

# Effects of canopy nitrogen addition on soil fauna and litter decomposition rate in a temperate forest and a subtropical forest

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## ABSTRACT

Traditionally, experiments testing the impacts of nitrogen (N) deposition on ecosystem processes have been conducted by adding N directly to forest floors, yet, in reality, atmospheric N passes through the canopy layer before it reaches the forest floor. Furthermore, we know little about how N-deposition mediated changes in soil fauna communities affect litter decomposition. Here, we use a novel experiment to contrast canopy addition of N (CAN) to understory addition of N (UAN) in a subtropical forest and a temperate forest to investigate the impact of N-deposition approaches and N-concentrations on soil fauna and leaf litter decomposition rate. We found that CAN increased the soil fauna diversity and accelerated litter decomposition whereas UAN did not significantly affect soil fauna or litter decomposition. In addition, N-deposition concentration significantly influenced soil fauna density, H' diversity, and species richness at the subtropical forest, but only affected H' diversity of soil fauna at the temperate forest. The high N-deposition concentration treatment in the subtropical forest and low N-deposition concentration treatment in our temperate forest had significantly higher decomposition rates compared with the control (no N) treatment. We assert that addition of N through the canopy is a more realistic approach for simulating the actual processes of atmospheric N-deposition in forests. Future experiments using CAN methods will provide an improved understanding of the effect of N-deposition on soil fauna-decomposition interactions and inform reliable predictions of the consequences of global change on soil ecosystems.

## 1. Introduction

Atmospheric nitrogen (N) deposition has increased dramatically over the last century due to fossil fuel burning and fertilization, leading to a substantial increase in inputs of N across a broad range of forest ecosystems (Holland et al., 2005; Liu et al., 2013). A meta-analysis of <sup>15</sup>N tracer addition experiments across ecosystems showed that much of the deposited N (approximately 40% in forest ecosystems) accumulated in soil ecosystems (Templer et al., 2012). Given these massive inputs, understanding how N-deposition impacts soil biodiversity and soil ecosystem functions is necessary to improve our knowledge of soil ecosystem responses to global change (Templer et al., 2012).

Conventional experiments simulating N-deposition have been

conducted by applying N solutions directly onto forest floors (Wortman et al., 2012; Zhang et al., 2015). However, in reality, atmospheric N contacts and passes through the canopy layer before it reaches the forest floor (Wortman et al., 2012; Zhang et al., 2015). During this process, part of the deposited N is retained by the forest canopy (Wortman et al., 2012), absorbed by epiphytes, microorganisms, and tree leaves, retained on tree bark, and transformed into other N forms (Adriaenssens et al., 2012). Consequently, N-deposition through the canopy alters the quantity and quality of N solutions that reach the forest floor (Zhang et al., 2015). While logistically tractable, previous experiments that applied an understory addition of N ignore many processes occurring in the canopy layer; a recent study suggests that understory N-addition overestimated the negative effects of N-

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deposition on asymbiotic N fixation by 375–472% compared to canopy N-addition (Zheng et al., 2018). Therefore, addition of N through the canopy is a more realistic approach for simulating the actual processes of atmospheric N-deposition that occurs in forests.

Increasing evidence indicates that elevated N-deposition can modify litter decomposition by changing biotic and abiotic factors in soil ecosystems (Berg and Matzner, 1997). However, no consistent patterns of N-deposition effects on decomposition have emerged, and previous studies have produced variable results from negative (Frey et al., 2014; Treseder, 2008) to positive (Mo et al., 2006; Hobbie et al., 2012), to neutral effects (Keeler et al., 2009). For example, Hättenschwiler and Vitousek (2000) and Keeler et al., (2009) showed that the inhibition of lignin or tannin degrading enzyme activity in response to N-deposition can significantly slow the breakdown of more resistant material and reduce litter decomposition rates. High N input may cause soil acidification, nutrient base cation loss, and toxic metal ion accumulation (Lu et al., 2014), which could decrease the litter decomposition rate (Gessner et al., 2010; Hättenschwiler et al., 2005). In contrast, increased N-deposition can lead to accelerated litter decomposition rates, because increased N availability for decomposers in the soil ecosystems induced by N-deposition are important drivers of litter decomposition (Mo et al., 2006; Hobbie et al., 2012). However, our knowledge is incomplete regarding the response of soil fauna to N-deposition and potential feedbacks on litter decomposition rates.

Deposited N can change the density, diversity, and community assemblage of soil fauna by changing soil conditions, such as nutrient content and soil pH (Liu et al., 2018). A long-term study at a subalpine forest site found that N-deposition increased soil nematode abundance by 40–96% by increasing food quality and availability (Shaw et al., 2019). In contrast, Sun et al., (2013) showed that N-deposition significantly reduced the abundance and diversity of soil fauna through changes in soil properties in a temperate forest. These inconsistent responses may be caused by N limitation of different forest types (Schulte-Uebbing and de Vries, 2018). In the context of decreasing N limitation from boreal to tropical forests, the response of soil fauna to N-deposition is expected to vary across different forest types (Schulte-Uebbing and de Vries, 2018). Nevertheless, we know little about how N-deposition influences soil fauna communities across different forest types.

Here, we use a novel experimental design to contrast canopy addition of N (CAN) to understory addition of N (UAN) to address the following questions about the relationships between soil fauna, litter decomposition and N-deposition: (1) is soil fauna affected by different N-deposition approaches and concentrations? (2) is the rate of litter decomposition affected by different N-deposition approaches and concentrations?

## 2. Materials and methods

### 2.1. Study site

Due to the differences in the propensities of the canopies of different forest types to retain N (Adriaenssens et al., 2012; Wortman et al., 2012), we conducted our study in a temperate forest and a subtropical forest. The temperate forest is located in Jigongshan (JGS) National Nature Reserve (168 m in elevation, 31°46′–31°52′ N, 114°01′–114°06′ E) in Xinyang, Henan Province, Central China (Supplementary Fig. S1). JGS has a warm temperate climate and the background N-deposition rate in precipitation is approximately 19.6 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Zhang et al., 2015). The soil properties in temperate forest JGS are: soil total N concentrations 1.88 ± 0.28 (g kg<sup>-1</sup> dry soil), soil organic carbon 101.76 ± 22.04 (g kg<sup>-1</sup> dry soil), soil pH 4.40 (see more detail in Liu et al., 2020). JGS dominated by approximately 45-year-old tree species, composed mainly of *Liquidambar formosana* Hance, *Quercus acutissima* Carruth, and *Quercus variabilis* Bl. The subtropical forest is conducted in Shimentai (SMT) National Nature Reserve (255 m in elevation, 24°22′–24°31′ N, 113°05′–113°31′ E), Yingde, Guangdong Province,

Southern China (Supplementary Fig. S1). SMT has a subtropical monsoon climate and background N-deposition rate in precipitation is approximately 34.1 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Zhang et al., 2015). SMT is dominated by approximately 53-year-old tree species, composed mainly of *Castanea henryi* Skam, *Cryptocarya concinna* Hance, *Schima superba* Gardn, *Machilus chinensis* Bentham, and *Engelhardtia roxburghiana* Wallich. The soil properties in subtropical forest SMT are: soil total N concentrations 1.95 ± 0.19 (g kg<sup>-1</sup> dry soil), soil organic carbon 69.50 ± 4.87 (g kg<sup>-1</sup> dry soil), soil pH 3.55 (see more detail in Shi et al., 2016).

### 2.2. Experimental design

In April 2013, we used a randomized block design to establish four blocks and five circular plots (907 m<sup>2</sup> in area and 17 m in radius) within each block at both the temperate JGS and subtropical SMT study locations. Within each block, we randomly assigned the circular plots to the following five N treatments: (1) canopy addition of NH<sub>4</sub>NO<sub>3</sub> solution at 25 kg N ha<sup>-1</sup> yr<sup>-1</sup> (CAN25); (2) canopy addition of NH<sub>4</sub>NO<sub>3</sub> solution at 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> (CAN50); (3) understory addition of NH<sub>4</sub>NO<sub>3</sub> solution at 25 kg N ha<sup>-1</sup> yr<sup>-1</sup> (UAN25); (4) understory addition of NH<sub>4</sub>NO<sub>3</sub> solution at 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> (UAN50); (5) control (CK) (Supplementary Fig. S1). Due to the breakdown of spraying equipment, we lost one UAN25 and one UAN50 plot at subtropical SMT.

CAN was applied with a spraying system (35 m high) built in the center of plots. The NH<sub>4</sub>NO<sub>3</sub> solution was transferred to the top of forest canopy with polyvinyl chloride (PVC) pipes which were installed to a supporting tower. Understory N-addition was applied with a 1.5 m high automatic irrigation system made up of five evenly spaced sprinklers that distributed the N solution onto the forest floor in each UAN plot. Both CAN and UAN nitrogen addition systems had sprinklers that turned 360° automatically ensuring N-addition solutions were evenly diffused over the forest floor and canopy. From April to October (the growing season), the treatment plots were sprayed with 3 mm of precipitation containing solution of NH<sub>4</sub>NO<sub>3</sub> to make the target concentration. The total solution (21 mm per year) used in each treated plot was < 1–2% of annual rainfall at JGS and SMT, thus the confounding effect of water addition was considered marginal (Zhang et al., 2015).

### 2.3. Sample collection and analyses

The litterbags method was used to assess leaf litter decomposition rate (Swift et al., 1979). In detail, we used 2 mm nylon mesh litterbags (20 cm × 20 cm) based on the body width of soil fauna (Swift et al., 1979). Litterbags were filled with air-dried leaf litter (10.0 g) from a dominant tree species at each forest: *Liquidambar formosana* (C:N ratio = 45.6 ± 3.61) at JGS and *Castanea henryi* (C:N ratio = 31.76 ± 1.77) at SMT. The newly fallen leaves were collected from the same forest (but not in the N treatment plots) using litter traps. In each plot, we placed twelve litterbags below the litter layer and attached to the soil surface in April 2015. At two-month intervals over the course of a year, two litterbags were randomly collected from each plot, and immediately returned to the laboratory. In the laboratory, soil invertebrates from the litterbags were extracted using Tullgren (“Berlese”) funnels for seven days and preserved in 75% ethanol (Edwards, 1991). Then leaf residues in the bags were oven-dried at 60 °C for 48 h to constant weights to determine remaining dry mass.

Soil fauna were identified to the lowest taxonomic group possible according to external morphological characteristics (Yi, 2000). We identified Collembola to the family level and Acari to Oribatida and Parasitiformes, and macrofauna to taxonomic orders (Table 1).

**Table 1**  
Density (ind./m<sup>2</sup>) of soil fauna taxonomic groups under different N-deposition treatments at temperate JGS and subtropical SMT (mean ± S.E.).

Name	Temperate forest JGS					Proportion
	CAN	UAN	High	Low	CK	
<b>Total macrofauna</b>	459.64 ± 44.00c	599.22 ± 87.66a	520.83 ± 63.77bc	538.02 ± 75.20b	490.63 ± 99.68c	12.69%
– Araneae	15.89 ± 3.49a	14.84 ± 2.44a	13.54 ± 2.74a	17.19 ± 3.26a	15.63 ± 4.63a	0.37%
– Pseudoscorpiones	39.06 ± 8.34a	23.18 ± 4.48c	34.64 ± 8.21ab	27.60 ± 4.83bc	9.90 ± 3.85d	0.65%
– Eurypteroidea	15.89 ± 5.42b	72.14 ± 47.49a	67.45 ± 47.30	20.57 ± 7.29	12.50 ± 4.40b	0.92%
– Thysanoptera	14.58 ± 5.36a	4.43 ± 1.28b	6.51 ± 2.62b	12.50 ± 4.88a	19.79 ± 6.14a	0.28%
– Homoptera	47.92 ± 7.67ab	40.36 ± 6.71ab	51.04 ± 8.73a	37.24 ± 5.18b	39.06 ± 12.56ab	1.05%
– Coleoptera (adult + larvae)	29.17 ± 4.63b	51.82 ± 7.37a	41.67 ± 5.46ab	39.32 ± 6.97b	35.94 ± 6.40b	0.96%
– Lepidoptera larvae	17.97 ± 3.26a	14.84 ± 2.53a	16.41 ± 3.05a	16.41 ± 2.79a	20.31 ± 4.57a	0.42%
– Diptera larvae	145.83 ± 16.01a	156.51 ± 17.23a	155.73 ± 17.12a	146.61 ± 16.13a	96.88 ± 12.69b	3.41%
– Formicidae	72.14 ± 19.36b	150.26 ± 64.62a	58.85 ± 17.99b	163.54 ± 64.88a	168.75 ± 85.18a	2.98%
– Other macrofauna	61.198 ± 7.52ab	70.83 ± 9.43ab	75.00 ± 10.05a	57.03 ± 6.58b	71.88 ± 12.27a	1.63%
<b>Acari</b>	1895.83 ± 153.50b	1674.74 ± 119.42d	1780.21 ± 138.50c	1790.36 ± 137.46c	2017.19 ± 196.06a	44.55%
– Parasitiformes	651.82 ± 61.98bc	670.83 ± 61.96b	625.26 ± 56.65c	697.4 ± 66.68b	814.58 ± 119.15a	16.83%
– Oribatida	1244.01 ± 148.49a	1003.91 ± 88.76c	1154.95 ± 126.16a	1092.97 ± 119.56b	1202.6 ± 125.28a	27.72%
<b>Collembola</b>	1465.63 ± 164.74e	1790.63 ± 206.15b	1584.64 ± 200.02d	1671.61 ± 173.62c	2278.13 ± 441.81a	42.76%
– Entomobryidae	325.52 ± 41.74b	312.24 ± 37.47b	332.55 ± 40.59b	305.21 ± 38.68b	441.15 ± 87.01a	8.35%
– Onychiuridae	26.04 ± 9.17a	15.36 ± 5.11b	14.58 ± 5.15b	26.82 ± 9.13a	17.19 ± 5.38b	0.49%
– Neanura	30.73 ± 6.05bc	41.15 ± 8.43ab	43.49 ± 9.06a	28.39 ± 5.00c	46.35 ± 11.96a	0.92%
– Tomoceridae	53.91 ± 12.10a	30.47 ± 6.43bc	27.34 ± 6.92c	57.03 ± 11.74a	41.67 ± 19.01b	1.02%
– Isotomidae	937.5 ± 140.72d	1291.67 ± 193.61b	1115.89 ± 187.61c	1113.28 ± 150.83c	1604.69 ± 396.61a	29.49%
– Sminthuridae	91.93 ± 32.93b	99.74 ± 19.34b	50.78 ± 11.05c	140.89 ± 35.96a	127.08 ± 32.25a	2.48%
<b>Predator</b>	65.89 ± 9.96a	48.44 ± 5.87c	58.59 ± 9.42b	55.73 ± 6.83b	41.15 ± 5.06c	
<b>Decomposer</b>	3782.03 ± 333.04d	4115.10 ± 323.58b	3946.35 ± 348.87c	3950.78 ± 307.39c	4718.75 ± 430.28a	
Subtropical forest SMT						
Name	CAN	UAN	High	Low	CK	Proportion
<b>Total macrofauna</b>	575.71 ± 49.90b	700.86 ± 95.06a	633.57 ± 59.02a	631.03 ± 87.96a	389.50 ± 34.49c	5.54%
– Araneae	40.36 ± 4.94ab	40.52 ± 14.57ab	35 ± 4.08b	46.98 ± 14.91a	39.50 ± 6.55ab	0.39%
– Pseudoscorpiones	29.29 ± 4.77b	31.9 ± 6.56b	41.79 ± 6.47a	16.81 ± 3.04c	25.50 ± 5.65bc	0.25%
– Isopoda	10.36 ± 3.61ab	12.50 ± 3.15ab	16.43 ± 4.13a	5.17 ± 1.71b	9.50 ± 2.46ab	0.26%
– Thysanoptera	35.00 ± 13.47bc	114.22 ± 48.29a	30.36 ± 6.37c	119.83 ± 50.21a	50.50 ± 18.30b	0.64%
– Homoptera	47.86 ± 22.03a	17.67 ± 3.64b	25 ± 6.86b	45.26 ± 25.65a	8.50 ± 2.64c	0.29%
– Coleoptera (adult + larvae)	25.71 ± 3.63a	24.57 ± 3.50a	26.79 ± 3.64a	23.28 ± 3.47a	25.00 ± 4.52a	0.11%
– Lepidoptera larvae	46.07 ± 5.77a	41.81 ± 5.68ab	52.14 ± 5.91a	34.48 ± 5.21b	32.50 ± 5.36b	0.40%
– Diptera larvae	177.14 ± 21.00b	130.17 ± 19.53b	191.07 ± 22.67a	113.36 ± 15.42c	105.00 ± 18.50c	1.39%
– Formicidae	81.79 ± 34.27c	209.05 ± 74.50a	123.93 ± 47.64b	158.19 ± 63.98b	42.50 ± 16.54d	1.10%
– Other macrofauna	82.14 ± 8.06ab	78.45 ± 11.18b	91.07 ± 9.98a	67.67 ± 8.32bc	51.00 ± 8.66c	0.71%
<b>Acari</b>	8550 ± 1018.37b	7734.05 ± 720.53c	8750 ± 727.82a	7492.67 ± 1118.76d	5865.00 ± 626.10e	73.99%
– Parasitiformes	1438.57 ± 165.65b	1104.31 ± 132.01c	1466.07 ± 149.98a	1071.12 ± 155.65c	888.50 ± 124.87d	11.55%
– Oribatida	7106.43 ± 922.59b	6624.14 ± 641.57c	7280 ± 644.44a	6414.66 ± 1021.49c	4974.50 ± 547.96d	62.40%
<b>Collembola</b>	2055.71 ± 319.89bc	2206.9 ± 415.70a	2081.79 ± 322.18b	2175.43 ± 413.27ab	1974.50 ± 323.90c	20.46%
– Entomobryidae	369.64 ± 85.25a	352.59 ± 91.89b	437.14 ± 81.64a	271.12 ± 95.20b	395.50 ± 94.93a	3.65%
– Isotomidae	1465.36 ± 277.36bc	1159.98 ± 240.22d	1365.00 ± 305.23c	1718.10 ± 357.10a	1358.50 ± 277.79c	14.53%
– Sminthuridae	131.43 ± 21.87b	184.05 ± 35.31a	168.21 ± 27.83ab	139.66 ± 28.86b	98.00 ± 20.38c	1.37%
– Other Collembola	89.29 ± 15.11b	73.28 ± 17.95b	111.43 ± 18.88a	46.55 ± 9.85c	122.50 ± 42.78a	0.92%
<b>Predator</b>	235.12 ± 27.27b	220.83 ± 38.90b	281.55 ± 34.70a	166.67 ± 28.04d	189.17 ± 23.25c	
<b>Decomposer</b>	10808.75 ± 1130.27b	10542.24 ± 1017.5c	11350.36 ± 939.76a	10023.9 ± 1254.73d	7973.75 ± 700.02e	

Different letters indicate that the differences of means among N-deposition treatments was significant ( $p < 0.05$ ). Predator include Araneae, Pseudoscorpiones, Diplura, Opiliones, Lithobiomorpha, Geophilomorpha, Scolopendromorpha. Decomposer include Acari, Collembola, Isopoda, Thysanoptera, Diptera larvae etc. Other macrofauna include Diplopoda, Psocoptera, Protura, Diplura etc. CAN = canopy addition of N, UAN = understory addition of N, High = high N-deposition concentration, Low = low N-deposition concentration.

#### 2.4. Statistical analyses

We first determined how the density, Shannon-Wiener index of diversity ( $H'$ ), taxonomic richness and different taxonomic groups of soil fauna responded to N-deposition approaches and concentrations. To achieve this, we performed a repeated measures Generalized Linear Mixed Model (GLMM) analysis with N-deposition approach, N concentration, sampling time and their interactions as a fixed effects and block as a random effect. We used a GLMM with a Poisson distribution and a log link function for density data and Gaussian distribution and identity link for diversity data. The GLMM were performed using the “lme4” package (Bates et al., 2015) in the R software (version 3.6.1, R Core Team, 2019).

Litter decomposition rate (k value) from the litterbags was evaluated with Olson’s formula (Olson, 1963):  $X_t = X_0 \times e^{-kt}$ , where  $X_t$  is mass remaining at time t,  $X_0$  was litter mass at t = 0, and k is annual

mass loss rate. Then, we again performed GLMM with a Gaussian distribution and identity link to test the N treatments impact on the k value at six months and 12 months.

We assessed soil fauna community composition across the different N treatments using non-metric multidimensional scaling (NMDS) with Bray–Curtis distances. Then, a one-way analysis of similarity (ANOSIM) with 999 permutations was conducted to test differences in soil fauna assemblage composition among N treatments. These analyses were performed with the R “vegan” package (Dixon, 2003; Oksanen et al., 2019).

To test how soil fauna assemblage affected litter decomposition rate under N treatment, we used a linear regression with k value as the response variable, and the abundance, and  $H'$  diversity of soil fauna as the independent variables.

**Table 2**

Summary of the Generalized Linear Mixed Model (GLMM) used to test for effects of N-deposition approach, N-concentration, sampling time and their interactions on the density, diversity and richness of soil fauna and decomposition rate (k value).

Temperate forest JGS											
	df	Density		H' diversity		Taxonomic richness		Decomposition rate (six months)		Decomposition rate (12 months)	
		F value	P	F value	P	F value	P	F value	P	F value	P
N approach (NA)	2	5.68	<b>0.02</b>	3.07	<b>0.04</b>	0.22	0.80	0.34	0.71	2.94	<b>0.05</b>
N concentration (NC)	1	0.12	0.81	5.69	<b>0.02</b>	0.08	0.77	1.20	0.28	12.22	<b>0.001</b>
Time	5	50.67	< <b>0.001</b>	31.86	< <b>0.001</b>	35.25	< <b>0.001</b>	–	–	–	–
NA*Time	10	3.91	<b>0.03</b>	2.88	<b>0.04</b>	1.00	0.44	–	–	–	–
NA*NC	1	8.27	<b>0.01</b>	0.08	0.78	0.16	0.69	2.09	0.16	2.17	0.16
Subtropical forest SMT											
	df	Density		H' diversity		Taxonomic richness		Decomposition rate (six months)		Decomposition rate (12 months)	
		F value	P	F value	P	F value	P	F value	P	F value	P
N approach (NA)	2	4.35	<b>0.02</b>	4.75	<b>0.03</b>	3.71	<b>0.05</b>	0.76	0.48	2.81	<b>0.05</b>
N concentration (NC)	1	0.91	0.55	0.12	0.73	4.25	<b>0.04</b>	5.22	<b>0.03</b>	0.11	0.74
Time	5	46.07	< <b>0.001</b>	21.72	< <b>0.001</b>	14.73	< <b>0.001</b>	–	–	–	–
NA*Time	10	3.05	<b>0.03</b>	0.85	0.58	6.38	<b>0.01</b>	–	–	–	–
NA*NC	1	6.19	<b>0.01</b>	1.28	0.26	0.42	0.52	2.83	0.10	12.27	<b>0.001</b>

### 3. Results

#### 3.1. Effect of N-deposition approach and concentration on soil fauna density, diversity and taxonomic richness

A total of 39,470 individuals from 35 taxonomic groups of soil fauna were recorded at temperate JGS, and 72,455 individuals from 37 taxonomic groups of soil fauna were recorded at subtropical SMT (Table 1). The average densities of soil fauna in CK treatments at JGS and SMT are  $4759.90 \pm 433.26$  (ind./m<sup>2</sup>) and  $9411.40 \pm 668.61$  (ind./m<sup>2</sup>) (mean  $\pm$  SE, n = 96), respectively (see more detail in Table 1 and Supplementary Fig. S2).

We found that N-deposition approach significantly affected soil fauna density, H' diversity, and taxonomic richness at both forests (Table 2). Compared to the CAN treatments, the density of soil fauna was highest in the CK treatment ( $P < 0.05$ ) at temperate forest JGS (Fig. 1), because the CK treatment had a higher density of Isotomidae and Parasitiformes (Table 1). In contrast, at subtropical forest SMT, the highest density of soil fauna was found in the CAN treatment (Fig. 1), because CAN treatment had a high density of Oribatida and Parasitiformes (Table 1). Furthermore, CK treatments at temperate forest JGS and UAN50 treatments at subtropical forest SMT had the highest density of soil fauna (Supplementary Fig. S3). For the H' diversity of soil fauna, the CAN treatment significantly increased H' diversity compared to the CK treatment at both forests ( $p = 0.04$  at temperate JGS;  $p = 0.05$  at subtropical SMT) (Fig. 1). For the taxonomic richness of soil fauna, CAN treatment only significantly increased richness compared to the CK treatment at subtropical forest SMT ( $p = 0.64$  at temperate JGS;  $p = 0.05$  at subtropical SMT) (Fig. 1). However, we observed no differences in soil fauna density, diversity and taxonomic richness between UAN and CK treatments at both forests (Fig. 1).

In addition, N-deposition concentration significantly influenced soil fauna density, H' diversity, and taxonomic richness at subtropical forest SMT, but only affected H' diversity of soil fauna at temperate JGS (Fig. 1) (Table 2). Compared to the CK treatments, the high N-deposition concentration treatment significantly increased density ( $p = 0.03$ ), H' diversity ( $p = 0.04$ ) and taxonomic richness ( $p = 0.02$ ) of soil fauna at subtropical forest SMT (Fig. 1). Furthermore, we did not detect the differences in soil fauna density, diversity and taxonomic richness between low N-deposition concentration and CK treatments at both forests (Fig. 1). There were not strong differences in soil fauna assemblage composition among the N treatments at both forest sites (one-way ANOSIM:  $R = -0.02$ ,  $p = 0.60$  at temperate JGS;  $R = 0.03$ ,  $p = 0.27$  at subtropical SMT) (Supplementary Fig. S4).

#### 3.2. Effect of N-deposition approach and concentration on mass remaining and decomposition rate

Compared with control, in the CAN treatments we found a significantly higher decomposition rates (k value) after 12 months at JGS ( $p = 0.028$ ) and SMT ( $p = 0.04$ ), respectively (Fig. 2c, d). The high N-deposition concentration treatment in subtropical SMT and low N-deposition concentration treatment in temperate JGS had significantly higher decomposition rates compared with CK treatment after 12 months ( $p = 0.05$ ;  $p = 0.003$ , respectively) (Fig. 2c, d). For the initial decomposition rate (k value) after six months, we only observed high N-deposition concentration treatment significantly increased the decomposition rate compared with low N-deposition concentration ( $p = 0.028$ ) and CK treatments ( $p = 0.028$ ) (Fig. 2a, b). In general, decomposition rates gradually increased over time in temperate JGS, but slowed down over time in subtropical SMT, and after 12 months about 69.2%–78.5% (6.92–7.85 g) litter in temperate JGS and 64.2%–91.8% (6.42–9.18 g) litter in tropical SMT were decomposed, respectively (Fig. 2e, f).

#### 3.3. Abundance and diversity of soil fauna impact on decomposition rate

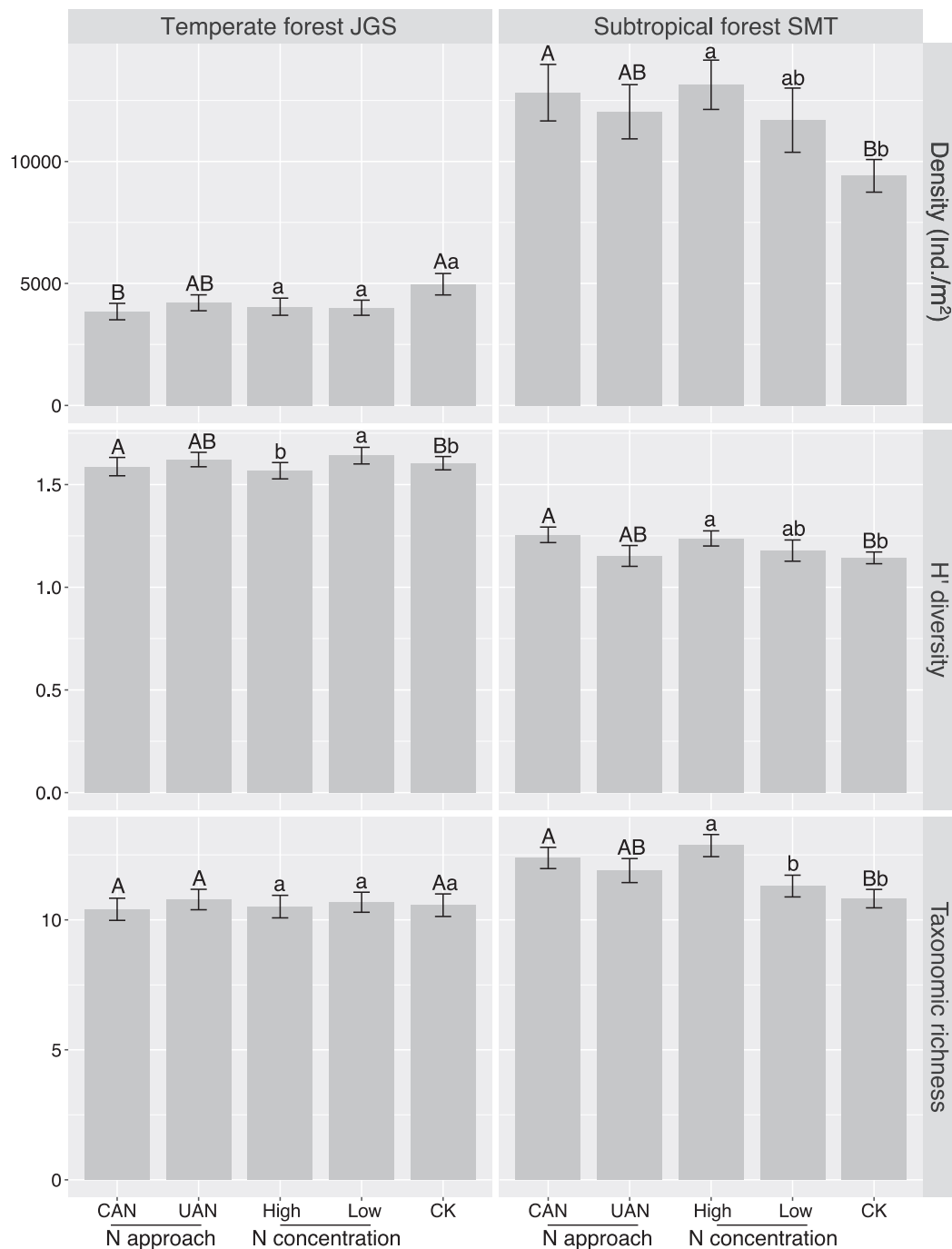
The linear regressions showed that H' diversity of total soil fauna was positively correlated with litter decomposition rates (JGS:  $R^2 = 0.21$ ,  $p = 0.04$ ; SMT:  $R^2 = 0.41$ ,  $p = 0.004$ ; Supplementary Fig. S5), and no significant correlation was found between total soil fauna abundance and decomposition (JGS:  $R^2 = 0.03$ ,  $p = 0.69$ ; SMT:  $R^2 = 0.002$ ,  $p = 0.86$ ; Supplementary Fig. S5).

### 4. Discussion

In the present study, we found that CAN accelerated the litter decomposition rate and increased diversity of soil fauna. In contrast, UAN had no significant impact on soil fauna index and litter decomposition compared with control. These results are important for our accurate estimate and understanding of soil fauna-decomposition interactions under N-deposition scenarios.

#### 4.1. N Treatment impacts on soil fauna

In this study, CAN had positive effect on the H' diversity of soil fauna at both forests. Our finding directly contrasts previous studies, in which negative effects on soil biota diversity have been detected in elevated N treatments that were applied using an understory N-addition



**Fig. 1.** Impacts of N-addition approach and N-concentration treatments on the annual mean value of soil fauna density, H' diversity and taxonomic richness at temperate forest JGS and subtropical forest SMT. Values are expressed as mean  $\pm$  SE. Different capital letters indicate that the differences of means among N-addition approach and CK treatments was significant ( $p < 0.05$ ) and different lowercase letters indicate the significant differences among N-concentration and CK treatments ( $p < 0.05$ ). CAN = canopy addition of N, UAN = understorey addition of N, High = high N-deposition concentration, Low = low N-deposition concentration.

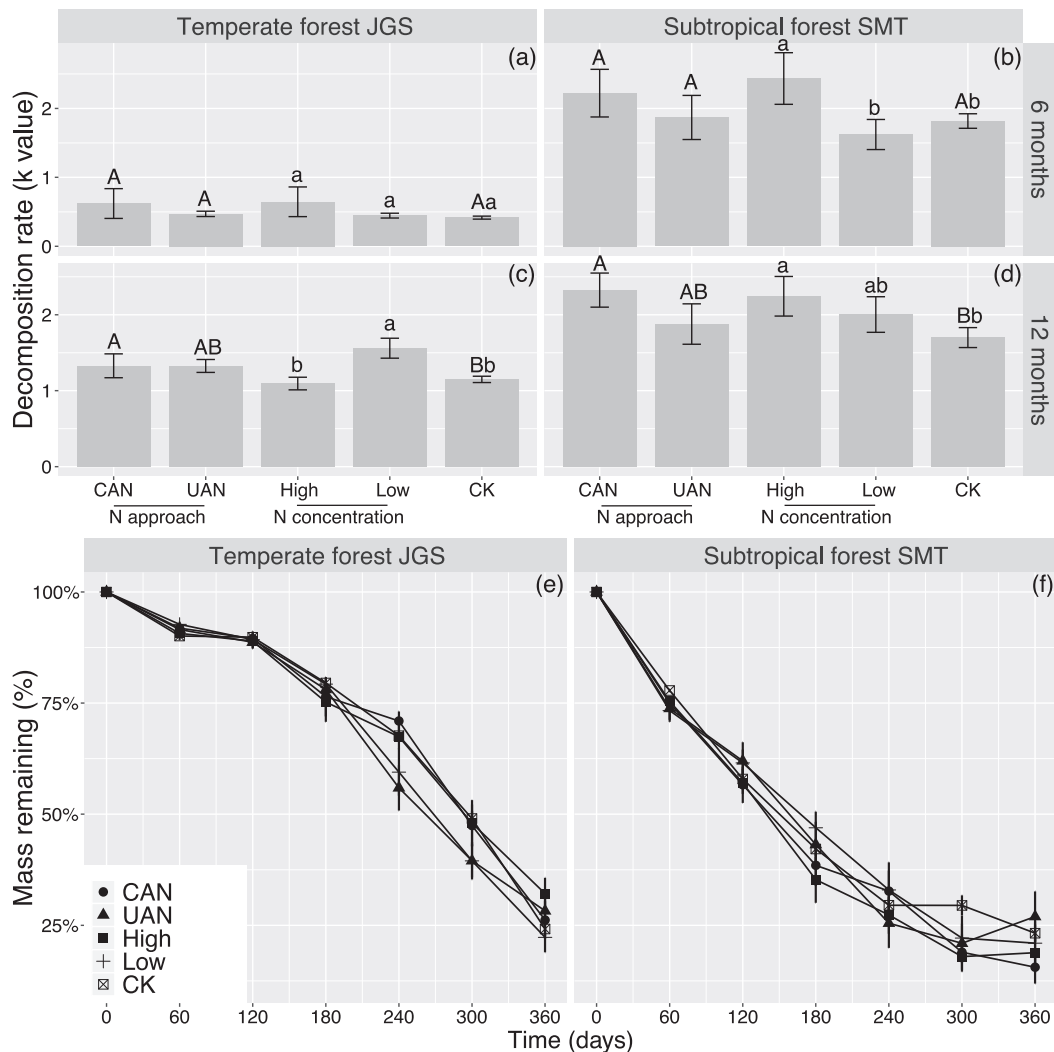
technique (Xu et al., 2009; Eisenhauer et al., 2012; Sun et al., 2013). In the CAN process, a substantial proportion of the deposited N is retained by the forest canopy (Wortman et al., 2012) which could buffer the negative impacts of N-deposition on soil fauna. The mechanisms underlying the positive effects of CAN on soil fauna in our study are currently unclear. We speculate that the canopy retained inorganic N absorbed by foliage, bark or epiphytes, which thereby reduced the N-concentrations and altered the ratio of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  reaching the forest floor (Adriaenssens et al., 2012). This change in  $\text{NH}_4^+/\text{NO}_3^-$  ratio may then lead to increased soil fauna diversity (Eisenhauer et al.,

2012). In this study, we did not find a significant difference in the diversity of soil fauna between UAN and control treatments, which contrasts with other studies. This may be because the amounts of N added in previous studies ( $80\text{--}100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) were much higher than those in our study ( $25\text{--}50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) (Knorr et al., 2005).

#### 4.2. N Treatment effect on litter decomposition

Our CAN data support the N limitation theory that N-deposition would accelerate decomposition rates by increasing N concentration





**Fig. 2.** Impacts of N-addition and N-concentration treatments on the litter decomposition rate ( $k$  value) after six months (a, b) and 12 months (c, d), and temporal dynamics of litter mass remaining (e, f) at temperate forest JGS and subtropical forest SMT. Values are expressed as mean  $\pm$  SE. Different capital letters indicate that the differences of means among N-addition approach and CK treatments was significant ( $p < 0.05$ ) and different lowercase letters indicate the significant differences among N-concentration and CK treatments ( $p < 0.05$ ). CAN = canopy addition of N, UAN = understory addition of N, High = high N-deposition concentration, Low = low N-deposition concentration.

(Berg and Matzner, 1997). Knorr et al. (2005) revealed that N-deposition generally accelerated decomposition when N fertilization rates were  $< 2$  times the ambient N-deposition level. The rate of background N-deposition is about 19.6 and 34.1 kg N ha<sup>-1</sup> yr<sup>-1</sup> at temperate JGS and subtropical SMT, respectively, so the amount of N added in CAN at both forests is about 1.3–2 times than the background rate. In addition, the responses of litter decomposition to N-addition can be subtle and non-linear, with decomposition increasing with low rates of N-addition, but no effect or decreasing with high N availability (Knorr et al., 2005). Therefore, the CAN treatment increased the rate of decomposition but UAN treatment had no effect on decomposition in the temperate or subtropical forests.

Our data show that CAN treatment had contrasting effects on initial (six months) and later stage (12 months) decomposition rates by increasing later stage decomposition rates. Our results are inconsistent with previous work, which found that externally supplied N stimulates early-stage decomposition rates by increasing initial leaf litter N concentrations (Hobbie, 2005). Meanwhile, the effects of N on decomposition may vary, depending on litter quality, N addition amount and rates (Knorr et al., 2005). In the later stage of decomposition, CAN treatment significantly enhances the diversity of soil fauna which has

positive effects on litter decomposition rates (Hättenschwiler et al., 2005).

#### 4.3. Correlation between soil fauna and litter decomposition

Our results suggest that a greater diversity of soil fauna species, not abundance or community composition, was associated with faster decomposition, thereby confirming the importance of species diversity in litter decomposition processes (Gessner et al., 2010). Experimental manipulations using microcosms suggest that the changes of detritivorous species diversity within or between trophic groups drives community compositional effects on litter decomposition rate (Gessner et al., 2010; Kitz et al., 2015; Liu et al., 2016). The soil fauna community in our study site was predominantly Oribatida, Isotomidae and Entomobryidae, which are active in the litter layer of forest floor (Liu et al., 2016). Therefore, Oribatida, Isotomidae and Entomobryidae may be the key groups driving litter decomposition in our study forest sites. Previous work demonstrated that litter decomposition was not correlated with the abundance of soil fauna, because soil fauna abundance showed an asymptotic response with litter decomposition, with maximal effects attained with few species (Heemsbergen et al., 2004). This

may explain why in our study, no significant correlation was found between soil fauna abundance and decomposition.

## 5. Conclusions

In conclusion, our study revealed that CAN deposition increased the diversity of soil fauna and stimulated the litter decomposition at both temperate and subtropical forests, and rates of litter decomposition were positively correlated with soil fauna diversity. Therefore, our data suggest that increasing in the diversity of soil fauna under CAN deposition would consequently accelerate the rate of litter decomposition. An improved understanding of how soil fauna-decomposition interactions responses to N-deposition is important for ensuring reliable predictions of the soil ecological consequences of global change.

## 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.geoderma.2020.114703>.

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