ECOSYSTEMS

The Response of Soil Nematode Community to Nitrogen, Water, and Grazing History in the Inner Mongolian Steppe, China

Wei-bin Ruan,[1] Yuan Sang,[1] Qing Chen,[2] Xiang Zhu,[3] Shan Lin,[2] and Yu-bao Gao[1]*

[1]College of Life Sciences, Nankai University, Tianjin 300071, People's Republic of China; [2]College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China; [3]School of Mathematical Sciences, Nankai University, Tianjin 300071, People's Republic of China

ABSTRACT

Nutrient nitrogen and water availability are co-limiting factors for grassland productivity in the Inner Mongolian steppe. The objective of this study was to evaluate the interactive effects of water and nitrogen (N) addition on soil abiotic factors and soil nematode community composition. A 3-year experiment with addition of water (with and without irrigation simulating wet year precipitation) and nitrogen (0, 25, and 50 kg N ha^{-1}) was conducted at two sites in Inner Mongolia with histories of heavy grazing (HG) and moderate grazing (MG). The results showed that HG had several lower nematode ecological indicators; namely, the Simpson index ($P < 0.01$), maturity index (MI, including free-living nematodes), and plant parasite index (PPI, including plant-parasitic nematodes). In addition, HG had a lower proportion of omnivores–predators and higher proportion of bacterivores ($P < 0.01$), with reduced soil moisture, total nitrogen and total carbon (C), and increased C/N ratio. A relatively low addition of N did not influence soil total carbon and total nitrogen and had only a slight effect on the composition and structure of the soil nematode community. Water addition increased the proportion of plant parasites, reduced the proportion of bacterivores and omnivores–predators and increased the soil total carbon and total nitrogen. Redundancy analysis revealed that grazing history alone explained more variation in nematode taxon composition (17.4%, $P < 0.01$) and PPI (35.6%, $P < 0.01$) and MI (17.5%, $P < 0.01$) and Simpson index (10.5%, $P < 0.01$). Water addition explained more variation in the nematode feeding group (24.4%, $P < 0.01$), total nematodes (7.9%, $P < 0.01$) and PPI (78.7%, $P < 0.01$). Nitrogen addition explained variation in the nematode taxon composition (6.2%, $P < 0.01$). These results suggested that HG caused soil degradation, and water addition facilitated plant parasites and enhanced soil properties, whereas application of small amounts of nitrogen to N-limited semiarid grasslands did not induce clear changes in this system in the short term.

Key words: soil abiotic properties; redundancy analysis; maturity index; plant parasite index; degradation.

INTRODUCTION

Anthropogenic-driven global change is affecting natural ecosystem structure and function. Nitrogen is the element that most commonly limits biological productivity in grasslands, and applying N could be a useful approach to restoring degraded grasslands via enhancing primary production and ground cover (Bai and others 2010). However, low levels of N addition reduce plant species richness (10 kg N ha^{-1} y^{-1}) (Clark and Tilman 2008) and cause the loss of plant species in mature grasslands (<17.5 kg N ha^{-1} y^{-1}) (Bai and others 2010). Community level plant species loss associated with low N addition is mainly due to the loss of perennial grasses and forbs and increased aboveground competition (Pan and others 2011).

Water limitation of plant primary production was reported in semiarid steppe (Bai and others 2004). Aboveground net primary productivity is positively related to annual precipitation in grasslands (Bai and others 2008). The strength of N limitation in degraded grasslands depends strongly on water availability (Chen and others 2011). Soil moisture profoundly impacts microbial-mediated processes of the N cycle (Paul and others 2003; Wang and others 2006) because water availability controls soil microbial activity.

Understanding the relevance of water and N availability might be helpful to understanding the response mechanisms of grassland ecosystems. Soil moisture can play a crucial role in N leaching and N uptake in plants and in turn, enhanced plant growth due to N addition could affect soil moisture and water availability by photosynthesis and transpiration, as well as evaporation from the soil surface. In arid and semiarid grassland ecosystems, where plant growth is usually co-limited by nutrient and water availability (Xia and others 2009), changes in N and water availability can have a profound influence on aboveground communities. Most studies focus on the aboveground plant community, plant primary productivity (Li and others 2011), shifts in plant species composition (Chen and others 2011), and tradeoffs between water use efficiency and N use efficiency (Gong and others 2011). However, much less attention has been paid to changes in belowground communities under conditions of N and water addition.

Soil biota constitutes a significant component of terrestrial ecosystems by governing essential ecosystem functions (De Deyn and others 2003; Wardle and others 2004). Nematodes are dominant soil biota in all terrestrial ecosystems with various trophic groups. Nematodes have profound effects on plant community structure and the structure and function of the soil ecosystem (Yeates and others 1993; Ferris 2010). Grasslands support large, herbivore-dominated, nematode populations in the soil (Porazinska and others 2003), and plant parasites are major controllers of plant production in these ecosystems. Root herbivory by nematodes can increase the allocation of photoassimilated carbon to roots, leading to increased root exudation and microbial activity in the rhizosphere. Plant-parasitic nematodes have been shown to be responsible for the shifting mosaic of the two dominant plant species in grasslands (Olff and others 2000). Bacteriovous and fungivorous nematodes are responsible for stimulating microbial activity and nitrogen mineralization, and alleviating N-deficiency (Ferris and others 2004). It has been estimated that approximately 40% of nutrient mineralization in certain ecosystems is due to nematodes and other soil fauna (De Ruiter and others 1993). In addition, the soil food web has been shown to be regulated from the top-down by omnivores and predators (Khan and Kim 2005). Moreover, the abundance of nematodes is also presumed to mirror that of other important consumers (Ferris and Matute 2003). Consequently, the nematode community may serve as a proxy for soil food web structure and composition (Liang and others 2009; Kardol and others 2010). The soil nematode community composition was found to be affected by grazing via changes in the plant community composition (Veen and others 2010). The soil nematode community is closely related to the plant community, especially plant species identity (De Deyn and others 2004; Viketoft and others 2009). Therefore, it is assumed that soil nematodes might respond to changes in the plant community induced by N addition (Pan and others 2011). In addition, the soil nematode community has been shown to be substantially affected by soil moisture because nematodes cannot move without water (Landesman and others 2011). Owing to the above responses of soil nematodes to N, water and grazing, the soil nematode community has the potential for use in evaluation of the effects of these factors on soil biota with potential consequences for ecosystem properties and processes.

We tested the effects of N and water addition in temperate steppe, which is a typical vegetation type in the central-east part of the Eurasian continent. Seriously degraded grassland was observed due to overgrazing and long-term drought (Nan 2005). Nitrogen is an important limiting resource in Inner Mongolian steppe and N fertilization has been used as a management tool for enhancing primary production and ground cover (Bai and others 2010).

Water is another limiting factor in this region, and plant species richness, plant productivity and species composition are sensitive to N enrichment and increased precipitation (Chen and others 2011). Moreover, interactions among multiple factors can affect ecosystems in ways that are not easily predicted from measuring a single factor (Kardol and others 2010). To examine the effects of N, water addition and grazing intensity on the functioning of a grassland ecosystem, we set up a water and N addition experiment in a grassland moderately grazed with 2 sheep ha^{-1} (MG) and in a grassland heavily grazed for more than 30 years with at least 4 sheep ha^{-1} (HG). The objectives of this study were to address: (1) whether the soil nematode communities were sensitive to changes in N and water addition; (2) whether grazing intensity history has resulted in different soil nematode community and soil characteristics; and (3) whether soil nematode communities respond differently to N addition, water addition and grazing history, and could thus act as indicators of changes caused by environmental factors and anthropogenic disturbance.

MATERIALS AND METHODS

Study Area

The study was conducted at the Inner Mongolian Grassland Ecosystem Research Station (IMGERS), located in the Xilin River Basin (43°26′–44°9′N, 115°2′–117°2′E), Inner Mongolia, China. The area has a semiarid steppe climate, the winter is cold and dry and the summer is warm and humid. The average annual temperature is 2.0°C. The average annual precipitation is 343 mm, more than 80% of which falls between May and September. The soil type is calcic chernozems.

Experimental Design

Two sites (3 km distance) in the same region, a representative moderate grazing (MG) site with 1–3 sheep ha^{-1} (MG) and a representative heavy grazing (HG) site with 4 sheep ha^{-1} (HG), were simultaneously fenced in 2005. There were substantial differences in the plant function groups between the two sites, with perennial bunchgrasses, perennial rhizome grasses, perennial forbs in MG, and perennial bunchgrasses, perennial forbs, shrubs and semi-shrubs, perennial annuals and a very small proportion of rhizome grasses (2.4%) in HG. The experimental design has been reported previously (Gong and others 2011). Briefly, the experiment was designed as a two-factorial split-plot with four replicates. The main plots were

natural precipitation (W0) and simulated wet year precipitation (W1). The subplots consisted of three N fertilizer (urea) application rates: 0 (N0), 25 (N25), and 50 kg N ha^{-1} (N50). Each subplot measured 5 × 8 m and was positioned at least 1 m from any fence to avoid edge effects. The watering treatments and subplots were separated by 3 and 0.8 m walkways, respectively. The experiment was a total of 6 treatments with 4 replicates, and each sample had a total of 24 plots. The same experimental design was established at two sites with contrasting grazing history. The area of each site was 0.2 ha. The experiment was initiated in 2005 and two sites were fenced to avoid animal grazing. We carried out manual cutting 3 cm above the ground level at the end of September every year after 2005.

N Application and Irrigation

To apply fertilizer evenly, we mixed granular urea (1.5 mm diameter) with air-dried and fine-sieved (<2 mm) soil particles at a ratio of 1:10 and spread this mixture by hand on May 15th each year.

The amount of water in the simulated wet year precipitation treatment was 431 mm to simulate wet year precipitation based on long-term rainfall data (1982–2003) obtained from the meteorological station at IMGERS (Chen and others 2011). Additional irrigation was applied to simulate the amount and distribution of the long-term wet year precipitation from May to September. The fields were irrigated at 10-day intervals with the amount of the average wet year 10-day precipitation during the same period using a pump-line injector system when wind was at a minimum (often at sunset). If the actual rainfall in a given 10-day interval during the experimental period was greater than the historical wet year precipitation in the same period, no additional irrigation for this 10-day interval and the amount of irrigation in the following 10-day interval was adjusted based on the actual precipitation in the previous 10 days.

Soil Sampling

Soil samples were extracted on August 5th 2008, August 16th 2009, and August 17th 2010. In each plot, three cores (diameter 3.5 cm) were randomly taken with a soil auger to a depth of 25 cm and mixed roughly to get a composite sample of about 400 g. Soil samples were placed into individual plastic bags, transported to the laboratory in Nankai University and stored at 4°C until use.

A subsample (~150 g) of the soil was used to analyze soil total nitrogen (TN), soil total carbon

(TC), soil pH and moisture content. Another subsample (~150 g) of soil was used to analyze the soil nematode community.

Soil Physicochemical Measurement

Soil pH was measured in a 1:2 soil–distilled H_2O suspension using a glass electrode (Sartorius PB-10). Soil TC and TN were measured using the method of Bao (2000). The soil moisture content was measured by oven-drying a subsample of 10 g of soil until the weight remained constant.

Soil Nematode Community Analyses

Nematodes were extracted from 150 g (fresh weight) of soil from each sample using sugar flotation and centrifugation. Nematode populations are expressed as number of nematodes per 100 g of dry soil. At least 150 nematodes from each sample were identified to genus level using an inverted compound microscope. The nematodes were categorized as follows: bacterivores (Ba), fungivores (Fu), plant parasites (PP), or omnivores + predators (OP) based on known feeding habitats or esophageal morphology (Yeates and others 1993). The nematode communities were analyzed using the following indices:

(1) Absolute abundance of individuals per 100 g dry soil (total nematodes, Tnem); (2) Shannon–Wiener diversity index $H = (-\sum Pi[lnPi])$, where Pi is the proportion of each taxon in the total population; (3) Simpson index: $D = 1/\Sigma pi^2$ (Yeates and Bongers 1999); (4) maturity index: $MI = \Sigma c(i) \times pi$, where $c(i)$ is the $c–p$ value of taxon i according to their r and K characteristics following Bongers (1990), pi is the frequency of taxon i in a sample. Maturity index (MI) indicates disturbance based on nematode life strategies (Bongers 1990). Nematodes were assigned to colonizer–persister (cp) groups based on Bongers (1990). The cp scale classifies nematodes into five groups from microbial feeders with short life cycles and high fecundity (cp 1 and 2) to omnivores–predators with long life cycles and greater sensitivity to perturbation (cp 3–5); (5) Plant parasite index: $PPI = \Sigma c(i) \times pi$, which was determined in a similar manner for plant parasitic genera (Bongers 1990); (6) PPI/MI = PPI/MI; (7) The ratio of fungivores (F) to bacterivores (B) was calculated, as F/B (Freckman and Ettema 1993).

Statistical Analysis

Repeated measurements were tested for direct and interactive effects of year as within-subjects effect, and grazing history, water and N addition as between-subject effects, using four-way repeated-measures, split-plot analysis of variance (ANOVA) via PROC MIXED, SAS version 8.0 (SAS Institute, Cary, NC, USA). In addition, all interactions among between-subjects effects were tested. Data were well pre-transformed to meet assumptions of normality and homogeneity of variance.

For soil nematode community, the effect of N, water addition and grazing history on total nematodes, Shannon diversity, PPI, MI, nematode feeding group and nematode taxon were analyzed by redundancy analysis (RDA), using N, water addition, grazing history and their interactions as explanatory variables. Significance of effects was tested using Monte Carlo permutation tests (499 permutations). Marginal effects (that is, the independent effect of each variable) were tested by auto-selection of each individual variable. The RDA analyses were performed using CANOCO, version 4.5 (ter Braak and Šmilauer 2002). Also, correlation analysis was performed to test for relationships between soil properties and the indexes of soil nematode community.

RESULTS

Soil Properties

Effects of N, water addition, grazing history, and year on soil properties are shown in Table 1. There was no significant change in soil moisture, soil pH, TN, TC, and C/N ratio among 0, 25, and 50 N ha^{-1} treatments. The addition of water resulted in soil moisture ($F_{1,108} = 94.73$, $P < 0.001$), soil pH ($F_{1,108} = 138.05$, $P < 0.01$), TN ($F_{1,108} = 28.72$, $P < 0.01$), and C/N ratio ($F_{1,108} = 13.83$, $P < 0.01$) significantly higher than the control sites. When compared with heavily grazed sites, moderately grazed sites had higher soil moisture ($F_{1,108} = 76.08$, $P < 0.01$), soil TN ($F_{1,108} = 102.20$, $P < 0.01$), and TC ($F_{1,108} = 25.23$, $P < 0.01$), but lower pH ($F_{1,108} = 45.21$, $P < 0.01$). The soil moisture, pH, TN, TC, and C/N ratio showed significant variation among the 3 years.

Water addition interacted with grazing history to affect soil TN ($F_{1,108} = 25.27$, $P < 0.001$) and TC ($F_{1,108} = 8.56$, $P < 0.01$). Water addition interacted with year to affect soil moisture ($F_{1,108} = 4.21$, $P = 0.017$) and soil pH ($F_{1,108} = 36.8$, $P < 0.01$). The interaction between water addition and grazing history was varied among years with regard to soil moisture ($F_{1,108} = 4.52$, $P = 0.013$), soil pH ($F_{1,108} = 4.05$, $P = 0.05$), and soil TC ($F_{1,108} = 8.94$, $P < 0.01$).

Table 1. Overview of Main Effects of Nitrogen, Water and Grazing History on Soil Properties Based on the Results of Repeated Measurements ANOVA (mean ± SE)

Treatment	Moisture (%)	pH	Soil TN (%)	Soil TC (%)	C/N
N					
N0	4.54 ± 0.30a	7.28 ± 0.07a	0.11 ± 0.003a	1.76 ± 0.07a	16.06 ± 0.36a
N25	4.65 ± 0.34a	7.20 ± 0.06a	0.11 ± 0.003a	1.75 ± 0.07a	15.97 ± 0.34a
N50	4.80 ± 0.38a	7.22 ± 0.06a	0.11 ± 0.003a	1.83 ± 0.08a	16.07 ± 0.39a
$F_{2,108}$	0.70	1.39	0.89	0.47	0.05
P	NS	NS	NS	NS	NS
W					
W0	3.80 ± 0.25b	7.02 ± 0.03b	0.10 ± 0.003b	1.60 ± 0.05b	15.40 ± 0.27b
W1	5.52 ± 0.27a	7.45 ± 0.06a	0.12 ± 0.002a	1.97 ± 0.06a	16.67 ± 0.30a
$F_{1,108}$	94.73	138.05	28.72	29.73	13.83
P	<0.01	<0.01	<0.01	<0.01	<0.01
G					
MG	5.43 ± 0.26a	7.11 ± 0.05b	0.12 ± 0.002a	1.95 ± 0.05a	15.82 ± 0.25
HG	3.89 ± 0.27b	7.36 ± 0.05a	0.10 ± 0.002b	1.61 ± 0.07b	16.25 ± 0.33
$F_{1,108}$	76.08	45.21	102.20	25.53	1.53
P	<0.01	<0.01	<0.01	<0.01	NS
Y					
2008	7.11 ± 0.26a	6.96 ± 0.04c	0.12 ± 0.003a	2.03 ± 0.09a	17.27 ± 0.35a
2009	3.45 ± 0.25b	7.17 ± 0.04b	0.10 ± 0.003b	1.66 ± 0.06b	16.14 ± 0.29b
2010	3.44 ± 0.18b	7.57 ± 0.07a	0.11 ± 0.003a	1.66 ± 0.05b	14.70 ± 0.34c
$F_{2,108}$	120.76	93.16	15.13	9.40	17.60
P	<0.01	<0.01	<0.01	<0.01	<0.01

N nitrogen (N0, N25, N50); W water (W0 no additional water supply; W1: simulated wet year precipitation); G grazing history (HG heavily grazed site; MG moderately grazed site); Y year (from 2008 to 2010); TN soil total nitrogen; TC soil total organic carbon. Means within each factor with the same letter are not significantly different at the 5% level.

Soil Nematode Community

Nitrogen addition did not affect the indexes of the structure and composition of soil nematode community, with the exception of bacterivores ($F_{1,108}$ = 3.83, P = 0.02) (Table 2). For the main effect of water addition, total nematodes ($F_{1,108}$ = 25.26, P < 0.01) were significantly increased. The Shannon–Wiener diversity index (H) ($F_{1,108}$ = 3.39, P < 0.07) and MI ($F_{1,108}$ = 3.04, P < 0.08) tended to increase, but PPI/MI ($F_{1,108}$ = 3.63, P < 0.06) tended to decrease due to the addition of water. In the heavily grazed plots, there were significant decreases in the Simpson index ($F_{1,108}$ = 20.5, P < 0.01), MI ($F_{1,108}$ = 36.52, P < 0.01), and F/B ($F_{1,108}$ = 7.99, P < 0.01), but an increase in Tnem ($F_{1,108}$ = 20.66, P < 0.01) and PPI ($F_{1,108}$ = 77.49, P < 0.01), and PPI/MI ($F_{1,108}$ = 66.32, P < 0.01). In addition, the Shannon diversity index tended to decrease in the heavily grazed sites ($F_{1,108}$ = 3.73, P < 0.06).

There was a significant change in the proportion of bacterivores under the N addition treatment (Table 2). Water addition strongly increased the proportion of plant parasites ($F_{1,108}$ = 77.40, P < 0.01), but decreased the proportion of bacterivores ($F_{1,108}$ = 49.39, P < 0.01), and omnivores–predators ($F_{1,108}$ = 4.61, P < 0.03). Higher proportions of bacterivores ($F_{1,108}$ = 18.87 P < 0.01) and lower omnivores–predators ($F_{1,108}$ = 38.10, P < 0.01) were observed in heavily grazed sites than moderately grazed sites.

Grazing history and water interaction significantly affected PPI ($F_{1,108}$ = 8.27, P < 0.01) and PPI/MI ($F_{1,108}$ = 4.86, P = 0.03) (Figure 1). Water addition significantly increased MI in moderately grazed plots, but not in heavily grazed plots (Figure 1A). Water addition significantly decreased the value of PPI/MI in moderately grazed plots, but not in heavily grazed plots (Figure 1B). For all tested indices, N addition showed no significant interaction effect with grazing history or water addition, with the exception of the interaction between N and water addition in the Shannon diversity index (F = 3.02, P = 0.05).

Soil nematode trophic group composition varied among treatments and soil properties (Figure 2). For example, Tnem, bacterivores, PPI, and PPI/MI were associated with HG. Conversely, MI and omnivores–predators were associated with MG sites. Bacterivores and omnivores–predators were

Table 2. Overview of the Main Effects of Nitrogen, Water and Grazing History on the Soil Nematode Community Based on the Results of Repeated Measurements ANOVA (mean ± SE)

	Tnem	H	Simpson	MI	PPI	PPI/MI	F/B	Ba%	Fu%	PP%	OP%
N											
N0	974.6 ± 88.3a	2.49 ± 0.04a	9.50 ± 0.44a	3.14 ± 0.09a	2.86 ± 0.03a	0.95 ± 0.021a	0.15 ± 0.03a	25.1 ± 1.96b	2.8 ± 0.37a	58.6 ± 2.03a	13.6 ± 1.43a
N25	1097.2 ± 97.5a	2.49 ± 0.03a	9.14 ± 0.38a	2.96 ± 0.08b	2.87 ± 0.02a	1.00 ± 0.03a	0.12 ± 0.03a	30.8 ± 2.18a	2.6 0.28a	54.0 ± 2.28a	12.6 ± 1.35a
N50	1098.6 ± 88.9a	2.52 ± 0.04a	9.35 ± 0.42a	2.92 ± 0.06b	2.85 ± 0.06b	1.00 ± 0.02a	0.11 ± 0.02a	29.4 ± 1.99ab	2.4 ± 0.28a	56.7 2.27a	11.5 ± 1.01a
$F_{2,108}$	1.67	0.21	0.26	3.17	0.22	2.08	1.19	3.83	0.46	2.24	1.18
P	NS	NS	NS	**0.045**	NS	NS	NS	**0.02**	NS	NS	NS
W											
W0	896.7 ± 72.1b	2.47 ± 0.03b	9.18 ± 0.30a	2.94 ± 0.07a	2.87 ± 0.02a	1.01 ± 0.02a	0.12 ± 0.02a	34.7 ± 1.75a	2.9 ± 0.30a	48.7 ± 1.70b	13.7 ± 1.11a
W1	1216.8 ± 72.8a	2.53 ± 0.03a	9.48 ± 0.37a	3.08 ± 0.06a	2.85 ± 0.01a	0.96 ± 0.02a	0.14 ± 0.017a	22.2 ± 1.24b	2.3 ± 0.21a	64.1 ± 1.39a	11.4 ± 0.95b
$F_{1,108}$	25.26	3.39	0.55	3.04	0.41	3.63	0.81	49.39	2.28	77.40	4.61
P	**<0.01**	0.07	NS	0.08	NS	0.060	NS	**<0.01**	NS	**<0.01**	**0.03**
G											
MG	912.1 ± 70.0b	2.54 ± 0.03 a	10.26 ± 0.34a	3.24 ± 0.07a	2.77 ± 0.02b	0.88 ± 0.02b	0.16 ± 0.019a	24.6 ± 1.58a	2.8 ± 0.29a	56.7 ± 1.84a	15.9 ± 1.19a
HG	1201.5 ± 75.8a	2.46 ± 0.03 a	8.41 ± 0.30b	2.78 ± 0.05b	2.95 ± 0.01a	1.09 ± 0.02a	0.097 ± 0.012b	32.3 ± 1.68a	2.4 ± 0.22a	56.1 ± 1.76a	9.17 ± 0.66b
$F_{1,108}$	20.66	3.73	20.50	36.52	77.49	66.32	7.99	18.87	1.31	0.10	38.10
P	**<0.01**	0.06	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	NS	NS	**<0.01**
Y											
2008	849.8 ± 65.6b	2.48 ± 0.04b	9.13 ± 0.46b	2.91 ± 0.06a	2.84 ± 0.02a	1.00 ± 0.02a	0.14 ± 0.021	25.5 1.59b	2.7 ± 0.27a	62.6 ± 1.78a	9.3 ± 0.84b
2009	1659.0 ± 68.0a	2.62 ± 0.03a	10.58 ± 0.38a	3.09 ± 0.09a	2.84 ± 0.03a	0.96 ± 0.03a	0.11 ± 0.017	33.6 ± 2.05a	2.8 ± 0.34a	46.1 ± 1.86b	17.5 ± 1.62a
2010	661.5 ± 67.5c	2.41 ± 0.03b	8.28 ± 0.33b	3.03 ± 0.09a	2.90 ± 0.09a	1.00 ± 0.03a	0.14 ± 0.023	26.2 ± 2.32b	2.4 ± 0.33a	60.5 ± 2.15a	10.9 ± 0.89b
$F_{2,108}$	92.34	11.43	13.63	2.32	2.79	1.19	0.90	8.55	0.42	35.28	16.52
P	**<0.01**	**<0.01**	**<0.01**	NS	NS	NS	NS	**<0.01**	NS	**<0.01**	**<0.01**

Tnem total nematodes, H Shannon–Wiener diversity index, Simpson Simpson diversity index, MI maturity index, PPI plant parasitic index, PPI/MI the ratio of PPI to MI, Ba bacterivores, Fu fungivores, PP plant parasites, OP omnivores–predators. Means within each factor with the same letter are not significantly different at the 5% level.
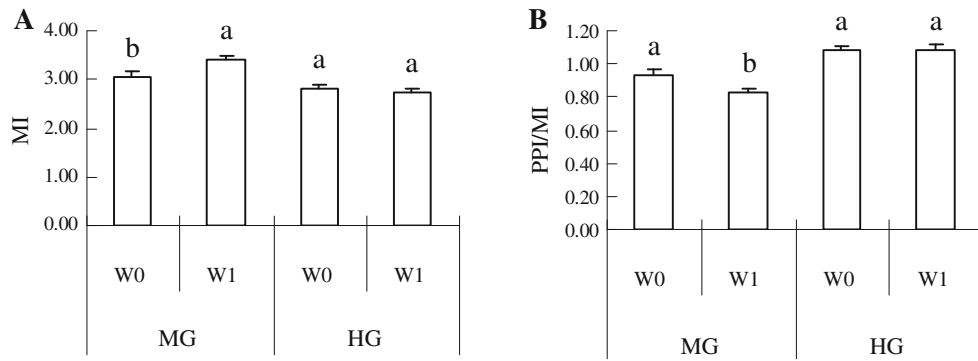
Figure 1. Soil nematode community indexes, **A** maturity index (MI), and **B** MI/PPI as influenced by water and grazing history. *Bars* labeled with the same lowercase letters were not significantly different ($P > 0.05$) between watering treatments within each grazing history. *HG* heavy grazing sites, *MG* moderate grazing sites, *W0* natural precipitation, *W1* simulated wet year precipitation.

associated with natural precipitation, whereas plant parasites were associated with simulated wet year precipitation.

For nematode taxon composition (Figure 3), plant parasites, that is, *Rotylenchus*, *Helicotylenchus*, *Tylenchorhynchus*, and *Criconema*, responded most strongly to the simulated wet year precipitation, suggesting they are closely associated with moisture. In contrast, bacterivores, that is, *Chiloplacus*, *Cervidellus*, *Acrobeloides*, *Alaimus*, and *Acrobeles*, were associated with natural precipitation.

The full RDA model, including grazing history, water and N addition as explanatory factors, explained 24.4% of the variation for nematode feeding group (Table 3). Of them, water (W) and grazing history (G) explained 19.6 and 5.1% of the variance, respectively. Nitrogen addition (N+) did not affect soil nematode feeding group composition. However, the interactions between N+ and G ($F = 37.67$, $P < 0.01$), W ($F = 38.03$, $P < 0.002$) and G × W (F = 39.17, $P < 0.01$) explained a significant amount of the variation in nematode
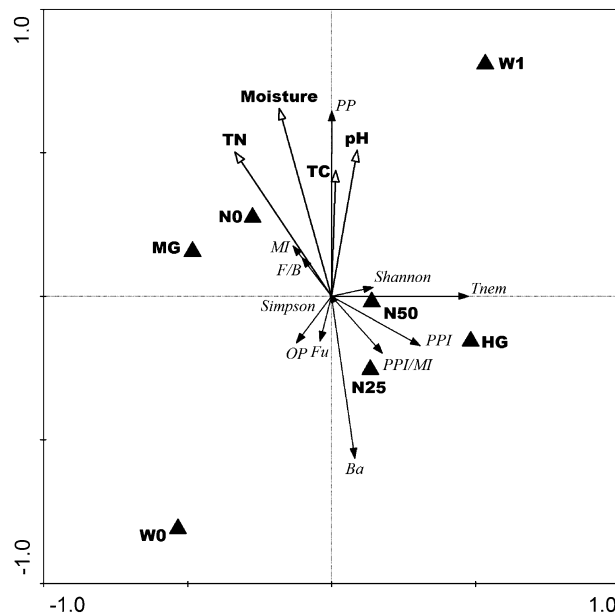

Figure 2. Redundancy analysis bi-plot of indexes of soil nematode community and environmental variables. Environmental variables included treatments and soil properties. Treatments included grazing history (*HG* heavy grazing sites, *MG* moderate grazing sites), nitrogen addition (N0, 0 kg/ha; N25, 25 kg/ha, N50, 50 kg/ha), and water (*W0* natural precipitation; *W1* simulated wet year precipitation). Soil properties included soil moisture, pH, TN, and TC. Eigenvalues (0.225 for horizontal axis, 0.001 for vertical axis) along the axes indicate the amount of variability explained in the nematode genus. *MI* maturity index, *PPI* plant parasites index, *Tnem* total nematodes, *Ba* bacterivores, *Fu* fungivores, *PP* plant parasites, and *OP* omnivores–predators, *F/B* Fu/Ba.
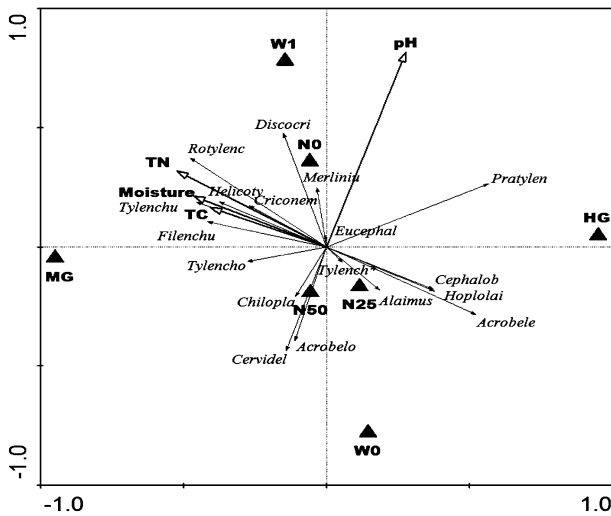
Figure 3. Redundancy analysis bi-plot of nematode taxon composition and environmental variables. Environmental variables included treatments and soil properties. Treatments included grazing history (*HG* heavy grazing sites, *MG* moderate grazing sites), nitrogen addition (N0, 0 kg/ha; N25, 25 kg/ha, N50, 50 kg/ha), and water (*W0* natural precipitation; *W1* simulated wet year precipitation). Soil properties included soil moisture, pH, TN, and TC. Eigenvalues (0.180 for horizontal axis, 0.062 for vertical axis) along the axes indicate the amount of variability explained in the nematode genus. Plant parasites: Tlyencho (*Tylenchorhynchus*), Pratylen (*Pratylenchus*), Merliniu (*Merlinius*), Discocri (*Discocriconemella*), Hoplolai (*Hoplolaimus*), Rotylenc (*Rotylenchus*), Criconem (*Criconemella*), Helicoty (*Helicotylenchus*), Tylenchu (*Tylenchus*), Filenchu (*Filenchus*). Bacterivores: Chilopla (*Chiloplacus*), Cervidel (*Cervidellus*), Acrobelo (Acrobeloides), Alaimus, Acrobele (*Acrobeles*), Eucephal (*Eucephalobus*), Cephalob (*Cephalobus*). Fungivores: Tylench*(*Tylencholaimus*).

feeding group composition. For nematode taxon, PPI, MI, Simpson index and total nematodes, the same full RDA model explained the variation by 17.6, 35.6, 17.5, 12.2, and 17.6%, respectively (Table 3). For nematode taxon composition, all explanatory variables explained a significant amount of variation, especially G (17.4%), G × W (19.8%), G × N+ (17.5%) and G × W × N+ (18.1%). Variations in the Simpson index, PPI and MI were best explained by G alone and G in combination with W and N+, and G × N+. In addition, N+ ($F = 3.93$, $P < 0.05$) explained 2.7% of the variation in MI. With the exception of N+ alone, N+ all variables explained the significant variation in total nematodes.

The Correlation Between Soil Properties and Soil Nematode Community

Soil moisture, pH, TC, and TN were negatively correlated with the percentage of bacterivores, but

Table 3. Redundancy Analyses of Treatment Effects and Their Interactions on the Soil Nematode Community Composition in 2008–2010

E.V.	Nematode feeding group			Nematode taxon			PPI			MI			Simpson			Tnem		
	E.V.(%)	F	P	E.V.(%)	F	P	E.V.(%)	F	P	E.V.(%)	F	P	E.V.(%)	F	P	E.V.(%)	F	P
G	5.1	**7.64**	**<0.01**	17.4	**29.64**	**<0.01**	35.6	**78.47**	**<0.01**	17.5	**30.0**	**<0.01**	10.5	**16.61**	**<0.01**	6.7	**10.20**	**<0.01**
W	19.6	**34.33**	**<0.01**	7.3	**11.14**	**<0.01**	0.2	0.27	0.64	1.5	2.09	0.14	0.3	0.40	0.50	7.9	**12.12**	**<0.01**
N	0.9	1.30	0.52	6.2	**9.38**	**<0.01**	0	0.01	0.93	2.7	**3.93**	**0.05**	0	0.06	0.84	2.1	3.06	0.22
G × W	22.1	**39.22**	**<0.01**	19.8	**34.30**	**<0.01**	12.7	**20.73**	**<0.01**	6.3	**9.48**	**<0.01**	3.5	**5.2**	**0.04**	13.7	**21.98**	**<0.01**
G × N	21.4	**37.67**	**<0.01**	17.5	**29.45**	**<0.01**	13	**21.21**	**<0.01**	13.8	**22.72**	**<0.01**	5	**7.44**	**0.01**	7.6	**11.38**	**0.024**
W × N	21.6	**38.03**	**<0.01**	7.4	**11.16**	**<0.01**	0	0.03	0.88	0.3	0.37	0.53	0	0.046	0.83	9.1	**13.91**	**0.012**
G × W × N	22.2	**39.17**	**<0.01**	18.1	**30.32**	**<0.01**	7.2	**10.98**	**<0.01**	8.0	**12.43**	**<0.01**	4	**5.87**	**0.07**	15.3	**24.80**	**<0.01**
Full Model	24.4	**41.41**	**<0.01**	17.6	**29.67**	**<0.01**	35.6	**78.47**	**<0.01**	17.5	**30.2**	**<0.01**	12.2	19.39	**<0.01**	17.6	27.39	0.04

Notes: Each factor was tested as an interactive term with year, reflecting the repeated measurements. Marginal effects (that is, effects when a term is used as the only explanatory variable) and results of the full model including all explanatory variables are presented. E.V. (%) is the percentage of variation explained. P values are based on Monte Carlo permutation tests.
PPI plant parasitic index, MI maturity index, Simpson Simpson index, Tnem total nematodes.
Bold values indicate significant values

positively correlated with the percentage of plant parasites. PPI/MI was negatively correlated with soil moisture and soil TC. Soil moisture was positively correlated with soil TC ($P < 0.001$) and soil TN ($P < 0.001$), and C/N ratio ($P < 0.001$), but negatively with pH ($P = 0.009$) (Table 4). Soil TN was positively correlated with TC ($P < 0.001$) (Table 4).

DISCUSSION

The sites with HG history had lower Simpson diversity, MI, and omnivores–predators, and a higher PPI, which indicated the negative effect of HG on the soil nematode community in Inner Mongolia steppe. The results of this study suggested that HG has caused the soil degradation and poor soil properties associated with a disturbed soil food web.

Our results indicated that relatively low N addition, in a short time frame, did not distinctly affect the composition and structure of the soil nematode community and soil properties (soil moisture, pH, TC, TN and C/N ratio). In contrast, water addition (simulated wet year precipitation) increased the proportion of plant parasites and increased the soil TC, TN and C/N ratio, as well as soil moisture. Nematode feeding group, nematode taxon and total nematodes (Tnem) were heavily impacted by grazing history and water, but not by nitrogen over the course of the study. In addition, grazing history substantially influenced PPI and MI. In this study, N addition did not cause significant changes in soil nematode community, with the exception of nematode taxon and MI. The results indicated that grazing history had a clearly negative effect on soil nematode community, followed by water addition, and then N addition.

Discrepancy of Soil Nematode Community and Soil Abiotic Properties Between Sites with Moderate and HG History

In this study, the representative sites subjected to MG and HG over the past 30 years were selected to study the effects of N and water addition on the restoration of degraded steppes. During the course of treatments, the difference in grazing intensity between the two sites became disconnected temporally. In addition, substantial differences in the soil nematode community and soil abiotic properties were observed between MG and HG over the course of the study. The reduced Shannon–Wiener diversity index ($P = 0.06$), Simpson index ($P < 0.01$), and MI ($P < 0.01$) and the percentage of omnivores–predatory ($P < 0.01$) in heavily grazed sites collectively indicated that the reduced diversity and altered composition of the soil nematode community was due to HG in the past (Table 2). A positive relationship between plant and nematode diversity indices was detected after a 13-year experiment containing ungrazed and grazed treatments, and plant community composition was the main determinant of the nematode community composition (Veen and others 2010). Different plant functional groups and species were also observed between the MG and HG sites (Steffens and others 2008; Chen 2011). Therefore, in this study, the differences in the plant functional groups might have partially contributed to the changes in the soil nematode community.

The reduced diversity indicated that HG had negative effects because diversity is thought to be a prerequisite for the maintenance of stability, resistance, and resilience of ecosystem properties (Wardle and others 2004). In addition, the reduced MI in heavily grazed sites also indicated the negative effects of HG on the soil nematode community, as the MI index is an ecological measure of disturbance (Bongers 1990). Similarly, the MI for the nematode community in a floodplain along a river was lower in the grazed plots (Veen and others 2010). To some extent, low MI indicates low stability of a soil ecosystem in HG sites due to long-term overgrazing.

Predatory and omnivorous nematodes are in higher colonizer–persister groups and are more sensitive to soil perturbation. Bacterial feeders were inhibited whereas predators and omnivores were stimulated by intermittent fallow, which is characterized by less soil perturbation than tillage (Sánchez-Moreno and others 2006). In this study, the lower MI and lower percentage of omnivores–predators in heavily grazed sites in comparison with MG indicated more perturbation in the former sites. In contrast, omnivore numbers were highest in the extensive and semi-intensive grazing treatments in other studies (Mills and Adl 2011). The artificial vegetation in Mills and Adl's study and natural vegetation in the present study may partially explain the differences between the two studies.

In this study, sites with a history of HG had significantly higher percentages of bacterivores than MG. The increase in bacterivores in heavily grazed sites indicated that the manner of decomposition changed toward bacterial-based energy channels. Similarly, Bardgett and others (2001) reported that soil microbial communities of heavily grazed sites are dominated by bacterial-based energy channels of decomposition. Bacterivores are closely related to N transformation (Ferris and others 1998). It was

Table 4. Pearson's Correlation Analysis of the Relationship Between Soil Characteristics and Soil Nematode Community in Plots ($n = 144$)

	Soil moisture	pH	Soil TN	Soil TC	C/N
Soil moisture	1.000				
pH	−0.216**	1.000			
Soil TN	0.552***	0.008	1.000		
Soil TC	0.634***	−0.094	0.822***	1.000	
C/N	0.426***	−0.151	0.251**	0.749***	1
Tnem	−0.087	0.042	−0.159	0.007	0.172*
H	0.025	−0.103	−0.019	−0.030	−0.043
Simpson	0.084	−0.201*	0.115	0.065	−0.007
MI	0.100	0.044	0.155	0.087	−0.029
PPI	−0.278***	0.167*	−0.356***	−0.200*	0.078
PPI/MI	−0.191*	0.04	−0.239**	−0.158	0.011
F/B	0.121	0.008	0.142	0.114	0.017
Ba (%)	−0.371***	−0.244**	−0.331***	−0.252**	−0.037
Fu (%)	−0.075	−0.187*	−0.008	−0.031	−0.058
PP (%)	0.428***	0.349***	0.312***	0.278***	0.114
OP (%)	−0.119	−0.161	0.001	−0.064	−0.122

Tnem total nematodes, H Shannon–Wiener diversity index, Simpson Simpson diversity index, MI maturity index, PPI plant parasitic index, PPI/MI the ratio of PPI to MI, Ba bacterivores, Fu fungivores, PP plant parasites, OP omnivores–predators, F/B the ratio of Ba to Fu.
*, **,*** Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

suggested that grazing increases N limitation in grasslands (Pineiro and others 2010). Given that bacterivores are important to the source of available N for plant growth, higher numbers of bacterivores may achieve the high rate of N turnover required for plant growth under conditions of N limitation caused by HG. The total abundance of nematodes in this study was higher in heavily than moderately grazed sites. This is consistent with findings of other studies in the same region, in which soil nematodes increased with increasing grazing intensity (Qi and others 2011). Theoretically, higher soil C/N ratios might facilitate the growth of fungi and thus lead to more fungal feeding nematodes. However, in this study, HG sites with a higher soil C/N ratio had a lower F/B ratio (ratio of fungivores to bacterivores) than MG sites. In addition, top-down regulation of microbial- and plant-feeding nematodes by omnivores–predators has been documented (Khan and Kim 2005). The low number of omnivores–predators might reduce the functioning of top-down regulation resulting in higher numbers of bacterivores and total nematodes in heavily grazed sites when compared with moderately grazed sites.

The soil moisture at heavily grazed sites was lower than at moderately grazed sites in the present study. This coincides with findings of other studies, in which soil moisture was significantly influenced by grazing intensity and thus reduced available

water and plant productivity (Zhao and others 2011). The soil physical and chemical properties differed between MG and HG (Steffens and others 2008), and this study also confirmed that the carbon and nitrogen storage declined in heavily grazed grasslands.

N Addition had Slight Effect on Soil Nematode Community

Low N addition did not affect soil abiotic properties in this study. This may have occurred for two reasons. One possible reason is the increased aboveground net primary productivity and low allocation of primary productivity to the root system. Previous work in this system showed that N addition did increase aboveground net primary production (ANPP) over 5 years (Chen and others 2011). Unchanged belowground biomass and proportionally reduced root allocation could reasonably explain the insignificant changes in soil TC under N addition (Chen and others 2011). Mowed vegetation was removed from the site, which could represent N output from the system. The annual removal of mowed vegetation with greater ANPP under N addition treatment means the corresponding amount of N output from the system might partially contribute to the unchanged soil TC and TN. A second possible reason is that N was applied at rates too low to cause clear changes in soil abiotic

properties. For soil abiotic properties, a study performed in the area near the present experimental sites demonstrated that the contents of soil $NO_3^- - N$ and $NH_4^+ - N$ in treatments with N addition rates lower than 105 kg ha^{-1} were not significantly changed (Bai and others 2010).

Low rate nitrogen addition caused a slight effect on the soil nematode community in the present study. Specifically, a decreased MI ($P = 0.045$) and an increase in Ba% ($P = 0.02$) were observed in response to N addition. Cheng and others (2008) reported that MI was significantly lower under high (223 kg N ha^{-1}) and medium (171 kg N ha^{-1}) N input management than low N (98 kg N ha^{-1}) input management of turf grass.

The identity of single plant species and plant functional groups are the most important factors influencing the nematode community (Viketoft and others 2009). In this study, N addition resulted in a substantial increase in the biomass of annuals/biennials in both HG and MG in the W0 treatments. However, under N and water addition, the biomass of shrubs and semi-shrubs in HG and perennials in MG bunch-grass increased. The variations in functional groups in the plant community associated with N addition might have partially contributed to the changes observed in the soil nematode community. Changes in community composition and losses of plant species would subsequently influence ecosystem functioning (Niu and others 2009). For instance, changes in plant community composition have significant impacts on microbial enzyme activities and soil nematodes (Kardol and others 2010). In addition, grassland ecosystems on low-N soils are sensitive to chronic N inputs even at low rates (for example,10 kg N ha^{-1} y^{-1} for 23 years) (Clark and Tilman 2008).

Considering the N input at the rate of 50 kg N ha^{-1} y^{-1} in this study, fivefold greater than in Clark and Tilman's study, changes in plant community composition might be expected to result in substantial changes in nematode community composition over a longer period of time.

Water Addition Apparently Increased Total Nematodes and Plant Parasites

As expected, soil water content was significantly higher after water addition, which simulated wet year precipitation. We detected high abundances of soil nematodes in natural precipitation plots with an average soil water content of 3.8%. The soil moisture was so low at the sampling date that the soil was not bound together and looked like air-dried soil, which cannot be sampled from the soil profile using a normal soil auger. In addition, nematodes may migrate to a more favorable soil moisture environment, enter anhydrobiosis or remain within plant roots during dry conditions (Freckman and others 1987). Considering the crucial role of the soil nematode community, the presence of high densities of nematodes in soils could be essential once the environmental conditions become optimal for plant growth.

Increased ANPP was associated with water addition (Chen and others 2011), which might partially result in increased numbers of plant parasites, as was found in the present study. Aboveground net primary productivity is positively related to annual precipitation in the studied region (Bai and others 2008). Greater net nitrification and N mineralization rates were observed at the moisture levels of 25 and 35% than 15% (Wang and others 2006), which subsequently causes high ANPP due to more N availability. Due to low allocation to belowground biomass, relatively low amounts of organic material enter the soil as substrate, which might be another reason we detected relatively low numbers of free-living nematodes in the water addition treatment. When compared to MG, there was more variation in the shoot biomass of dominant plant species under natural precipitation treatments in the HG sites, and these values changed annually, whereas the dominant plant species was relatively stable under water addition treatments (Chen 2011). These results indicated that the plant community in HG was still less stable than that in MG over the course of the study. In addition, the insignificant changes in Shannon–Wiener diversity, Simpson index, and MI indicated that the water addition did not exert an apparent effect on the diversity of the soil nematode community, whereas it had a significant effect on trophic groups of the soil nematode community (Table 2). Increased pH was also observed after water addition. Because potential evaporation rates exceed precipitation by 4–5 times in this region, the salt in the added water might contribute to the increased pH due to evaporation. There was very little rainfall in July 2010, so the relatively high amount of water added to simulate wet year precipitation may have caused the greater difference in the soil pH between natural and artificial precipitation treatments in 2010 than in 2008 and 2009.

In this study, the soil nematode community was studied at the genus level. However, the changes in the lowest taxonomic level might be revealed by means of molecular assays utilizing conserved 18S rRNA. In addition, the effects of the N and water addition treatments on soil nematode community

were evaluated only in the short term. N and water addition, especially N addition, might exert greater effects on the soil nematode community and soil abiotic properties with time. Accordingly, continuous research evaluating the effects of length of N and water addition treatments on the soil nematode community and soil abiotic properties should be conducted. Furthermore, studies improving our understanding of the relationship between the soil microbe community and soil nematode community would be advantageous. Understanding long-term soil ecosystem responses and feedbacks to N and water addition could benefit sustainable grazing management strategies in the Inner Mongolia steppe.

CONCLUSIONS

Our results indicated that sites with a HG history had suffered soil degradation, and were thus characterized by poor soil properties and a disturbed soil food web. Water addition (simulating wet year precipitation) increased the proportion of plant parasites, lowered the proportion of bacterivores and omnivores–predators, and enhanced soil TC and TN. The addition of N did not affect soil abiotic properties and had only a slight effect on the soil nematode community. However, the duration of this study did not allow detection of potential effects of N addition that might occur in the long term. Further analysis of changes in the soil nematode community based on species via molecular techniques might give insight into the effects of N and water addition on the functioning and composition of soil food webs in sites with different grazing histories.

REFERENCES

Bai YF, Han XG, Wu JG, Chen ZZ, Li LH. 2004. Ecosystem stability and compensatory effects in the Inner Mongolia grassland. Nature 431:181–4.

Bai YF, Wu JG, Xing Q, Pan QM, Huang JH, Yang DL, Han XG. 2008. Primary production and rain use efficiency across a precipitation gradient on the Mongolia plateau. Ecology 89:2140–53.

Bai YF, Wu JG, Clark CM, Naeem S, Pan QM, Huang JH, Zhang LX, Han XG. 2010. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from Inner Mongolia Grasslands. Global Change Biol 16:358–72.

Bao SD. 2000. Soil and agricultural chemistry analysis. Beijing: China Agricultural Press (in Chinese).

Bardgett RD, Jones AC, Jones DL, Kemmitt SJ, Cook R, Hobbs PJ. 2001. Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. Soil Biol Biochem 33:1653–64.

Bongers T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14–19.

Chen Q. 2011. The response of net primary production and plant species composition to the interaction of water and nitrogen in typical steppe, Inner Mongolia. Dissertation. pp 40–66.

Chen Q, Hooper DU, Lin S. 2011. Shifts in species composition constrain restoration of overgrazed grassland using nitrogen fertilization in Inner Mongolian steppe. China. Plos One 6(3):e16909.

Cheng Z, Grewal PS, Stinner BR, Hurto KA, Hamza HB. 2008. Effects of long-term turfgrass management practices on soil nematode community and nutrient pools. Appl Soil Ecol 38: 174–84.

Clark CM, Tilman D. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451:712–15.

De Deyn GB, Raaijmakers CE, Zoomer HR, Berg MP, de Ruiter PC, Verhoef HA, Bezemer TM, van der Putten WH. 2003. Soil invertebrate fauna enhances grassland succession and diversity. Nature 422:711–13.

De Deyn GB, Raaijmakers CE, van Ruijven J, Berendse F, van der Putten WH. 2004. Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. Oikos 106:576–86.

De Ruiter PC, Moore JC, Zwart KB, Bouwman LA, Hassink J, Bloem J, De Vos JA, Marinissen JCY, Didden WAM, Lebbink G, Brussaard L. 1993. Simulation of nitrogen mineralization in the below-ground food webs of two winter wheat fields. J Appl Ecol 30:95–106.

Ferris H. 2010. Form and function: metabolic footprints of nematodes in the soil food web. Eur J Soil Biol 46:97–104.

Ferris H, Matute MM. 2003. Structural and functional succession in the nematode fauna of a soil food web. Appl Soil Ecol 23:93–110.

Ferris H, Venette RC, van der Meulen HR, Lau SS. 1998. Nitrogen mineralization by bacterial-feeding nematodes: verification and measurement. Plant Soil 203:159–71.

Ferris H, Venette RC, Scow KM. 2004. Soil management to enhance bacterivore and fungivore nematode populations and their nitrogen mineralisation function. Appl Soil Ecol 25:19–35.

Freckman DW, Ettema CH. 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agric Ecosyst Environ 45:239–61.

Freckman DW, Whitford WG, Steinberger Y. 1987. Effect of irrigation on nematode population dynamics and activity in desert soils. Biol Fert Soils 3:3–10.

Gong XY, Chen Q, Lin S, Brueck H, Dittert K, Taube F, Schnyder H. 2011. Tradeoffs between nitrogen- and water-use efficiency in dominant species of the semiarid steppe of Inner Mongolia. Plant Soil 340:227–38.

Kardol P, Cregger MA, Campany CE, Classen AT. 2010. Soil ecosystem functioning under climate change: plant species and community effects. Ecology 91:767–81.

Khan Z, Kim YH. 2005. The predatory nematode, *Mononchoides fortidens* (Nematoda: Diplogasterida), suppresses the root-knot nematode, *Meloidogyne arenaria*, in potted field soil. Biol Control 35:78–82.

Landesman WJ, Treonis AM, Dighton J. 2011. Effects of a one-year rainfall manipulation on soil nematode abundances and community composition. Pedobiologia 54:87–91.

Li JZ, Lin S, Taube F, Pan QM, Dittert K. 2011. Above and belowground net primary productivity of grassland influenced by supplemental water and nitrogen in Inner Mongolia. Plant Soil 340:253–64.

Liang WJ, Lou YL, Li Q, Zhong S, Zhang XK, Wang JK. 2009. Nematode faunal response to long-term application of nitrogen fertilizer and organic manure in Northeast China. Soil Biol Biochem 41:883–90.

Mills AAS, Adl MS. 2011. Changes in nematode abundances and body length in response to management intensive grazing in a low-input temperate pasture. Soil Biol Biochem 43:150–8.

Nan ZB. 2005. The grassland farming system and sustainable agricultural development in China. Grassland Sci 51:15–19.

Niu SL, Yang HJ, Zhang Z, Wu MY, Lu Q, Li LH, Han XG, Wan SQ. 2009. Non-additive effects of water and nitrogen addition on ecosystem carbon exchange in a temperate steppe. Ecosystems 12:915–26.

Olff H, Hoorens B, de Goede RGM, van der Putten WH, Gleichman JM. 2000. Small-scale shifting mosaics of two dominant grassland species: the possible role of soil-borne pathogens. Oecologia 125:45–54.

Pan QM, Bai YF, Wu JG, Han XG. 2011. Hierarchical plant responses and diversity loss after nitrogen addition: testing three functionally-based hypotheses in the Inner Mongolia grassland. PLoS ONE 6(5):e20078.

Paul KI, Polglase PJ, O'Connell AM, Carlyle JC, Smethurst PJ, Khanna PK. 2003. Defining the relation between soil water content and net nitrogen mineralization. Eur J Soil Sci 54:39–47.

Pineiro G, Paruelo JM, Oesterheld M, Jobbagy EG. 2010. Pathways of grazing effects on soil organic carbon and nitrogen. Rangel Ecol Manag 63:109–19.

Porazinska DL, et al., 2003. Relationships at the aboveground-belowground interface: plants, soil biota, and soil processes. Ecol Monogr 73:377–395.

Qi S, Zheng HX, Lin QM, Li GT, Xi ZH, Zhao XR. 2011. Effects of livestock grazing intensity on soil biota in a semiarid steppe of Inner Mongolia. Plant Soil 340:117–26.

Sánchez-Moreno S, Minoshima H, Ferris H, Jackson LE. 2006. Linking soil properties and nematode community composition: effects of soil management on soil food webs. Nematology 8:703–15.

Steffens M, KÖlbl A, Totsche KU, KÖgel-Knabner I. 2008. Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (P.R. China). Geoderma 143:63–72.

ter Braak CJF, Šmilauer P. 2002. Canoco for Windows 4.5. Wageningen: Biometris.

Veen GF, Olff H, Duyts H, van der Putten WH. 2010. Vertebrate herbivores influence soil nematodes by modifying plant communities. Ecology 91:828–35.

Viketoft M, Bengtsson J, Sohlenius B, Berg MP, Petchey O, Palmborg C, Huss-Danell K. 2009. Long-term effects of plant diversity and composition on soil nematode communities in model grasslands. Ecology 90:90–9.

Wang CH, Wan SQ, Xing XR, Zhang L, Han XG. 2006. Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. Soil Biol Biochem 38:1101–10.

Wardle DA, Bardgett RD, Klironomos JN, Setala H, van der Putten WH, Wall DH. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629–33.

Xia JY, Niu SL, Wan SQ. 2009. Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. Global Change Biol 15:1544–56.

Yeates GW, Bongers T. 1999. Nematode diversity in agroecosystems. Agric Ecosyst Environ 74:113–35.

Yeates GW, Bongers T, de Goede RGM, Freckman DW, Georgieva SS. 1993. Feeding habits in soil nematode families and genera: an outline for soil ecologists. J Nematol 25:315–31.

Zhao Y, Peth S, Reszkowska A, Gan L, Krummelbein J, Peng XH, Horn R. 2011. Response of soil moisture and temperature to grazing intensity in a Leymus chinensis steppe, Inner Mongolia. Plant Soil 340:89–102.