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Canopy nitrogen addition affects ground-dwelling spider assemblages and trophic position

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Abstract

1. While it is clear that nitrogen (N) deposition impacts the plant diversity of major terrestrial ecosystems, how ground-dwelling fauna respond to N deposition is less understood.

2. Here, we use a novel experimental methodology of canopy addition of N (CAN) to simulate realistic N deposition and contrast this method to standard understory addition of N (UAN) in a temperate forest and a subtropical forest. We investigated the shifts in community composition and trophic position for ground-dwelling spiders under different N deposition treatments.

3. We found that the CAN deposition treatment increased the diversity of sit-and-wait spiders and the abundance of actively hunting spiders at the subtropical forest, but N treatments had no effect on spider abundance or diversity at the temperate forest. In contrast, UAN treatments had no significant impact on spider communities compared to control treatment. Furthermore, N treatments had significant effects on spider assemblage at subtropical forest. Overall, spiders in the CAN treatment had a higher trophic position as indicated by $\delta^{15}N$ isotopic signatures at both forest types.

4. In conclusion, our study suggests that the effects of N deposition on ground-dwelling spider assemblages depend on N addition approaches. Furthermore, our results suggest that CAN is a more realistic method for understanding the effects of atmospheric N deposition on soil arthropods in forest ecosystems.

KEYWORDS
^{15}N and ^{13}C stable isotope, atmospheric nitrogen, canopy and understory, foraging strategy, global change, subtropical and temperate forest

INTRODUCTION

China has experienced a rapid increase in N deposition rates since the 1980s; the N deposition rate in Chinese forest ecosystems increased from 4.6 kg N ha^{-1} year^{-1} in the 1980s to 23.2 kg N ha^{-1} year^{-1} in the 2000s (Gu et al., 2015). Liu et al. (2013) estimated N deposition rates continue to increase at a rate of 0.41 year^{-1} and N deposition in some regions of China, such as temperate and subtropical regions, could reach more than 50 kg N ha^{-1} year^{-1} in the next decade (Liu et al., 2011, 2013; Lü & Tian, 2007; Pan et al., 2012). Atmospheric deposition of nitrogen (N) affects a wide range of ecosystem structures and functions, including nutrient cycling and storage (Quinn Thomas et al., 2010), litter decomposition (Knorr et al., 2005) and biodiversity loss (Bobbink et al., 2010; Niu et al., 2016). Across major terrestrial ecosystems worldwide, high N inputs may lead to variation in soil nutrient availability and soil acidification, which then can drive changes in plant species diversity and community composition (Bobbink et al., 2010; Payne et al., 2017). In China, high N deposition rates to forest ecosystems have resulted in soil acidification (Liu et al., 2011), plant biodiversity loss (Liu et al., 2013), carbon storage changes (Gu et al., 2015), changes in fluxes of greenhouse gases and increased susceptibility to environmental stresses (Huang et al., 2021; Liu et al., 2011). In contrast, the effects of N deposition on soil fauna have been less explored, especially ground-dwelling fauna (Gan et al., 2014).

In forest floor ecosystems, spiders represent a large fraction of the arthropod predator biomass and have high diversity (Cardoso et al., 2011). Spiders play an important role in regulating decomposer populations and cascading effects on soil functions, such as litter decomposition and nutrient cycling (Koltz et al., 2018; Liu et al., 2018). Our previous work found that N deposition can change the abundance, diversity and community structure of decomposers by altering soil pH and nutrient storage (Liu et al., 2018; Liu et al., 2021). Changes in the decomposer community are likely to affect spider communities in the forest floor, because spiders are very sensitive to alterations of decomposer diversity and abundance (Meunier et al., 2016). Furthermore, the trophic position of spiders is likely determined by prey availability, because generalist spiders are more flexible in their choice of prey, which may cause different stable isotope signatures (Sanders & Platner, 2006). Therefore, N deposition has the potential to change the ground-dwelling spider assemblages and trophic position indirectly by changing the decomposer community. Despite this likely relationship, no empirical studies have explicitly investigated the effect of N deposition on the spider community and trophic position in forest floor ecosystems.

Our current understanding of N deposition on forest ecosystems is based on a conventional understory addition of N (UAN) which directly adds N solutions to the understory vegetation or forest floors (Gaige et al., 2007; Zhang et al., 2015). This UAN experimental approach, however, ignores many key processes occurring in the forest canopy. Forest canopies could retain part of deposited N before it enters the soil ecosystem. For instance, canopy branches and foliage can retain approximately 80% of atmospheric N deposition in conifer forests (Sievering et al., 2007) and 44%–52% in temperate forests (Liu, Mao, Shi, Wang, et al., 2020). Therefore, canopy addition of N (CAN) is a more realistic approach to assess the effects of atmospheric N deposition on biodiversity and ecological functions in forest ecosystems. Furthermore, increasing evidence suggests that compared to CAN, UAN overestimated the negative effects of N deposition on soil fauna diversity (Liu et al., 2021; Liu, Mao, Shi, Wang, et al., 2020), soil organic carbon (Lu et al., 2020; Shi et al., 2016), plant leaf traits (Tang et al., 2020), water use efficiency (Hu et al., 2019) and asymbiotic N fixation (Zheng et al., 2019).

Different forest types have divergent interception rates of atmospheric N deposition because the canopy size, branches and foliage traits are expected to vary across different forest types. Meanwhile, N limitation generally decreases from boreal to tropical forests (Menge et al., 2012), which indicates that different forest types may have varied responses to atmospheric N deposition. For example, Zheng et al. (2019) found that N deposition differed significantly in its negative effect on the asymbiotic N fixation between tropical (−35%) and temperate forests (−420%). Similarly, some soil taxa (e.g., Acarina and Collembola) showed different responses to N addition in a temperate forest and a subtropical forest (Liu et al., 2021). Thus, focusing on different forest types could offer new insights into the assessment of ground-dwelling arthropod responses to N deposition.

In the present study, we compare a novel and more realistic manipulation N deposition through CAN to the conventional UAN in a temperate forest and a subtropical forest. We investigated the ground-dwelling spider community and trophic position using stable isotope analysis under different N deposition conditions to test the following hypotheses: (1) the CAN approach will have less of a change in diversity and abundance on ground-dwelling spider assemblages relative to UAN since substantial amount of N from the N addition could be retained by the forest canopy; (2) the trophic position of spiders may have different responses to the N deposition treatments due to shifting prey composition; (3) spiders with different foraging strategies will have different responses to N addition due to differences in prey choice and visibility, capture efficiency and mobility (Liu et al., 2018; Schmitz, 2008).

MATERIALS AND METHODS

Study site

This experiment was conducted in two contrasting forest types: temperate forest Jigongshan (JGS) and subtropical forest Shimentai (SMT). The temperate JGS is located in JGS National Nature Reserve (31°46′–31°52′N, 114°01′–114°06′E, 168 m in elevation) in Henan Province, Central China, which has a warm temperate climate (Figure 1). The annual mean precipitation is 1119 mm and mean annual air temperature is 15.2°C. The soil type in JGS site is yellow-brown soil. JGS is dominated by approximately 45-year-old tree species, composed mainly of *Liquidambar formosana*, *Quercus acutissima*, and *Quercus variabilis*. The subtropical forest is in SMT National Nature Reserve (24°22′–24°31′N, 113°05′–113°31′E, 255 m in elevation), Guangdong Province, Southern China, which has a subtropical monsoon climate (Figure 1). The monsoon climate indicates that large proportion of precipitation and wet N deposition occurs during the rainy
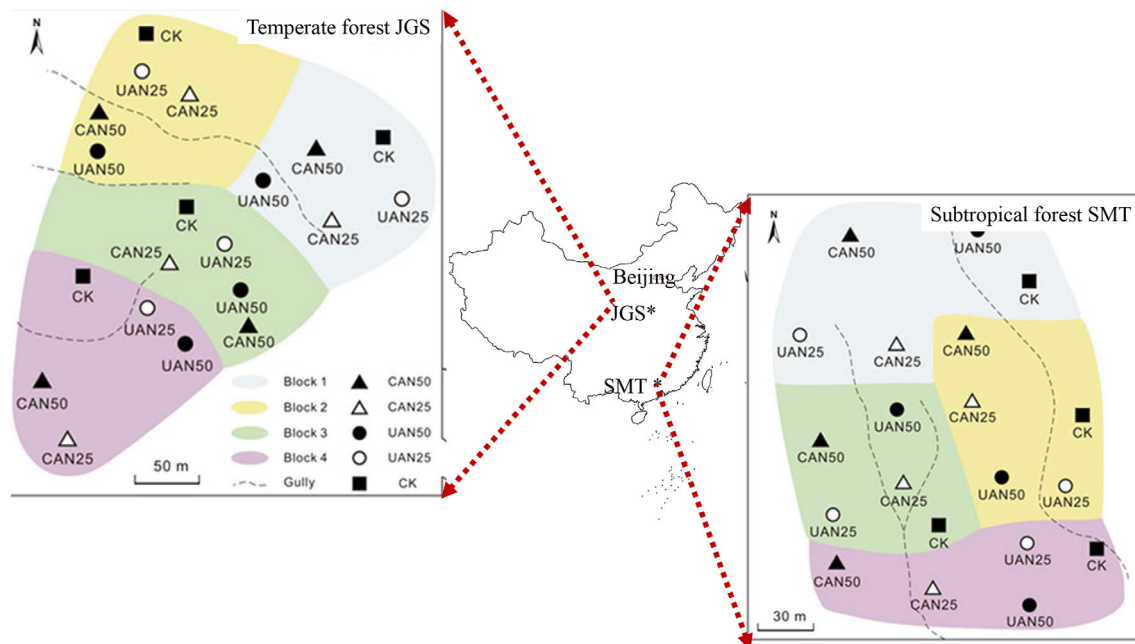
FIGURE 1 Location of study sites and layout of nitrogen treatment plots at temperate Jigongshan (JGS) forest and subtropical Shimengtai (SMT) forest

season (from April to November) (Lü & Tian, 2007). The annual mean precipitation is 2364 mm and mean annual air temperature is 20.8°C. The soil type in SMT is Latosolic red soil. SMT is dominated by approximately 53-year-old tree species, composed mainly of *Castanea henryi*, *Cryptocarya concinna*, *Schima superba*, *Machilus chinensis* and *Engelhardtia roxburghiana*. The ambient N deposition rate in precipitation is approximately 19.6 kg N ha^{-1} year^{-1} in the JGS region and 34.1 kg N ha^{-1} year^{-1} in SMT (Zhang et al., 2015), and the dry N deposition in JGS and SMT are about 4.05 kg N ha^{-1} year^{-1} and 3.46 kg N ha^{-1} year^{-1}, respectively (Lü & Tian, 2007; Wen et al., 2020).

Experimental design

At both the temperate JGS and subtropical SMT study locations, the experiment was a randomised block design with four blocks and five circular plots (907 m^2 in area and 17 m in semi diameter) within each block. Within each block, two concentrations of N were added through two approaches: CAN and UAN. The following five treatments were used at both sites: (1) CAN at 25 kg N ha^{-1} year^{-1} (CAN25); (2) CAN at 50 kg N ha^{-1} year^{-1} (CAN50); (3) UAN at 25 kg N ha^{-1} year^{-1} (UAN25); (4) UAN at 50 kg N ha^{-1} year^{-1} (UAN50); and (5) control (CK) (Figure 1). We lost one UAN25 and one UAN50 plot at SMT due to the breakdown of spraying equipment. The low N deposition concentration (25 kg N ha^{-1} year^{-1}) is approximately twice the current average N deposition rate and the high N deposition concentration (50 kg N ha^{-1} year^{-1}) is approximately triple the current N deposition in temperate and subtropical China (Gu et al., 2015; Lü & Tian, 2007; Wen et al., 2020). To simulate the wet deposition, the target amount of NH$_4$NO$_3$ pellets was added to water to set the concentration in each N treatment plot. Starting in April

2013, the treatment plots were applied with the equivalent of 3 mm of precipitation containing NH$_4$NO$_3$ solution at monthly intervals during the growing season (April to November) each year. The N addition amount in every month (April to November) was based on the actual amount that occurs in each forest type. Water was not sprayed into the CK plots because the effect of adding incremental water to the plots was considered negligible since the total amount was less than 1%–2% of annual precipitation in our study sites (Zhang et al., 2015). See more detail in Appendix S1, Table S1.

CAN was achieved by using a 35 m high spraying system built in the centre of the plots. The NH$_4$NO$_3$ solution was pumped to the top (about 5 m above the forest canopy) through polyvinyl chloride pipes which were fixed to a supporting tower. UAN was applied with a 1.5 m high automatic irrigation system, which represented the conventional method of N addition (Zhang et al., 2015). The automatic irrigation system is made of five evenly spaced sprinklers that distributed the N solution onto the forest floor in each UAN plot. The nitrogen addition systems had sprinklers that turned 360° automatically to ensure N addition solutions were evenly sprayed on the forest canopy and forest floor. More detailed descriptions of the working principles and validity of the spraying system can be found in Zhang et al. (2015).

Spider collection and analyses

In 2016, 3 years into the N-addition treatments, we used a leaf-litter sampling method to collect spiders at 2-month intervals over the course of the year (from January to December) in each forest type. One leaf-litter sample (50 × 50 cm) including surface debris was taken from two randomly selected locations per N plot at both JGS and

TABLE 1 Summary of the generalised linear mixed models (abundance) and linear mixed models (diversity) used to test for effects of different N deposition treatments on the spider community parameters at temperate Jigongshan (JGS) and subtropical Shimentai (SMT) (F value/Wald χ^2)

|	d.f.	Parameters	Temperate JGS	Subtropical SMT
Total spiders	4	Abundance	2.76	12.62**
	4	H' diversity	0.90	2.68*
	4	Richness	0.85	3.31**
	4	Evenness	0.71	2.94*
Actively hunting spiders	4	Abundance	3.20	11.22*
	4	H' diversity	1.08	1.47
	4	Richness	0.62	1.88
	4	Evenness	0.45	3.05*
Sit-and-wait spiders	4	Abundance	8.11	7.36
	4	H' diversity	0.62	6.09***
	4	Richness	1.66	2.41*
	4	Evenness	0.20	3.10*

*$p < 0.05$;

**$p < 0.01$;

***$p < 0.001$.

SMT. The large spiders were first collected by hand, then the remaining spiders were extracted from the samples using Tullgren ('Berlese') funnels for 5 days or until the litter was completely dry (Edwards, 1991). Spiders were identified to species level or morphospecies according to external morphological characteristics and divided into two functional guilds: sit-and-wait (SW) spider and actively hunting (AH) spider according to Schmitz (2008). Specimen vouchers were deposited at the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, in the authors' personal collection.

Generally, stable isotopes of carbon ($\delta^{13}C$) are used to discriminate resource use of species, and nitrogen stable isotopes ($\delta^{15}N$) to assess trophic position (Peterson & Fry, 1987). At both temperate JGS and subtropical SMT forest, we first selected the three most common spider species in each functional guild that were observed in all the N deposition plots. There were three independent replicates for each species and N deposition treatments at both sites, resulting in a total of 180 samples (3 replicates × 3 spider species × 2 foraging strategies × 5 N deposition treatments × 2 study sites). We used the prosoma for stable isotope analysis. We selected spiders from the summer season (May to September) for stable isotope analysis due to strong seasonality variation (Mestre et al., 2013). For each replicate, we combined prosomas from a minimum of four individuals to help level out possible variation between individuals in ^{15}N and ^{13}C signatures. Spider samples were oven-dried at
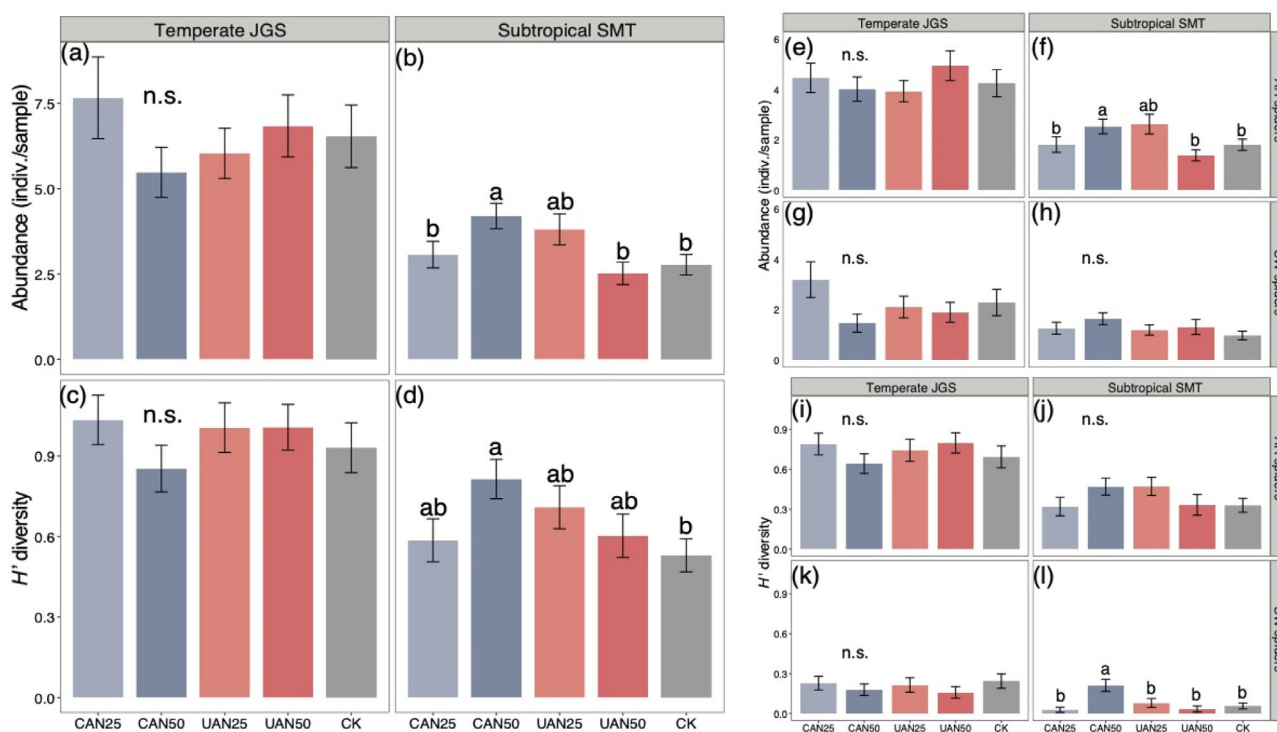
FIGURE 2 Impacts of nitrogen treatments on the abundance and diversity (H') of total spiders (a–d), actively hunting (AH) spiders (e, f, i, j) and sit-and-wait (SW) spiders (g, h, k, l) at temperate forest Jigongshan (JGS) and subtropical forest Shimengtai (SMT). Values are mean ± SE. Different letters indicate significant differences among nitrogen treatments ($p < 0.05$) and n.s. indicates no significance ($p > 0.05$). CAN25 = canopy addition of N at 25 kg N ha^{-1} year^{-1}; CAN50 = canopy addition of N at 50 kg N ha^{-1} year^{-1}; UAN25 = understory addition of N at 25 kg N ha^{-1} year^{-1}; UAN50 = understory addition of N at 50 kg N ha^{-1} year^{-1}; CK = control treatment

60°C for at least 48 h until weights were constant and then ground to a fine powder to homogenization. Afterwards, 1 ± 0.05 mg of ground spider tissue was weighed and stored at 60°C until isotope analysis. The natural abundances of dual isotope analysis ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were performed by an elemental analyser (Flash 2000 EA-HT) coupled with a stable isotope ratio mass spectrometer (Finnigan MAT 253 Thermo Fisher Scientific, Inc., USA). Natural abundances of stable isotope ratios were reported as $\delta^{15}N$ and $\delta^{13}C$ values (in ‰) and calculated as the following formula (Peterson & Fry, 1987):

$$\delta_{sample} (‰) = \left[R_{sample}/R_{standard} - 1 \right] \times 10^3,$$

where δ_{sample} is the isotope ratio of sample relative to a standard, and R_{sample} and $R_{standard}$ is the ratio of heavy/light isotope content for the element ($R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$). The atmospheric N for $\delta^{15}N$ and Pee Dee Belemnite carbonate for $\delta^{13}C$ are international standards.

Statistical analyses

In brief, our study included 2 forest sites × 5 N treatments × 5 blocks × 6 sampling times × 2 samples/plot. The response variable were abundance and diversity parameters of total spider and two spider species type (AH vs. SW). To determine the effects of different N deposition treatments on the Shannon–Wiener Index (H'), species richness and Pielou's evenness ($J = H'/\ln[S]$, where S is the total species number in a sample) of spiders, we used linear mixed models (LMM), where N treatment was treated as a fixed effect, and random slope model was used in the random effects, where plots were nested within blocks at each sampling time. Then we performed generalised linear mixed models (GLMM) with a Poisson distribution and log link for the spider abundance data, where N treatment was treated as a fixed effect, and where plots were nested within blocks at each sampling time as random effects. To account for multiple comparisons, we used Tukey honestly significant difference post-hoc test to assess differences among the N deposition treatments. For the comparison of each type of spider species (AH vs. SW) among different N treatments, we applied a Bonferroni correction to p-values to assess the significance of each level comparison in the LMM and GLMM. We conducted separate LMM and GLMM for each forest site. LMM and GLMM were performed using the 'lme4' package in the R software (Bates et al., 2015).

We used non-metric multidimensional scaling (NMDS) to assess spider community composition across the five N deposition treatments at both study forests. Then, one-way analysis of similarity (ANOSIM) with 4999 permutations was conducted to test differences in spider community composition among treatments. To account for multiple comparisons, we used the pairwise adonis function to assess differences among N deposition treatments (Anderson, 2001).

We used linear model (LM) to test whether the isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$ values) of spider species varied among the N treatments, spider species and foraging strategies. We performed two different LMs and its main factors are N treatment, spider species and their interactions, and foraging strategies, respectively. We applied a Bonferroni

correction to assess the significance of each level comparison in the spider species model for *Ozyptila praticola*, *Camillina pulchra*, *Antistea brunnea*. See more detail on the analyses in Appendix S2.

RESULTS

Effect of N deposition treatments on the abundance and diversity of spiders

A total of 1823 individuals from 28 spider species or morphospecies were recorded at temperate JGS (Appendix S1, Table S2), and
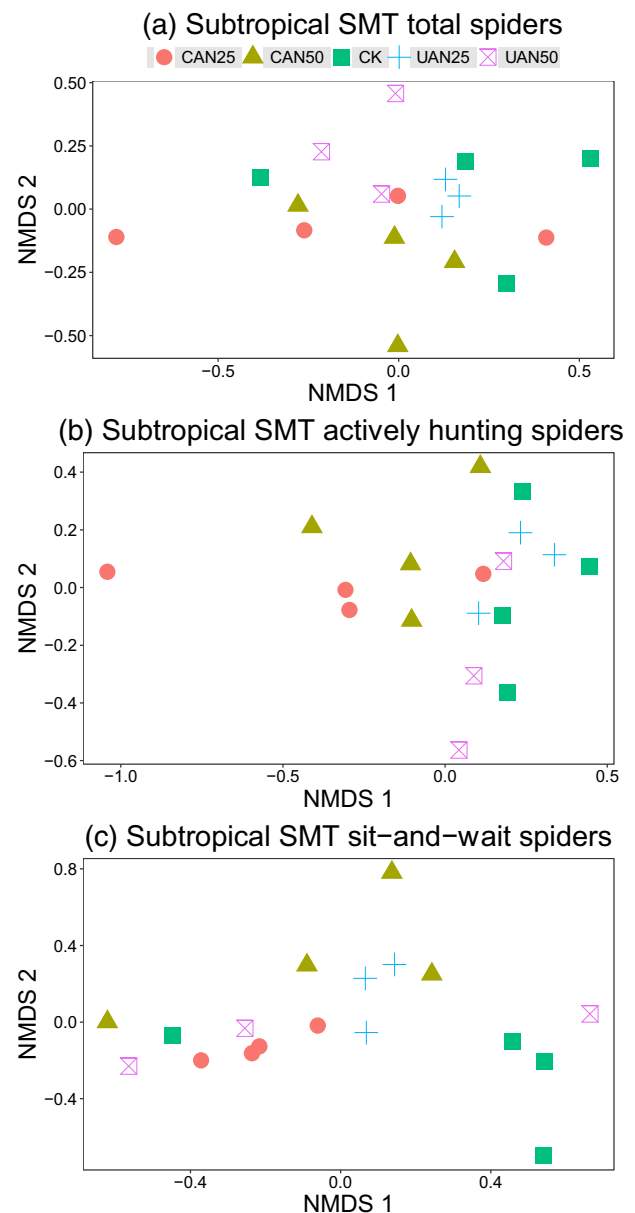
FIGURE 3 The non-metric multidimensional scaling (NMDS) of species assemblage composition of total spiders (a), actively hunting spiders (b) and sit−and−wait spiders (c) at subtropical Shimengtai (SMT)
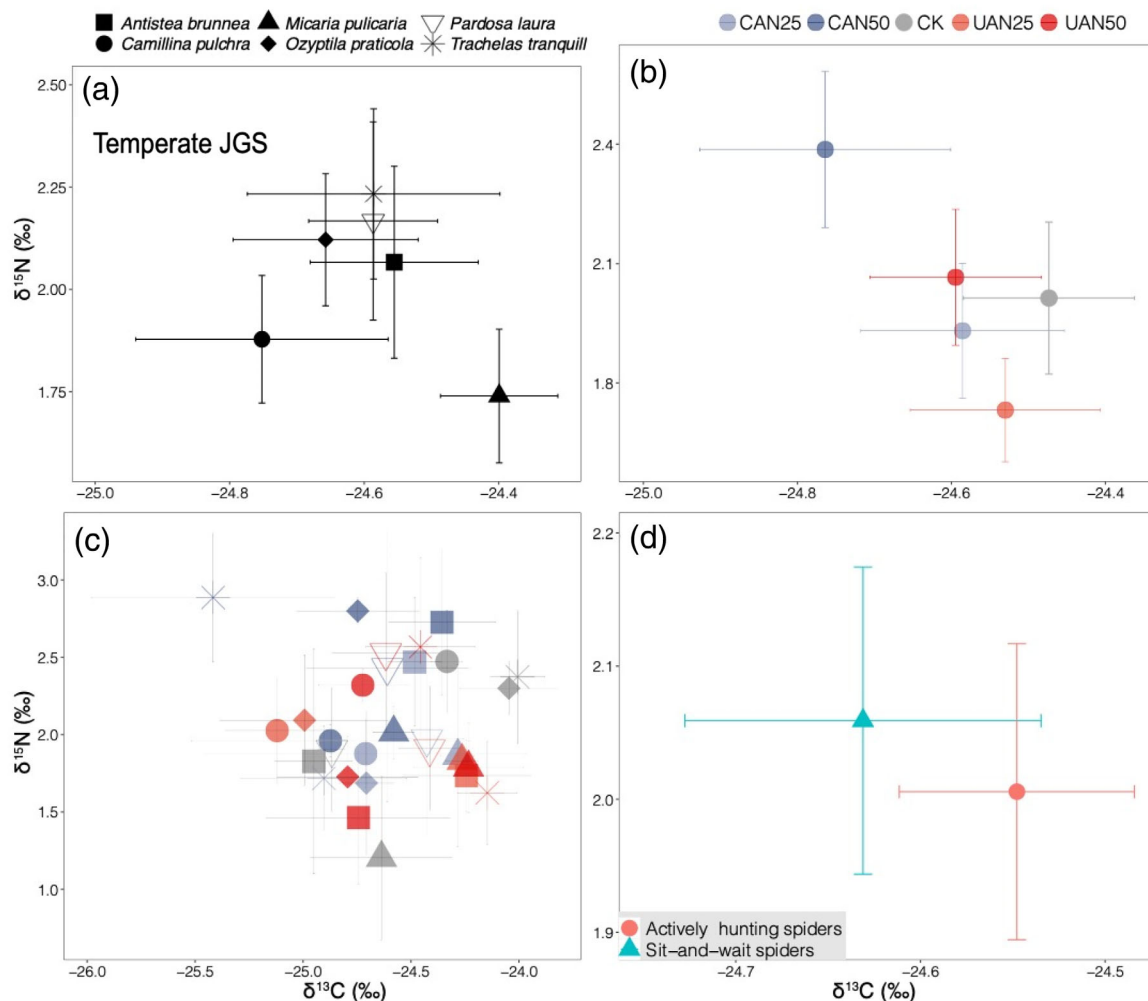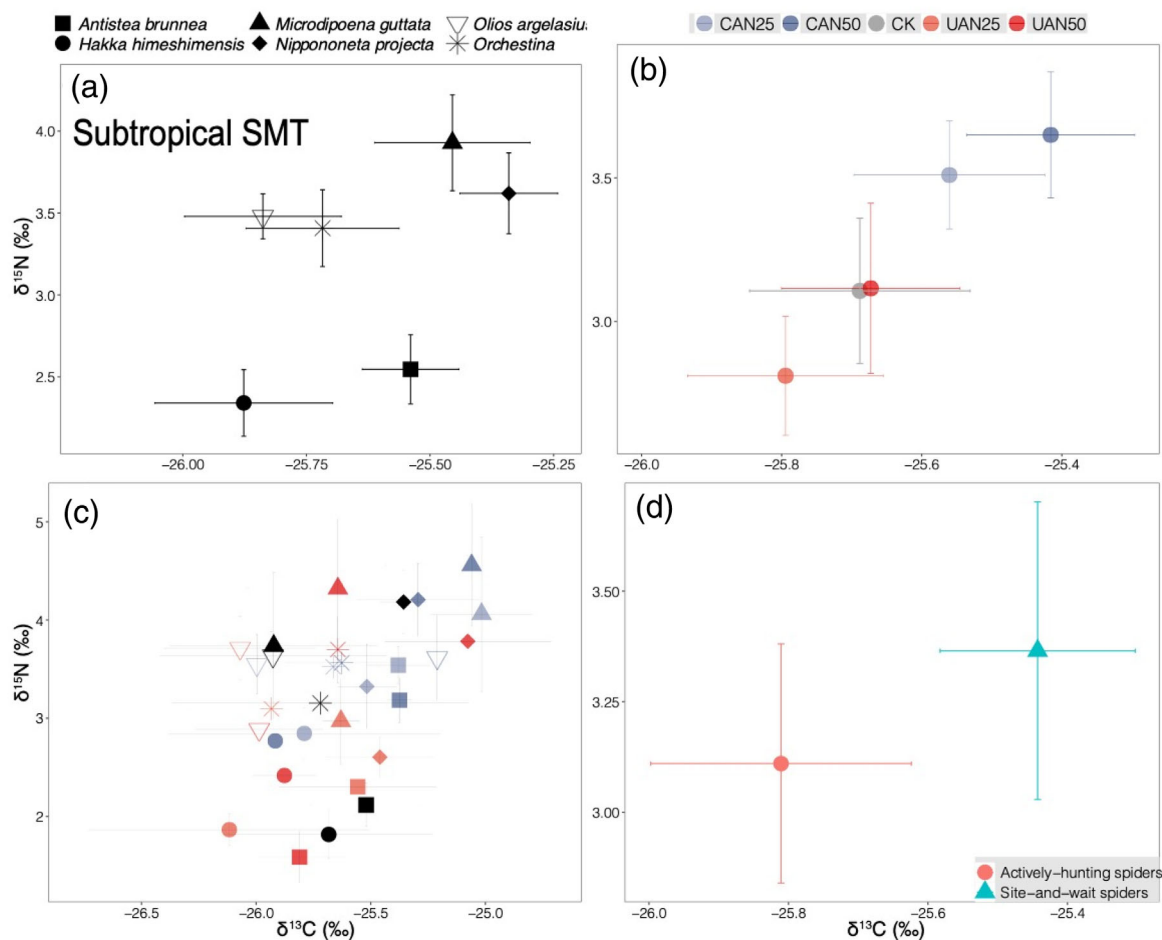
FIGURE 4 Impacts of spider species (a), nitrogen treatments (b), interaction between spider species and nitrogen treatments (c), and foraging strategy (d) on the δ^{13}C and δ^{15}N values at temperate Jigongshan (JGS). Values are mean ± SE.

820 individuals from 26 spider species or morphospecies were recorded at subtropical SMT (Appendix S1, Table S3). In addition, our species rarefaction curves indicate that our sampling was sufficient to collect the majority of spider species present at both forest sites (Appendix S1, Figure S1). Our results showed that there were no significant differences among N deposition treatments for spider abundance (Wald $\chi^2 = 2.76$, d.f. $= 4$, $p = 0.59$) and H' diversity ($F_{d.f.=4} = 0.90$, $p = 0.48$) at temperate JGS (Table 1; Appendix S1, Table S4). In contrast, N deposition treatments significantly affected total spider abundance (Wald $\chi^2 = 12.62$, d.f. $= 4$, $p = 0.01$) and H' diversity ($F_{d.f.=4} = 2.68$, $p = 0.03$) at subtropical SMT (Table 1; Appendix S1, Table S4). At subtropical SMT, the highest spider abundance was found in the CAN50 treatment (Figure 2b) due to a high abundance of *Orchestina* sp., *Microdipoena guttata*, *Heser schmitzi*, and others (Appendix S1, Table S2). The CAN50 treatment at SMT also had higher H' diversity compared to the CK treatments ($p = 0.02$) (Figure 2d). Similar trends were found among different N treatments for spider evenness and richness for JGS and SMT (Appendix S1, Figure S2).

For subsequent analyses, we divided total spiders into SW spiders and AH spiders. We observed no differences in abundance or diversity of AH or SW spiders across the N treatments at temperate JGS (Figure 2e,g,i,k), but we found that compared to the CK treatment, the CAN50 treatments had significantly higher abundance of AH spiders ($p = 0.05$) and H' diversity of SW spiders ($p = 0.001$) at subtropical SMT (Figure 2f,l).

The NMDS and ANOSIM analyses showed that N addition treatments did not have significant effects on the composition of total spiders or AH and SW spiders at temperate JGS (total spiders: $R = 0.10$, $p = 0.89$; AH spiders: $R = 0.09$, $p = 0.90$; SW spiders: $R = 0.04$, $p = 0.64$) (Appendix S1, Figure S3). In comparison, N treatments had significant effects on AH and SW spider assemblages at subtropical SMT (AH spiders: $R = 0.19$, $p = 0.03$; SW spiders: $R = 0.20$, $p = 0.04$) (Figure 3). The multiple comparisons showed that AH spider assemblage composition in CAN25 treatment was different from CK ($R^2 = 0.35$, $p = 0.04$), UAN25 ($R^2 = 0.32$, $p = 0.05$) and UAN50 treatment ($R^2 = 0.31$, $p = 0.05$) (Figure 3b; Appendix S1, Table S5). In addition, SW spider assemblage composition in CAN25 treatment was

FIGURE 5 Impacts of spider species (a), nitrogen treatments (b), interaction between spider species and nitrogen treatments (c) and foraging strategy (d) on the $\delta^{13}C$ and $\delta^{15}N$ values at subtropical Shimengtai (SMT). Values are mean ± SE.

different from UAN25 treatments ($R^2 = 0.35$, $p = 0.04$) (Figure 3c; Appendix S1, Table S5).

Effect of N deposition treatments on the trophic position of spiders

At temperate JGS, there were no significant differences in $\delta^{13}C$ ($F = 1.07$, $p = 0.38$) and $\delta^{15}N$ values ($F = 1.45$, $p = 0.22$) among the six spider species we tested (Figure 4a). For the $\delta^{15}N$ values among N treatments, we found that CAN50 treatment had higher $\delta^{15}N$ values than UAN25 treatments ($p = 0.05$), whereas no such differences were found for $\delta^{13}C$ value among N treatments ($F = 1.12$, $p = 0.35$) (Figure 4b). The interaction between spider species and N treatments revealed that there were no significant differences in $\delta^{15}N$ values for the spider species among N treatments ($F = 2.02$, $p = 0.17$) (Figure 4c). Furthermore, no statistically significant differences were found among $\delta^{13}C$ and $\delta^{15}N$ isotope ratios for the AH spider and SW spiders (Figure 4d).

At subtropical SMT, there was a significant effect of spider species on $\delta^{15}N$ values ($F = 12.31$, $p < 0.001$; Figure 5a). The SW spider *M. guttata* ($3.93 \pm 0.29‰$), SW spider *Nippononeta projecta*

($3.62 \pm 0.25‰$) and AH spider *Olios argelasius* ($3.48 \pm 0.14‰$) were more enriched in $\delta^{15}N$ than SW spider *A. brunnea* ($2.55 \pm 0.21‰$) and AH spider *Hakka himeshimensis* ($2.34 \pm 0.20‰$) (Figure 5a). For the $\delta^{15}N$ values among N treatments, we found that the CAN50 treatment ($3.65 \pm 0.22‰$) had higher $\delta^{15}N$ values than the UAN25 ($2.81 \pm 0.21‰$) and CK treatments ($3.11 \pm 0.25‰$) ($p = 0.02$ and $p = 0.05$, respectively), whereas no such differences were found for $\delta^{13}C$ values ($F = 1.50$, $p = 0.21$) (Figure 5b). The interaction between spider species and N treatments showed that $\delta^{15}N$ values of *A. brunnea* differed among N treatments ($F = 11.27$, $p = 0.001$); the *A. brunnea* in CAN25 ($3.54 \pm 0.19‰$) and CAN50 treatment ($3.18 \pm 0.23‰$) had higher $\delta^{15}N$ values than *A. brunnea* in UAN50 ($1.59 \pm 0.27‰$) and CK treatment ($2.12 \pm 0.22‰$) ($p = 0.01$ and $p = 0.05$, respectively) (Figure 5c; Appendix S1, Table S7). There were no significant differences in $\delta^{15}N$ values for the other spider species among N treatments (Figure 5c; Appendix S1, Table S6). Furthermore, no statistically significant differences were found among $\delta^{13}C$ and $\delta^{15}N$ isotope ratios for the AH spider and SW spider (Figure 5d). In addition, $\delta^{13}C$ signatures of six spider species did not differ among N treatments at both study sites (Figures 4 and 5; Appendix S1, Table S7).

DISCUSSION

In this study, we demonstrated that the method of simulated nitrogen deposition matters and that these effects differ depending on N-deposition approaches and spider-foraging strategy. In particular, our results showed that the CAN50 deposition treatment which simulates canopy-level deposition of about triple the ambient N deposition, increased the diversity of SW spiders and the abundance of AH spiders at our subtropical forest site. In contrast, we observed no differences in spider abundance or diversity across the N treatments at our temperate forest site. At both study sites, the trophic position (δ^{15}N values) of spiders was higher in CAN50 treatments compared to the UAN25 treatments. Furthermore, our data showed significant differences in trophic position (δ^{15}N values) for some spider species among N treatments at our subtropical but not our temperate forest site.

Canopy and understory N addition impacts on the spider assemblages

Contrary to our first hypothesis, the CAN50 treatment increased the abundance and diversity of ground-dwelling spider assemblages but UAN treatments had no significant impact on spider communities compared to CK. The different impacts of CAN versus UAN on spiders maybe attributable to the interception effects of the forest canopy on N deposition. As previous work has shown that the forest canopy can retain about 50% of the N added to the forest in our study site (Liu, Mao, Shi, Eisenhauer, et al., 2020), this N retained and absorbed by the forest canopy may increase the N concentration in leaves, and thus promote plant growth rate (Tang et al., 2020) and litter decomposition (Liu et al., 2021). This could thereby increase the energy flow through detrital food webs which may cascade up to affect subsequent predator levels via bottom-up effects (Dyer & Letourneau, 2002). Our finding of higher spider abundance and diversity in the CAN treatment is consistent with Liu et al. (Liu et al., 2021; Liu, Mao, Shi, Eisenhauer, et al., 2020) who found that CAN increased the abundance and diversity of decomposers. Increased decomposer density has the potential to change the abundance and diversity of soil fauna at predator levels through bottom-up effects (Gan et al., 2013), which may explain why CAN increased the diversity and abundance of spiders.

Our UAN effects on spider assemblages were inconsistent with previous studies on soil fauna using conventional N addition approaches (Chen et al., 2015; Gan et al., 2013) which showed negative effects of N addition on soil fauna. This may be because the magnitude of N effects on soil fauna are highly dependent on the amounts of N added and treatment duration (Eisenhauer et al., 2012). The N concentration (25 and 50 kg N ha^{-1} year^{-1}) in our study is much lower than other studies (80–300 kg N ha^{-1} year^{-1}) (Knorr et al., 2005; Schuldt et al., 2008) because we wanted to simulate only slightly higher deposition rates beyond ambient levels. In addition, N addition experiments have immediate short-term effects and long-term effects with large time intervals in forest ecosystems (Schuldt

et al., 2008). For example, Zak et al. (2004) found that short- and long-term N deposition had very different impacts on the N content in each ecosystem component such as leaves, roots, soil N and microbial N.

In our study, we found that AH spiders and SW spiders assemblage composition did not respond in the same way to N addition experiments at our subtropical forest. Previous studies showed that the AH and SW spiders had different dispersal ability and life-history traits which may influence their responses to environmental change (Scharf et al., 2011). In addition, a range of some ecological processes (such as prey community changes, competition and predation) could affect the pattern of spider community composition (Scharf et al., 2011), which appear to explain why CAN25 treatment had significant effects on AH and SW spider assemblage composition.

N deposition treatment effects on the trophic position of spiders

Our results support our second hypothesis of differences in spider trophic positions among N addition treatments in our subtropical SMT forest. Overall, results of the present study showed that the spiders in the CAN50 treatment had a higher trophic position (δ^{15}N values) than spiders from the UAN25 treatment and CK treatment at subtropical SMT forest. This difference was probably driven by fluctuations in prey availability that caused changes in spiders' diets because different prey isotopic signatures (δ^{15}N values) can lead to variation in spider δ^{15}N enrichment (Oelbermann et al., 2008). Previous research in the same study sites showed differences in Collembola that may explain the changes in spider trophic position we observed. Specifically, the CAN50 treatment plots had a higher abundance of Isotomidae and Entomobryidae than the UAN25 and CK treatments, but lower abundance of Sminthuridae than UAN25 and CK treatments (Liu et al., 2021). Collembolans are one of the usual types of prey of ground-dwelling spiders, and different Collembola species vary in δ^{15}N signature. For example, Entomobryidae and Isotomidae are primary decomposers and secondary decomposers respectively, which have higher δ^{15}N values (Entomobryidae range from -2.4‰ to -4.4‰; Isotomidae approximately 1.2‰) (Chahartaghi et al., 2005), but Sminthuridae mainly feed on plant tissues which have lower δ^{15}N values (from -7.2‰ to -4.8‰) (Chahartaghi et al., 2005; Moore et al., 1987). Thus, CAN50 treatments increased the abundance of Collembolans with higher δ^{15}N value and UAN25 and CK treatments increased the Collembolans with lower δ^{15}N value (Chahartaghi et al., 2005), which may explain the varying patterns of ^{15}N enrichment in spider among N deposition treatments.

Comparatively, we did not detect the differences in spider trophic positions among N addition treatments and CK treatments at temperate JGS forest. A possible explanation is that N addition treatments may not significantly change the Collembola community composition compared to the CK treatment. Furthermore, increased N deposition can directly alter plant chemistry and isotopic composition (δ^{15}N values) of potential spider prey, and these chemical changes can affect

the trophic position of predators (Nijssen et al., 2017), but we did not directly test this effect in our study. Therefore, further research will aim to integrate the plant chemistry and isotopic composition of the potential prey to evaluate the mechanism behind N deposition impacts on the trophic position of spiders. In addition, differences in $\delta^{13}C$ for the two forest sites suggest that different Collembola species feeding on different food sources (Chahartaghi et al., 2005).

At subtropical SMT, individuals of the SW spider *A. brunnea* in the CAN25 (3.54 ± 0.19‰) and CAN50 treatments (3.18 ± 0.23‰) had a higher $\delta^{15}N$ value than individuals of *A. brunnea* in CK treatment (2.12 ± 0.22‰), which displayed about 1.06‰–1.42‰ increase in CAN deposition compared to CK treatment. CAN impacts on *A. brunnea* $\delta^{15}N$ value may be mediated by changes in the $\delta^{15}N$ signature of prey (Oelbermann et al., 2008), and *A. brunnea* needs a suitable microhabitat to build its specialised web in the upper layer of leaf-litter which may make *A. brunnea* more sensitive to abiotic changes such as the N concentration of leaf-litter, rainfall and temperature (Mallis & Hurd, 2005).

At subtropical SMT, *M. guttata*, *N. projecta* and *O. argelasius* were more enriched in $\delta^{15}N$ than *A. brunnea* and *H. himeshimensis* (Figure 4a). This might be explained by the fact that $\delta^{15}N$ signatures were affected by the body size of spiders (Sanders et al., 2015). Large spiders tend to feed on prey from higher trophic levels, leading to a higher trophic position in the food web (Sanders et al., 2015). Sanders et al. (2015) showed a significant relationship between body size and $\delta^{15}N$ enrichment for different spider species. The exception was *M. guttata* which has a small body size but high trophic position, possibly due to cannibalism, which may change their trophic position (Mestre et al., 2013).

Spider-foraging strategies response to N deposition treatments

Our results agree with our third hypothesis of spiders with different foraging strategies will have different responses to N addition. Our results showed that CAN50 treatments significantly increased the abundance of AH spiders and diversity of SW spiders at our subtropical forest site. Most of the dominant Collembola species in our study system, such as Entomobryidae and Isotomidae species (Liu et al., 2021) have furcae which permit high mobility in the litter layer (Bilde et al., 2000). Previous work predicted that mobile prey were more likely to be encountered and captured by AH spiders (Liu et al., 2015). That possibly explains the increased AH abundance in CAN50 treatments. Meanwhile, the increased abundance of AH spiders may intensify intraguild predation or conspecific competition among AH spiders, which may constrain the increase of AH spider diversity (Willemart & Lacava, 2017). Our finding is similar to Oelbermann et al. (2008) who found out that the density of AH spiders (e.g., wolf spiders) was increased in prey-enhancement treatments, but densities of SW spider (e.g., web-building linyphiids) were not affected by the treatments. By contrast, SW spiders often occur at high diversity, owing to the uneven distribution of suitable

microhabitats for web building (Scharf et al., 2011), and the CAN50 treatment may increase the microhabitat heterogeneity in forest floor by changing litter N concentration and biomass, which may have influenced the increased diversity of SW spiders we observed (Rao, 2009). Alternatively, SW spiders may decrease their web size to reduce the conspecific density and avoid cannibalism which possibly can increase the species diversity (Scharf et al., 2011).

N deposition treatment effects on the spider assemblages in two contrasting forests

Our results showed that N addition (CAN50) affected spider abundance and diversity at our subtropical but not our temperate forest. Our previous research at the same study sites showed that N deposition suppressed forest floor and foliar N fixation in the temperate JGS, but there were no inhibitory effects in the subtropical SMT (Zheng et al., 2019). Thus, temperate forest N addition reduced N fixation in forest floor may result in no different N cycling among N treatments, which may partly explain why there were no significant effects on spider abundance and diversity at our temperate forest site. While the extensive N-deposition simulation setup of our experimental design precluded replicating the experiment across multiple temperate and subtropical locations, the differences we found between the effects of N-addition at the temperate and subtropical sites warrant further investigation to determine if they are consistent trends.

CONCLUSIONS

Our study suggests that the effects of N deposition on ground-dwelling spider assemblages is dependent on N addition approaches. To the best of our knowledge, our study is one of few studies that formally assesses the impact of N deposition on the trophic position of spider assemblages in a subtropical and a temperate forest using stable isotope analysis. Our study revealed that spider assemblages in temperate and subtropical forests had different responses to N deposition, and also different spider-foraging strategies had different response to N deposition. Furthermore, our results suggest that combining biodiversity and trophic position indicators provides fuller insights when assessing the impact of N deposition on soil arthropods.

N ADDITION AFFECTS SPIDERS

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(lead); supervision (equal); writing – original draft (supporting); writing – review and editing (supporting).

CONFLICT OF INTEREST

The authors do not have any conflicts to declare.

DATA AVAILABILITY STATEMENT

Data are available from Dryad, Dataset, https://doi.org/10.5061/dryad.g79cnp5n4

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Supporting Information.

Table S1. The amount of NH_4NO_3 (kg N ha^{-1}) from rainfall in the ambient N deposition and N addition treatment plots in every month.

Table S2. Spider species abundance across nitrogen deposition treatments at temperate forest JGS (mean $\pm$ SE, $n = 4$).

Table S3. Spider species abundances across nitrogen deposition treatments at subtropical forest SMT (mean $\pm$ SE, $n = 4$).

Table S4. Summary of generalised linear mixed model (abundance) and linear mixed model (diversity) used to test for effects of N deposition treatments on the spider community parameters at temperate JGS and subtropical SMT.

Table S5. The pairwise comparison of non-metric multidimensional scaling (NMDS) of species assemblage composition of actively hunting spiders and sit-and-wait spiders at subtropical SMT (p value).

Table S6. Summary of the linear model used to test for effects of different N deposition treatments on the $\delta^{15}N$ values (‰) among the six spider species at temperate JGS and subtropical SMT (mean $\pm$ SEM).

Table S7. Summary of the linear model used to test for effects of different N deposition treatments on the $\delta^{13}C$ values (‰) among the six spider species at temperate JGS and subtropical SMT (mean $\pm$ SEM).

Figure S1. The rarefaction curves of spider diversity among different N treatments in temperate JGS and subtropical SMT.

Figure S2. Effects of nitrogen treatments on the evenness and richness of different spider foraging strategies at temperate forest JGS and subtropical forest SMT. Values are mean $\pm$ SE. Different letters indicate significant differences among nitrogen treatments ($p < 0.05$) and n.s. indicates no significance ($p > 0.05$). TS = total spider, AH = actively hunting spider, SW = sit-and-wait spider. CAN25 = canopy addition of N at 25 kg N ha^{-1} year^{-1}, CAN50 = canopy addition of N at 50 kg N ha^{-1} year^{-1}, UAN25 = understory addition of N at

25 kg N ha^{-1} year^{-1}, UAN50 = understory addition of N at 50 kg N ha^{-1} year^{-1}, CK = control treatment.

Figure S3. The non-metric multidimensional scaling (NMDS) of species assemblage composition of total spider (a), actively hunting spider (b) and sit-and-wait spider (c) at temperate JGS.

Appendix S2. Supporting Information.