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Forest canopy maintains the soil community composition under elevated nitrogen deposition

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ABSTRACT

As an important agent of environmental change, atmospheric nitrogen (N) deposition could have profound effects on terrestrial ecosystems. However, previous studies simulating N deposition in forest ecosystems were mostly based on understory manipulations, often neglecting canopy processes (e.g., N retention). Here, we employed a novel field experiment simulating N deposition through the canopy addition of N (CAN), and explored how soil nematode communities change in response to elevated N deposition in comparison with the conventional approach of understory addition of N (UAN), at two levels of N concentration. We found that 52% and 44% of the N added to the forest canopy at two N concentration levels were retained by the forest canopy. The soil nematode community showed contrasting responses to different approaches of N addition. The conventional UAN approach decreased the abundance of most nematode trophic groups and community diversity compared with CAN approach. This detrimental effect was probably due to changes in fine root biomass and/or nematode community composition caused by the high concentration of N directly entering the soils without the canopy N retention process. Our results suggest that the conventional UAN approach might result in an incomplete and potentially misleading understanding of the effects of N deposition on forest ecosystems. The results show that previous studies might have overestimated the negative effects of N deposition on forest ecosystems by overlooking forest canopy processes. In conclusion, forest canopy N-interceptions contribute to maintaining the composition of soil communities and soil biodiversity under elevated N deposition. Our study helps reconcile some of the discrepancies in the existing literature, and demonstrate the urgent need to consider canopy processes in future N deposition studies.

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1. Introduction

Nitrogen (N) deposition has rapidly increased in recent decades due to industrialization and anthropogenic activities (Vitousek et al., 1997; Galloway et al., 2004, 2008). In particular, the mean rate of wet N deposition in China increased by about 25% from the 1990s to the 2000s (Jia et al., 2014), and reached an average rate of 19 kg N ha^{-1} yr^{-1} from 2010 to 2014 (Xu et al., 2015). As an agent of global change, N deposition profoundly influences the biodiversity and ecosystem functioning of terrestrial ecosystems, e.g., forest ecosystems (Sala et al., 2000; Liu et al., 2011; Zhou et al., 2018). As a major limiting nutrient for plant growth in most ecosystems, N supports the productivity of vegetation. However, excessive N inputs might be harmful to the balance of plant nutrition, which could reduce plant diversity and productivity (van Dijk and Roelofs, 1988; Aber et al., 1989; Gilliam, 2006; Mo et al., 2008; Lu et al., 2010; Basto et al., 2015), with cascading effects on higher trophic levels.

Given that soil food webs depend on plant inputs (i.e., plant litter, root exudates; Wardle et al., 2004), N deposition might induce cascading effects on the community structure of soil fauna (Boxman et al., 1998). In fact, previous studies simulating N deposition in forest ecosystems showed that elevated N deposition has significant detrimental effects on the structure of soil microbial (Frey et al., 2004) and faunal communities (Ruess et al., 1996; Xu et al., 2009; Zhao et al., 2014; Shao et al., 2017). Moreover, a recent meta-analysis indicated that soil nematode diversity and functions are significantly reduced under elevated N inputs (Liu et al., 2016).

The conventional approach to simulate N deposition is to directly add N to the forest floor or understory plants to investigate the ecosystem responses (Boxman et al., 1998; Frey et al., 2004; Mo et al., 2008; Sun et al., 2013). Therefore, most of the N added reaches the soil surface directly and rapidly. Consequently, N inputs often suppress the density and diversity of soil biota by directly changing the soil environmental conditions, e.g., soil acidification (Chen et al., 2015) and ammonium toxicity (Wei et al., 2012). However, Sievering et al. (2007), Gaige et al. (2007) and Nair et al. (2016) showed that 80%, 70%, and 61% of N added to the canopy is retained and recovered, respectively, by the forest canopy foliage and branches. Furthermore, foliar N-uptake has been proved to directly affect plant metabolism and tree growth (Sparks, 2009; Wortman et al., 2012). Therefore, if a large proportion of N is retained and utilized by the canopy, this might mitigate the direct influence of N on understory plants and soil biota. Consequently, the N retention and uptake processes occurring in the canopy have been largely overlooked when using the conventional approach to simulate N deposition. To explore this potential caveat, Zhang et al. (2015) employed the novel approach of canopy addition of N (CAN) to investigate the effects of N deposition on the structure and functions of forest ecosystems. In this case, soil biota might be mostly affected by N-induced changes in the resource inputs of arbor trees, and less so by detrimental changes to soil chemistry.

Nematodes are the most abundant group of soil invertebrates, representing ~80% of all multicellular animals (Bongers and Ferris, 1999), and they have the capacity to respond rapidly to changes in the nutrient status of soils (Bongers and Ferris, 1999). Nematodes are exceptionally diverse (Hugot et al., 2001) and represent various components of most trophic levels of soil food webs (Yeates et al., 1993), providing information on the structure and function of food webs (Bongers and Ferris, 1999). For example, nematodes provide information on soil energy channels through comparisons of "green" versus "brown energy channels", as well as main decomposition pathways in the soil (Ferris et al., 2001; Yeates, 2003). Furthermore, they are also used to assess the disturbance level and structural complexity of soil food webs (Moore and Hunt, 1988; Ferris et al., 2001). Thus, the diversity and community indices of soil nematodes are common and powerful predictors of soil biodiversity and soil food web structure (Yeates, 2003). Considering the great diversity and bioindicator potential of soil nematodes, investigating the responses of soil nematode communities to elevated N deposition could improve our understanding of how soil food webs respond to environmental disturbances induced by anthropogenic activities. Additionally, it would allow us to predict the effects of the consequences of this response for ecosystem functioning.

The present study aimed to improve our understanding of how soil nematode communities respond to elevated N deposition. Specifically, a novel and more realistic manipulation of N addition (canopy addition of N, CAN) was compared with the conventional approach of N application (understory addition of N, UAN). We hypothesized that: 1) the novel CAN approach might have less significant impacts on soil nematode communities than UAN, 2) the conventional UAN approach overestimates the effects of N deposition on soil nematode communities, and 3) higher concentrations of N addition might have greater impacts on soil nematode communities than lower levels of N addition. Our results are expected to challenge the conclusions drawn from previous studies in forests using the conventional approach of simulating N deposition effects.

2. Materials and methods

2.1. Study site

This experiment was conducted in a mixed deciduous forest in Jigongshan (JGS) National Natural Reserve (31°46′–31°52′ N, 114°01′–114°06′ E), Henan Province, Central China. The site at JGS has a warm temperate climate. The mean annual temperature is 15.2 °C, and the mean annual precipitation is 1119 mm (according to temperature and precipitation records of the last 60 years). The ambient N deposition rate in rainfall is 19.6 kg N ha^{-1} yr^{-1} (Zhang et al., 2015). This site has a yellow-brown sandy-loam soil with a pH value of 4.2. The forest is 45 years old, and the canopy tree species are dominated by *Quercus acutissima* Carruth., *Quercus variabilis* Blume, and *Liquidambar formosana* Hance (Zhang et al., 2015).

2.2. Experimental design

The experiment was a randomized block design with four blocks. Two concentrations of N (25 kg N ha^{-1} yr^{-1} and 50 kg N ha^{-1} yr^{-1}) were added through two approaches: canopy addition of N (CAN) and understory addition of N (UAN). Treatments included: 1) canopy addition of N 25 kg N ha^{-1} yr^{-1} (CAN25); 2) canopy addition of N 50 kg N ha^{-1} yr^{-1} (CAN50); 3) understory addition of N 25 kg N ha^{-1} yr^{-1} (UAN25); 4) understory addition of N 50 kg N ha^{-1} yr^{-1} (UAN50); and 5) control (CK, without N addition) (Zhang et al., 2015). The four treatments and control were randomly assigned to five circular plots in each of the blocks. Twenty plots were established in total, and each plot covered an area of 907 m^2 (with a diameter of 34 m).

Canopy addition of N was achieved by placing spraying systems on the top of towers. Towers were built in the center of the CAN25 and CAN50 treatment plots at a height of 35 m (5–8 m above the forest canopy) to support the PVC pipelines (10 cm in diameter), which transferred N solution. Understory addition of N was achieved by an automatic irrigation system at a height of 1.5 m above the ground (Zhang et al., 2015), which represented the conventional method of N addition (Shi et al., 2016). From 2013, treatments were applied monthly through each year from the start of the growing season, i.e., from mid-April, until mid-October, according to the phenology of the JGS forest (Zhang et al., 2015). The same frequency and duration of treatments were used for both approaches.

For each N application event, an appropriate amount of NH_4NO_3 pellets was added to water to reach the target concentration. The total volume of the solution used in each plot per year was less than 2% of the equivalent mean annual rainfall. Therefore, the potentially confounding effect caused by water addition was negligible (Zhang et al., 2015).

2.3. Sampling and analysis of throughfall and stemflow

To quantify the amount of N input to forest floor under the treatments of CAN and UAN, throughfall and stemflow in each treatment plot were collected and measured during the growing season of 2016. For throughfall collection, three L-shaped PVC troughs (diameter 185 mm and length 1 m at each side) were connected with a drain-hosepipe (diameter 35 mm), which led to opaque plastic buckets that were randomly placed beneath the crown of dominant tree species (*Quercus acutissima* Carruth., *Quercus variabilis* Blume and *Liquidambar formosana* Hance.) in each plot (Fig. S1). The trough was fixed by Y-brackets 1 m above ground, with a sampling area of 0.387 m^2. A downward bent stainless net (bore diameter = 18 mm and wire diameter = 1.2 mm) was installed above the trough to prevent contamination by litterfall.

In order to collect stemflow, three stemflow collection devices were installed separately on the trunk of dominant tree species. Stemflow was collected by an ethylene-vinyl acetate copolymer (EVA) encircling collection device, which has been patented (Patent No. ZL201621395698.2). Briefly, this device includes a 25 cm width EVA material with its 5 cm basal edge surrounding tree trunk to intercept stemflow and an inserted pipe draining the intercepted stemflow to another opaque plastic bucket with the same size as used for throughfall collection. All buckets contained an opaque barrelhead to minimize evaporation and photolytic degradation.

Throughfall collection troughs and storage buckets were rinsed with deionized water after every sampling to avoid subsequent sample contamination. Samplings were conducted according to the amount of precipitation and the dates of N application. If the collected throughfall volume approached or exceeded half of the max volume (71 L) of the bucket, then the throughfall and stemflow were recorded and collected. Generally, there were one to three times sampling during the interval of N application. The total volume of throughfall and stemflow were recorded through the inner volume scale of the bucket. About 60 mL of throughfall or stemflow from each bucket was sealed in HDPE bottles and stored at 4 °C for later chemical analysis. The throughfall and stemflow samples were filtered using Whatman 0.45-μm filter paper. The concentrations of total dissolved N (TDN) in filtered samples were determined using a TOC analyzer (Shimazu, Japan).

To calculate per unit area N input into the forest floor via throughfall and stemflow, we used the amount of total dissolved N divided by the corresponding sampling area. The amount of total dissolved N was calculated as the concentration multiplied the volume of each corresponding sample. Since the study site is a mature forest with a closed canopy, per unit canopy area and per unit sample area are almost identical (Tomaszewski et al., 2003). Thus, we simply deemed the sample tree crown area to be the sampling area of stemflow. Moreover, we calculated the monthly cumulative amount of N input by throughfall and stemflow of the different treatments during the whole growing season.

2.4. Sampling and analysis of plant material

A composite litter sample for each plot was collected in May 2016, July 2016, and November 2016. Specifically, two leaf litter samples were collected from the soil surface of two randomly selected areas (25 cm × 25 cm) and combined into a single composite sample in each plot. As a result, 20 samples (5 treatments × 4 blocks × 1 composite sample) were collected during each litter sampling event.

October 2014, fine root samples were collected in a selection of plots. To avoid too much soil disturbance due to the fine root collection, we randomly selected 13 out of the 20 available plots (CK, 2 plots; CAN25, 3 plots; CAN50, 3 plots; UAN25, 3 plots; UAN50, 2 plots). In each of the selected plots, 2–3 samples (an area of 30 cm × 30 cm and a depth of 20 cm) were collected, and fine roots were picked out by hand and combined in a composite sample for fine root biomass analyses. Therefore, 34 fine root samples (CK, 6 samples; CAN25, 9 samples; CAN50, 7 samples; UAN25, 7 samples; UAN50, 5 samples) were collected in total. After cleaning and oven-drying the plant material at 70 °C for 48 h, biomass of litter and fine root were measured. Afterwards, all litter samples were ground to fine powder to measure litter carbon (LC), and litter N (LN) with a Flow-Injection Autoanalyzer (FIA, Lachat Instruments, USA). The biomass of litter and fine root was standardized to g dry weight m^{-2}.

2.5. Sampling and analysis of soil properties and biodiversity

Soil was sampled to extract nematodes in June 2013, August 2013, October 2013, June 2014, August 2014, October 2014, June 2015, October 2015, and June 2016. For each sampling event, eight soil cores (5 cm in diameter) were collected and combined into a single composite sample from each plot at 0–10 cm and 10–20 cm soil depths. As a result, 40 samples (5 treatments × 4 blocks × 2 depths) were collected during each soil sampling event. Litter was removed from the soil surface before soil samples were taken. Visible roots in the soil samples were picked out by hand. In addition, microbial biomass and soil properties were measured using the soil samples collected in June 2016.

Nematodes were extracted from 50 g fresh soil with Baermann funnels for each composite soil sample (Barker, 1985). The number of nematodes was counted with an inverted microscope (Nikon TS100, Tokyo, Japan) after fixation in 4% formalin solution. The first 100 individuals (nematodes) encountered were identified to the genus level using a differential interference contrast (DIC) microscope (Nikon 80i, Tokyo, Japan). All nematodes were identified, when there were fewer than 100 individuals in a sample. The identified nematodes were classified into four trophic groups (bacterivores, fungivores, omnivore-predators, and herbivores) according to Yeates et al. (1993) except *Filenchus* into fungivores (McSorley and Frederick, 1999; Okada et al., 2005) and into functional guilds (Bongers, 1990; http://nemaplex.ucdavis.edu/Ecology/EcophysiologyParms/GenusParmsQuery.aspx (November 2018)).

Soil water content was measured by placing samples in an oven at 105 °C for 48 h. Soil pH was determined in a soil/deionized water suspension (1:2.5) with a pH meter (Mettler Toledo, Shanghai, China). Soil organic carbon (SOC, g kg^{-1} dry soil) was measured by dichromate oxidation. Soil total N (STN, g kg^{-1} dry soil) was measured using an ultraviolet spectrophotometer after Kjeldahl digestion (Liu, 1996). Soil ammonium (NH_4^+-N, mg kg^{-1} dry soil) and nitrate (NO_3^--N, mg kg^{-1} dry soil) were measured with a Flow-Injection Autoanalyzer (FIA, Lachat Instruments, USA) after 2 M KCl extraction. Soil microbial biomass carbon (MBC, mg kg^{-1} dry soil) and soil microbial biomass N (MBN, mg kg^{-1} dry soil) were measured by the method described by Vance et al. (1987).

2.6. Data analysis

Canopy N interception rate (CNIR) were calculated with the N intercepted by canopy (NIC) and the N applied to the forest canopy (NAC) as follows:

$$CNIR = NIC/NAC$$

$$NIC_x = NAC_x - Stemflow_x - (Throughfall_x - Throughfall_{CK})$$

NAC_x, the N applied to the forest canopy under CAN25 and CAN50 were 3.76 kg N ha^{-1} and 7.52 kg N ha^{-1} in each N application event; x represents the specific treatment.

The abundance of each nematode trophic group was calculated and converted to individuals per 100 g dry soil. Nematode community indices including enrichment index (EI), structure index (SI), channel index (CI), maturity index (MI), and plant parasite index (PPI) were calculated according to Ferris et al. (2001) and Bongers (1990).

The diversity of the nematode community at the genus level was

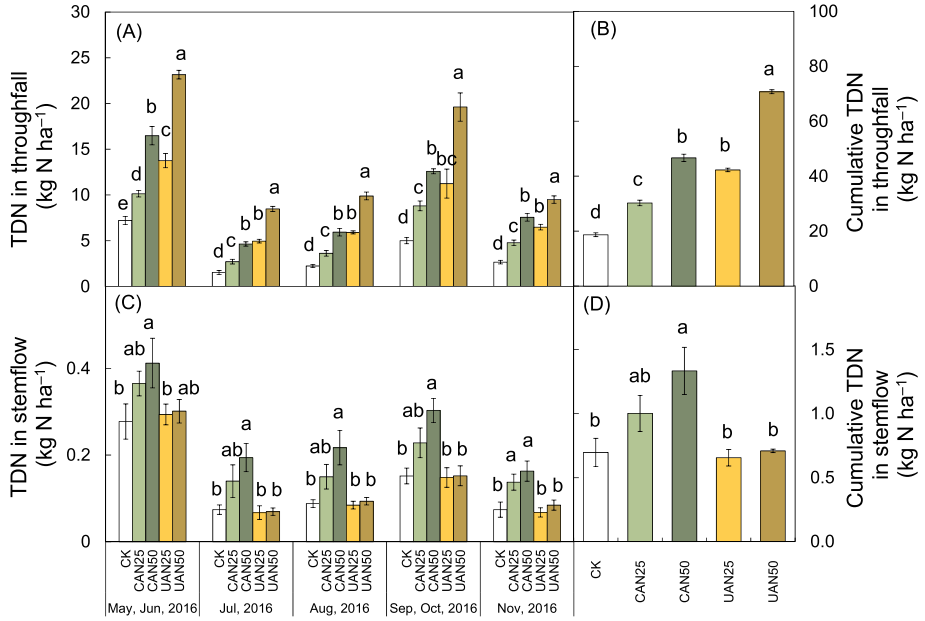

Fig. 1. Temporal dynamics and cumulative values of total dissolved nitrogen (TDN) in throughfall and stemflow under the control (CK), canopy addition of N with 25 kg N ha^{-1} yr^{-1} (CAN25), canopy addition of N with 50 kg N ha^{-1} yr^{-1} (CAN50), understory addition of N with 25 kg N ha^{-1} yr^{-1} (UAN25), and understory addition of N with 50 kg N ha^{-1} yr^{-1} (UAN50). Data are means ± SE (n = 4).

assessed using the Shannon-Wiener index, $H' = -\sum_{i=1}^{S} Pi(lnPi)$ and the Richness index, SR = $(S-1)/\ln N$; where *Pi* is the proportion of the individual genus (*i*) in the total nematode community, *S* is the number of total genera in the community, and *N* is the total number of nematodes in the community (Neher and Darby, 2009).

Post-hoc tests (LSD) were applied on TDN in stemflow and throughfall in each sampling event, and on cumulative TDN to compare differences among all N addition treatments and CK.

Two-way repeated measures ANOVAs were applied to test the effects of N addition approach (A: CAN and UAN), N addition concentration (C: 25 kg N ha^{-1} yr^{-1} and 50 kg N ha^{-1} yr^{-1}), and their interaction on litter quantity, LC, LN, soil nematode abundances (bacterivores, fungivores, omnivore-predators, herbivores, and total nematodes), nematode community indices (EI, SI, CI, MI, and PPI), nematode diversity indices (H' and SR), and dominant genera which apparently responded to the treatments (Bacterivores: *Acrobeloides* and *Prismatolaimus;* Fungivores*: Aphelenchoides* and *Filenchus;* Omnivore-Predators*: Pungentus* and *Aporcelaimellus;* Herbivores*: Basiria* and *Hoplotylus*; Table S4). Post-hoc tests (LSD) were applied to compare differences among all N addition treatments and CK.

Two-way ANOVAs were applied to test the effects of N addition approach (A: CAN and UAN), N addition concentration (C: 25 kg N ha^{-1} yr^{-1} and 50 kg N ha^{-1} yr^{-1}), and their interaction on fine root biomass, soil properties (soil pH, SOC, STN, NH_4^+-N, and NO_3^--N), and microbial biomass (MBC and MBN). Post-hoc tests (LSD) were applied to compare differences among all N addition treatments and CK. Pearson correlation analyses were used to explore the potential relationships between soil microbial biomass, abundance of nematode trophic groups, and soil properties for the final sampling event (June, 2016). All data were log transformed before the analyses to allow for parametric statistical tests. Differences were considered significant at $P < 0.05$. Statistical analyses were performed using SPSS 19 (SPSS Inc., Chicago, IL).

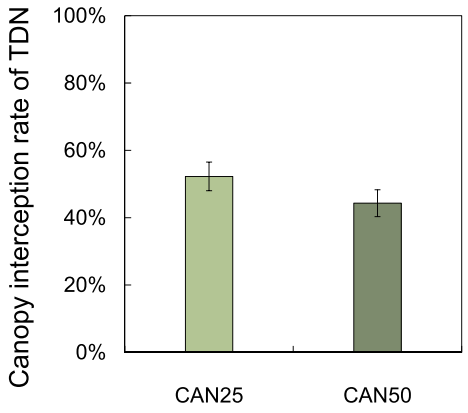

Fig. 2. The ratio of the N intercepted by the canopy to the N applied to the forest canopy under the canopy addition of N with 25 kg N ha^{-1} yr^{-1} (CAN25), canopy addition of N with 50 kg N ha^{-1} yr^{-1} (CAN50). Data are means ± SE (n = 4).

3. Results

3.1. Throughfall, stemflow, and interception rate

The high N concentration treatment (with N addition of 50 kg N ha^{-1} yr^{-1}) increased the mean and cumulative content of total dissolved N in throughfall compared to the control and low N concentration treatment (with N addition of 25 kg N ha^{-1} yr^{-1}) in both CAN and UAN (Fig. 1A and B). Furthermore, the content of the total dissolved N in UAN was higher than in CAN at the same N concentration level (Fig. 1A and B). In addition, the mean and cumulative content of total dissolved N in stemflow under CAN was higher than in UAN and control (Fig. 1C and D). The high N concentration treatment in CAN increased the mean content of the total dissolved N in stemflow in comparison with the low N concentration treatment in CAN (Fig. 1C and D). Overall, mean canopy interception rate of total dissolved N of the forest canopy under CAN25 and CAN50 was 52% and 44%, respectively (Fig. 2).

Table 1
Effects of N addition approach (A), N concentration (C), sampling time (t), and their interactions on plant litter properties assessed by two-way repeated measures ANOVAs.

	A		C		A × C		t		t × A		t × C		t × A × C	
	$F_{(1,12)}$	P	$F_{(1,12)}$	P	$F_{(1,12)}$	P	$F_{(2,24)}$	P	$F_{(2,24)}$	P	$F_{(2,24)}$	P	$F_{(2,24)}$	P
Litter	0.872	0.369	0.620	0.446	0.001	0.978	4.179	**0.028**	0.283	0.756	1.394	0.267	3.738	**0.039**
LC	0.132	0.722	1.373	0.264	0.033	0.859	0.006	0.994	1.090	0.352	0.804	0.459	0.024	0.977
LN	0.146	0.709	5.971	**0.031**	0.906	0.360	105.874	**<0.001**	2.044	0.151	0.867	0.433	4.951	**0.016**

Litter: leaf litter quantity; LC: litter carbon; LN: litter nitrogen; significant ($P < 0.05$) effects are presented in bold.

Table 2
Effects of N addition approach (A), N concentration (C), and their interactions on root, soil properties and microbial biomass assessed by two-way ANOVAs.

Variable	Soil depth (cm)	A		C		A × C	
		$F_{(1,12)}$	P	$F_{(1,12)}$	P	$F_{(1,12)}$	P
Root	0–20	5.690	**0.025**	1.829	0.189	0.088	0.769
	–	–	-	–	–	–	–
Soil pH	0–10	0.077	0.786	1.604	0.229	0.024	0.880
	10–20	4.538	0.055	0.580	0.461	0.122	0.732
SOC	0–10	0.014	0.907	1.013	0.334	0.750	0.403
	10–20	0.487	0.499	0.041	0.842	4.155	0.064
STN	0–10	0.100	0.757	2.469	0.142	0.875	0.368
	10–20	1.832	0.201	3.268	0.096	0.000	0.992
NH_4^+-N	0–10	0.117	0.738	0.638	0.440	2.349	0.151
	10–20	0.822	0.382	0.005	0.948	1.837	0.200
NO_3^--N	0–10	4.611	0.053	1.696	0.217	1.713	0.215
	10–20	0.336	0.573	0.011	0.918	0.797	0.390
MBC	0–10	0.003	0.958	1.012	0.334	1.520	0.241
	10–20	0.208	0.656	1.038	0.328	1.606	0.229
MBN	0–10	0.189	0.671	1.862	0.197	0.240	0.633
	10–20	0.824	0.382	0.021	0.887	3.073	0.105

Root: fine root biomass (df value = 1, 24); SOC: soil organic carbon; STN: soil total nitrogen; NH_4^+-N: ammonium; NO_3^--N: nitrate; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; significant ($P < 0.05$) effects are presented in bold.

3.2. Litter and fine root material

N addition approach had no significant effects on leaf litter quantity, LC and LN (Table1). However, UAN approach decreased fine root biomass compared with the CAN approach ($F = 5.690$, $P = 0.025$; Tables 2, 3). Furthermore, high N concentration (with N addition of 50 kg N ha^{-1} yr^{-1}) increased LN in comparison with low N concentration (with N addition of 25 kg N ha^{-1} yr^{-1}) ($F = 5.971$, $P = 0.031$; Tables 1, 3).

3.3. Soil properties and microbial biomass

UAN approach tended to increase the content of NO_3^--N in the 0–10 cm soil layer ($F = 4.611$, $P = 0.053$; Tables 2, 3) compared with CAN approach. No significant effects of N addition approach or concentration on microbial biomass were found (Table 2).

3.4. Nematode abundance, community indices, and diversity indices

Abundances of all taxa found in the present study are provided in Table S3. Generally, 90 and 84 genera were found in the 0–10 cm and 10–20 cm soil layer, respectively. The CAN approach increased the abundance of bacterivores in the 0–10 cm soil layers ($F = 17.231$, $P = 0.001$; Table 4; Fig. 3A). Specifically, the CAN approach increased the abundance of the dominant bacterivorous nematode genera of *Acrobeloides* ($F = 5.841$, $P = 0.033$; Table S4; Fig. S2) and *Prismatolaimus* ($F = 6.611$, $P = 0.024$; Table S4; Fig. S2) in the 0–10 cm soil layer compared with UAN. Also, The CAN approach increased the abundance of fungivores in the 10–20 cm soil layer ($F = 6.400$, $P = 0.026$; Table 4; Fig. 3D) compared with UAN. However, the UAN approach decreased the abundance of bacterivores in the 10–20 cm soil layers ($F = 5.887$, $P = 0.032$; Table 4; Fig. 3B), omnivore-predators ($F = 5.420$, $P = 0.038$; Table 4; Fig. 3E), and total nematodes ($F = 4.868$, $P = 0.048$; Table 4; Fig. 3I) in the 0–10 cm soil layer compared with CAN. In addition, high N concentration tended to increase the abundance of herbivores ($F =$

Table 3
Plant, soil, and microbial properties.

Variable	Depth (cm)	Treatment				
		CK	CAN25	CAN50	UAN25	UAN50
Plant properties						
Litter	–	619.34 (36.03)	616.83 (44.35)	665.31 (49.48)	569.53 (39.53)	612.91 (47.81)
LC	–	388.54 (6.84)	377.33 (7.92)	387.21 (7.07)	379.13 (7.51)	392.88 (7.14)
LN	–	15.43 (0.55)ab	14.95 (0.47)ab	15.84 (0.63)ab	14.33 (0.68)a	16.18 (0.69)b
Root	0–20	107.31 (30.85)a	85.74 (14.11)a	67.86 (5.39)ab	59.84 (8.83)ab	46.96 (9.59)b
Soil properties						
Soil pH	0–10	4.19 (0.12)	4.03 (0.08)	4.13 (0.13)	3.99 (0.06)	4.11 (0.04)
	10–20	4.23 (0.10)	4.03 (0.07)	4.12 (0.07)	4.23 (0.06)	4.27 (0.12)
SOC	0–10	101.76 (22.04)	76.51 (7.67)	123.44 (36.40)	86.57 (3.42)	89.28 (6.79)
	10–20	30.49 (1.67)ab	35.99 (6.10)ab	25.30 (2.10)a	27.83 (2.98)ab	44.41 (12.97)b
STN	0–10	1.88 (0.28)	1.60 (0.14)	2.36 (0.45)	1.93 (0.19)	2.13 (0.30)
	10–20	0.65 (0.07)a	0.73 (0.14)a	1.13 (0.23)ab	1.01 (0.16)ab	1.50 (0.37)b
NH_4^+-N	0–10	3.05 (0.29)	2.99 (0.15)	2.75 (0.20)	2.61 (0.06)	3.37 (0.60)
	10–20	1.66 (0.10)	2.73 (0.84)	2.04 (0.28)	1.63 (0.25)	2.26 (0.31)
NO_3^--N	0–10	29.18 (1.92)ab	23.64 (2.59)a	23.63 (3.57)a	27.06 (4.29)ab	37.71 (5.34)b
	10–20	5.20 (0.76)	6.16 (0.61)	5.39 (1.31)	5.86 (1.00)	6.84 (0.89)
Microbial biomass						
MBC	0–10	362.75 (49.26)	346.23 (33.17)	337.11 (24.98)	310.16 (29.43)	383.37 (38.67)
	10–20	57.17 (15.95)	112.90 (24.48)	72.80 (24.39)	87.57 (4.69)	99.53 (23.37)
MBN	0–10	108.84 (12.16)	94.05 (8.92)	101.80 (3.10)	93.75 (9.83)	115.52 (15.62)
	10–20	23.81 (2.81)	37.11 (7.62)	26.92 (5.06)	21.92 (3.76)	30.85 (4.70)

CK: Control; CAN25: canopy addition of N with 25 kg N ha^{-1} yr^{-1}; CAN50: canopy addition of N with 50 kg N ha^{-1} yr^{-1}; UAN25: understory addition of N with 25 kg N ha^{-1} yr^{-1}; UAN50: understory addition of N with 50 kg N ha^{-1} yr^{-1}; Litter: leaf litter quantity (g dry weight m^{-2}); LC: litter carbon (g kg^{-1} dry weight); LN: litter nitrogen (g kg^{-1} dry weight); n = 12. Root: fine root biomass (g dry weight m^{-2}), n = 6, 9, 7, 7, and 5 by the order of the treatment. SOC: soil organic carbon (g kg^{-1} dry soil); STN: soil total nitrogen (g kg^{-1} dry soil); NH_4^+-N: ammonium (mg kg^{-1} dry soil); NO_3^--N: nitrate (mg kg^{-1} dry soil); MBC: microbial biomass carbon (mg kg^{-1} dry soil); MBN: microbial biomass nitrogen (mg kg^{-1} dry soil); n = 4. Values are means (with one SE in parentheses). Different letters in the same row indicate significant ($P < 0.05$) differences between treatments tested by post-hoc test (Tables S1 and S2).

Table 4
Effects of N addition approach (A), N concentration (C), sampling time (t), and their interactions on nematode abundances and diversity indices assessed by two-way repeated measures ANOVAs.

	Depth (cm)	A		C		A × C		t		t × A		t × C		t × A × C	
		$F_{(1,12)}$	P	$F_{(1,12)}$	P	$F_{(1,12)}$	P	$F_{(8,96)}$	P	$F_{(8,96)}$	P	$F_{(8,96)}$	P	$F_{(8,96)}$	P
Abundance															
Ba	0–10	17.231	**0.001**	2.927	0.113	1.599	0.230	10.842	**<0.001**	0.614	0.765	1.187	0.315	0.326	0.954
	10–20	5.887	**0.032**	0.544	0.475	0.109	0.747	3.170	**0.003**	0.801	0.603	0.454	0.885	2.010	0.053
Fu	0–10	1.232	0.289	0.120	0.735	0.635	0.441	9.949	**<0.001**	1.860	0.075	1.083	0.381	0.524	0.836
	10–20	6.400	**0.026**	3.224	0.098	0.999	0.337	6.417	**<0.001**	1.467	0.180	0.598	0.777	0.769	0.631
OP	0–10	5.420	**0.038**	0.005	0.946	0.012	0.914	6.317	**<0.001**	1.428	0.195	1.807	0.085	1.306	0.250
	10–20	4.662	0.052	0.927	0.355	0.322	0.581	4.736	**<0.001**	0.507	0.848	1.003	0.439	1.105	0.367
He	0–10	0.509	0.489	2.732	0.124	0.549	0.473	4.815	**<0.001**	0.963	0.469	1.464	0.181	1.534	0.156
	10–20	0.066	0.801	3.700	0.078	0.899	0.362	3.608	**0.001**	1.038	0.413	2.875	**0.007**	1.092	0.376
Total	0–10	4.868	**0.048**	3.262	0.096	0.777	0.395	9.471	**<0.001**	0.971	0.463	1.579	0.141	0.862	0.551
	10–20	0.650	0.436	1.160	0.303	0.909	0.359	2.304	**0.026**	0.420	0.906	1.790	0.088	1.623	0.128
Diversity															
H'	0–10	0.459	0.511	4.010	0.068	0.052	0.824	4.871	**<0.001**	1.167	0.327	0.570	0.801	0.590	0.784
	10–20	3.859	0.073	3.066	0.105	0.047	0.832	3.644	**0.001**	1.200	0.308	2.430	**0.019**	0.493	0.859
SR	0–10	0.923	0.356	0.498	0.494	0.005	0.946	3.866	**0.001**	0.373	0.933	0.682	0.707	0.756	0.642
	10–20	5.991	**0.031**	0.517	0.486	0.147	0.709	3.174	**0.003**	0.536	0.827	1.556	0.148	0.356	0.941

Ba: bacterivores; Fu: fungivores; OP: omnivore-predators; He: herbivores; Total: total nematodes; H': Shannon-Wiener index; SR: Richness index; significant ($P < 0.05$) effects are presented in bold.

3.700, $P = 0.078$; Table 4; Fig. 3H) compared with low N concentration in the 10–20 cm soil layer and a significant interaction of sampling time and N concentration ($F = 2.875$, $P = 0.007$; Table 4; Fig. 3H) was found, with the greatest differences among treatments in August 2014. Nematode community mainly showed no significant change in response to the treatments at genus level (single dominant genus of fungivores, omnivore-predators, and herbivores), but showed significant responses to the treatments at the trophic group level (multiple genera).

The UAN approach decreased the richness index ($F = 5.991$, $P = 0.031$; Table 4; Fig. 4D) and tended to decrease the Shannon-Wiener index ($F = 3.859$, $P = 0.073$; Table 4; Fig. 4B) in the 10–20 cm soil layer compared with CAN. Furthermore, high N concentration tended to decrease the Shannon-Wiener index in the 0–10 cm soil layer ($F = 4.010$, $P = 0.068$; Table 4; Fig. 4A) compared with low N concentration. Furthermore, a significant interaction between N concentration and sampling time ($F = 2.430$, $P = 0.019$; Table 4; Fig. 4B) was found, with the greatest difference among treatments in October 2015. Nematode community indices were not significantly affected by any of the treatments with an exception that high levels of N addition decreased the Channel Index compared with low levels of N addition in the 10–20 cm soil layer ($F = 5.254$, $P = 0.041$; Fig. S3; Table S6).

3.5. Relationships between soil properties and microbial biomass with nematode abundance and diversity

In the 0–10 cm soil layer, NH_4^+-N was positively correlated with MBC ($r = 0.66$, $P < 0.01$; Table S8), abundance of fungivores ($r = 0.64$, $P < 0.01$; Table S8), and total nematode abundance ($r = 0.46$, $P < 0.05$; Table S8). NO_3^--N was positively correlated with MBC ($r = 0.54$, $P < 0.05$; Table S8), MBN ($r = 0.64$, $P < 0.01$; Table S8), abundance of herbivores ($r = 0.51$, $P < 0.05$; Table S8), and total nematode abundance ($r = 0.56$, $P < 0.05$; Table S8).

In the 10–20 cm soil layer, NO_3^--N was positively correlated with MBC ($r = 0.57$, $P < 0.01$; Table S9). Furthermore, MBC and NH_4^+-N were positively correlated with the abundance of fungivores ($r = 0.46$, $P < 0.05$; $r = 0.48$, $P < 0.05$; Table S9).

4. Discussion

Our results showed that the soil nematode community was mainly and negatively affected by the conventional approach of understory addition of N (UAN) compared with canopy addition of N (CAN). When using the conventional approach of N addition, our findings were consistent with those of previous studies (e.g., Ruess et al., 1996; Zhao et al., 2014). This is of great importance, because N addition approaches used in previous studies were mainly based on UAN, while the significant role of canopy processes was overlooked, which might have resulted in an incomplete understanding of how N deposition affects soil communities in forest ecosystems.

The negative effects of N deposition on nematode abundance and diversity using UAN might be misleading. In the present study, CAN increased the abundance of fungivores in the 10–20 cm soil layer compared with UAN and control, while UAN caused the abundance (bacterivores, fungivores, omnivore-predators, and total nematodes) and diversity (Richness index, SR) of the soil nematode community to decline compared with CAN, supporting our hypotheses. The contrasting effects of CAN and UAN on soil nematodes might be attributable to the canopy processes, which retained 44% and 52% of the N added to the forest canopy by CAN50 and CAN25, respectively, and thereby mitigated the effects of N addition on root resource input and soil chemical status.

Previous studies have indicated that conventional simulated N deposition significantly decreases fine root (diameter < 2 mm) biomass by shifting the plant growth strategy (e.g., root: shoot ratio), increasing environmental stresses, and injuring root tissues (Li et al., 2015; Shao et al., 2017; Yan et al., 2017). The biomass of fine roots decreased in UAN, which was in line with these previous studies. Therefore, soil bacteria might be inhibited by the decease of food resources, i.e. root exudates and dead root material. Consequently, UAN might decrease soil bacterivorous nematodes by inhibiting the growth of fine roots. Fungivorous nematodes decreased in UAN possibly also due to the decrease of fine root biomass. For instance, changes in the growth of symbiotic fungi (e. g., arbuscular mycorrhizal fungi), which typically correlates with fine root biomass, could induce cascading effects on the abundance of fungivores. Meanwhile, a previous study suggested that conventional N addition decreases the fungal biomass (Frey et al., 2004). While we did not find any significant changes in soil microbial biomass in the present study, the reader should note that we did not differentiate bacterial and fungal biomass.

Furthermore, many previous studies have shown that the forest canopy could retain and take up N, and most of the N added to the forest canopy tends to be recovered in aboveground plant material (Gaige et al., 2007; Sievering et al., 2007; Nair et al., 2016), which might alter the quantity and quality of plant-derived resources available to the soil food web. For instance, densities of bacterivorous nematodes, fungivorous nematodes, and microbes may be regulated by leaf litter through

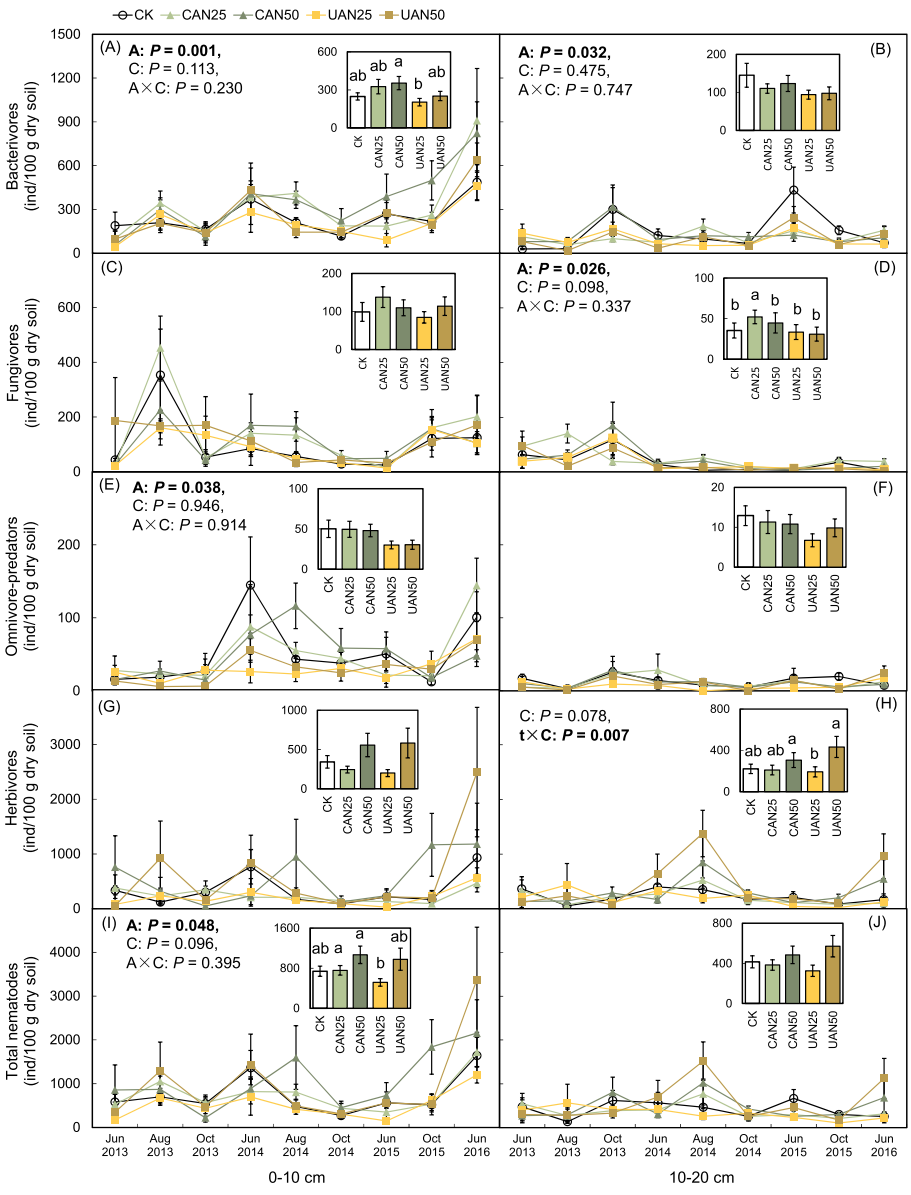

Fig. 3. Temporal dynamics and the means of abundance for bacterivores, fungivores, omnivore-predators, herbivores, and total nematodes under the control (CK), canopy addition of N with 25 kg N ha^{-1} yr^{-1} (CAN25), canopy addition of N with 50 kg N ha^{-1} yr^{-1} (CAN50), understory addition of N with 25 kg N ha^{-1} yr^{-1} (UAN25), and understory addition of N with 50 kg N ha^{-1} yr^{-1} (UAN50) at 0–10 cm depth and 10–20 cm depth. Data are means ± SE (n = 4); insets data are means ± SE (n = 36). Significant ($P < 0.05$) effects from two-way repeated measures ANOVA are presented in bold. Results of post hoc tests (Table S7) among treatments are provided with lowercase letters only when the effects of N addition approach (A) or N concentration (C) in the ANOVA are significant ($P < 0.05$) or marginally significant ($0.05 < P < 0.1$).

bottom-up effects (Wardle et al., 2004). Although we did not detect any significant changes in the leaf litter quantity and quality between CAN and UAN, it could be a critical factor which needs to be considered in the future studies.

Omnivores and predators are more sensitive to disturbances and soil condition changes (Ferris et al., 2001). In our hypothesis, if a large proportion of N is retained and utilized by the canopy, this might mitigate the direct influence of N on soil properties and biota. In the present study, UAN decreased the abundance of omnivore-predators (0–10 cm soil layer) and soil nematode community richness index (10–20 cm soil layer), and tended to increase the content of NO_3^--N (0–10 cm soil layer) compared with CAN. However, no significant changes of soil properties and soil nematodes abundance were found between CAN and the control. These results indicate that UAN caused more significant changes of soil environmental conditions and stronger disturbances on those trophic groups than CAN did. However, a previous study at the same study site has shown that both CAN and UAN increased soil Al^{3+} concentration (Shi et al., 2016), which might be toxic to soil nematodes (Lucas et al., 2011). Furthermore, ammonium toxicity on soil nematodes (Wei et al., 2012) may be more likely to be caused by directly spraying of N solution (NH_4NO_3) onto the forest floor through UAN. In the present study, considering the background input of N deposition (19.6 kg N ha^{-1} yr^{-1}) and the fact that total N (50 kg N ha^{-1} yr^{-1}) was applied in many small batches, the toxicity of ammonium may have only weak effects, but we are still unable to eliminate the potential impact of ammonium toxicity. However, no significant change of the content of $NH4^+$-N was found in the present study, possibly due to the time lag of sampling events compared with the N application events, i.e. soil was sampled one month after the N addition. A previous study showed that most ammonium is easily and rapidly oxidized to nitrate by nitrification after N addition in the forest floor (Stams et al., 1991). Consequently, given that the quantity of N that directly affects the forest floor under CAN is less than that under UAN, we speculate that this possibly exerted unfavorable conditions and caused the significant decline of omnivore-predators and the richness index. Overall, the conventional approach of UAN tended to reduce the abundance of most nematode trophic groups and the diversity of the nematode community, which is in contrast to the results obtained using the novel CAN approach. This finding provides empirical evidence for the notion that the negative effects of N deposition on soil nematodes are overestimated when using the conventional UAN approach. Our results, thus, indicate that the CAN approach is a more realistic simulation of N deposition and

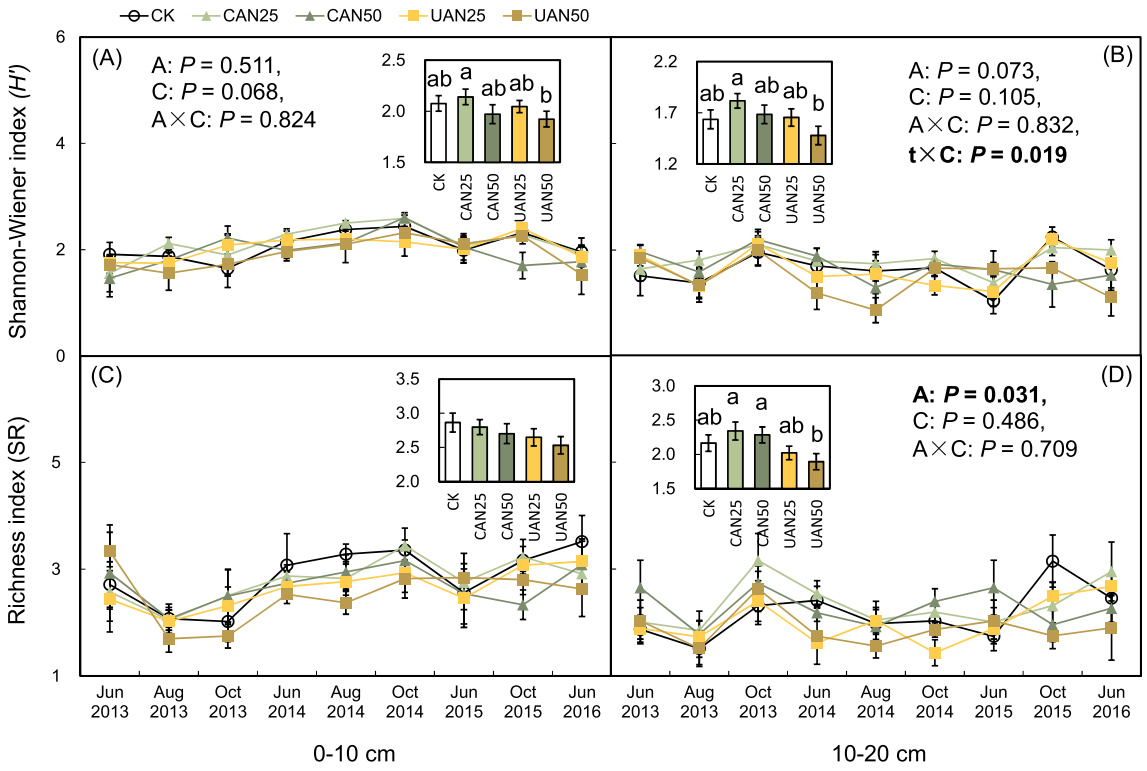

Fig. 4. Temporal dynamics and means of diversity indices of the nematode community under the control (CK), canopy addition of N with 25 kg N ha^{-1} yr^{-1} (CAN25), canopy addition of N with 50 kg N ha^{-1} yr^{-1} (CAN50), understory addition of N with 25 kg N ha^{-1} yr^{-1} (UAN25), and understory addition of N with 50 kg N ha^{-1} yr^{-1} (UAN50) at 0–10 cm depth and 10–20 cm depth. Data are means ± SE (n = 4); insets data are means ± SE (n = 36). Significant ($P < 0.05$) effects from two-way repeated measures ANOVA are presented in bold. Results of post hoc tests (Table S7) among treatments are provided with lowercase letters only when the effects of N addition approach (A) or N concentration (C) in the ANOVA are significant ($P < 0.05$) or marginally significant ($0.05 < P < 0.1$).

has no detrimental effects on nematode communities in the studied temperate forest. Therefore, the forest canopy may play a critical role in maintaining soil biota under elevated N deposition.

Previous studies have found that high concentrations of N addition can decrease soil biodiversity. For instance, Xu et al. (2007) showed that soil fauna diversity and density decreased when N addition was >100 kg N ha^{-1} yr^{-1} in a subtropical forest. In the present study, in line with our last hypothesis, relatively high concentration of N addition (50 kg N ha^{-1} yr^{-1}) tended to decrease the nematode community diversity (Shannon-Wiener index). However, N addition concentration did not influence the abundance of most of the soil nematode trophic groups except herbivores. In fact, temperate forests are often considered as N-limited ecosystems (Suding et al., 2005). Therefore, the relatively high concentration (50 kg N ha^{-1} yr^{-1}) used in the present experiment might not reach the threshold N concentration (i.e., N saturation concentration) of the studied forest ecosystem. Furthermore, high concentration of N deposition may stimulate the herbivores and therefore exacerbate the decrease of fine root biomass.

Moreover, we found that interception rates of N addition of 25 kg N ha^{-1} yr^{-1} (52%) and 50 kg N ha^{-1} yr^{-1} (44%) in the present study were lower than those observed in previous studies: e.g., Gaige et al. (2007) found an interception rate of 70% in a spruce-hemlock forest and Sievering et al. (2007) found an interception rate of 80% in a conifer forest. Besides, the highest N addition concentration among the studies mentioned above is 20 kg N ha^{-1} yr^{-1} (Gaige et al., 2007). Therefore, the forest type and N deposition intensity should be considered when evaluating the interception effects of canopy processes. Moreover, it should be noted that the dynamics of soil nematode communities typically varies across seasons (Ferris et al., 1996), which may be hard to capture by a few sampling campaigns. In field studies, time might be required for experimental treatment effects to materialize, with long-term effects often being considered the most representative (e.g., Eisenhauer et al., 2012; Thakur et al., 2015; Shaw et al., 2019).

5. Conclusions

In summary, our results indicate that without forest canopy processes, conventional simulated N deposition decreased the plant resources input (root biomass), and tended to negatively affect the soil nematode community. Furthermore, high N concentration levels tended to reduce soil nematode diversity. In our study, abundances of most trophic groups and diversity indices differed in their response to the CAN and UAN treatments, underlining the sensitivity of soil nematodes to environmental changes. Additionally, canopy fauna (e.g., some r-selected taxa, bacterivorous nematodes, which colonize leaves) plays an important role in the first stage of leaf litter decomposition (de Goede, 1996; Kitagami et al., 2019) and may also be influenced by canopy N deposition. Thus, canopy N deposition may have profound effects on ecosystem functioning via canopy responses (e.g., leaf litter production and decomposition), which are overlooked by conventional simulated N deposition studies. Notably, other systems (e.g., more N-rich forest ecosystems) could have different resistance or tolerance to the N addition levels used in this experiment (i.e. 25 and 50 kg N h $^{-1}$ year $^{-1}$), which could lead to different conclusions. Our results provide the first important insights into the dissimilar effects of canopy and understory addition of N to explore the effect of N deposition and highlight the significance of canopy processes in N deposition studies. While these findings help reconcile some of the discrepancies in the existing literature, they also demonstrate the urgent need to consider canopy processes in future N deposition studies.

Author contributions

S. F. and Y. S. designed and supervised the experiment, T. L., P. M., L.

S., S. L., Z. L., L. Z., and Z. W. collected the data, T. L., P. M., X. H., and X. W. analysed the data, T. L., P. M., and N. E. prepared and wrote the manuscript with contributions from all the authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2020.107733.

References

Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M., 1989. Nitrogen saturation in northern forest ecosystems. BioScience 39, 378–386.
Barker, K.R., 1985. Nematode extraction and bioassays. In: Barker, K.R., Carter, C.C., Sasser, J.N. (Eds.), An Advanced Treatise on Meloidogyne, vol. 2. North Carolina State University Graphics, Methodology, pp. 19–35.
Basto, S., Thompson, K., Phoenix, G., Sloan, V., Leake, J., Rees, M., 2015. Long-term nitrogen deposition depletes grassland seed banks. Nature Communications 6, 6185.
Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14–19.
Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution 14, 224–228.
Boxman, A.W., Blanck, K., Brandrud, T.E., Emmett, B.A., Gundersen, P., Hogervorst, R.F., Janne Kjønaas, O., Persson, H., Timmermann, V., 1998. Vegetation and soil biota response to experimentally-changed nitrogen inputs in coniferous forest ecosystems of the NITREX project. Forest Ecology and Management 101, 65–79.
Chen, D., Lan, Z., Hu, S., Bai, Y., 2015. Effects of nitrogen enrichment on belowground communities in grassland: relative role of soil nitrogen availability vs. soil acidification. Soil Biology and Biochemistry 89, 99–108.
de Goede, R.G.M., 1996. Effects of sod-cutting on the nematode community of a secondary forest of *Pinus sylvestris L.* Biology and Fertility of Soils 22, 227–236.
Eisenhauer, N., Reich, P.B., Scheu, S., 2012. Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. Basic and Applied Ecology 13, 571–578.
Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Applied Soil Ecology 18, 13–29.
Ferris, H., Venette, R.C., Lau, S.S., 1996. Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems, and their impact on soil fertility. Applied Soil Ecology 3, 161–175.
Frey, S.D., Knorr, M., Parrent, J.L., Simpson, R.T., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196, 159–171.
Gaige, E., Dail, D.B., Hollinger, D.Y., Davidson, E.A., Fernandez, I.J., Sievering, H., White, A., Halteman, W., 2007. Changes in canopy processes following whole-forest canopy nitrogen fertilization of a mature spruce-hemlock forest. Ecosystems 10, 1133–1147.
Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S. P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A. F., Porter, J.H., Townsend, A.R., Vorosmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153–226.
Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892.
Gilliam, F.S., 2006. Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. Journal of Ecology 94, 1176–1191.
Hugot, J.P., Baujard, P., Morand, S., 2001. Biodiversity in helminths and nematodes as a field of study: an overview. Nematology 3, 199–208.
Jia, Y., Yu, G., He, N., Zhan, X., Fang, H., Sheng, W., Zuo, Y., Zhang, D., Wang, Q., 2014. Spatial and decadal variations in inorganic nitrogen wet deposition in China induced by human activity. Scientific Reports 4, 3763.
Kitagami, Y., Kanzaki, N., Tanikawa, T., Matsuda, Y., 2019. Free-living nematodes associated with pine cones of *Pinus thunbergii* and *P. taeda* at Japanese coastal and inland forest sites. Nematology 21, 389.
Li, W., Jin, C., Guan, D., Wang, Q., Wang, A., Yuan, F., Wu, J., 2015. The effects of simulated nitrogen deposition on plant root traits: a meta-analysis. Soil Biology and Biochemistry 82, 112–118.
Liu, G., 1996. Analysis of Soil Physical and Chemical Properties and Description of Soil Profiles. China Standard, Beijing.
Liu, T., Chen, X., Hu, F., Ran, W., Shen, Q., Li, H., Whalen, J.K., 2016. Carbon-rich organic fertilizers to increase soil biodiversity: evidence from a meta-analysis of nematode communities. Agriculture, Ecosystems & Environment 232, 199–207.
Liu, X., Duan, L., Mo, J., Du, E., Shen, J., Lu, X., Zhang, Y., Zhou, X., He, C., Zhang, F., 2011. Nitrogen deposition and its ecological impact in China: an overview. Environmental Pollution 159, 2251–2264.
Lu, X., Mo, J., Gilliam, F.S., Zhou, G., Fang, Y., 2010. Effects of experimental nitrogen additions on plant diversity in an old-growth tropical forest. Global Change Biology 16, 2688–2700.
Lucas, R.W., Klaminder, J., Futter, M.N., Bishop, K.H., Egnell, G., Laudon, H., Högberg, P., 2011. A meta-analysis of the effects of nitrogen additions on base cations: implications for plants, soils, and streams. Forest Ecology and Management 262, 95–104.
McSorley, R., Frederick, J.J., 1999. Nematode population fluctuations during decomposition of specific organic amendments. Journal of Nematology 31, 37–44.
Mo, J., Zhang, W., Zhu, W., Gundersen, P., Fang, Y., Li, D., Wang, H., 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Global Change Biology 14, 403–412.
Moore, J.C., Hunt, H.W., 1988. Resource compartmentation and the stability of real ecosystems. Nature 333, 261–263.
Nair, R.K.F., Perks, M.P., Weatherall, A., Baggs, E.M., Mencuccini, M., 2016. Does canopy nitrogen uptake enhance carbon sequestration by trees? Global Change Biology 22, 875–888.
Neher, D.A., Darby, B.J., 2009. General community indices that can be used for analysis of nematode assemblages. In: Wilson, M.J., Kakouli-Duarte, T. (Eds.), Nematodes as Environmental Indicators. CABI, Wallingford, UK, pp. 107–123.
Okada, H., Harada, H., Kadota, I., 2005. Fungal-feeding habits of six nematode isolates in the genus *Filenchus*. Soil Biology and Biochemistry 37, 1113–1120.
Ruess, L., Sandbach, P., Cudlín, P., Dighton, J., Crossley, A., 1996. Acid deposition in a spruce forest soil: effects on nematodes, mycorrhizas and fungal biomass. Pedobiologia 40, 51–66.
Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. Science 287, 1770–1774.
Sievering, H., Tomaszewski, T., Torizzo, J., 2007. Canopy uptake of atmospheric N deposition at a conifer forest: part I -canopy N budget, photosynthetic efficiency and net ecosystem exchange. Tellus B: Chemical and Physical Meteorology 59, 483–492.
Shao, Y., Zhang, W., Eisenhauer, N., Liu, T., Xiong, Y., Liang, C., Fu, S., 2017. Nitrogen deposition cancels out exotic earthworm effects on plant-feeding nematode communities. Journal of Animal Ecology 86, 708–717.
Shaw, E.A., Boot, G.M., Moore, J.C., Wall, D.H., Baron, J.S., 2019. Long-term nitrogen addition shifts the soil nematode community to bacterivore-dominated and reduces its ecological maturity in a subalpine forest. Soil Biology and Biochemistry 130, 177–184.
Shi, L., Zhang, H., Liu, T., Zhang, W., Shao, Y., Ha, D., Li, Y., Zhang, C., Cai, X., Rao, X., Lin, Y., Zhou, L., Zhao, P., Ye, Q., Zou, X., Fu, S., 2016. Consistent effects of canopy vs. understory nitrogen addition on the soil exchangeable cations and microbial community in two contrasting forests. The Science of the Total Environment 553, 349–357.
Sparks, J.P., 2009. Ecological ramifications of the direct foliar uptake of nitrogen. Oecologia 159, 1–13.
Stams, A.J.M., Booltink, H.W.G., Lutke-Schipholt, I.J., Beemsterboer, B., Woittiez, J.R. W., van Breemen, N., 1991. A field study on the fate of ^{15}N-ammonium to demonstrate nitrification of atmospheric ammonium in an acid forest soil. Biogeochemistry 13, 241–255.
Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., Gross, K.L., Milchunas, D. G., Pennings, S., 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences of the United States of America 102, 4387–4392.
Sun, X., Zhang, X., Zhang, S., Dai, G., Han, S., Liang, W., 2013. Soil nematode responses to increases in nitrogen deposition and precipitation in a temperate forest. PloS One 8, 82468.
Thakur, M.P., Milcu, A., Manning, P., Niklaus, P.A., Roscher, C., Power, S., Reich, P.B., Scheu, S., Tilman, D., Ai, F., Guo, H., Ji, R., Pierce, S., Ramirez, N.G., Richter, A.N., Steinauer, K., Strecker, T., Vogel, A., Eisenhauer, N., 2015. Plant diversity drives soil microbial biomass carbon in grasslands irrespective of global environmental change factors. Global Change Biology 21, 4076–4085.
Tomaszewski, T., Boyce, R.L., Sievering, H., 2003. Canopy uptake of atmospheric nitrogen and new growth nitrogen requirement at a Colorado subalpine forest. Canadian Journal of Forest Research 33, 2221–2227.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707.

van Dijk, H.F.G., Roelofs, G.M., 1988. Effects of excessive ammonium deposition on the nutritional status and condition of pine needles. Physiologia Plantarum 73, 494–501.
Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Technical report: human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7, 737–750.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D. H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.
Wei, C., Zheng, H., Li, Q., Lü, X., Yu, Q., Zhang, H., Chen, Q., He, N., Kardol, P., Liang, W., Han, X., 2012. Nitrogen addition regulates soil nematode community composition through ammonium suppression. PloS One 7, 43384.
Wortman, E., Tomaszewski, T., Waldner, P., Schleppi, P., Thimonier, A., Eugster, W., Buchmann, N., Sievering, H., 2012. Atmospheric nitrogen deposition and canopy retention influences on photosynthetic performance at two high nitrogen deposition Swiss forests. Tellus B: Chemical and Physical Meteorology 64 (1), 17216.
Xu, G., Mo, J., Fu, S., Gundersen, P., Zhou, G., Xue, J., 2007. Response of soil fauna to simulated nitrogen deposition: a nursery experiment in subtropical China. Journal of Environmental Sciences 19, 603–609.
Xu, G., Schleppi, P., Li, M., Fu, S., 2009. Negative responses of Collembola in a forest soil (Alptal, Switzerland) under experimentally increased N deposition. Environmental Pollution 157, 2030–2036.
Xu, W., Luo, X.S., Pan, Y.P., Zhang, L., Tang, A.H., Shen, J.L., Zhang, Y., Li, K.H., Wu, Q. H., Yang, D.W., Zhang, Y.Y., Xue, J., Li, W.Q., Li, Q.Q., Tang, L., Lu, S.H., Liang, T., Tong, Y.A., Liu, P., Zhang, Q., Xiong, Z.Q., Shi, X.J., Wu, L.H., Shi, W.Q., Tian, K., Zhong, X.H., Shi, K., Tang, Q.Y., Zhang, L.J., Huang, J.L., He, C.E., Kuang, F.H., Zhu, B., Liu, H., Jin, X., Xin, Y.J., Shi, X.K., Du, E.Z., Dore, A.J., Tang, S., Collett, J.L., Goulding, K., Sun, Y.X., Ren, J., Zhang, F.S., Liu, X.J., 2015. Quantifying atmospheric nitrogen deposition through a nationwide monitoring network across China. Atmospheric Chemistry and Physics 15, 12345–12360.
Yan, G., Chen, F., Zhang, X., Wang, J., Han, S., Xing, Y., Wang, Q., 2017. Spatial and temporal effects of nitrogen addition on root morphology and growth in a boreal forest. Geoderma 303, 178–187.
Yeates, G.W., 2003. Nematodes as soil indicators: functional and biodiversity aspects. Biology and Fertility of Soils 37, 199–210.
Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and genera— an outline for soil ecologists. Journal of Nematology 25, 315–331.
Zhang, W., Shen, W., Zhu, S., Wan, S., Luo, Y., Yan, J., Wang, K., Liu, L., Dai, H., Li, P., Dai, K., Zhang, W., Liu, Z., Wang, F., Kuang, Y., Li, Z., Lin, Y., Rao, X., Li, J., Zou, B., Cai, X., Mo, J., Zhao, P., Ye, Q., Huang, J., Fu, S., 2015. CAN canopy addition of nitrogen better illustrate the effect of atmospheric nitrogen deposition on forest ecosystem? Scientific Reports 5, 11245.
Zhao, J., Wang, F., Li, J., Zou, B., Wang, X., Li, Z., Fu, S., 2014. Effects of experimental nitrogen and/or phosphorus additions on soil nematode communities in a secondary tropical forest. Soil Biology and Biochemistry 75, 1–10.
Zhou, X., Bowker, M.A., Tao, Y., Wu, L., Zhang, Y., 2018. Chronic nitrogen addition induces a cascade of plant community responses with both seasonal and progressive dynamics. The Science of the Total Environment 626, 99–108.