematode abundance, plant parasitic nematode abundance, EI, and PPI were significantly greater in fertilized plots (Table 1), while family richness, Shannon diversity, and MI were significantly greater in control plots (Table 1). Total nematode abundance was 40–96% greater and plant parasitic nematode abundance was 136–236% greater in the fertilized plots than the control. Post hoc tests showed that the community structure (e.g., relative abundances) was more variable by year in the control plot (Table 2), while the absolute abundance was more variable by year in the fertilized plots despite relative abundances remaining constant (Table 1). We enumerated additional soil fauna – rotifers, tardigrades, and enchytraeids – that were present in the sample extracts, and there was a significant main effect of treatment on rotifers, which were also more abundant in the fertilized plots (Table 1).

Thirteen nematode families were identified in subalpine soils (Table 2). All families were found in both treatments, except Aporcelaimidae, which was only found in control plots. Treatment was a significant main effect on the relative abundance of all bacterivore families: Plectidae, Rhabditidae, Cephalobidae; plant parasitic Tylenchidae; fungivorous Aphelenchidae; and predaceous Aporcelaimidae. In fertilized plots, Rhabditidae (22–25%) and Tylenchidae (12–15%) made up a greater proportion of the community compared to control plots (6–7% and 3–6%, respectively) (Table 2, LSMeans, $p < 0.05$), while Aphelenchidae, Aporcelaimidae, Cephalobidae, and Plectidae were relatively more abundant in the control (Table 2).

We observed an inverse relationship between the nematode MI and the PPI (Fig. 1), where the MI was significantly lower but the PPI was significantly greater in fertilized plots compared to control plots

Table 1

Mean values of nematode abundance, richness, diversity, nematode trophic group abundances, nematode community indices, and other soil fauna groups (\pm standard error, $n = 12$). Significant results from the mixed model are shown ($p < 0.05$). Different letters denote significant differences across treatments and years (LSMeans, $p < 0.05$). Groups with no letters had no significant differences between years/treatments.

	Control		Fertilized		Model Summary
	2014	2015	2014	2015	
Nematode abundance (kg dry soil ⁻¹)	20484 \pm 3343ac	11356 \pm 2108b	40168 \pm 9227a	15854 \pm 1458bc	T, Y
Nematode family richness	10.67 \pm 0.38a	9.67 \pm 0.48ac	8.17 \pm 0.55b	8.33 \pm 0.45bc	T
Shannon diversity (H')	2.04 \pm 0.04a	1.90 \pm 0.05ac	1.64 \pm 0.06b	1.82 \pm 0.07bc	T, T*Y
Nematode trophic group abundance (kg dry soil⁻¹)					
Bacterivore	7245 \pm 1123a	2366 \pm 385b	12507 \pm 3179a	5436 \pm 317a	Y, T*Y
Fungivore	6154 \pm 1244abc	3096 \pm 550ac	13318 \pm 4368b	3541 \pm 746c	Y
Plant parasite	2440 \pm 407 ab	1628 \pm 430a	8194 \pm 2232b	3838 \pm 560b	T
Omnivore	3030 \pm 655a	2423 \pm 712 ab	4073 \pm 1474a	1116 \pm 199b	
Predator	932 \pm 217	1288 \pm 305	876 \pm 180	1405 \pm 294	
Nematode community indices					
MI	2.05 \pm 0.10a	2.28 \pm 0.08a	1.46 \pm 0.08b	1.52 \pm 0.11b	T
PPI	0.35 \pm 0.06ac	0.25 \pm 0.04 ab	0.47 \pm 0.07bd	0.54 \pm 0.09cd	T
EI	53.61 \pm 1.80a	54.17 \pm 1.96a	74.55 \pm 3.07b	77.25 \pm 3.71b	T
SI	60.99 \pm 3.36 ab	73.00 \pm 2.94ac	50.85 \pm 7.34b	64.02 \pm 4.50bc	
Other soil fauna abundances (kg dry soil⁻¹)					
Rotifers	960 \pm 227a	1373 \pm 242 ab	2297 \pm 449b	1627 \pm 398 ab	T
Tardigrades	763 \pm 254	442 \pm 81	976 \pm 409	864 \pm 214	
Enchytraeids	114 \pm 30	217 \pm 57	187 \pm 62	133 \pm 31	

(Table 1). Additionally, EI values were significantly greater in fertilized plots while there was no significant difference in SI values between control and fertilized (Table 1 and Fig. 2). On the structure-enrichment plot, both control and fertilized communities were structured and enriched (quadrant B, Fig. 2).

After 19 years of N addition to the fertilized plots, dissimilarity tests based on the Bray-Curtis distance showed that nematode communities from the fertilized plots were significantly different from the control plots when assessed at the family level (Fig. 3, PERMANOVA $p = 0.001$). There were interannual differences in community structure and the community composition shifted between 2014 and 2015 (Fig. 3, PERMANOVA $p = 0.006$) with a significant decrease in the relative abundance of Aphelenchidae and an increase in Dorylaimidae and Monochidae for control plots (Table 2).

4. Discussion

Despite their critical role in regulating terrestrial ecosystem processes, such as nutrient cycling and energy flow, higher trophic levels of

Table 2

Nematode families shown by their trophic group, colonizer-persister value ($c-p$), and relative abundance (%) by year and treatment (mean \pm standard error, $n = 12$). Significant results from the mixed model for each family are shown ($p < 0.05$); T = treatment and Y = year. Different lowercase letters denote significant differences across treatments and years (LSMeans, $p < 0.05$). Groups with no letters had no significant differences between years/treatments.

Trophic group	$c-p$	Family	Control		Fertilized		Model Summary
			2014 (%)	2015 (%)	2014 (%)	2015 (%)	
Bacterivore	2	Plectidae	0.08 \pm 0.01a	0.04 \pm 0.01a	0.04 \pm 0.01bc	0.05 \pm 0.01ac	T, Y, T*Y
	1	Rhabditiidae	0.07 \pm 0.01a	0.06 \pm 0.01a	0.22 \pm 0.04b	0.25 \pm 0.04b	T
	2	Cephalobidae	0.10 \pm 0.02a	0.11 \pm 0.01a	0.03 \pm 0.01b	0.06 \pm 0.01b	T
Fungivore	2	Aphelenchidae	0.10 \pm 0.02a	0.02 \pm 0.00b	0.01 \pm 0.00b	0.01 \pm 0.01b	T
	2	Aphelenchoididae	0.28 \pm 0.02 ab	0.29 \pm 0.02 ab	0.31 \pm 0.03a	0.20 \pm 0.02b	T*Y
Plant parasite	2	Tylenchidae	0.03 \pm 0.01a	0.06 \pm 0.01a	0.15 \pm 0.02b	0.12 \pm 0.02b	T
	3	Hoplolaimidae	0.02 \pm 0.00	0.01 \pm 0.00	0.02 \pm 0.00	0.04 \pm 0.01	
	3	Dolichodoridae	0.04 \pm 0.01	0.01 \pm 0.00	0.02 \pm 0.00	0.05 \pm 0.02	T*Y
	3	Criconeematidae	0.03 \pm 0.02	0.02 \pm 0.00	0.02 \pm 0.01	0.01 \pm 0.00	
	3	Pratylenchidae	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	
Predators/Omnivores	4	Dorylaimidae	0.08 \pm 0.01a	0.16 \pm 0.02b	0.09 \pm 0.02a	0.08 \pm 0.02a	Y, T*Y
	5	Aporcelaimidae	0.07 \pm 0.02a	0.04 \pm 0.01a	0.00 \pm 0.00b	0.00 \pm 0.00b	T
	4	Monochidae	0.05 \pm 0.01a	0.12 \pm 0.02bc	0.03 \pm 0.01a	0.08 \pm 0.02ac	Y

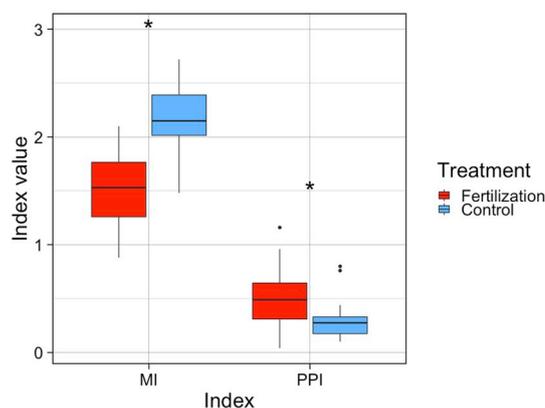


Fig. 1. Maturity index and plant parasite index. Colors show treatment: fertilized is red and control is blue. Asterisks denote significant differences in treatment effects ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

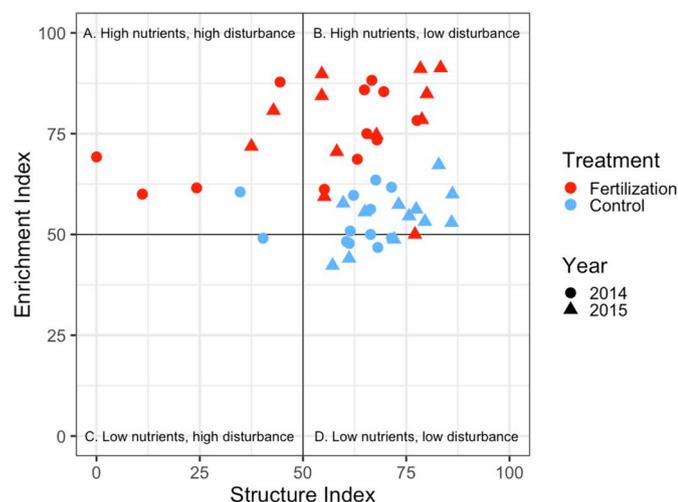


Fig. 2. Structure-Enrichment plot by treatment and year. Quadrants are labeled A thru D after Ferris et al. (2001). Colors show treatment: fertilized is red and control is blue. Shapes show year: circles are 2014 and triangles are 2015. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the belowground food web have rarely been considered in studies of N addition. This is likely due to the enormous diversity belowground and highly complex food web structure, which is difficult to identify and characterize. Additionally, both top down and bottom up forces act on soil food web structure where the strength of trophic relationships and the relative dominance of bacterial or fungal-driven structure are closely tied to disturbances, ecosystem type, and soil nutrient status (de Ruiter et al., 1995; Moore et al., 2003). In turn, these relationships influence ecosystem function and structure, and affect a range of ecosystem components from microbial biomass to aboveground productivity (Moore et al., 2003; Sackett et al., 2010). Fertilization has a positive effect on soil fauna across ecosystems such as the subarctic (Sjursen et al., 2005), temperate grasslands (Cole et al., 2005), and coniferous forests (Marshall, 1974; Sohlenius and Wasilewska, 1984) via bottom-up effects of enhanced food availability and quality. After

18–19 years of 25 kg N ha⁻¹ yr⁻¹ N addition, our results show that N addition positively affects the abundance of soil nematodes – and opportunistic bacterivores in particular – for subalpine spruce and fir forests of the Colorado Rocky Mountains. These results indicate high levels of grazing and bacterial turnover, which could be a mechanism for ecosystem microbial biomass and C loss.

The relative dominance of the fungal component of forest soil food webs has been generally accepted and shown for both deciduous and coniferous forests in locations throughout the world (Kageyama et al., 2008; Ananyeva et al., 2010), but this can depend on factors from altitude (Siles and Margesin, 2016) to soil horizon (Baldrian et al., 2012). However, fertilization can reduce fungal biomass (Demoling et al., 2008) and induce shifts from fungal-based to bacterial-based soil food webs (Bardgett and Chan, 1999; Bardgett and McAlister, 1999), with important consequences for resource availability for soil fauna and food web stability (Moore and de Ruiter, 2000). In a study of maple forests in the Great Lakes Region with similar N addition (30 kg N ha⁻¹) and length (17 years) to our study, microarthropod abundance declined with fertilization and this change was attributed to bottom-up effects through the decline in fungal hyphae (Gan et al., 2013). In other long term experiments (13–14 years), the total microbial biomass and the fungal:bacterial ratio decreased in fertilized experimental plots compared to control plots in Northeastern United States forests (Wallenstein et al., 2006). Treseder (2008) found that total microbial community and fungal abundance decrease as N load and duration of N addition treatments increase in a meta-analysis of N addition studies. At our study site, Boot et al. (2016) showed that both control and fertilized plots were bacterial dominant (high bacterial:fungal ratios) with lower soil C and microbial biomass for fertilized plots in this subalpine forest. We expected that this loss could be attributed to trophic cascades in the soil food web where elevated grazing by microbivore fauna leads to increased turnover of microbial biomass.

We hypothesized that fertilized plots would have greater total nematode abundance, be bacterivore dominated, and would exhibit lower diversity due to loss of predators and omnivores, which respond negatively to ecosystem disturbance. The fertilized plots had 40–96% greater total abundance of nematodes, while the nematode community was less diverse and ecologically mature (e.g., lower MI) compared to the control, confirming this hypothesis. These changes were driven by

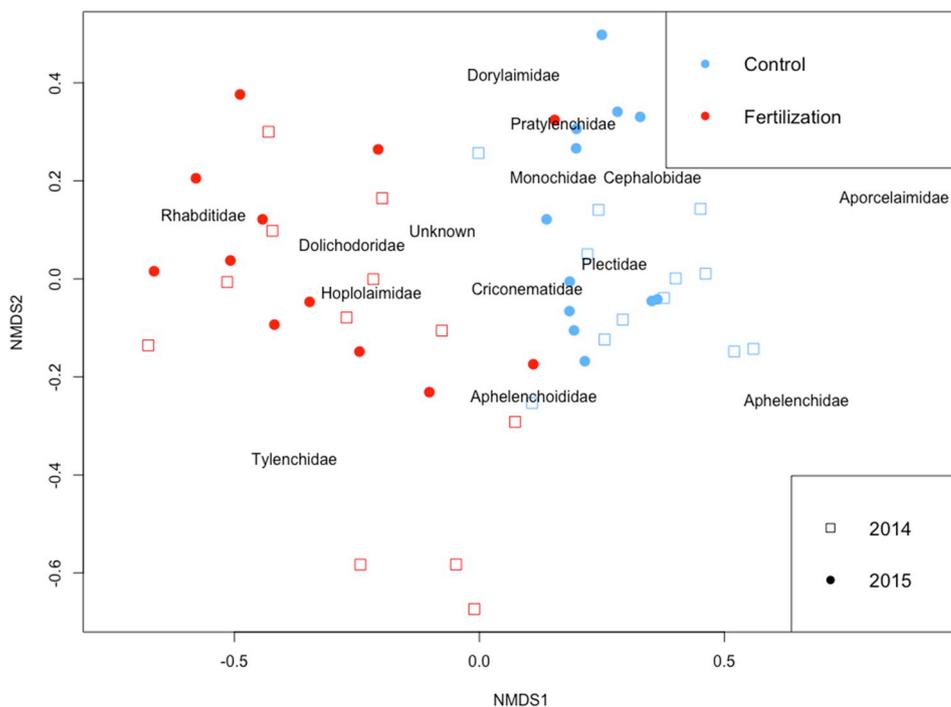


Fig. 3. NMDS ordination (Bray-Curtis dissimilarity) of nematode communities based on relative abundances of nematode families. Each point reflects the community found in an individual sample (n = 12 per treatment x 2 years). Points that are close together have more similar communities than points that are far apart. Colors show treatment: fertilized is red and control is blue. Shapes show year: open squares are 2014 and filled circles are 2015. Names of nematode families are overlaid. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the relative dominance of rhabditid bacterivores in fertilized plots. This group is opportunistic and responds rapidly to flushes of resources. In the subarctic heath and fellfields, Ruess et al. (2002) observed similar dominance of rhabditids in the nematode community after 7 years of N addition (100 kg N ha^{-1}). In a Swedish pine forest, Sohlenius and Wasilewska (1984) found that rhabditid bacterivores increased in fertilized vs. control plots – especially when these plots were also irrigated – after 7 years of N addition at $70\text{--}200 \text{ kg N ha}^{-1}$. Such changes also occur over shorter time scales. After 3 years of a poplar microcosm experiment, both total nematode abundance and total rhabditid abundance was greater in high N compared to low N soil (Hoeksema et al., 2000). The response of *c-p* 1 bacterivores to nutrient flushes is typically considered transient, and for this group to dominate 19 years after N addition began suggests that the enrichment and turnover through the bacterial pathway is either sustained over time or the flush of resources does not become available immediately upon N addition. Bongers et al. (1997) found similar results and showed that differences in nematode communities (as evidenced by MI) persisted for 19 years after nutrient addition ceased.

The MI and PPI are two indices that often respond in opposite directions to increased nutrient availability (Bongers et al., 1997). Indices for plant parasitic nematodes (e.g., the PPI) are calculated separately from the total nematode community (e.g., the MI) because of differing life strategies, which result in contrasting responses to disturbance (Bongers, 1990). While other studies have found that the MI and PPI respond in the same direction to disturbance (Neher and Lee Campbell, 1994), this was to physical disturbance and not nutrient status. The MI decreases as a result of an increasing proportion of bacterivore enrichment colonizers (*c-p* 1), while the PPI increases due to higher quality plant tissue which results in an increased carrying capacity for plant parasitic nematodes (*c-p* 3 to 5; Bongers et al., 1997). We observed a significant increase in the PPI in fertilized plots compared to control plots. An increase in the PPI is related positively to soil nutrient enrichment, and has been observed in systems undergoing N amendments (Bongers et al., 1997). However, some studies have found no changes in the PPI with N addition (Li et al., 2013; Song et al., 2016), but these studies took place on grasslands, were shorter (4 and 1 year), and applied higher concentrations of N (100 kg N ha^{-1}). Differences in plant parasitic responses to N could also be due to plant species identity and their differing responses to N addition (Wardle et al., 2003). In our study, the tylenchid root associates (Yeates et al., 1993) increased in abundance in the fertilized plots. Sohlenius and Wasilewska (1984) also observed an increase in tylenchids in a Swedish pine forest undergoing fertilization ($70\text{--}200 \text{ kg N ha}^{-1}$), which was possibly due to elevated root growth. Higher root turnover in high N compared to low N soil was associated with greater abundance of microarthropods for a microcosm study examining poplar cuttings over one season (Lussenhop et al., 1998). However, coniferous roots are likely longer-lived and much slower to turn over compared to deciduous roots (Matamala et al., 2003). In deciduous forests of the Eastern United States, root exudation is affected by fertilization and increases with N addition despite loss of fine root biomass (Phillips et al., 2010). Further work on root exudation, biomass, and turnover is needed to understand the mechanism of plant parasite response and feedback to the subalpine forest.

We expected the fertilized nematode communities to have a higher EI and a lower SI than the control. While fertilized communities had a significantly greater EI than control, there were no differences in SI (Table 1). Thus, fertilization treatment resulted in an increased abundance of enrichment opportunists (bacterivore colonizers, *c-p* 1) as expected, without affecting the absolute abundance of the omnivores and predators (persisters, *c-p* 4 and 5), which was unexpected since omnivores and predators are sensitive to disturbance (Bongers, 1990). Only the predator Aporcelaimidae was negatively affected by N addition (Table 2). Other omnivore and predator groups such as Dorylaimidae and Monochidae were not affected by N addition, and overall, there was no difference in total omnivore and predator

abundance between the treatments (Table 1). As a result, both fertilized and control plots were structured (no significant differences between SI, Table 1 and Fig. 2), with equal trophic links. In other studies using greater rates of N application ($70\text{--}100 \text{ kg N ha}^{-1}$), the abundance of omnivore and predator nematodes declines with N addition (Sohlenius and Wasilewska, 1984; Li et al., 2013; Song et al., 2016). Our study did not find declines in higher trophic level abundance with fertilization. Some plausible explanations include: 1) our application rate was low enough to avoid effects on sensitive groups, 2) our application time was long enough for these groups to adapt, or 3) background deposition of N on the control plots causes similar effects on sensitive groups so detection was not possible. On the structure-enrichment plot, both control and fertilized treatments are in quadrant B (Fig. 2). These communities are characterized as diverse, mature food webs, occur in ecosystems that are N-enriched, and experience low levels of disturbance (Ferris et al., 2001). We expected control communities in the structure-enrichment plot to fall into quadrant C, which is typical for undisturbed forests (Ferris et al., 2001). The MI and PPI were lower while the EI and SI were higher for all treatments in our study compared to results from a study of unfertilized pine and sweetgum forests in the Eastern United States (Neher and Weicht, 2013). The high enrichment of the control communities in our study may be due to the natural atmospheric N deposition, which is an order of magnitude greater than pre-European settlement background values (Baron, 2006). This background N deposition could be driving the subalpine system towards a bacterially dominant one, atypical for forests soil food webs, but often found in fertilized systems (Treseder, 2008).

Despite soil sampling on the same calendar date each year, we observed temporal variation in nematode communities. In particular, total abundance of nematodes in 2015 was reduced to only 0.39 to 0.55 times the abundance for 2014 (for control and fertilized, respectively), and this loss was especially evident for fungivore abundance (Table 1). These differences could be due to differences in season, such as total precipitation, timing of snowmelt, or soil moisture, which varies from year to year in the subalpine ecosystem. Total annual precipitation was lower in 2015 than 2014, (117 vs 138 cm, respectively; NADP: <http://nadp.slh.wisc.edu/>). However, soil moisture was 2.74 times greater in 2015 than 2014 (132.18 ± 15.44 vs $48.25 \pm 3.27\%$, respectively), and recent precipitation (1.69 vs 0.35 cm rain in 2015 and 2014, respectively; NADP: <http://nadp.slh.wisc.edu/>) was greater for the week prior to soil sampling. The overall reduction in nematode abundance could be due to a dry summer (12.5 vs 22.2 cm rain in 2015 and 2014, respectively; NADP: <http://nadp.slh.wisc.edu/>) despite the high soil moisture at sampling in 2015. The lower relative abundance of omnivores and higher relative abundance of bacterivores in 2014 control plots responded similarly as other studies, which have shown that omnivore nematode populations (e.g., dorylaimids) decline during winter (Wasilewska, 1971) and in seasons of high precipitation (Sun et al., 2016) while bacterivores increase during wet periods (Sohlenius, 1985).

In conclusion, we characterized the nematode communities in fertilized and control plots in a long-term N-addition study in a subalpine forest. Nematode communities were distinct between treatments and were more diverse and successional mature in control plots. Higher abundance of nematodes and an enriched food web characterized by opportunistic bacterivore and plant parasitic taxa – which are typical for transient flushes of resources – were present 19 years after treatments began. These changes in nematode community have ecosystem implications. A high abundance of bacterivore nematodes are indicative of a high rate of N mineralization (Wasilewska, 1979), which has been shown to increase in these subalpine forests in response to N deposition (Baron et al., 2000). Such a change in soil fauna abundance could then impact the growth of coniferous plants (Sackett et al., 2010). Further, our study complements findings of Boot et al. (2016) and suggests that lower microbial biomass in N-amended plots may be linked to top-down control by soil fauna.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2018.12.007>.

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