



# Community variation characteristics and driving pathways of soil microarthropods in meadow steppe under different nitrogen application durations

Zhendan Wu<sup>a</sup>, Shangfei Ma<sup>d</sup>, Renheng Wu<sup>a</sup>, Siyuan Bao<sup>a</sup>, Meijun Sheng<sup>a</sup>, Dianlin Yang<sup>e</sup>, Mei Hong<sup>a,b,c,\*</sup>

<sup>a</sup> College of Resources and Environment Sciences, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia 010018, China

<sup>b</sup> Inner Mongolia Key Laboratory of Soil Quality and Nutrient Resources, Hohhot, Inner Mongolia 010018, China

<sup>c</sup> Key Laboratory of Agricultural Ecological Security and Green Development at Universities of Inner Mongolia Autonomous Region, Hohhot, Inner Mongolia 010018, China

<sup>d</sup> Institute of Grassland Research, Chinese Academy of Agricultural Sciences, Hohhot, Inner Mongolia 010018, China

<sup>e</sup> Agro-Environmental Protection Institute, Ministry of Agriculture and Rural Affairs, Tianjin 300191, China



## ARTICLE INFO

### Keywords:

Nitrogen application

Soil microarthropods

Threshold effect

Meadow steppe

## ABSTRACT

Soil microarthropods are important links between aboveground and belowground food webs in grassland ecosystems and are sensitive to changes in environmental conditions. However, the continuous changes in soil microarthropod community characteristics under the threat of increasing global nitrogen (N) deposition remain unclear. On the basis of the N deposition simulation experiment design in 2010 for the *Stipa baicalensis* meadow steppe, 8 N treatments (0 (control group), 15, 30, 50, 100, 150, 200, and 300 kg ha<sup>-1</sup> yr<sup>-1</sup>) were used to analyse the changes in the soil microarthropod community structure and diversity characteristics and their potential driving pathways after 10, 11, 13 and 15 years of N addition. Microdispidae of Prostigmata (prostigmatal mites) consistently appeared as a dominant taxon across multiple years (the 10th, 11th, 13th, and 15th years of N application). With increasing N application rates and duration, the abundance and taxa of Prostigmata gradually increased, whereas the abundance and taxa of Poduromorpha (poduromorph springtails) and Entomobryidae (entomobryid springtails) decreased. Therefore, Prostigmata gradually dominated the composition of soil microarthropod community. There were significant differences in the soil microarthropod community structure among the different N rates and durations of application. However, with increasing N application rates and durations, the Jaccard index gradually increased, and the difference in the soil microarthropod community structure gradually decreased. Long-term N addition had a threshold effect on the abundance and diversity of soil microarthropods, with a threshold of 200 kg ha<sup>-1</sup> yr<sup>-1</sup>. Precipitation and pH values were the main factors driving the characteristics of changes in the soil microarthropod community in the meadow steppe under different years of N application. In summary, long-term N application significantly changed the soil microarthropod community in the *S. baicalensis* meadow steppe, and the interannual changes in the soil microarthropod community were regulated by both climatic and environmental factors under N application.

## 1. Introduction

Grassland ecosystems are important carriers in the construction of ecological civilizations. They play an important ecological role in protecting biological resources, regulating carbon (C) and nitrogen (N) cycles, and influencing the productivity of agriculture and animal husbandry (Bardgett et al., 2021; Yang et al., 2024). N is the main limiting

factor for grassland ecosystems (Du et al., 2020). A long-term low N supply limits the ecological service functions of grassland ecosystems (LeBauer and Treseder, 2008). Since the industrial revolution, the burning of fossil fuels and the application of chemical fertilizers in farmlands have led to a rapid increase in atmospheric N deposition in China and around the world (Wen et al., 2022). It is estimated that from the 1860s to the 1990s, the amount of N input caused by human

\* Corresponding author at: College of Resources and Environment Sciences, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia 010018, China.  
E-mail address: [nmczhm1970@126.com](mailto:nmczhm1970@126.com) (M. Hong).

activities increased from 15 Tg yr<sup>-1</sup> to 156 Tg yr<sup>-1</sup>, and it is expected to increase to 270 Tg yr<sup>-1</sup> by 2050 (Galloway et al., 2004). When N in the atmosphere falls to the soil in the form of dry and wet depositions, it increases the availability of N in grassland ecosystems and affects important ecosystem functions such as grassland productivity and soil nutrient cycling (De Vries, 2021; Wang et al., 2024). The soil fauna are among the most abundant groups in the grassland ecosystem. They are vital for improving soil structure, promoting litter decomposition and maintaining the structure and function of the grassland ecosystem (Liu et al., 2016). They are also ideal indicators of soil health and ecological restoration (Wei et al., 2022). However, the changes in the community characteristics of soil fauna in grassland ecosystems under increased N loading remain unclear, which limits the feasibility of formulating sustainable grassland management strategies with a further increase in N input in the future.

As an important part of soil fauna, soil microarthropods are 'microengineers' of grassland ecosystems (Peng et al., 2023). They affect grassland productivity and ecological stability by decomposing organic matter, regulating nutrient cycling, and improving soil structure (Doblas-Miranda et al., 2014). Previous studies noted out that once the soil microarthropod community changes due to N deposition changes, key ecosystem functions may be strengthened or weakened (Peng et al., 2022b). In recent years, against the background of increasing global N deposition (Galloway et al., 2004), the response of soil microarthropods to N addition has attracted the attention of many researchers due to their sensitivity to environmental changes. A global-scale meta-analysis revealed that N addition generally increased the abundance and diversity of soil microarthropods in grassland ecosystems. It showed that appropriate N application increased the food sources of soil microarthropods by meeting plant nutrient requirements and increasing primary productivity, promoted the community reproduction of soil microarthropods, and strengthened the anti-interference ability of grassland ecosystems (Peng et al., 2022a; Wang et al., 2024). However, some studies have noted that excessive N addition has an inhibitory effect on the abundance and diversity of soil microarthropods (Tipping et al., 2013; Wang et al., 2021). Other research shows that the increase in ammonium N under excessive N addition will not only cause soil acidification, but also has toxic effects on the growth of plants and roots (Wu et al., 2024; Yang et al., 2023). Soil fauna prefer to survive in a neutral or slightly acidic environments. The decrease in pH and plant productivity after excessive N addition forms a habitat that is not conducive to the survival of soil microarthropods, which weakens the decomposition and nutrient cycling ability of soil microarthropods, reduces the tolerance of soil microarthropods to habitat changes, and emphasizes the threshold effect of N application on the ecological function of the soil microarthropod community (Kuperman, 1996; Xia and Wan, 2008).

Previous studies have shown that with increasing N application time and N application level, the impact on plant communities and soil properties become greater and a threshold appears (Xu et al., 2021). For example, the negative effects of N on soil pH and microbial biomass carbon, as well as the promotion of aboveground biomass and soil organic carbon, are enhanced with increasing experimental treatment time, and both negative and positive effects show an inflection point with increasing N application level (Bian et al., 2019; Xu et al., 2007), indicating that the ecological needs and feeding strategies of soil microarthropods also vary with experimental time increases. However, most of the current studies on the duration of N application have been conducted for less than five years, with analyses restricted to short-term changes ( $\leq 1$  year) and low-level postapplication ( $< 50 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ) (Liu et al., 2021; Ochoa-Hueso et al., 2014). There is a lack of continuous monitoring of the changes in soil microarthropod communities after long-term multilevel N addition (Dan-yan1 et al., 2015; Zhang et al., 2023). Many studies have noted that the response of soil microarthropod communities to N addition is a long-term stable process. Understanding the long-term continuous changes in soil microarthropod communities

and their existing threshold effects is the key to understanding the effects of N input on grassland ecosystems (Li et al., 2024; Xu et al., 2009). Therefore, a better understanding of the response of grassland ecosystems to long-term N addition, especially, changes in soil microarthropod community characteristics, is critical.

The *Stipa baicalensis* meadow steppe is an important part of the Eurasian grassland biota and plays an important role in the development of animal husbandry in China (Gong et al., 2020). However, in recent years, due to the overgrazing of grasslands and the aggravation of global environmental change, this area has experienced the dual pressure of human disturbance and environmental change (Li et al., 2023). As important regulators of the material cycle and energy flow in meadow steppes, soil microarthropods are extremely sensitive to environmental changes (Wei et al., 2022). Exploring the continuous changes in soil microarthropod community characteristics under long-term N addition can effectively reflect the impact of N deposition changes on grassland ecosystems. Therefore, on the basis of the current and projected N deposition trends, we conducted long-term N addition manipulative experiments to investigate temporal dynamics in the soil microarthropod community, analyse their nonlinear response dynamics to N deposition, and identify key mechanisms driving N-induced succession of the soil microarthropod community structure. We hypothesized that (1) the community composition of soil microarthropods significantly differs among different N application rates and durations and that N addition has a positive effect on the abundance and diversity of soil microarthropods. (2) There is a threshold effect of N addition on the increase in soil microarthropod abundance and diversity, and (3) the abundance, taxa and diversity changes in soil microarthropods in meadow steppe are regulated mainly by pH and plant biomass as a result of different N application durations.

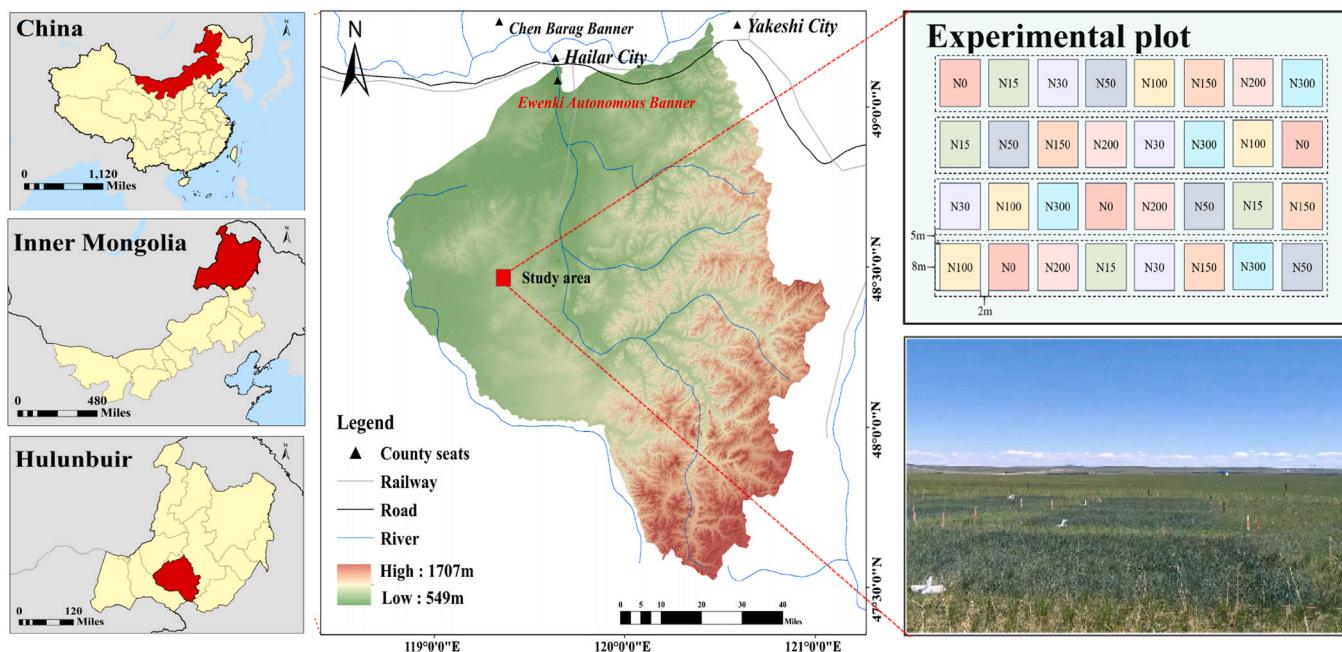
## 2. Materials and methods

### 2.1. Study site description

The experimental area is located in the *S. baicalensis* meadow steppe ( $48^{\circ}27'N$ – $48^{\circ}35'N$ ,  $119^{\circ}35'E$ – $119^{\circ}41'E$ ) in Yiminhe Town, Ewenki Autonomous Banner, Hulun Buir City, Inner Mongolia, China (Fig. 1). The altitude is 760–770 m, and the terrain is flat. It has a continental temperate climate. The average annual temperatures in 2019 (10 yr), 2020 (11 yr), 2022 (13 yr) and 2024 (15 yr) were  $0.8^{\circ}\text{C}$ ,  $0.8^{\circ}\text{C}$ ,  $-0.3^{\circ}\text{C}$  and  $-1.0^{\circ}\text{C}$ , respectively. The annual precipitation amounts in 2019, 2020, 2022 and 2024 were 352.4 mm, 546.1 mm, 142.1 mm and 326.4 mm, respectively. The precipitation is mainly concentrated from June to September. The average temperatures during the growth period (May–September) were  $16.14^{\circ}\text{C}$ ,  $16.34^{\circ}\text{C}$ ,  $16.30^{\circ}\text{C}$  and  $17.1^{\circ}\text{C}$ ; the precipitation amounts were 317.4 mm, 463.3 mm, 123.0 mm and 282.3 mm (Fig. S1) in 2019, 2020, 2022 and 2024, respectively. The annual evaporation is approximately 1400 mm, and the average annual frost-free period is 100 days. The soil type is a dark chestnut soil. At the beginning of the experiment, the soil pH was 7.07, the amount of organic matter was  $35.92 \text{ g}\cdot\text{kg}^{-1}$ , the total N content was  $1.85 \text{ g}\cdot\text{kg}^{-1}$ , and the total phosphorus content was  $0.45 \text{ g}\cdot\text{kg}^{-1}$ .

### 2.2. Experimental design

The long-term experimental field site was established in 1981. N fertilizer was applied in June 2010 to simulate an increase in N deposition. A random block design with 4 replications was implemented, with eight levels of N application: N0 (control), N15, N30, N50, N100, N150, N200, and N300, which represented net N amounts ranging from 0, 15, 30, 50, 100, 150, 200, 300  $\text{kg ha}^{-1} \text{ yr}^{-1}$  (excluding atmospheric N deposition), respectively. The N addition gradient setting refers to the previous N addition experiment in the Inner Mongolia grassland (Bai et al., 2010; Yang et al., 2023; Zhang et al., 2020). Each plot measured 8 m × 8 m, with a 2-m alley between adjacent plots and a 5-m separation



**Fig. 1.** The test site located in Hulunbeier, Inner Mongolia, China.

between the blocks (Fig. 1). From 2010 onwards, N fertilizer ( $\text{NH}_4\text{NO}_3$ ) was applied to designated plots in two equal splits annually—once in June and again in mid-July. To minimize N volatilization, the fertilizer was first dissolved in water. The water volume was 1900 ml per application to ensure complete dissolution, even for the highest N300 treatment (300 kg N  $\text{ha}^{-1}$   $\text{yr}^{-1}$ ). This was based on the solubility of  $\text{NH}_4\text{NO}_3$  (150 g dissolves in 100 ml of water at 20 °C; Bao, 2000). The resulting solution was evenly applied onto the plot using a watering can. An equal volume of water (without N) was applied to the control plots to standardize the moisture conditions.

### 2.3. Sampling methods

The samples of soil microarthropods were collected in mid-August in the 10th, 11th, 13th, and 15th years of N addition. To avoid the boundary effects, four unperturbed points were selected in each experimental plot, and a ring knife ( $R=5$  cm,  $V=200 \text{ cm}^3$ ) was used for sampling. The sampling depth was 0–10 cm, and 400 ml of soil was taken from each plot to collect soil microarthropods. The soil microarthropods were isolated by the Tullgren dry funnel method (Macfadyen, 1953) for 48 h. The collected soil microarthropods were placed in a bottle with a 75 % alcohol solution and labelled. The collection of soil microarthropod samples was conducted simultaneously with the collection of 0–10 cm soil samples. In each plot, soil samples were collected from 10 points in an "S" shape and mixed into one composite soil sample, which was used for determining the physical and chemical properties of the soil.

The plant community survey was carried out in the middle and late August of each year. To avoid edge effects, representative quadrats were selected in each community for investigation, and the sampling area was 1 m × 1 m. The species composition, height, coverage and density of the plant communities in each quadrat were investigated. After the vegetation survey, the plants were cut using the harvest method and taken back to the laboratory in an envelope. After being heated at 105 °C for 30 min, the plants were dried to a constant weight at 75 °C and weighed to obtain aboveground biomass (AGB).

### 2.4. Measurement methods

The classification of soil microarthropods was based on the 'Pictorial Keys to Soil Animals of China' (Yin, 1998), and the collected soil microarthropod specimens were identified under an Olympus CKX41 microscope and a SZ78 series stereomicroscope at the family level. The groups were divided into the following categories on the basis of their relative abundance: dominant taxa ( $\geq 10\%$ ), common taxa (1 %–10 %), and rare taxa ( $\leq 1\%$ ) (Nogueira et al., 2015).

The soil physical and chemical properties were determined according to 'Soil and Agricultural Chemistry Analysis 3rd ed.' (Bao, 2000). The soil pH was determined by potentiometry (with a soil-to-water ratio of 1:5). The soil moisture content (SMC) was determined by the drying method and the soil organic carbon (SOC) was determined by the potassium dichromate external heating method. The soil total nitrogen (TN) content was determined by the semimicro Kjeldahl method (K9840 Kjeldahl apparatus); the soil available potassium (AK) content was determined by the ammonium acetate extraction-flame photometric method. Ammonium N ( $\text{NH}_4^+$ -N) and nitrate N ( $\text{NO}_3^-$ -N) were determined by a 2 mol·L<sup>-1</sup> potassium chloride extraction-flow analyser (AA3, Germany).

### 2.5. Data analysis

The Shannon–Wiener index, Simpson index, Pielou index, Margalef index, and Jaccard similarity index were used as the characteristic indices of the soil arthropod community under N addition. The calculation formula can be found in Supplementary Material 2.5. The calculation of all the community characteristic indices was based on the family level of the soil microarthropods. The names, abundances and taxon numbers of the soil microarthropod groups are detailed in Supplementary material Table S2. The mean method was used to process the four sets of repeated data. First, the index of each repetition was calculated separately (Table S1), and then the mean of the four repetitions was taken as the representative value of the processing. The coefficient of variation (CV) was calculated to evaluate the degree of dispersion between replicates. The CVs of all the indices were less than 30 %, indicating that the variation between replicates was controllable and that the data were reliable (Table S1). One-way ANOVA was used to

detect the differences in the community structure and diversity characteristics of soil microarthropods with different durations of N addition. The data are presented as the means  $\pm$  standard errors ( $n = 4$ ). Before the analysis of variance, the premise hypothesis (normality and homogeneity of variance) of the analysis of variance was verified, and Duncan multiple comparisons were performed. The significance level of all analyses was set to  $P < 0.05$ .

The specimens were used as the unit for measuring the abundance of soil microarthropods (at a depth of 0–10 cm) (abundance/specimens), and the families were used as the taxa unit of soil microarthropods (taxa/families). Principal component analysis (PCA) was used to analyse the responses of the dominant and common taxa of the soil fauna to N addition and the number of years of N application. The vegan package in R was used to construct the principal response curve (PRC) to analyse the dynamic responses of dominant and common taxa of soil microarthropods to N addition in the 10th, 11th, 13th, and 15th years. The random forest package and the rfPermute package in R 4.5.0 were used to construct a random forest model to analyse the environmental factors related to soil microarthropods at different levels of N addition and in different N application years. The importance value of each environmental factor was evaluated on the basis of the mean square error (MSE). Using the piecewise SEM package in R 4.5.0, a structural equation model was established to analyse how N application and climate change affect changes in the soil microarthropod community in different ways.

Data analysis and processing were performed using Excel 2016; variance analysis was carried out using SPSS 22.0; principal component analysis was conducted using Canoco 5.0, and graphical visualizations were generated with Origin 2022.

### 3. Results

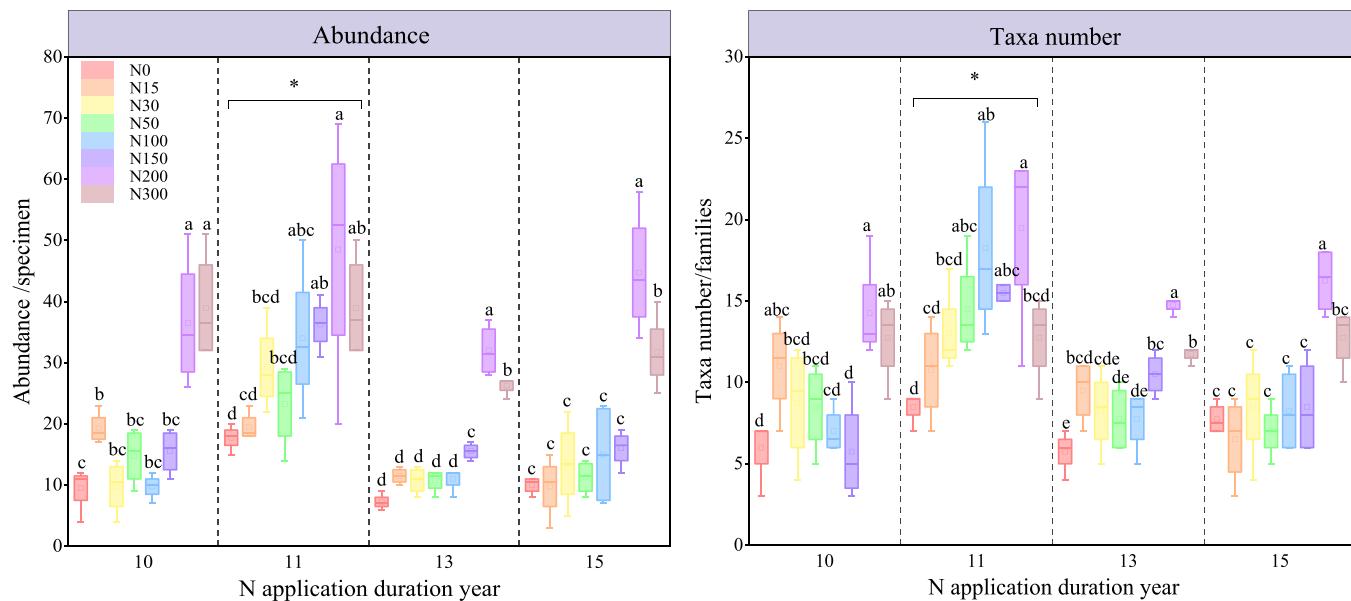
#### 3.1. Distribution characteristics of soil microarthropods

A total of 2714 soil microarthropod specimens belonging to 13 orders and 57 families were captured in the study area in the 10th, 11th, 13th, and 15th years of N addition. The dominant taxon was Microdispidae (S5), accounting for 16.40 % of the total catches. In the 10th, 11th, 13th, and 15th years of N addition, 616, 990, 500, and 608 soil microarthropod specimens, respectively, were captured in the study

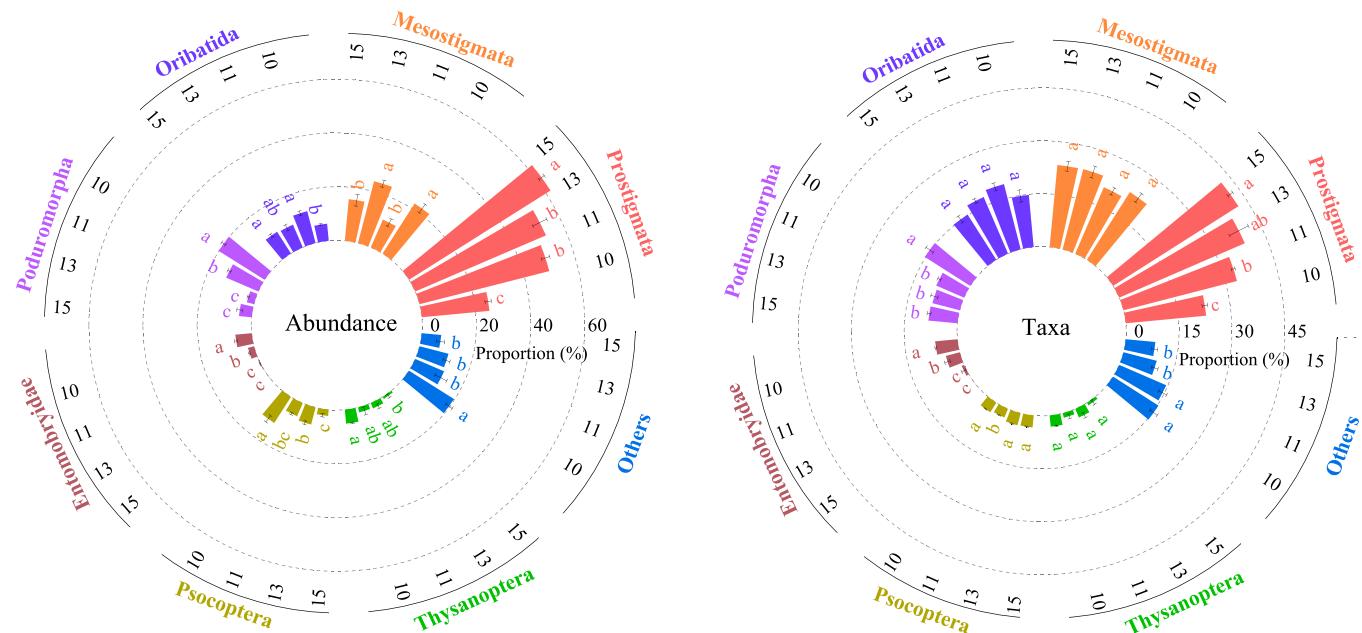
area (Table S1). These specimens belonged to 47 families of 13 orders, 46 families of 13 orders, 37 families of 8 orders, and 38 families of 9 orders. The dominant taxa were Microdispidae (S5) of Prostigmata, Hypogastruridae (S42) of Poduromorpha, Isotomidae (S54) of Poduromorpha (Fig. S2a), Microdispidae (Fig. S2b), Microdispidae (Fig. S2c), Microdispidae and Cheyletidae (S12) (Fig. S2d), accounting for 38.96 %, 13.23 %, 20.20 %, and 30.59 % of the total catches in each year, respectively. Microdispidae was the dominant taxon in each year.

The abundance and taxa of soil microarthropods in each year showed a trend of 2020 (11) > 2019 (10) > 2024 (15) > 2022 (13), and the abundance and taxa in the 11th year of N addition were significantly greater than those in other years ( $P < 0.05$ ) (Fig. 2). Compared with those under N0, the abundance and taxa of soil microarthropods increased with increasing the N application level. The abundances of N200-N300, N100-N300, N150-N300, and N200-N300 reached significant levels in the 10th, 11th, 13th, and 15th years of N application, respectively ( $P < 0.05$ ). The number of taxa in N200–300, N100-N200, N150-N300, and N200 significantly differed ( $P < 0.05$ ) (Fig. 2). Overall, the distribution of soil microarthropods was consistent across years, and the abundance and taxa increased significantly under high-level N addition (N100-N300) ( $P < 0.05$ ).

Through the analysis of the distribution characteristics of soil microarthropod communities at the family level, we found that the abundance of many soil microarthropod taxa gradually decreased or that the taxa disappeared with increasing the number of years of N application at the family level (Phloeothenripidae, Poduridae, Entomobryidae, Sminthuridae, Kalotermitidae, Geophilidae, Liposcelididae, Hypogastruridae, etc.). These soil microarthropods with reduced abundance or that have disappeared are distributed across multiple orders (Mesostigmata, Poduromorpha, Entomobryidae, Psocoptera, Thysanoptera) (Table S2). Therefore, to more intuitively understand the macroscale changes in soil microarthropod abundance and groups, we analysed soil microarthropods at the order level. The results revealed significant differences in the community composition of soil microarthropods at the order level under different durations of N application. According to the proportion of soil microarthropod abundance at the order level to the total abundance in that year, the proportion of Prostigmata and Oribatida abundance to the total abundance increased significantly with increasing N application duration ( $P < 0.05$ ) (Fig. 3).



**Fig. 2.** Changes in the distribution characteristics of soil microarthropods under different N application durations. Different lowercase letters indicate significant differences among different N addition treatments for the same index ( $P < 0.05$ ). The asterisk indicates that the same index is significantly different between different N application years,  $*P < 0.05$ .



**Fig. 3.** Changes in the soil microarthropod community composition under different N application durations at the order level. The soil microarthropods represented by S1-S57 in the figure are shown in Table S2. Prostigmata: S1-S16; Mesostigmata: S17-S28; Oribatida: S29-S40; Poduromorph: S41-S45; Entomobryidae: S46, S47; Psocoptera: S54; Thysanoptera: S55; Others: S48-S53, S56, S57. Statistically significant relationships are indicated by different letters using Duncan's test with the  $P < 0.05$  threshold.

The proportions of the total abundance of Mesostigmata, Poduromorpha, Entomobryidae, Psocoptera, Thysanoptera and other soil microarthropods decreased significantly as the number of years of N application increased ( $P < 0.05$ ) (Fig. 3). According to the proportion of the number of taxa to the total number of taxa in that year, the proportion of Prostigmata taxa to the total number of groups increased significantly with increasing N application duration, and the proportions of Poduromorpha, Entomobryidae and other soil microarthropod taxa to the total number of taxa decreased significantly with increasing N application duration (Fig. 3).

### 3.2. Differences in the soil microarthropod community structure

The community structure of the soil microarthropods was significantly differed across the for durations of N application. PCA was carried out basis of the distribution characteristics of the dominant and common taxa of soil microarthropods along different N gradients in the same year. The results revealed that PC1 explained 54.74 %, 46.40 %, 58.19 %, and 45.06 % of the variation in the soil microarthropod community structure in the 10th, 11th, 13th, and 15th years of N application, respectively (Fig. 4). The main taxon affecting soil microarthropod community structure on the PC1 axis was Microdispidae each year (Fig. 4). PC2 explained 21.22 %, 11.43 %, 6.24 %, and 23.03 % of the variation in the 10th, 11th, 13th, and 15th years of N application, respectively. The main groups affecting the community structure of the soil microarthropods were Hypogastruridae (S42), Ceratozetidae (S36), Liposcelididae (S54), and Ameroseiidae (S18) (Fig. 4). The cumulative variances of PC1 and PC2 were 75.96 %, 57.83 %, 64.43 %, and 68.09 %, in the 10th, 11th, 13th, and 15th years of N application, respectively, indicating that the first two principal components could represent most of the effective information on the soil microarthropod community distribution. According to the community distribution of the soil microarthropods, PC1 and PC2 divided the different N addition treatments into several groups with significant differences. In the 10th year of N application, according to the aggregation of soil microarthropod sample points, the N addition treatment was divided into three groups: N0-N150 was considered one group, and the N200 and

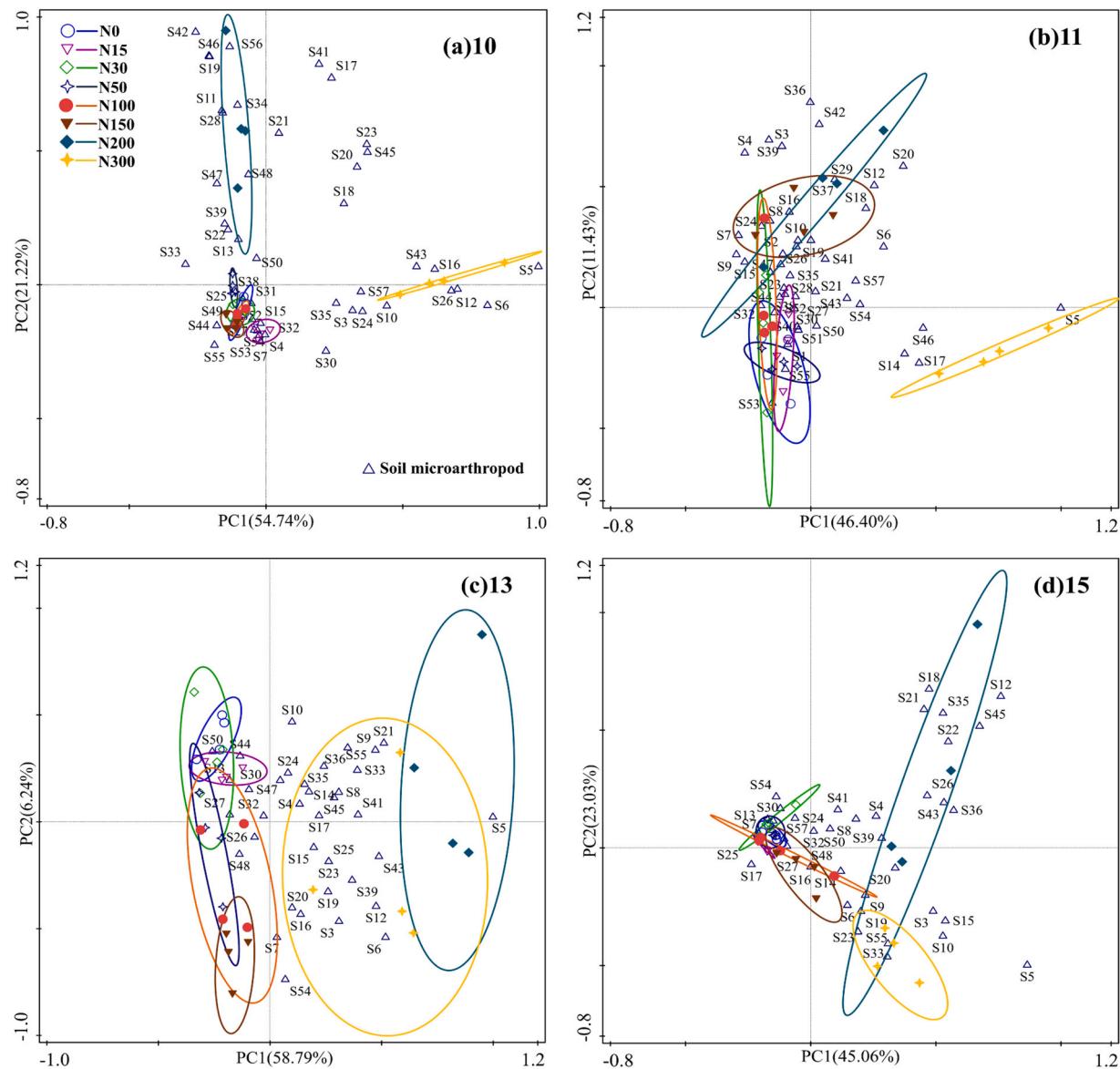
N300 treatments each formed a group (Fig. 4a). In the 11th year, they were divided into three groups: N0-N30 constituted one group, and the N150-N200 and N300 treatments each formed a group (Fig. 4b). In the 13th year, they were divided into three groups: N0-N30 constituted one group, and the N150 and N200-N300 treatments each formed a group (Fig. 4c). In the 15th year, the plants were divided into two groups: the N0-N150 treatment group and the N200-N300 treatment group (Fig. 4d). In general, compared with that in the N0 treatment, the soil microarthropod community structure in the N150-N300 treatments groups significantly changed.

According to the PCA of the dominant and common taxa of soil microarthropods in different years under the same N addition, PC1 explained 34.31 %, 33.29 %, 29.30 %, 37.06 %, 29.20 %, 46.85 %, 43.47 %, and 26.49 % of the variation in the soil microarthropod community structure in the N0, N15, N30, N50, N100, N150, N200, and N300 treatments, respectively (Fig. 5). The scores of PC2 explained 20.54 %, 28.07 %, 27.01 %, 18.62 %, 26.66 %, 21.69 %, 18.86 %, and 15.37 % of the variation, and cumulatively explained 54.85 %, 61.36 %, 56.31 %, 55.68 %, 52.86 %, 68.54 %, 62.33 %, and 41.86 % of the variation, respectively (Fig. 5). According to the aggregation of soil microarthropods, PC1 and PC2 divided soil microarthropods in different years under the same N addition into three groups. Among them, in the N0-N150 treatment, the 10th year of N application formed one group, and the 11th year and the 13th–15th years each formed a group. In the N200-N300 treatment, the 10th–11th years of N application formed one group, and the 13th and 15th years each formed a group. The differences in the community structure of the soil microarthropods among the three groups were obvious.

### 3.3. Characteristics of soil microarthropod diversity

#### 3.3.1. Diversity changes

There were significant differences in the diversity characteristics of the soil microarthropods across the different N application durations. Compared with those in other years, the Shannon–Wiener index and Margalef index of the soil microarthropods in the 11th year of N addition increased significantly, and the Simpson index decreased significantly



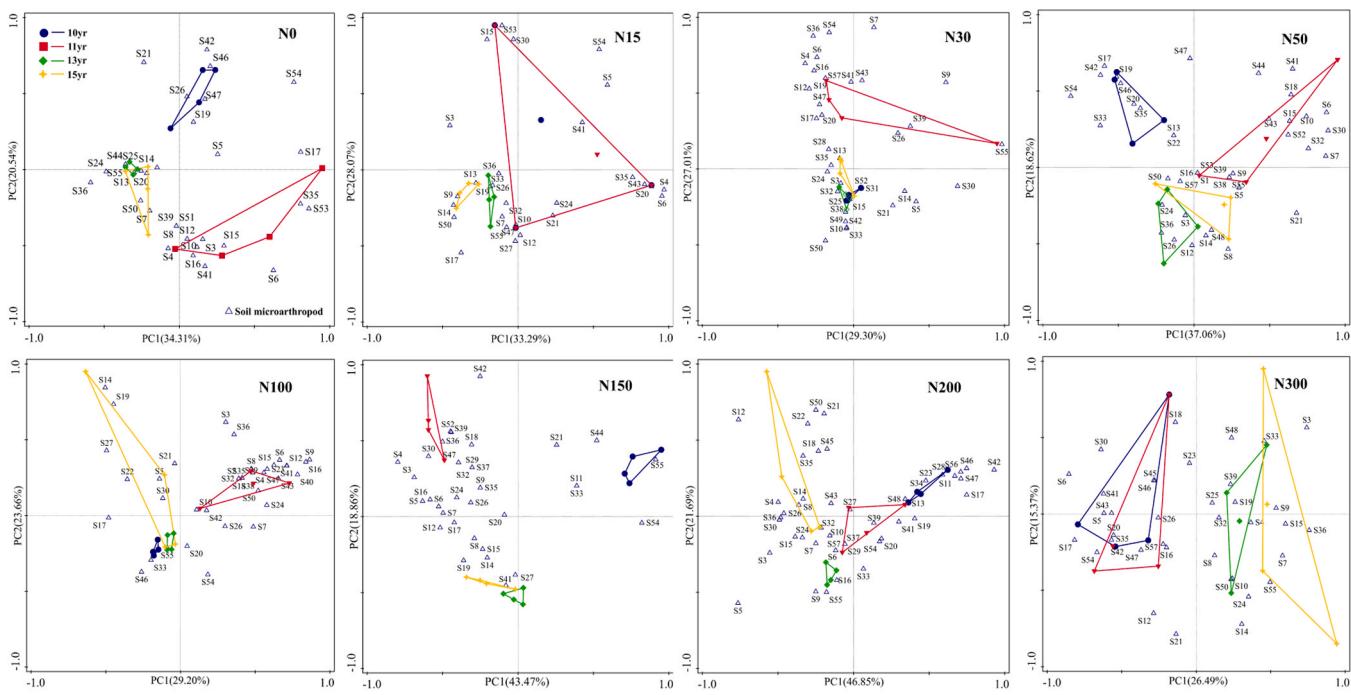
**Fig. 4.** Differences in the soil microarthropod community structure under different levels of N addition across the years evaluated. N0, N15, N30, N50, N100, N150, N200 and N300 represent nitrogen addition amounts of 0, 15, 30, 50, 100, 150, 200 and 300 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively. The ellipses in the analysis represent 95 % confidence intervals. The same four symbols in the confidence interval represent four replicates of different N treatments. S1-S57 refer to the taxa of soil microarthropods at the family-level classification unit. The soil microarthropod groups they represent are shown in Table S2.

( $P < 0.05$ ) (Fig. 6). The Pielou index increased as the number of years of N application increased. In addition, the diverse characteristics of the soil microarthropods presented different responses to long-term N addition (Fig. 6). The Pielou index first decreased but then increased with increasing the N addition level in the 10th and 11th years of N addition, and there was no significant change in the 13th and 15th years of N addition (Fig. 6). The Shannon–Wiener index increased with increasing N addition level at the 10th, 11th and 15 years of N addition, and the highest value occurred in the N200 treatment, which was significantly greater than that in the N0 treatment ( $P < 0.05$ ). In the 11th year of N addition, compared with the N0 treatment, the Shannon–Wiener index first increased but then decreased as the N addition level increased, and the inflection point occurred at N200. Compared with the N0 treatment, the Pielou index in the 10th, 11th and 15 years of N addition tended to decrease. The Margalef index first increased but then decreased as the N addition level increased in each year, and the highest value occurred in the N200 treatment, which was significantly different from that in the N0 treatment ( $P < 0.05$ ) (Fig. 6). In general,

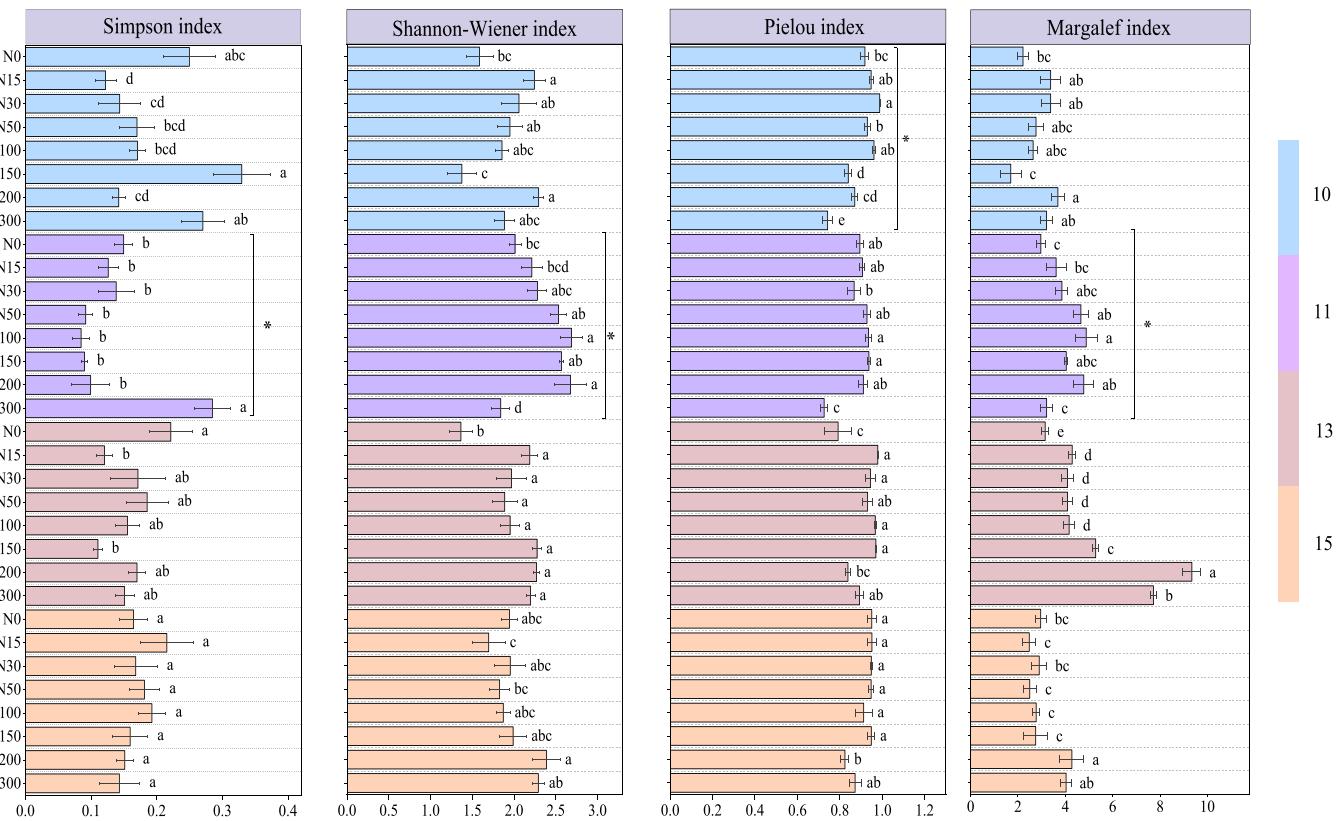
compared with the N0 treatment, the diversity of soil microarthropods in each year was significantly differed, which was mainly concentrated in the N200 treatment.

### 3.3.2. Similarity change

The Jaccard ( $q$ ) similarity coefficient reflects the similarity of soil microarthropod groups among different years under different N treatments. The results of the  $q$  similarity coefficient revealed that the 10th year of N addition was moderately dissimilar to the 11th ( $q=0.44$ ), the 13th ( $q=0.41$ ) and 15th ( $q=0.28$ ) years (Fig. S3a); and the 11th year was moderately similar to the 13th ( $q=0.58$ ) and the 15th ( $q=0.58$ ) years; the 13th year was moderately similar to the 15 year ( $q=0.58$ ) (Fig. S3b). In the 13th year of N addition, compared with those in  $q_{13-10}$ , those in  $q_{13-11}$  and  $q_{13-15}$  increased significantly ( $P < 0.05$ ) (Fig. S3c); In the 15th year, compared with those in  $q_{15-10}$ ,  $q_{15-11}$ , those in  $q_{15-13}$  and  $q_{15-12}$  increased significantly ( $P < 0.05$ ) (Fig. S3d), indicating that with increasing N application duration, the similarity of the soil microarthropods gradually increased (Fig. S3).



**Fig. 5.** Differences in the soil microarthropod community structure with different N application durations.



**Fig. 6.** Changes in the diversity of soil microarthropods under different N application durations. Different lowercase letters indicate significant differences among different N addition treatments for the same index ( $P < 0.05$ ). The asterisk indicates that the same index is significantly different between different N application years,  $*P < 0.05$ .

The  $q$  value of the soil microarthropods changed significantly with N addition. In the 10th year of N addition,  $q_{10-11}$  presented moderate similarity in the N15 and N300 treatments, whereas  $q_{10-13}$  and  $q_{10-15}$

presented moderate dissimilarity in the N300 treatment (Fig. S2a). In the 11th year,  $q_{11-13}$  and  $q_{11-15}$  were moderately dissimilar in the N150-N300 treatment (Fig. S2b). In the 13th year,  $q_{13-15}$  showed moderate

dissimilarity in the N100-N300 treatment (Fig. S2c). The remaining years and treatments were extremely dissimilar (Fig. S2d). Overall, the similarity of soil microarthropods increased as the N addition level increased, and the range of high level N addition treatments covered by soil microarthropods groups at medium dissimilar levels gradually expanded as the N application duration increased.

### 3.4. Responses of major soil microarthropod taxa to N addition

Through the principal response curve (PRC), the variation in the soil microarthropod abundance over time can be comprehensively reflected, and the difference between different levels of N addition and the control at each time point can be intuitively presented, and the dominant factors driving the difference can be reflected. With N0 as the control baseline, the number of soil microarthropod taxa changed as the number of N application years increased. In different N application years, Microdispidae (S5), Oppiidae (S30), Phloeoethripidae (S55), Isotomidae (S41), Ceratozetidae (S36) and Cryptognathidae (S12) were dominant (Fig. 7). The main contributors to the dynamic changes in the soil microarthropod abundance in the N15-N300 treatment during the 10th, 11th and 13th years of N addition were S5, S30 and S55. The main contributors to the N15 and N30 treatments in the 15th year were S41, S36 and S12, and the main contributors to the N100-N300 treatments were S5, S30 and S55 (Fig. 7). In addition, the distribution of soil microarthropods each year tended to be similar to that of N0 in the N15-N50 treatments, and separation was obvious in the N100-N300 treatments (Fig. 7). The effects of different levels of N addition on soil microarthropods were different, and S5, S30, S55, S41, S36, and S12 were sensitive to N addition.

### 3.5. The main driving factors and pathways affecting the changes in the soil microarthropod community

#### 3.5.1. Changes in environmental factors

Compared with the N0 treatment, annual N addition promoted the aboveground biomass, ammonium-N ( $\text{NH}_4^+$ -N) and nitrate-N ( $\text{NO}_3^-$ -N) contents in the meadow steppe, and the promoting effect increased with increasing N application duration and N application level ( $P < 0.05$ ). The soil pH decreased significantly with N addition in all the evaluated years, and the magnitude of the pH decrease increased with increasing N application duration and rates ( $P < 0.05$ ) (Table S3). Compared with that in the N0 treatment, the available phosphorus (AP) content in 2020 increased significantly in the N300 treatment ( $P < 0.05$ ) (Table S3). In

the 10th year of N addition, the soil moisture content (SMC) decreased significantly under the N15-N300 treatment ( $P < 0.05$ ), while there were no significant changes in the AP or soil water content in other years (Table S3). The soil organic carbon (SOC), total N (TN) and the soil C/N ratio did not change significantly with N addition across all years (Table S3).

#### 3.5.2. The main factors driving the changes in the soil microarthropod community

The importance of the environmental factors affecting soil microarthropod community changes at different levels of N addition and with different N application durations was analysed by the random forest method. The results revealed that, considering different N addition levels, the soil microarthropod community was regulated by different environmental factors in the 10th year ( $\text{NO}_3^-$ -N and pH), the 11th year ( $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, pH and C/N), the 13th year ( $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N and pH) and the 15th year ( $\text{NH}_4^+$ -N, SOC,  $\text{NO}_3^-$ -N and C/N) (Fig. S4a, b, c, d). Considering N application years, the interannual variation in soil microarthropod communities was affected by AGB,  $\text{NH}_4^+$ -N, SOC,  $\text{NO}_3^-$ -N and precipitation (Fig. S4e). Specifically, AGB,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, pH, C/N and SOC were the main environmental factors driving the changes in the soil arthropod community, and precipitation was the main climatic factor. A structural equation model (SEM) was subsequently used to explore the main ways that affected soil microarthropods under long-term N addition. The results revealed that the soil microarthropod abundance was affected mainly by the indirect effect of decreasing pH and the direct effect of changes in precipitation during different N application periods (Fig. 8).

## 4. Discussion

### 4.1. The community variation characteristics of soil microarthropods in meadow steppe under different N application durations

The community composition of the soil microarthropods in the *S. baicalensis* meadow steppe changed significantly with increasing N application duration. The results revealed that the total abundance and total taxa of the soil microarthropods in the 11th year of N application were significantly greater than those in the other years. The increase in total abundance showed a common increase in the abundance of multiple groups, whereas the increase in the total number of taxa showed a prominently increased in a certain group compared with other years (Prostigmata, Psocoptera, etc.). Compared with other years, the growth

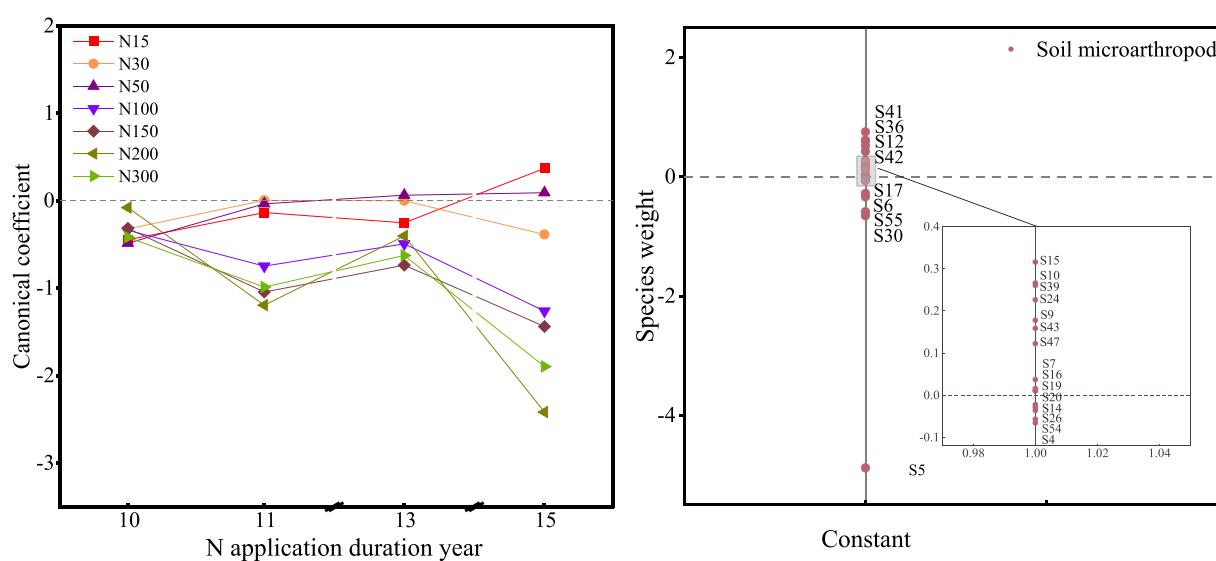
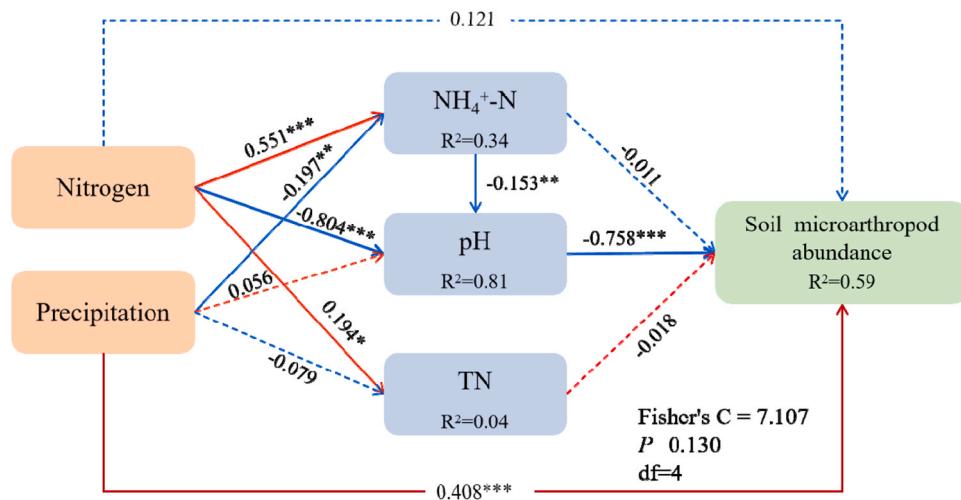


Fig. 7. Changes in the main response curve of the soil microarthropods changes with increasing N application duration under long-term N addition.



**Fig. 8.** The main pathways affecting soil microarthropod abundance under long-term N addition. The red and blue arrows represent positive and negative correlations, respectively. The solid line and the dotted line represent significant and insignificant correlations, respectively. The thickness of the lines represents the size of the relationship, and the numbers near them represent the path coefficients. The asterisks indicate statistical significance (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). TN: total nitrogen; NH<sub>4</sub><sup>+</sup>-N: ammonium-N.

period in 2020 had favourable hydrothermal conditions (with an average temperature of 16.34°C and precipitation of 463.3 mm) (Fig. S1). Our study revealed that an increase in precipitation can increase the abundance of soil microarthropods (Fig. 8). On the one hand, it may be that the Poduromorpha, Entomobryidae and Psocoptera may prefer a high-temperature and high-humidity living environments (A'Bear et al., 2014; Jiang et al., 2008). Good hydrothermal conditions can provide suitable habitats for soil microarthropods. On the other hand, suitable temperature and precipitation are conducive to the reproduction of soil microorganisms (Shi et al., 2020), which increases the food accessibility of saprophagous soil microarthropods (Prostigmata and Oribatida). Therefore, compared with other years, the 11th year of N application promoted the coexistence of multiple groups of soil microarthropods by increasing the food supply and creating a suitable living environment. In addition, among the soil microarthropods captured in this study, Microdispidae of Prostigmata were the common dominant group for the 4 years investigated (Fig. S2, Table S2). With increasing annual N application duration, the proportion of the abundance and taxa of Prostigmata to the total abundance and taxa increased significantly (Fig. 3), indicating that the Prostigmata groups in the meadow steppe were abundant and relatively stable under long-term N addition. This may be because, compared with that in the 11th year of N application, the decrease in precipitation in the 13th and 15th years of N application was not conducive to the survival of strongly water-loving soil microarthropods such as the original Poduromorpha, Entomobryidae, and Psocoptera. The Prostigmata suborder has a large tolerance range for precipitation changes, and the decrease in precipitation does not adversely affect its survival and reproduction (Jiang et al., 2008; Joly et al., 2021). Mites in Prostigmata are usually positively correlated with organic matter content and prefer to live in soils with a lower pH (5.0–6.5) than those of springtails (pH: 6.0–7.5) in Poduromorpha and Entomobryidae (Maraun et al., 2003; Patel, 2023; Ponge, 2003; Zhou et al., 2023). The organic carbon content of the *S. baicalensis* meadow steppe soil increased annually with increasing N application duration, whereas the soil pH gradually decreased with increasing N application level (Table S3). This gradually created a habitat environment conducive to the development of the Prostigmata community, broke the original competition mode of the soil microarthropods, and made the Prostigmata, dominated by the increase in Microdispidae, gradually occupied the dominant position.

In different years of N application, by aggregating soil microarthropod sample points under different levels of N addition each year,

we can observe that the community distribution of soil microarthropods can be divided into three groups across different years: the 10th and 11th years of N addition form two groups, and the 13th and 15th years form one group. The community structure of the soil microarthropods in the first two groups was significantly different from that in other treatments under the N200 and N300 applications, and there was a significant difference between the N200 and N300 treatments (Figs. 4, 5). These findings are consistent with our first hypothesis. However, with increasing N application duration, the N200 and N300 treatments were still significantly different from the other treatments, but there was no significant difference between the two treatments (Figs. 4, 5). This finding with the calculation results of the Jaccard index; that is, with increasing N application duration and N application level, the similarity of the community distribution of soil microarthropods increases (Fig. S3). It is possible that with increasing N application rates and durations, an ecological environment conducive to the survival and reproduction of Prostigmata gradually developed. In particular, under the N200 and N300 treatments, the abundance and groups of Prostigmata increased significantly (Fig. 3, Table S1), which limited the community development of Mesostigmata, and so on. The soil microarthropods formed a community composition dominated by the former suborder, thereby reducing the interannual differences in community structure.

#### 4.2. Diversity changes and threshold effects of soil microarthropods in meadow steppe under different N application durations

The abundance and taxa of the soil microarthropods trend to increase first but then decreased with increasing N addition rates during the years evaluated, and the maximum appeared in N200 (Fig. 2). The diversity characteristics showed different trends each year, and most of the changes occurred in the N200 treatment (Fig. 6). Among them, the Shannon—Wiener index and Margalef index of soil microarthropods increased significantly in the N200 treatment (Fig. 6), indicating that although the number of soil microarthropod groups in Poduromorpha and Entomobryidae decreased with increasing N application duration in the N200 treatment, the increase in the number of soil microarthropod groups in Prostigmata could still increase the diversity of soil microarthropods in the meadow steppe. We then integrated the changes in the community composition, structure, and diversity characteristics of the soil microarthropods at different N application levels each year and found that the N200 treatment was an inflection point affecting the

changes in the soil microarthropod community characteristics (Figs. 2, 4, 5, 6). In the N15-N200 treatment, an appropriate amount of N addition could create a good environment for the growth and development of soil microarthropods. However, with increasing the N application level, the continuous high-N input in the N300 treatment reached or even exceeded the N saturation level of the meadow steppe, which may have negatively affected the soil microarthropods. This finding is consistent with the previous studies on the threshold effect of N addition on ecosystem function. That is, N addition to a certain extent is beneficial to the organisms (Laelapidae, Pachylaelapidae, Microdispidae, Parasitidae, and Cheyletidae) of grassland ecosystems, but excessive N deposition weakens this beneficial effect and even has negative effects (Phloeo-thripidae and Liposcelidida) (Aber et al., 1995; Magill et al., 2000; Wang et al., 2021). Therefore, it can be concluded that the threshold of the effect of N addition on soil microarthropod community characteristics in this study was 200 kg ha<sup>-1</sup> yr<sup>-1</sup>, which supports our second hypothesis. This is attributed to the fact that an appropriate amount of N addition (N15-N200) promoted vegetation growth (Table S3) and increased the soil soluble organic carbon content (Xu et al., 2015), thereby increasing soil microbial activity and accelerating litter turnover, increasing the accessibility of food to soil microarthropods. Furthermore, soil microarthropods may thrive in neutral to slightly acidic environments, i.e., pH 5.5–7.0 (Baker and Whitby, 2003). The significant decrease in pH under N addition provides a suitable habitat for soil microarthropods. However, with the continuous input of excessive N, the pH in the N300 treatment was too low (ranging from 4.76 to 5.32) for the optimum soil microarthropod community. When the pH was less than 5.5, toxic forms of Al attacked plants (Khan et al., 2024) and may have resulted in vegetation biomass loss (Table S2). This was not conducive to the acquisition and accumulation of soil microarthropod food, thus weakening the positive effect of N addition on soil microarthropods.

#### 4.3. Driving pathways of changes in the community characteristics of soilmicroarthropods in meadow steppe under different N application durations

The *S. baicalensis* meadow steppe is an N-limited ecosystem and is sensitive to N addition (Wu et al., 2024). In different N application years, the composition of the soil microarthropod community in the meadow steppe was affected by changes in environmental and climatic factors (Fig. 8). This difference indicates that although the decrease in pH under N addition is an important factor affecting the change in soil microarthropod abundance, the effect of the change in precipitation on soil microarthropod abundance is equally important across different years. This may be related to the habitat of soil microarthropods. In this study, an increase in precipitation had a positive effect on the abundance, taxa number and diversity of soil microarthropods in different N application years (Fig. 2, Fig. 6), and a change in precipitation directly affected the soil microarthropod community (Fig. 8), which is consistent with the meta-analysis results of Peng et al. (Peng et al., 2022b). From the perspective of the abundance of soil microarthropods, in years with relatively high precipitation (the 11th year of N application), sufficient water conditions promoted the coexistence and reproduction of groups of soil microarthropods (Table S2). In years with less precipitation (the 13th year of N application), only some of the soil microarthropods with strong drought resistance survive, and other soil microarthropods with strong hygroscopicity enter diapause. From the perspective of community composition, soil microarthropods with different feeding habits have different adaptabilities to precipitation. Previous studies have noted that saprophagous soil microarthropods are not sensitive to precipitation changes (Joly et al., 2021). The soil microarthropods in Prostigmata, Mesostigmata and Oribatida are mostly saprophagous (Table S2). Therefore, in the case of less precipitation in the 13th year of N application, the soil microarthropods in Prostigmata, Mesostigmata and Oribatida can still be affected by the positive effect of N addition, which increases their abundance and diversity.

In addition to precipitation, the decrease in soil pH (4.75–6.96) under long-term N addition also had a positive effect on the abundance of Prostigmata and Oribatida in the soil microarthropods (Fig. 8). It is possible that the increase in soil NH<sub>4</sub><sup>+</sup>-N content under N addition enhances soil nitrification and releases a large amount of H<sup>+</sup>, resulting in a continuous decrease in soil pH (Table S3) (Gao et al., 2020; Ren et al., 2024). Previous studies have indicated that acidic conditions are conducive to fungal growth and activity (Hou et al., 2024), whereas soil microarthropods in the soil mite suborder prefer to prey on fungi (Table S2). The decrease in pH after N addition promoted an increase in fungal biomass, thereby providing a richer food source for fungus-eating microarthropods and increasing the abundance of soil microarthropods. Furthermore, in an acidified environment, an increase in H<sup>+</sup> can reduce the stability of organic carbon on mineral surfaces by dissolving alkali metal ions (such as calcium and magnesium) adsorbed on mineral surfaces (Chen et al., 2014; Jilling et al., 2021), thereby accelerating the decomposition of organic carbon and ensuring an adequate supply of nutrients for soil microarthropods. In addition, as the duration and level of N application increase, the continuous decline in pH exceeds the soil pH neutral range (6.0–7.5) preferred by soil springtails (Baker and Whitby, 2003; Ponge, 2003), which is unfavourable for the growth and reproduction of soil springtails (Poduromorpha, Entomobryidae), thereby releasing more ecological niche resources and reducing inter-specific competition among soil microarthropods. Moreover, soil mites (Prostigmata and Oribatida) that prefer slightly acidic environments not only gain more suitable living environments but also obtain richer food resources, enabling Prostigmata and Oribatida to gradually dominate the soil microarthropod community as the number of years of N application increased (Fig. 3). In summary, with increasing N application years and N application levels, the community characteristics of soil microarthropods significantly differ. In different years of N application, the food source, living environment and reproductive capacity of soil microarthropods are affected mainly by the decrease in pH and the change in precipitation, and these two factors work together to affect the growth and reproduction of soil microarthropods.

## 5. Conclusion

The soil microarthropod community in the *S. baicalensis* meadow steppe changed significantly with increasing N application duration and N application level. Long-term N addition promotes the abundance and diversity of soil microarthropods, but there is a threshold effect with a threshold of 200 kg ha<sup>-1</sup> yr<sup>-1</sup>. In different years of N application, soil pH and precipitation are the main factors affecting changes in the soil microarthropod community. As the N application duration and N application-level increase, precipitation changes and the decrease in soil pH creates a favourable habitat for soil microarthropods in Prostigmata, gradually enhancing their dominant position. Consequently, the differences in the community distributions of the soil microarthropods gradually decreased with increasing N application duration and N application level, whereas the similarity index gradually increased. In general, under the condition of long-term N application, exploring the continuous dynamic changes in soil microarthropods can help us understand the effects of environmental changes on soil microarthropods in meadow steppes from the perspectives of N deposition and precipitation changes. This study is important for further understanding the role of global environmental change in grassland ecosystems.

## Author Contributions

Zhendan Wu and Shangfei Ma conceived the ideas and designed the methodology; Zhendan Wu, Shangfei Ma, Renheng Wu, Siyuan Bao and Meijun Sheng conducted sample collection; Zhendan Wu and Shangfei Ma analysed the data; Zhendan Wu led the writing of the manuscript; Mei Hong oversaw financial support and paper revision; Yang Dianlin provided guidance. All authors contributed critically to the drafts and

gave final approval for publication.

## CRediT authorship contribution statement

**Shangfei Ma:** Writing – review & editing, Investigation. **Mei Hong:** Resources, Funding acquisition. **Renheng Wu:** Investigation. **Zhendan Wu:** Writing – original draft. **Meijun Sheng:** Investigation. **Siyuan Bao:** Investigation. **Dianlin Yang:** Resources.

## 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.

## Acknowledgements

The author are grateful to the Grassland Research Institute of the Chinese Academy of Agricultural Sciences and the Institute of Environmental Protection Research and Monitoring Institute of the Ministry of Agriculture and Rural Affairs for their help in the field sampling process. The research was funded by the National Natural Science Foundation of China (nos. 32460315 and 31170435).

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2025.109818](https://doi.org/10.1016/j.agee.2025.109818).

## Data availability

Data will be made available on request.

## References

- A'Bear, A.D., Jones, T.H., Boddy, L., 2014. Potential impacts of climate change on interactions among saprotrophic cord-forming fungal mycelia and grazing soil invertebrates. *Fungal Ecol.* 10, 34–43. <https://doi.org/10.1016/j.funeco.2013.01.009>.
- Aber, J.D., Magill, A., McNulty, S.G., Boone, R.D., Nadelhoffer, K.J., Downs, M., Hallett, R., 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. *Water Air Soil Pollut.* 85, 1665–1670. <https://doi.org/10.1007/BF00477219>.
- Bai, Y., Wu, J., Clark, C.M., Naeem, S., Pan, Q., Huang, J., Zhang, L., Guohan, X., 2010. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands, 889–889 *Glob. Change Biol.* 16. <https://doi.org/10.1111/j.1365-2486.2009.02142.x>.
- Baker, G., Whithy, W., 2003. Soil pH preferences and the influences of soil type and temperature on the survival and growth of *Aporrectodea longa* (Lumbricidae)The 7th international symposium on earthworm ecology · Cardiff · Wales · 2002. *Pedobiologia* 47, 745–753. [https://doi.org/10.1016/S0031-4056\(04\)70263-2](https://doi.org/10.1016/S0031-4056(04)70263-2).
- Bao, S.D., 2000. *Soil and Agricultural Chemistry Analysis*, 3rd ed. Agriculture Press, Beijing: China, pp. 20–97.
- Bardgett, R.D., Bullock, J.M., Lavorel, S., Manning, P., Schaffner, U., Ostle, N., Chomel, M., Durigan, G., L. Fry, E., Johnson, D., Lavallee, J.M., Le Provost, G., Luo, S., Ping, K., Sankaran, M., Hou, X., Zhou, H., Ma, L., Ren, W., Li, X., Ding, Y., Li, Y., Shi, H., 2021. Combatting global grassland degradation. *Nat. Rev. Earth Environ.* 2, 720–735. <https://doi.org/10.1038/s43017-021-00207-2>.
- Bian, H., Geng, Q., Xiao, H., Shen, C., Li, Q., Cheng, X., Luo, Y., Ruan, H., Xu, X., 2019. Fine root biomass mediates soil fauna community in response to nitrogen addition in poplar plantations (*Populus deltoids*) on the East Coast of China. *Forests* 10, 122. <https://doi.org/10.3390/f10020122>.
- Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. *Glob. Change Biol.* 20, 2356–2367. <https://doi.org/10.1111/gcb.12475>.
- Dan-yan1, Z., Dan-rong1, B.U., Zhi-weil, G.E., Jing2, Y.A.N., Han-ran1, X., Hong-hua1\*\*, R., Guo-hua, C.A.O., 2015. Effects of nitrogen addition on soil fauna in poplar plantation with different ages in a coastal area of eastern China. *Chin. J. Ecol.* 34, 2553.
- De Vries, W., 2021. Impacts of nitrogen emissions on ecosystems and human health: a mini review. *Curr. Opin. Environ. Sci. Health* 21, 100249. <https://doi.org/10.1016/j.coesh.2021.100249>.
- Doblas-Miranda, E., Paquette, A., Work, T.T., 2014. Intercropping trees' effect on soil oribatid diversity in agro-ecosystems. *Agroforest Syst.* 88, 671–678. <https://doi.org/10.1007/s10457-014-9680-y>.
- Du, E., Terrer, C., Pellegrini, A.F.A., Ahlström, A., Van Lissa, C.J., Zhao, X., Xia, N., Wu, X., Jackson, R.B., 2020. Global patterns of terrestrial nitrogen and phosphorus limitation. *Nat. Geosci.* 13, 221–226. <https://doi.org/10.1038/s41561-019-0530-4>.
- 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., Vöösmarty, C.J., 2004. Nitrogen Cycles: Past, Present, and Future. *Biogeochemistry* 70, 153–226. <https://doi.org/10.1007/s10533-004-0370-0>.
- Gao, S., Zhou, G., Liao, Y., Lu, Y., Nie, J., Cao, W., 2020. Contributions of ammonia-oxidising bacteria and archaea to nitrification under long-term application of green manure in alkaline paddy soil. *Geoderma* 374, 114419. <https://doi.org/10.1016/j.geoderma.2020.114419>.
- Gong, J., Zhu, C., Yang, L., Yang, B., Wang, B., Baoyin, T., Liu, M., Zhang, Z., Shi, J., 2020. Effects of nitrogen addition on above-and belowground litter decomposition and nutrient dynamics in the litter-soil continuum in the temperate steppe of Inner Mongolia, China. *J. Arid Environ.* 172, 104036. <https://doi.org/10.1016/j.jaridenv.2019.104036>.
- Hou, Z., Zhang, X., Chen, W., Liang, Z., Wang, K., Zhang, Y., Song, Y., 2024. Differential responses of bacterial and fungal community structure in soil to nitrogen deposition in two planted forests in Southwest China in Relation to pH. *Forests* 15, 1112. <https://doi.org/10.3390/f15071112>.
- Jiang, H.-B., Liu, J.-C., Wang, Z.-Y., Wang, J.-J., 2008. Temperature-dependent development and reproduction of a novel stored product psocid, *Liposcelis badia* (Psocoptera: Liposcelidae). *Environ. Entomol.* 37, 1105–1112. [https://doi.org/10.1603/0046-225x\(2008\)37\[1105:tdaroa\]2.0.co;2](https://doi.org/10.1603/0046-225x(2008)37[1105:tdaroa]2.0.co;2).
- Jilling, A., Keilweitz, M., Gutknecht, J.L.M., Grandy, A.S., 2021. Priming mechanisms providing plants and microbes access to mineral-associated organic matter. *Soil Biol. Biochem.* 158, 108265. <https://doi.org/10.1016/j.soilbio.2021.108265>.
- Joly, F., McAvoy, E., Subke, J., 2021. Synergistic interactions between detritivores disappear under reduced rainfall. *Ecology* 102, e03299. <https://doi.org/10.1002/ecy.3299>.
- Khan, S., Li, R., Pan, R., Zhang, C., Lv, Y., Tang, H., Huang, J., Yin, L., 2024. Aluminium bioavailability and toxicity disrupted chloroplast structure and inhibited inorganic carbon utilization and nutrient uptake in *Vallisneria natans* at acidic and alkaline pH. *Environ. Exp. Bot.* 228, 105997. <https://doi.org/10.1016/j.envexpbot.2024.105997>.
- Kuperman, R.G., 1996. Relationships between soil properties and community structure of soil macroinvertebrates in oak-hickory forests along an acidic deposition gradient. *Appl. Soil Ecol.* 4, 125–137. [https://doi.org/10.1016/0929-1393\(96\)00108-4](https://doi.org/10.1016/0929-1393(96)00108-4).
- LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89, 371–379. <https://doi.org/10.1890/06-2057.1>.
- Li, C., Fu, B., Wang, S., Stringer, L.C., Zhou, W., Ren, Z., Hu, M., Zhang, Y., Rodriguez-Caballero, E., Weber, B., Maestre, F.T., 2023. Climate-driven ecological thresholds in China's drylands modulated by grazing. *Nat. Sustain.* 6, 1363–1372. <https://doi.org/10.1038/s41893-023-01187-5>.
- Li, J., Liu, Q., Yin, R., You, C., Zhang, L., Li, H., Wang, L., Xu, H., Xu, L., Liu, S., Tan, B., Xu, Z., 2024. Nitrogen addition and plant functional type mediate the mesofauna-driven litter element release of subtropical forest. *Plant Soil.* <https://doi.org/10.1007/s11104-024-06969-0>.
- Liu, C., Song, X., Wang, L., Wang, D., Zhou, X., Liu, J., Zhao, X., Li, J., Lin, H., 2016. Effects of grazing on soil nitrogen spatial heterogeneity depend on herbivore assemblage and pre-grazing plant diversity. *J. Appl. Ecol.* 53, 242–250. <https://doi.org/10.1111/1365-2664.12537>.
- Liu, S., Behm, J.E., Wan, S., Yan, J., Ye, Q., Zhang, W., Yang, X., Fu, S., 2021. Effects of canopy nitrogen addition on soil fauna and litter decomposition rate in a temperate forest and a subtropical forest. *Geoderma* 382, 114703. <https://doi.org/10.1016/j.geoderma.2020.114703>.
- Macfadyen, A., 1953. Notes on methods for the extraction of small soil arthropods. *J. Anim. Ecol.* 22, 65. <https://doi.org/10.2307/1691>.
- Magill, A.H., Aber, J.D., Berntson, G.M., McDowell, W.H., Nadelhoffer, K.J., Melillo, J. M., Steudler, P., 2000. Long-term nitrogen additions and nitrogen saturation in two temperate forests. *Ecosystems* 3, 238–253. <https://doi.org/10.1007/s100210000023>.
- Maraun, M., Salamon, J.-A., Schneider, K., Schaefer, M., Scheu, S., 2003. Oribatid mite and collembolan diversity, density and community structure in a moder beech forest (*Fagus sylvatica*): effects of mechanical perturbations. *Soil Biol. Biochem.* 35, 1387–1394. [https://doi.org/10.1016/S0038-0717\(03\)00218-9](https://doi.org/10.1016/S0038-0717(03)00218-9).
- Nogueira, V.L.R., Rocha, L.L., Colares, G.B., Angelim, A.L., Normando, L.R.O., Cantão, M. E., Agnez-Lima, L.F., Andreato, F.D., Melo, V.M.M., 2015. Microbiomes and potential metabolic pathways of pristine and anthropized Brazilian mangroves. *Reg. Stud. Mar. Sci.* 2, 56–64. <https://doi.org/10.1016/j.rsma.2015.08.008>.
- Ochoa-Hueso, R., Rocha, I., Stevens, C.J., Manrique, E., Lucíañez, M.J., 2014. Simulated nitrogen deposition affects soil fauna from a semiarid Mediterranean ecosystem in central Spain. *Biol. Fertil. Soils* 50, 191–196. <https://doi.org/10.1007/s00374-013-0838-y>.
- Patel, L.C., 2023. *Habit, Habitat, Distribution, Sampling and Identification of Mite Pests in Applied Entomology*. CRC Press.
- Peng, Y., Peñuelas, J., Vesterdal, L., Yue, K., Peguero, G., Fornara, D.A., Hedénec, P., Steffens, C., Wu, F., 2022b. Responses of soil fauna communities to the individual and combined effects of multiple global change factors. *Ecol. Lett.* 25, 1961–1973. <https://doi.org/10.1111/ele.14068>.

- Peng, Y., Holmstrup, M., Schmidt, I.K., De Schrijver, A., Schelhout, S., Hedénec, P., Zheng, H., Bachega, L.R., Yue, K., Vesterdal, L., 2022a. Litter quality, mycorrhizal association, and soil properties regulate effects of tree species on the soil fauna community. *Geoderma* 407, 115570. <https://doi.org/10.1016/j.geoderma.2021.115570>.
- Peng, Y., Vesterdal, L., Peñuelas, J., Peguero, G., Wu, Q., Hedénec, P., Yue, K., Wu, F., 2023. Soil fauna effects on litter decomposition are better predicted by fauna communities within litterbags than by ambient soil fauna communities. *Plant Soil* 487, 49–59. <https://doi.org/10.1007/s11104-023-05902-1>.
- Ponge, J.-F., 2003. Humus forms in terrestrial ecosystems: a framework to biodiversity. *Soil Biol. Biochem.* 35, 935–945. [https://doi.org/10.1016/S0038-0717\(03\)00149-4](https://doi.org/10.1016/S0038-0717(03)00149-4).
- Ren, J., Wang, C., Wang, Q., Song, W., Sun, W., 2024. Nitrogen addition regulates the effects of variation in precipitation patterns on plant biomass formation and allocation in a *Leymus chinensis* grassland of northeast China. *Front. Plant Sci.* 14, 1323766. <https://doi.org/10.3389/fpls.2023.1323766>.
- Shi, Y., Zhang, K., Li, Q., Liu, X., He, J.-S., Chu, H., 2020. Interannual climate variability and altered precipitation influence the soil microbial community structure in a Tibetan Plateau grassland. *Sci. Total Environ.* 714, 136794. <https://doi.org/10.1016/j.scitotenv.2020.136794>.
- Tipping, E., Henrys, P.A., Maskell, L.C., Smart, S.M., 2013. Nitrogen deposition effects on plant species diversity: threshold loads from field data. *Environ. Pollut.* 179, 218–223. <https://doi.org/10.1016/j.envpol.2013.04.008>.
- Wang, X., Wang, M., Tao, Y., Fang, N., Yang, G., Cai, J., Jiang, Y., Han, X., Yu, F.-H., Li, M.-H., 2021. Beneficial effects of nitrogen deposition on carbon and nitrogen accumulation in grasses over other species in Inner Mongolian grasslands. *Glob. Ecol. Conserv.* 26, e01507. <https://doi.org/10.1016/j.gecco.2021.e01507>.
- Wang, Y., Xiao, C., Liu, C., He, N., 2024. The response of grassland productivity to atmospheric nitrogen deposition in northern China. *Agric. Ecosyst. Environ.* 359, 108764. <https://doi.org/10.1016/j.agee.2023.108764>.
- Wei, X., Wu, F., Hedénec, P., Yue, K., Peng, Y., Yang, J., Zhang, X., Ni, X., 2022. Changes in soil faunal density and microbial community under altered litter input in forests and grasslands. *Fundam. Res.* 2, 954–963. <https://doi.org/10.1016/j.fmre.2022.01.029>.
- Wen, Z., Wang, R., Li, Q., Liu, J., Ma, X., Xu, W., Tang, A., Collett, J.L., Li, H., Liu, X., 2022. Spatiotemporal variations of nitrogen and phosphorus deposition across China. *Sci. Total Environ.* 830, 154740. <https://doi.org/10.1016/j.scitotenv.2022.154740>.
- Wu, Z., Ma, S., Lu, J., Ye, H., Yang, D., Hong, M., 2024. Vertical distribution and driving mechanisms of soil microarthropods in a *Stipa baicalensis* meadow steppe under long-term nitrogen addition. *Ecol. Indic.* 159, 111732. <https://doi.org/10.1016/j.ecolind.2024.111732>.
- Xia, J., Wan, S., 2008. Global response patterns of terrestrial plant species to nitrogen addition. *N. Phytol.* 179, 428–439. <https://doi.org/10.1111/j.1469-8137.2008.02488.x>.
- Xu, C., Xu, X., Ju, C., Chen, H.Y.H., Wilsey, B.J., Luo, Y., Fan, W., 2021. Long-term, amplified responses of soil organic carbon to nitrogen addition worldwide. *Glob. Change Biol.* 27, 1170–1180. <https://doi.org/10.1111/gcb.15489>.
- Xu, G., Mo, J., Fu, S., Per, G., Zhou, G., Xue, J.-H., 2007. Response of soil fauna to simulated nitrogen deposition: A nursery experiment in subtropical China. *J. Environ. Sci.* 19, 603–609. [https://doi.org/10.1016/S1001-0742\(07\)60100-4](https://doi.org/10.1016/S1001-0742(07)60100-4).
- Xu, G.-L., Schleppi, P., Li, M.-H., Fu, S.-L., 2009. Negative responses of Collembola in a forest soil (Alptal, Switzerland) under experimentally increased N deposition. *Environ. Pollut.* 157, 2030–2036. <https://doi.org/10.1016/j.envpol.2009.02.026>.
- Xu, Z., Ren, H., Li, M., Van Ruijven, J., Han, X., Wan, S., Li, H., Yu, Q., Jiang, Y., Jiang, L., 2015. Environmental changes drive the temporal stability of semi-arid natural grasslands through altering species asynchrony. *J. Ecol.* 103, 1308–1316. <https://doi.org/10.1111/1365-2745.12441>.
- Yang, G.-J., Stevens, C., Zhang, Z.-J., Lü, X.-T., Han, X.-G., 2023. Different nitrogen saturation thresholds for above-, below-, and total net primary productivity in a temperate steppe. *Glob. Change Biol.* 29, 4586–4594. <https://doi.org/10.1111/gcb.16803>.
- Yang, Y., Ma, E., Liao, L., Wu, M., 2024. Land Use Change in a Typical Transect in Northern China and Its Impact on the Ecological Environment. *Sustainability* 16, 9291. <https://doi.org/10.3390/su16219291>.
- Yin, W.Y., 1998. *Pictorial Keys to Soil Animals of China*. Science Press, Beijing.
- Zhang, B., Ma, W., Song, L., Liang, X., Xi, X., Wang, Z., 2023. Nitrogen addition and experimental drought simplified arthropod network in temperate grassland. *Funct. Ecol.* 37, 1815–1826. <https://doi.org/10.1111/1365-2435.14341>.
- Zhang, K., Ni, Y., Liu, X., Chu, H., 2020. Microbes changed their carbon use strategy to regulate the priming effect in an 11-year nitrogen addition experiment in grassland. *Sci. Total Environ.* 727, 138645. <https://doi.org/10.1016/j.scitotenv.2020.138645>.
- Zhou, S., Hu, J., Liu, X., Zou, X., Xiao, L., Cao, D., Tu, L., Cui, X., Huang, C., 2023. The response of mesofauna to nitrogen deposition and reduced precipitation during litter decomposition. *Forests* 14, 1112. <https://doi.org/10.3390/f14061112>.