



# Cattle grazing mitigates the negative impacts of nitrogen addition on soil nematode communities

Ying Chen<sup>a</sup>, Yao Zhang<sup>a</sup>, Uffe N. Nielsen<sup>b</sup>, Quanhui Ma<sup>a</sup>, Xiang Zhang<sup>a</sup>, Xuewen Huang<sup>c</sup>, Duofeng Pan<sup>d</sup>, Xiuquan Yue<sup>a</sup>, Jushan Liu<sup>a,\*</sup>, Deli Wang<sup>a</sup>

<sup>a</sup> Key Laboratory of Vegetation Ecology of the Ministry of Education, Jilin Songnen Grassland Ecosystem National Observation and Research Station, Institute of Grassland Science, Northeast Normal University, Changchun 130024, China

<sup>b</sup> Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia

<sup>c</sup> School of Life Science, Hulunbuir University, Hulunbuir 021008, China

<sup>d</sup> Institute of Forage and Grassland Sciences, Heilongjiang Academy of Agricultural Sciences, Harbin, China

## ARTICLE INFO

### Keywords:

Cattle grazing  
Grassland management  
Mixed grazing  
Nitrogen enrichment  
Sheep grazing  
Soil nematodes

## ABSTRACT

Livestock grazing and atmospheric nitrogen (N) deposition have been reported as important factors affecting soil communities. However, how different large herbivore grazing and N addition may interact to affect soil biota in grassland ecosystems is unclear. Nematodes are the most abundant metazoan in soil ecosystems, play critical roles in regulating carbon and nutrient dynamics, and are valuable bioindicators. We examined the independent and interactive effects of grazing regimes (no grazing; sheep grazing; cattle grazing; mixed grazing of sheep and cattle) and N addition (ambient N; N addition) on soil nematodes in a meadow steppe. We found that grazing and N addition interacted to influence total nematode abundance, trophic group abundance, generic richness, diversity and several nematode-based indices (maturity index, channel ratio, enrichment index). In cattle grazing treatment, N addition significantly increased total nematode abundance, and the abundance of bacterial feeders, plant feeders, and omnivore-predators, and generic richness. By contrast, in the sheep and mixed grazing treatments, N addition had a negative effect on the same variables. Moreover, N addition reduced nematode maturity, enrichment and structure indices, and enhanced nematode channel ratio, in most grazing treatments, except mixed grazing where N addition had no effect on these variables. Structural equation modeling (SEM) revealed that N addition indirectly reduced nematode abundance and richness through increased soil NO<sub>3</sub>-N content, whereas the effects of grazing were associated with increased relative biomass of grasses. Our results suggested that the response of soil nematodes to N addition strongly depended on herbivore assemblages. Nitrogen addition enhanced soil nematode diversity and maintained a relatively complex and mature soil food web in the presence of cattle rather than sheep grazing. Furthermore, our study highlighted that under N deposition, cattle grazing could benefit the soil nematode community.

## 1. Introduction

Nematodes are a crucial component of terrestrial ecosystems and are the most abundant and diverse animals in soil (Bardgett and van der Putten, 2014). They make up four-fifths of all animals on earth (Lorenzen, 1994), and occupy most of the vital positions in soil food web (Yeates et al., 1993; Bongers and Bongers, 1998). Soil nematodes play critical roles in maintaining soil biodiversity and regulating organic matter decomposition and nutrient mineralization (Hunt and Wall, 2002; Ferris, 2010; Nielsen et al., 2015), and are used as indicators of

environmental conditions (Bongers and Ferris, 1999). Therefore, it is imperative to understand how soil nematodes are affected by environmental changes, especially the potential mechanisms for assessing effects of land management practices and global change on terrestrial ecosystems. Grassland is the largest managed ecosystem of terrestrial ecosystems and supports a great abundance and diversity of soil nematodes (Ettema and Yeates, 2003). However, the effects of land management and global change on nematode communities in grassland ecosystems remain poorly understood.

Grassland ecosystems cover about 40% of Earth's terrestrial surface

\* Corresponding author at: Institute of Grassland Science, Northeast Normal University, Changchun, Jilin Province 130024, China.

E-mail address: [liujs606@nenu.edu.cn](mailto:liujs606@nenu.edu.cn) (J. Liu).

<https://doi.org/10.1016/j.ecolind.2021.107876>

Received 3 December 2020; Received in revised form 12 May 2021; Accepted 3 June 2021

Available online 7 June 2021

1470-160X/© 2021 The Authors.

Published by Elsevier Ltd.

This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

area and are beneficial to ecosystem services, including biodiversity conservation and carbon sequestration (White et al., 2000; Lorenz and Lal, 2018; Wang et al., 2018). Livestock grazing is the most extensive land management practice in grassland ecosystems (Asner et al., 2004) and has profound effects on the structure and functions of ecosystems (Ritchie, Tilman and Knops, 1998; Bardgett and Wardle, 2003; Filazzola et al., 2020). Previous studies have suggested that grazing affected nematode communities through altering plant characteristics and soil properties (Bardgett and Wardle, 2003; Veen et al., 2010; Chen et al., 2013; Andriuzzi and Wall, 2017). Grazing may alter plant biomass, species composition and plant nutrient content, thereby affecting root exudates and the quality and quantity of litter (Olf and Ritchie, 1998; Wang et al., 2010; Frank et al., 2018) that in turn can impact soil nematode communities (Veen et al., 2010). Selective foraging can also influence plant diversity through suppression of dominant plant species or a loss of rare plant species, thus altering the abundance and diversity of soil nematodes (Crawley, 1983; Eisenhauer et al., 2013). In addition, grazers influence the composition and distribution of resources through deposition of urine and faeces (Liu et al., 2018), which can create hotspots of microbial activity and enhance the abundance and biomass of microbial feeding nematodes (Wang et al., 2006). Grazing by multiple herbivores is common in natural grasslands, and it has been shown that the effect of herbivores on grassland ecosystems differ substantially based on feeding preferences and size (Liu et al., 2015a; Chang et al., 2018; Wang et al., 2019). Yet, few studies have considered how different herbivore assemblages affect belowground communities.

Terrestrial ecosystems are subjected to global change, such as atmospheric nitrogen (N) deposition caused by fossil fuels burning and fertilizer application (Eisenhauer et al., 2012). Atmospheric N deposition has increased to  $19.6 \pm 2.5 \text{ TgN yr}^{-1}$  and is predicted to increase further in China (Galloway et al., 2008; Yu et al., 2019). High N inputs can have a strong impact on plant community dynamics and soil properties (Bobbink et al., 2010; Du et al., 2014; Maaroufi et al., 2015; Yang et al., 2018). Nitrogen addition can also alter belowground communities by affecting plant and soil characteristics. There is growing evidence that N addition generally has a negative effect on the abundance and diversity of soil nematodes (Sarathchandra et al., 2001; Liu et al., 2019). This is because N enrichment tends to increase availability of nitrogen, inducing ammonium and aluminum toxicity to the detriment of soil nematode communities (Shao et al., 2012; Wei et al., 2012). Nitrogen addition can also cause soil acidification, which results in decreasing concentrations of soil base mineral cations and suppression soil microbes, thus affecting the physiological capabilities of soil nematodes and reducing nematode food resources (Van den Berg et al., 2005; Chen et al., 2015). In contrast, N addition can increase nematode abundance and diversity through increasing plant biomass and belowground carbon allocation and alleviating the carbon limitation for nematode communities (Lebauer and Treseder, 2008; van der Wal et al., 2009). Although the response of soil biota to N addition was well documented, it remains unknown how herbivore assemblages and N addition may interact to affect soil communities. In particular, there is a lack of experiments that simultaneously manipulate grazing and N addition treatments in grasslands. A strong interaction between N addition and herbivore assemblage composition is expected that herbivores have different nutrient requirements (Liu et al., 2015a) and N addition could improve plant nutrient content and thus alter belowground resource inputs (Bobbink et al., 2010).

Our aim was to examine how grazing regimes and N addition influence soil nematode communities, and assess the relationships between environment variables and soil nematodes. Specifically, we wanted to address the following questions: (a) How do different grazing regimes affect soil nematode communities? (b) Do the impacts of N addition on nematodes differ among grazing treatments? (c) How do grazing and N addition influence soil nematodes directly and indirectly through altering plant community and soil properties?

## 2. Materials and methods

### 2.1. Study site

Our experiment was carried out in a meadow steppe in Ewenke of Hulunber, Inner Mongolia, China ( $48^{\circ}43' \text{ N}$ ,  $119^{\circ}55' \text{ E}$ ). The site is characterized by semi-arid continental climate, annual mean temperature from  $-2.4$  to  $2.2^{\circ} \text{ C}$  and annual precipitation from 250 to 350 mm. The soil type is dark chestnut. A site with relatively homogenous vegetation and a history of moderate grazing intensity was enclosed to prohibit livestock grazing in 2012 (Zhang et al., 2020). The average annual dry and wet N deposition at the experimental site is approximately  $8.5 \text{ kg N ha}^{-1} \text{ year}^{-1}$  (Liu et al., 2015b). The vegetation is dominated by *Stipa baicalensis* Roshev. Other common species were found at the site including the grass *Leymus chinensis* Tzvel., the sedge *Carex pediformis* C. A. Mey., the legume *Melissitus ruthenicus* Peschkoua. and *Lespedeza davurica* Schindl., and the forb *Filifolium sibiricum* Kitam. and *Artemisia frigida* Willd (Zhang et al., 2020).

### 2.2. Experimental design and measurements

A long-term grazing and N addition experiments were conducted with a split-plot design in randomized blocks in June 2013, with grazing treatment as the main plot factors and N treatment as subplot factors (Fig. S1). Three replicate blocks were randomly allocated within the study site, and each block containing four main plots for grazing experiments (no grazing, NG; sheep grazing, SG; cattle grazing, CG; mixed grazing of sheep and cattle, MG) and two subplots within each main plot for N treatments (ambient N and N addition). The no-grazing and single-species grazing plots were  $50 \times 50 \text{ m}$  in size while mixed-grazing plots were  $50 \times 100 \text{ m}$  to maintain comparable stocking rates between single-species grazing and mixed-grazing. Six  $4 \times 4 \text{ m}$  subplots were established within each of the no-grazing and single-species grazing plots to assess the effects on N addition, with three control subplots and three N addition subplots. Twelve plots were established within each mixed-grazing plot, with six assigned to the N addition treatment, given the larger area used for the mixed-grazing treatment. The subplots with N treatments in each grazing plot were still grazed by sheep or cattle grazing during 2013–2014.

All grazing treatments were maintained at moderate grazing intensity. Thirty adult Ujumuqin sheep (body weight  $31.3 \pm 1.2 \text{ kg}$ , mean  $\pm$  SE) and six adult Mongolian cattle (body weight  $413.3 \pm 11.2 \text{ kg}$ , mean  $\pm$  SE) were arranged in grazing plots. Grazing experiments were implemented from June to August of each year, twice a day from 05:00 to 07:00 and from 15:00 to 17:00. Nitrogen was added as ammonium nitrate aqueous solution at a rate of  $5 \text{ g N m}^{-2} \text{ year}^{-1}$  in spring each year, which is lower than airborne nutrient deposition in Northern China.

Vegetation surveys were conducted in mid-August 2014. Twelve  $0.5 \times 0.5 \text{ m}$  quadrats were arranged along two 100-m cater-corner transects in each main plot and two were randomly placed in each subplot. Plant species richness was represented by the number of plant species within six quadrats of main plots and subplots. The plant species diversity was estimated using Shannon-Weiner diversity index:  $H' = -\sum P_i \ln(P_i)$ , where  $P_i$  is the proportional abundance of species  $i$ . Plant shoot biomass was determined by clipping aboveground living plants of each quadrat, and then drying to constant weight at  $65^{\circ} \text{ C}$  and weighing. Plant root biomass was collected with one soil core (diameter 10 cm, depth 30 cm) from per quadrat. Roots were rinsed by placing them under running water over a 1 mm screen, oven-dried at  $70^{\circ} \text{ C}$  to constant weight, and weighed.

Soil samples were collected in mid-August 2014. A composite sample of three soil cores with a diameter of 2 cm and a depth of 15 cm was taken within and outside each N addition subplot after removing litter, respectively. In each MG plot, the soil cores were randomly selected from three N treatment subplots. Thus, six soil samples were obtained in

each grazing plot, resulting in a total of 72 samples, 3 replicate blocks  $\times$  4 grazing treatments  $\times$  2 N treatments  $\times$  3 soil samples. The moist soil was separated into two parts after passing through a 2 mm mesh. One part was kept at 4 °C until nematode extraction and soil moisture, nitrate concentration ( $\text{NO}_3^-$ -N), and ammonium concentration ( $\text{NH}_4^+$ -N) measurement. The other part was air-dried to determine total carbon, total nitrogen, pH and electric conductivity. Soil moisture content was measured as mass loss by drying 10 g moist soil at 105 °C until constant weight. Soil  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N were measured by dissolving 10 g fresh soil in 50 mL of 2 mol L<sup>-1</sup> KCl, and the concentration was subjected to analysis using flow analyzer (Alliance Flow Analyzer; Futura, Frépillon, France). We determined soil total carbon and total nitrogen using an automated element analyzer (Vario EL cube, Elementar Analysensysteme GmbH, Hanau, Germany). Soil C:N ratio was calculated as the ratio of total C to N. Soil pH and electrical conductivity were measured by using a pH and conductivity meter in 1:2.5 (soil:water) suspension.

Nematodes were extracted from 50 g fresh soil for 48 h following modified Baermann funnel method (Barker, 1985), and then heat-killed at 65 °C, fixed in 4% formalin solution, counted and identified. The first 100 nematodes, or all nematodes in samples with less than 100 individuals, were identified to genus with a microscope. Nematodes were assigned to four feeding groups, including plant-feeders (PF), fungal-feeders (FF), bacterial-feeders (BF) and omnivores-predators (OP) (Yeates et al., 1993). The nematode generic richness was estimated according to the following formula:  $R = (S-1)/\ln(N)$ , where  $S$  is the total number of nematode taxa and  $N$  is the total number of nematodes. Shannon-Weiner diversity index was applied as plant diversity (Yeates and Bongers, 1999). Nematode maturity index, channel ratio, enrichment and structure indices were calculated to assess the responses of soil food web to resource and disturbance (Bongers, 1990; Ferris et al., 2001; Yeates, 2003).

### 2.3. Statistical analyses

We tested the influences of grazing regimes, N and their interaction on the soil, plant and nematode characteristics using mixed-effects model, with grazing and N as fixed factors and block as a random factor. Significant differences among grazing treatments were analyzed post hoc with Tukey's multiple comparison.

Piecewise structural equation modelling (SEM) was performed to distinguish between the direct impacts of grazing and N addition on nematodes and indirect effects by changing soil and plant variables (Lefcheck, 2016). According to a *priori* and theoretical knowledge, the SEM was carried out with the specification of a conceptual model of hypothetical relationships (Grace, 2006). The initial model included paths from N and grazing to soil  $\text{NO}_3^-$ -N, C:N ratio and relative biomass of grasses, from soil  $\text{NO}_3^-$ -N, C:N ratio and relative biomass of grasses to nematode abundance and richness, and from nematode abundance to richness (Fig. S2, Supporting Information). The fit of the models was measured by the Shipley's test of *d*-separation and Akaike information criterion (AIC) (Grace, 2006; Lefcheck, 2016). According to modification indices, the path from relative biomass of grasses to soil C:N ratio was added to the model, and the nonsignificant path from relative biomass of grasses to nematode abundance was removed from the model. The final test of *d*-separation (Fisher's  $C = 20.40$ ,  $P = 0.12$ ) showed a good model fit. Redundancy analysis (RDA) was conducted to explore the correlation between the abundance of nematode genera and environmental factors (plant characteristics; soil properties). Monte Carlo permutation test was used to evaluate relationships between nematodes and environmental factors.

All data were analyzed using R version 4.0.2 (R Development Core Team, 2013). A low probability value ( $P < 0.05$ ) suggests that the analyses have a statistical significance. The mixed-effects model analysis was done using the 'nlme' package (Pinheiro et al., 2018), the SEM using the 'piecewiseSEM' packages and the RDA using the 'vegan' package

(Lefcheck, 2016; Oksanen et al., 2015).

## 3. Results

### 3.1. Effects of grazing and N addition on soil and plant characteristics

Grazing significantly affected plant richness, shoot biomass and the relative biomass of grasses, and N addition affected the relative biomass of grasses and root biomass (Table 1, S1). Grazing and N addition interactively affect soil moisture, C:N ratio,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, total nitrogen and plant shoot biomass. In the NG and CG treatments, N addition significantly decreased soil moisture, soil total nitrogen and the relative biomass of grasses, and increased soil C:N ratio. In the SG and MG treatments, N addition reduced plant richness and shoot biomass, but did not affect soil C:N ratio. Soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N were significantly increased by N addition in all grazing treatments (Fig. 1). Without N addition, grazing treatment had little influence on most soil and plant variables except that SG and CG significantly decreased shoot biomass. With N addition, SG significantly increased soil moisture,  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N, and decreased soil C:N ratio. CG significantly increased soil C:N ratio and decreased soil total nitrogen. Plant richness was decreased and the relative biomass of grasses was increased by MG treatment. All grazing treatments decreased plant shoot biomass compared with no grazing in the plots with and without N addition plots (Fig. 1).

### 3.2. Effects of grazing and N addition on nematode communities

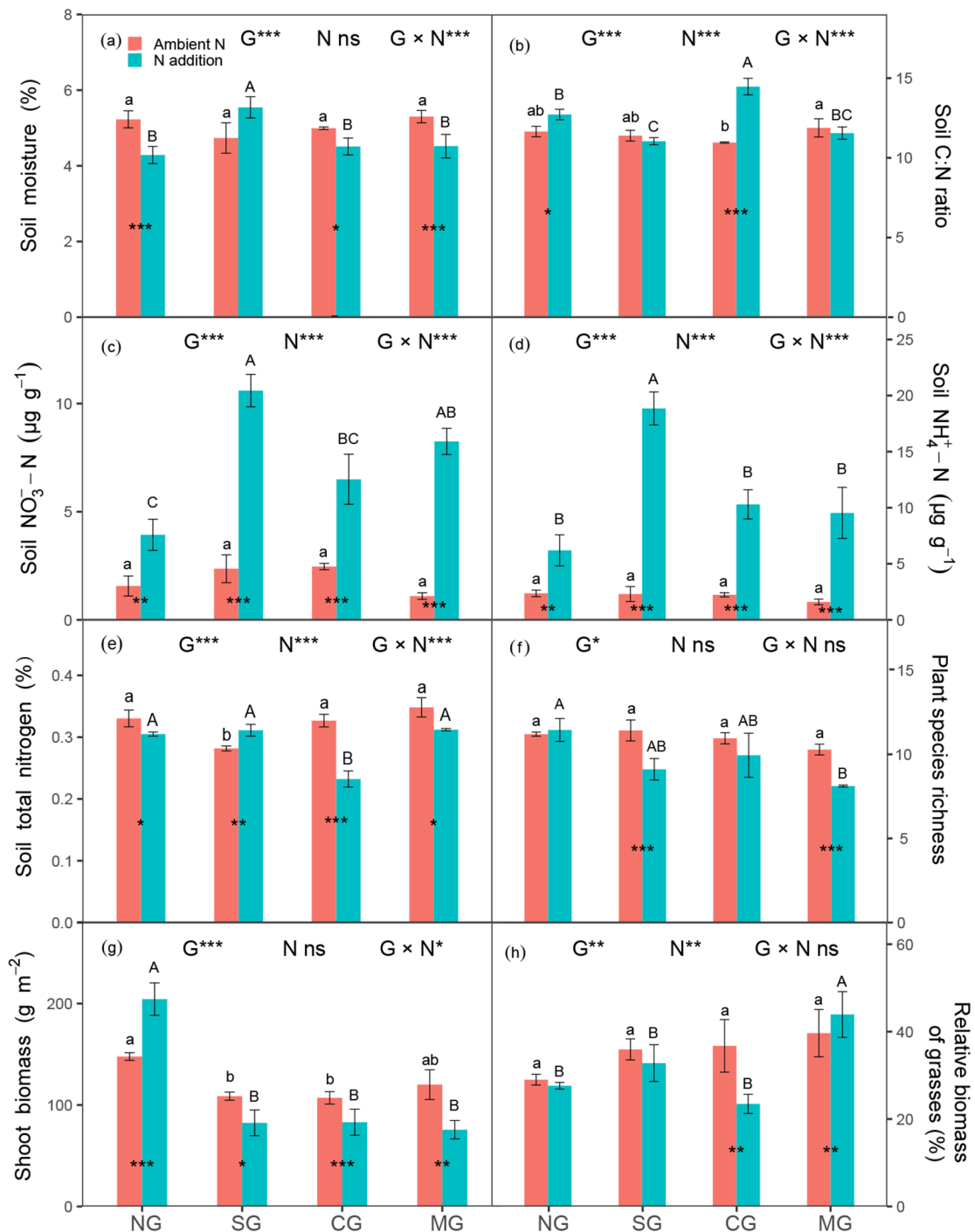
Grazing significantly influenced total nematode abundance, and the abundance of PF, BF and OP, and generic richness, Shannon-Wiener diversity and maturity index. Nitrogen addition affected almost all nematode variables except fungal-feeders and structure index (Table 1). Grazing and N addition had an interactive effect on the abundance of total nematodes and different trophic groups, generic richness, maturity index, channel ratio and enrichment index. Nitrogen addition significantly increased total nematode abundance, BF, PF and OP abundance, and generic richness in CG treatment, whereas decreased them in SG and MG treatments (Figs. 2 and 3). Nitrogen addition reduced nematode

**Table 1**

Two-way ANOVA testing the effects of grazing (G), N addition (N) and their interaction (G  $\times$  N) on soil (moisture, C:N ratio, ammonium concentration, nitrate concentration and total nitrogen), plants (species richness, shoot biomass and relative biomass of grasses), nematodes (total abundance, generic richness, Shannon-Wiener diversity, channel ratio, maturity index, enrichment index and structure index) and different trophic group abundance (plant-feeders, fungal-feeders, bacterial-feeders and omnivores-predators).

	Response variables	G	N	G $\times$ N
Soil	Moisture	9.775***	3.562	9.743***
	C:N ratio	16.451***	43.860***	11.725***
	$\text{NH}_4^+$ -N	22.420***	25.122***	11.025***
	$\text{NO}_3^-$ -N	18.306***	18.796***	8.531**
	Total nitrogen	14.533***	43.304***	12.519***
Plants	Species richness	4.960*	1.264	1.768
	Shoot biomass	32.682***	2.445	8.592*
	Relative biomass of grasses	9.371**	10.547**	3.202
Nematode communities	Total abundance	34.631***	60.516***	48.485***
	Generic richness	12.856***	10.031**	15.946***
	Shannon-Wiener diversity	13.031***	7.060*	27.431***
	Maturity index	9.598**	9.560**	10.355***
	Channel ratio	3.315	55.738***	10.230***
	Enrichment index	0.680	48.787***	10.396***
	Structure index	1.528	0.025	0.650
Trophic group abundance	Plant-feeders	40.559***	88.637***	51.259***
	Fungal-feeders	0.742	1.425	3.390**
	Bacterial-feeders	28.390***	49.639***	40.103***
	Omnivore-predators	15.618***	30.749***	22.938***

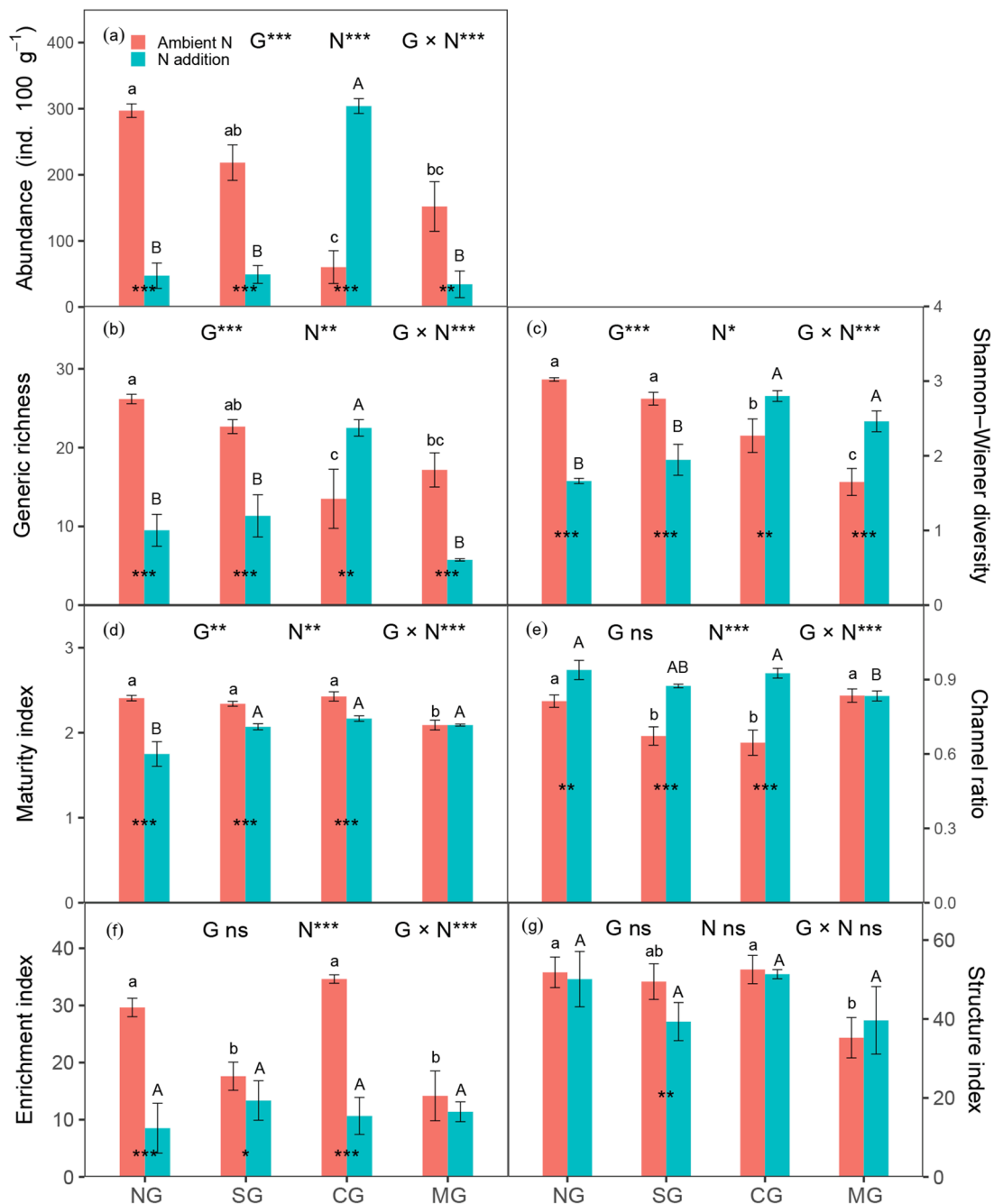
\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



**Fig. 1.** Effects of different grazing treatments (G), N addition (N) and their interaction (G × N) on soil characteristics, soil moisture (a), soil C:N ratio (b), soil NO<sub>3</sub>-N (c), soil NH<sub>4</sub>-N (d) and soil total nitrogen (e), and plant communities, plant species richness (f), shoot biomass (g) and relative biomass of grasses (h). Different grazing treatments include no grazing (NG), sheep grazing (SG), cattle grazing (CG), mixed grazing (MG) by sheep and cattle and N addition treatments include ambient N and N addition ( $P < 0.05$ ). Values are means ± SE. Different lowercase letters and capital letters represent significant differences among grazing treatments under ambient N and N addition ( $P < 0.05$ ), respectively. Asterisks indicate significant differences at different N levels (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , ns  $P > 0.05$ ).

maturity index, enrichment index and structure index and enhanced nematode channel ratio in most grazing treatments, except under MG treatment, in which N addition had no effect on these variables (Fig. 2). In ambient N treatment, CG and MG significantly decreased total nematode abundance, generic richness and diversity, and all grazing treatments decreased BF and OP abundance (Fig. 2a,2b,2c,3). SG markedly reduced the channel ratio and enrichment index while MG significantly decreased the maturity, enrichment and structure indices

(Fig. 2d,2e,2f,2g). In contrast, with N addition, CG increased total nematode abundance, every trophic group abundance, generic richness and Shannon-Wiener diversity. Moreover, total nematode abundance, BF, PF and OP abundance, generic richness were lower in CG plots than in the other grazing treatments under without N addition conditions, and were higher in CG with N addition (Figs. 2a,2b,2c,3). The maturity index was significantly higher in all grazing treatments.

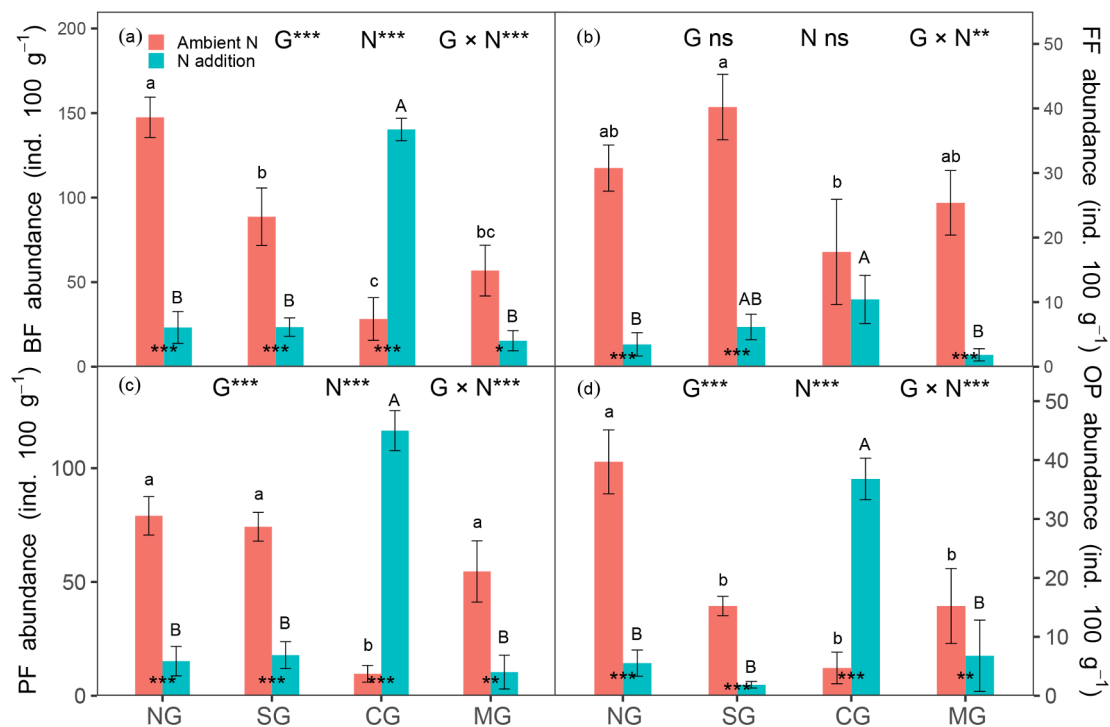


**Fig. 2.** Effects of different grazing treatments (G), N addition (N) and their interaction (G × N) on total nematode abundance (a), generic richness (b), Shannon-Wiener diversity (c), maturity index (d), channel ratio (e), enrichment index (f) and structure index (g). Different grazing treatments include no grazing (NG), sheep grazing (SG), cattle grazing (CG), mixed grazing (MG) by sheep and cattle and N addition treatments include ambient N and N addition. Data are presented as means ± SE. Different lowercase letters and capital letters express significant differences among grazing treatments under ambient N and N addition ( $P < 0.05$ ), respectively. Asterisks indicate significant differences at different nitrogen levels (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , ns  $P > 0.05$ ).

### 3.3. Relationships between nematodes and environmental factors

Redundancy analysis showed that there was a strong relationship between the abundance of nematode genera and environmental parameters based on the results of the Monte Carlo permutation tests for the first canonical axes ( $F = 6.740$ ,  $P = 0.002$ ) and all canonical axes ( $F = 1.574$ ,  $P = 0.003$ ) (Fig. 4). Relative biomass of grasses, plant shoot biomass, soil total nitrogen, soil total carbon, soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N were found to be significantly related to nematode community composition. The plant-feeding nematodes *Tylenchorhynchus* were positively correlated with the relative biomass of grasses, and other plant-feeding

nematodes *Helicotylenchus*, *Coslenchus*, *Tylenchus* and *Boleodorus* were positively correlated with soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N. The omnivore-predators *Mononchus* and *Aporcelaimus* were positively correlated with plant shoot biomass. The fungal-feeders *Filenchus* and bacterial-feeding nematodes *Eucephalobus* were positively correlated with soil total nitrogen and soil total carbon, respectively. The final SEM showed that grazing indirectly decreased nematode abundance and richness through an increase in the relative biomass of grasses and a decrease in soil C:N ratio. Nematode abundance was positively associated with soil C:N ratio and negatively associated with the relative biomass of grasses. Nitrogen addition reduced nematode abundance and richness mainly through



**Fig. 3.** Effects of different grazing treatments (G), N addition (N) and their interaction (G × N) on different trophic group abundance. BF, bacterial-feeders (a), FF, fungal-feeders (b), PF, plant-feeders (c) and OP, omnivore-predators (d). Different grazing treatments include no grazing (NG), sheep grazing (SG), cattle grazing (CG), mixed grazing (MG) by sheep and cattle and N addition treatments include ambient N and N addition. Values represent means ± SE. Different lowercase letters and capital letters indicate significant differences among grazing treatments under ambient N and N addition ( $P < 0.05$ ), respectively. Asterisks indicate significant differences at different nitrogen levels (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , ns  $P > 0.05$ ).

increasing soil  $\text{NO}_3^-$ -N concentration (Fig. 5).

## 4. Discussion

### 4.1. Effects of large herbivore grazing on nematode communities

Our results demonstrated that the effect of grazers on soil nematodes strongly depend on herbivore assemblages. The presence of cattle significantly decreased total nematode abundance, the abundance of bacterial-feeders, plant-feeders and omnivore-predators, generic richness and diversity and cattle grazing had greater negative effects than sheep grazing in ambient N plots (Figs. 2, 3). This is likely because cattle need more resources to meet their energy requirements, which results in greater consumption of plant tissues reducing plant biomass and resource allocation belowground, thereby decreasing nematode abundance (Fig. 1g). Large herbivore trampling could also cause soil compaction with adverse effects on nematode communities (Bouwman and Arts, 2000; Mikola et al., 2009). Cattle have larger body size than sheep, so the negative effects of the foraging and trampling on plant and soil properties are stronger, thus further suppressing nematode communities. Previous studies have also demonstrated that the responses of soil communities shifted from neutral to negative with increasing herbivore body size (Andriuzzi and Wall, 2017). Furthermore, our study also showed that mixed grazing had less negative effects on nematode communities than cattle grazing alone, likely because the existence of cattle influences the behaviour of sheep when cattle and sheep graze together (Ritchie and Olf, 1999). Sheep grazing significantly reduced the channel ratio and enrichment index and mixed grazing decreased the maturity, enrichment and structure indices (Fig. 2d,2e,2f,2g). These results suggested that the soil organic matter decomposition pathway shifted from bacteria-dominated to fungal-dominated and that resource availability for soil biota was lower in sheep grazing treatment. Mixed grazing could increase soil disturbance and decrease the complexity of

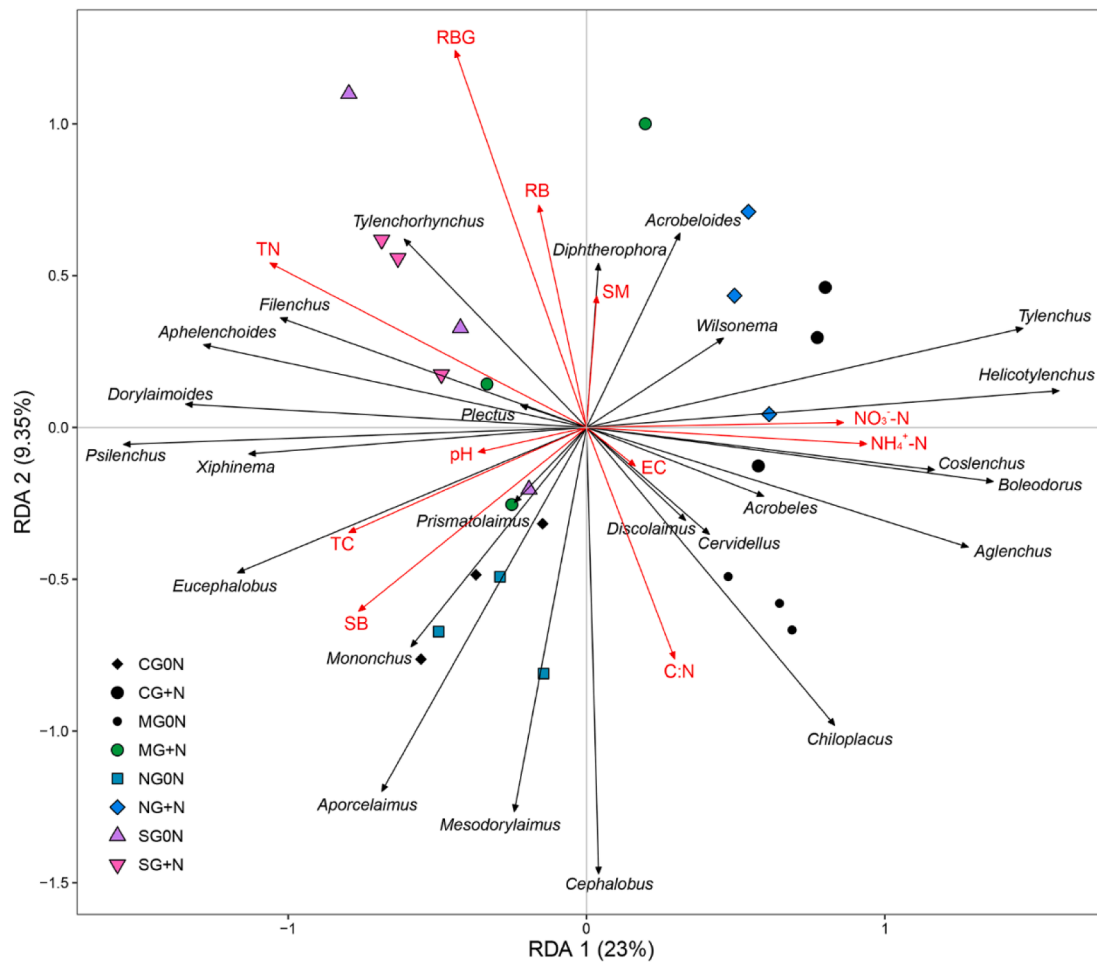
the nematode communities and consequently simplify soil food webs under control treatment.

### 4.2. Effects of N addition on nematode communities

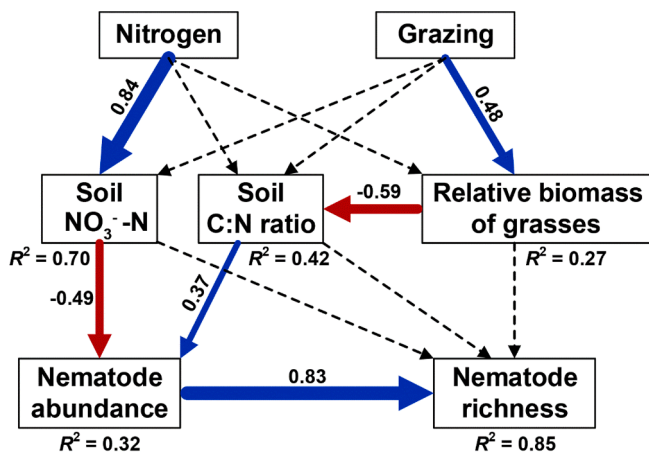
Our study showed that N addition reduced total nematode abundance, the abundance of all trophic groups and generic richness except when cattle were present, which have also been found in most previous studies in grasslands (Eisenhauer et al., 2012; Chen et al., 2015; Liu et al., 2019). Nitrogen addition negatively affected nematodes possibly due to ammonium toxicity and soil acidification, which was known to have detrimental to the survival of nematodes and consequently suppressed nematode communities (Wei et al., 2012; Chen et al., 2015). Nitrogen addition also affected nematode communities by altering plant communities. Previous studies have suggested that N addition reduced plant below-ground carbon allocation resulting in fewer resources to support belowground communities and thereby suppressing nematode communities (Högberg et al., 2010). The SEM indicated that N addition indirectly reduced total nematode abundance mainly through increasing soil nitrate concentration (Fig. 5). Nitrogen addition reduced maturity, enrichment and structure indices, whereas increased nematode channel ratio except under mixed grazing treatment, with similar patterns observed in a previous study (Liu et al., 2019). These results indicated significant negative effects on soil processes including a simplification of the soil food web. The positive influences of N addition on nematode channel ratio suggested that the decomposition pathway was dominated by bacteria.

### 4.3. Effects of grazing and N addition on nematode communities

Our study showed that there was a significant interaction between grazing and N addition on most of the nematode variables (Table 1), indicating that the effects of N addition on nematodes changed with



**Fig. 4.** Redundancy analysis of the relationship between the abundance of dominant nematode genera and environmental variables under different grazing treatments (NG, no grazing; SG, sheep grazing; CG, cattle grazing; MG, mixed grazing) and N addition (0 N, ambient N and + N, N addition). Environmental variables are abbreviated as: SM (soil moisture),  $\text{NH}_4^+\text{-N}$  (soil  $\text{NH}_4^+\text{-N}$ ),  $\text{NO}_3^-\text{-N}$  (soil  $\text{NO}_3^-\text{-N}$ ), C:N (soil C:N), pH (soil pH), EC (soil electric conductivity), TN (soil total nitrogen) and TC (soil total carbon), SB (plant shoot biomass), RBG (relative biomass of grasses) and RB (root biomass).



**Fig. 5.** Structural equation models for direct and indirect impacts of grazing treatment and N addition on total nematode abundance and nematode richness. Blue solid arrows indicate significant positive paths and red are significant negative paths, while dotted arrows denote non-significant relationships. The width of the solid arrows reflects the strength of the standardized path coefficients.  $R^2$  is the coefficient of all the paths.

different grazing regimes. Nitrogen addition markedly increased nematode abundance and generic richness, and had no effect on nematode functional indices under cattle and mixed grazing treatments, respectively (Figs. 2 and 3). These results indicated that the presence of cattle could alleviate the negative impacts of N addition on nematodes and resulted in a relatively complex and mature soil food web. In general, N addition could suppress forbs biomass and enhance grasses biomass (Stevens et al., 2006; Phoenix et al., 2012). Cattle are a less selective herbivore and need to forage low-nutrient grasses with higher biomass to meet their energetic requirements (Liu et al., 2015a), which may result in lower N inputs into soil and thereby mitigating the adverse effects of ammonium toxicity and soil acidification on nematodes. In addition, the reduction in dominance of grasses would enhance forbs survival rates thereby increasing plant diversity (Martin and Wilsey, 2006). Higher plant diversity would induce higher nematode diversity (Eisenhauer et al., 2013). We found that sheep grazing did not ameliorate the negative effect of N addition on nematodes (Figs. 2 and 3). Sheep preferentially feed on non-dominant rare forbs with high nutrient content (Wang et al., 2010; Liu et al., 2015a), which may not alter availability of nitrogen to soil biota and thus cannot mediate the negative effect of N addition on nematodes. Overall, these findings indicated that herbivore grazing could modify the impacts of N addition on nematodes, with the effect differing predictably between herbivore species.

#### 4.4. Grazing and N addition affected nematodes via changes in the plant and edaphic variables

Redundancy analysis (RDA) showed that individual nematode genera were correlated with different plant and soil characteristics (Fig. 4). In particular, the relative biomass of grasses, plant shoot biomass and soil total nitrogen were important in determining nematode community composition. Structural equation modelling suggested that grazing and N addition affected nematode communities mainly through changes in plant and soil properties (Fig. 5). Grazing indirectly decreased nematode abundance and richness through increases in the relative biomass of grasses and a reduction in the soil C:N ratio. A greater proportion of grasses biomass is associated with lower biomass of forbs and legumes as well as lower plant diversity (Fig. S3). The reduction in soil C:N ratio was associated with the decrease plant diversity. Previous studies have found that the reduction of plant diversity can decrease carbon input into soil (Chen et al., 2019; Yang et al., 2019). The increase in relative biomass of grasses and decrease soil C:N ratio would reduce resource input into soil, and the two pathways could both suppress nematode abundance and richness. Nitrogen addition indirectly reduced nematode abundance mainly by increasing soil  $\text{NO}_3^-$ -N (Fig. 5). The nitrate ion is negatively charged and absorbed by plant roots, which then release  $\text{OH}^-$  resulting in increased pH in the rhizosphere (Söderberg and Bååth, 2004). Most nematodes can live only in mildly acid and neutral conditions; therefore, higher rhizosphere pH can negatively impact nematode communities.

#### 5. Conclusions

Our study indicated that there are significant interactive influences of large grazers and N addition on soil nematodes, with the influences of N addition on nematodes depending strongly on which herbivore is present. Nitrogen addition markedly increased total nematode abundance, and the abundance of bacterial-feeders, plant-feeders and omnivore-predators, and generic richness in the cattle grazing treatment, while decreased them in sheep and mixed grazing treatments. The changes in the relative biomass of grasses, soil  $\text{NO}_3^-$ -N and C:N ratio played an important role in driving the responses of nematode abundance and richness to grazing and N addition. The positive effects of N addition on nematodes in cattle grazing treatment, suggested that cattle grazing could alleviate the negative impacts of N addition on nematodes and be served as an optimal grassland management under N deposition.

##### Authors' contributions

Y.C., Y.Z., J.S.L., D.L.W. designed experiments; Y.C., Y.Z., Q.H.M., X. Z. X.W.H., D.F.P and X.Q.Y conducted the experiments. Y.C. and J.S.L. analyzed the data and Y.C., J.S.L., D.L.W. and U.N. drafted the manuscript; All authors contributed critically to the drafts and gave final approval for publication.

##### CRedit authorship contribution statement

**Ying Chen:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing - original draft. **Yao Zhang:** Conceptualization, Data curation, Investigation, Methodology. **Uffe N. Nielsen:** . **Quanhui Ma:** Investigation. **Xiang Zhang:** Investigation. **Xuewen Huang:** Investigation. **Duofeng Pan:** Investigation. **Xiuquan Yue:** Resources. **Jushan Liu:** Conceptualization, Methodology, Funding acquisition, Project administration, Supervision, Visualization. **Deli Wang:** Conceptualization, Methodology, Funding acquisition, Project administration, Supervision, Visualization.

##### 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

This project was supported by the National Natural Science Foundation of China (31971744, 31770520, 32061143027), the State Basic Research Program (2012FY111900), the Program for Introducing Talents to Universities (B16011) and the National Key Research and Development Program of China (2016YFC0500602). All of the animal experiments complied with the current laws of China.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2021.107876>.

#### References

- Andriuzzi, W.S., Wall, D.H., 2017. Responses of belowground communities to large aboveground herbivores: Meta-analysis reveals biome-dependent patterns and critical research gaps. *Glob. Change Biol.* 23 (9), 3857–3868.
- Asner, G.P., Elmore, A.J., Olander, L.P., Martin, R.E., Harris, A.T., 2004. Grazing systems, ecosystem responses, and global change. *Annu. Rev. Environ. Resour.* 29 (1), 261–299.
- Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515 (7528), 505–511.
- Bardgett, R.D., Wardle, D.A., 2003. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* 84 (9), 2258–2268.
- Barker, K.R., 1985. Nematode extraction and bioassays. In: In: Barker, K.R., Carter, C.C., Sasser, J.N. (Eds.), *An Advanced Treatise on Meloidogyne*, vol. 2. North Carolina State University Graphics, Methodology, pp. 19–35.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinnerby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol. Appl.* 20 (1), 30–59.
- Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83 (1), 14–19.
- Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. *Appl. Soil Ecol.* 10 (3), 239–251.
- Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. *Trends Ecol. Evol.* 14 (6), 224–228.
- Bouwman, L.A., Arts, W.B.M., 2000. Effects of soil compaction on the relationships between nematodes, grass production and soil physical properties. *Appl. Soil Ecol.* 14 (3), 213–222.
- Chang, Q., Wang, L., Ding, S., Xu, T., Li, Z., Song, X., Zhao, X., Wang, D., Pan, D., Mukul, S., 2018. Grazer effects on soil carbon storage vary by herbivore assemblage in a semi-arid grassland. *J. Appl. Ecol.* 55 (5), 2517–2526.
- Chen, C., Chen, H.Y.H., Chen, X., Huang, Z., 2019. Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. *Nat. Commun.* 10, 1332.
- Chen, D., Lan, Z., Hu, S., Bai, Y., 2015. Effects of nitrogen enrichment on belowground communities in grassland: Relative role of soil nitrogen availability vs. soil acidification. *Soil Biol. Biochem.* 89, 99–108.
- Chen, D., Zheng, S., Shan, Y., Taube, F., Bai, Y., Briones, M.J., 2013. Vertebrate herbivore-induced changes in plants and soils: Linkages to ecosystem functioning in a semi-arid steppe. *Funct. Ecol.* 27 (1), 273–281.
- Crawley, M.J., 1983. *Herbivory: The Dynamics of Plant-Animal Interactions*. Blackwell Scientific Publications, Oxford, UK.
- Du, Y., Guo, P., Liu, J., Wang, C., Yang, N., Jiao, Z., 2014. Different types of nitrogen deposition show variable effects on the soil carbon cycle process of temperate forests. *Glob. Change Biol.* 20 (10), 3222–3228.
- Eisenhauer, N., Cesarz, S., Koller, R., Worm, K., Reich, P.B., 2012. Global change belowground: impacts of elevated  $\text{CO}_2$ , nitrogen, and summer drought on soil food webs and biodiversity. *Glob. Change Biol.* 18 (2), 435–447.
- Eisenhauer, N., Dobies, T., Cesarz, S., Hobbie, S.E., Meyer, R.J., Worm, K., Reich, P.B., 2013. Plant diversity effects on soil food webs are stronger than those of elevated  $\text{CO}_2$  and N deposition in a long-term grassland experiment. *PNAS* 110 (17), 6889–6894.
- Ettema, C.H., Yeates, G.W., 2003. Nested spatial biodiversity patterns of nematode genera in a New Zealand forest and pasture soil. *Soil Biol. Biochem.* 35 (2), 339–342.
- Ferris, H., 2010. Contribution of Nematodes to the Structure and Function of the Soil Food Web. *Journal of Nematology* 42, 63–67.
- Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Appl. Soil Ecol.* 18 (1), 13–29.
- Filazzola, A., Brown, C., Dettlaff, M.A., Batbaatar, A., Grenke, J., Bao, T., Peetoom Heida, I., Cahill, J.F., Seabloom, E., 2020. The effects of livestock grazing on biodiversity are multi-trophic: a meta-analysis. *Ecol. Lett.* 23 (8), 1298–1309.
- Frank, D.A., Wallen, R.L., Hamilton, E.W., White, P.J., Fridley, J.D., Rees, M., 2018. Manipulating the system: How large herbivores control bottom-up regulation of grasslands. *J. Ecol.* 106 (1), 434–443.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the Nitrogen



- Cycle: Recent Trends, Questions, and Potential Solutions. *Science* 320 (5878), 889–892.
- Grace, J.B., 2006. Structural equation modeling and natural systems. Cambridge University Press, New York, NY, USA.
- Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., Högberg, P., 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *The New phytologist* 187 (2), 485–493.
- Hunt, H.W., Wall, D.H., 2002. Modelling the effects of loss of soil biodiversity on ecosystem function. *Glob. Change Biol.* 8, 33–50.
- LeBauer, D.S., Treseder, K.K., 2008. Nitrogen Limitation of Net Primary Productivity in Terrestrial Ecosystems is Globally Distributed. *Ecology* 89 (2), 371–379.
- Lefcheck, J.S., 2016. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods Ecol. Evol.* 7, 573–579.
- Liu, C., Wang, L., Song, X., Chang, Q., Frank, D.A., Wang, D., Li, J., Lin, H., Du, F., Huenneke, L., 2018. Towards a mechanistic understanding of the effect that different species of large grazers have on grassland soil N availability. *J. Ecol.* 106 (1), 357–366.
- Liu, J., Feng, C., Wang, D., Wang, L., Wilsey, B.J., Zhong, Z., Firn, J., 2015a. Impacts of grazing by different large herbivores in grassland depend on plant species diversity. *J. Appl. Ecol.* 52 (4), 1053–1062.
- Liu, X., Ren, J., Li, S., Zhang, Q., 2015b. Effects of Simulated Nitrogen Deposition on Soil Net Nitrogen Mineralization in the Meadow Steppe of Inner Mongolia, China. *PLoS one* 10, 1–15.
- Liu, J., Chen, Y., Du, C., Liu, X., Ma, Q., Zhang, X., Wang, D., 2019. Interactive effects of nitrogen addition and litter on soil nematodes in grassland. *Eur. J. Soil Sci.* 70 (3), 697–706.
- Lorenz, K., Lal, R., 2018. Carbon sequestration in grassland soils. Springer International Publishing, Cham, Switzerland.
- Lorenzen, S., 1994. The phylogenetic systematics of free living nematodes. Ray Society.
- Maaroufi, N.I., Nordin, A., Hasselquist, N.J., Bach, L.H., Palmqvist, K., Gundale, M.J., 2015. Anthropogenic nitrogen deposition enhances carbon sequestration in boreal soils. *Glob. Change Biol.* 21 (8), 3169–3180.
- Martin, L.M., Wilsey, B.J., 2006. Assessing grassland restoration success: relative roles of seed additions and native ungulate activities. *J. Appl. Ecol.* 43, 1098–1109.
- Mikola, J., Setälä, H., Virkajärvi, P., Saarijärvi, K., Ilmarinen, K., Voigt, W., Vestberg, M., 2009. Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a dairy cow pasture. *Ecol. Monogr.* 79 (2), 221–244.
- Nielsen, U.N., Wall, D.H., Six, J., 2015. Soil Biodiversity and the Environment. *Annu. Rev. Environ. Resour.* 40 (1), 63–90.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2015. *Vegan: Community Ecology Package*. R Package Version 2.3-1.
- Olff, H., Ritchie, M.E., 1998. Effects of herbivores on grassland plant diversity. *Trends Ecol. Evol.* 13 (7), 261–265.
- Phoenix, G.K., Emmett, B.A., Britton, A.J., Caporn, S.J.M., Dise, N.B., Helliwell, R., Jones, L., Leake, J.R., Leith, I.D., Sheppard, L.J., Sowerby, A., Pilkington, M.G., Rowe, E.C., Ashmore, M.R., Power, S.A., 2012. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Glob. Change Biol.* 18 (4), 1197–1215.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2018. *Nlme: linear and nonlinear mixed effects models*. R package version 3, 1–137.
- R Core Team, 2013. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Ritchie, M.E., Olff, H., 1999. Herbivore diversity and plant dynamics: Compensatory and additive effects. In: Olff, H., Brown, V.K., Drent, R.H. (Eds.), *Herbivores: Between plants and predators*. Blackwell Science Ltd, Oxford, UK, pp. 175–204.
- Ritchie, M.E., Tilman, D., Knops, J.M.H., 1998. Herbivore Effects on Plant and Nitrogen Dynamics in Oak Savanna. *Ecology* 79 (1), 165–177.
- Sarathchandra, S.U., Ghani, A., Yeates, G.W., Burch, G., Cox, N.R., 2001. Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils. *Soil Biol. Biochem.* 33 (7–8), 953–964.
- Shao, Y., Zhang, W., Liu, Z., Sun, Y., Chen, D., Wu, J., Zhou, L., Xia, H., Neher, D.A., Fu, S., 2012. Responses of soil microbial and nematode communities to aluminum toxicity in vegetated oil-shale-waste lands. *Ecotoxicology (London, England)* 21 (8), 2132–2142.
- Söderberg, K.H., Bååth, E., 2004. The influence of nitrogen fertilisation on bacterial activity in the rhizosphere of barley. *Soil Biol. Biochem.* 36, 195–198.
- Stevens, C.J., Dise, N.B., Gowing, D.J.G., Mountford, J.O., 2006. Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. *Glob. Change Biol.* 12, 1823–1833.
- Veen, G.F., Olff, H., Duyts, H., van der Putten, W.H., 2010. Vertebrate herbivores influence soil nematodes by modifying plant communities. *Ecology* 91 (3), 828–835.
- van der Wal, A., Geerts, R.H.E.M., Korevaar, H., Schouten, A.J., op Akkerhuis, G.A.J.M. J., Rutgers, M., Mulder, C., 2009. Dissimilar response of plant and soil biota communities to long-term nutrient addition in grasslands. *Biol. Fertil. Soils* 45 (6), 663–667.
- Van Den Berg, L.J., Dorland, E., Vergeer, P., Hart, M.A., Bobbink, R., Roelofs, J.G., 2005. Decline of acid-sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH. *New Phytol.* 166 (2), 551–564.
- Wang, K.H., McSorley, R., Bohlen, P., Gathumbi, S.M., 2006. Cattle grazing increases microbial biomass and alters soil nematode communities in subtropical pastures. *Soil Biol. Biochem.* 38 (7), 1956–1965.
- Wang, D., Wang, L., Liu, J., Zhu, H., Zhong, Z., 2018. Grassland ecology in China: perspectives and challenges. *Front. Agric. Sci. Eng.* 5 (1), 24.
- Wang, L., Delgado-Baquerizo, M., Wang, D., Isbell, F., Liu, J., Feng, C., Liu, J., Zhong, Z., Zhu, H., Yuan, X., Chang, Q., Liu, C., 2019. Diversifying livestock promotes multidiversity and multifunctionality in managed grasslands. *PNAS* 116 (13), 6187–6192.
- Wang, L., Wang, D., He, Z., Liu, G., Hodgkinson, K.C., 2010. Mechanisms linking plant species richness to foraging of a large herbivore. *J. Appl. Ecol.* 47, 868–875.
- Wei, C., Zheng, H., Li, Q., Lü, X., Yu, Q., Zhang, H., Chen, Q., He, N., Kardol, P., Liang, W., Han, X., 2012. Nitrogen addition regulates soil nematode community composition through ammonium suppression. *PLoS one* 7, e43384.
- White, R.P., Murray, S., Rohweder, M., 2000. *Pilot analysis of global ecosystems: Grassland ecosystems*. World Resources Institute, Washington DC.
- Yang, Y.i., Tilman, D., Lehman, C., Trost, J.J., 2018. Sustainable intensification of high-diversity biomass production for optimal biofuel benefits. *Nat. Sustainability* 1 (11), 686–692.
- Yang, Y., Tilman, D., Furey, G., Lehman, C., 2019. Soil carbon sequestration accelerated by restoration of grassland biodiversity. *Nat. Commun.* 10, 718.
- Yeates, G.W., 2003. Nematodes as soil indicators: functional and biodiversity aspects. *Biol. Fertil. Soils* 37 (4), 199–210.
- Yeates, G.W., Bongers, T., 1999. Nematode diversity in agroecosystems. *Agric. Ecosyst. Environ.* 74, 113–135.
- Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and genera—an outline for soil ecologists. *Journal of Nematology* 25, 315–331.
- Yu, G., Jia, Y., He, N., Zhu, J., Chen, Z., Wang, Q., Piao, S., Liu, X., He, H., Guo, X., Wen, Z., Li, P., Ding, G., Goulding, K., 2019. Stabilization of atmospheric nitrogen deposition in China over the past decade. *Nat. Geosci.* 12 (6), 424–429.
- Zhang, M., Li, G., Liu, B., Liu, J., Wang, L., Wang, D., 2020. Effects of herbivore assemblage on the spatial heterogeneity of soil nitrogen in eastern Eurasian steppe. *J. Appl. Ecol.* 57 (8), 1551–1560.