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# Responses of soil nematodes to water and nitrogen additions in an old-field grassland

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ABSTRACT

Given that soil water and nitrogen (N) are two major limiting factors for plant growth, changing precipitation regime and atmospheric N deposition may have profound impacts on ecosystem structure and function. However, the effects of changing precipitation regime and atmospheric N deposition on soil fauna abundance and trophic complexity are still poorly understood. A field manipulation experiment was established to investigate the responses of soil nematode communities to water and N additions in an old-field grassland ecosystem. Results showed that water addition significantly increased soil nematode abundance and generic richness, but had no effects on the relative abundance of different trophic groups. Nitrogen addition did not affect nematode abundance, but reduced their generic richness, and significantly altered community structure by promoting the abundance of bacterivores and suppressing that of fungivores and omnivores-predators. Compared to water addition, N addition resulted in a community with higher enrichment index (EI), and lower maturity index (MI) and channel index (CI), consistent with a soil food web with domination of bacterial decomposition channel. The resource availability might be responsible for the observed changes in nematode population size, whereas the alterations in nematode community trophic structure could be attributed to the changes in soil nitrate concentration. Our findings reveal different but independent effects of water and N additions on soil nematode communities in an old-field grassland of North China Plain.


## 1. Introduction

Soil fauna constitute an essential component of terrestrial ecosystems and exert extensive impacts on soil nutrient processes (Ingham et al., 1985; Fu et al., 2005). Occupying multiple trophic levels in the soil food web, soil nematodes are highly diverse, and can be easily extracted and assigned to trophic groups (Yeates et al., 1993). Nematodes can serve as a model soil animal group that provides a holistic measure of the biotic and functional status of soils (Bongers, 1999; Neher, 2001). Given that soil nematodes rely almost entirely on plant-derived resources, changes in plant community may significantly influence resource availability and thus alter soil nematode communities (Yeates, 1999; Pollierer et al., 2007). Soil microhabitat is also a major factor affecting soil nematodes, which have been revealed to be sensitive to changes in soil pH and moisture (Korthals et al., 1996; Landesman et al., 2011; Chen et al., 2013). Thus, alterations of food source and habitat may affect soil nematode community composition and structure, and subsequently influence associated ecological functions.

Precipitation and nitrogen (N) are two important resources regulating plant growth (Li et al., 2011; Yang et al., 2011; Xu et al., 2012). It has been demonstrated that elevated precipitation can increase belowground net primary productivity (BNPP) (Bai et al., 2010a), stimulate soil microbial biomass, and provide more resources for soil fauna (Liu et al., 2009; Zhang et al., 2013). In addition, changes in precipitation will directly alter soil water availability and subsequently affect soil nematodes (Griffiths and Caul, 1993). Nitrogen enrichment due to atmospheric N deposition can also stimulate plant growth and increase belowground translocation of photosynthates, thereby benefiting soil biota (Xia and Wan 2008; Li et al., 2013). However, there are increasing evidences that N enrichment can restructure soil nematode community by affecting N availability, altering plant community, and increasing ammonium and aluminum toxicity (Okada and Harada, 2007; Wei et al., 2012; Sun et al., 2013). Nitrate ($NO_3^-$-N) and ammonium ($NH_4^+$-N) are the two main forms of N available for plant uptake. Soil nitrate concentration was found positively correlated with nematode community enrichment index and negatively with maturity index and structure index (Azpilicueta et al., 2014). In a multi-level N addition experiment in Inner Mongolia, soil nematode diversity has been shown to be negatively

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correlated with ammonium concentration (Wei et al., 2012). In addition, N addition can change microbial community composition (Frey et al., 2004; Waldrop et al., 2004; Wang et al., 2004) and consequently induce structural shifts in soil fauna communities (Fiscus and Neher, 2002; Chen et al., 2013).

Under climate change scenarios, changing precipitation regime and atmospheric N deposition occur simultaneously and may have interactive impacts on soil water and N availability. On the one hand, precipitation plays an important role in soil N leaching and mineralization (Schimel et al., 1997). On the other hand, alleviated N limitation can stimulate plant growth, increase photosynthesis and transpiration, which in turn influence soil moisture. Therefore, the interactive effects of precipitation change and N deposition on soil fauna communities may be more complex than the effects of single factors (Sun et al., 2013). Distinguishing between the effects of precipitation and N on soil nematode communities will facilitate sustainable managements of ecological goods and services.

A field manipulation experiment was established to examine the main and interactive effects of water and N additions on soil nematode communities in an old-field grassland ecosystem in Kaifeng, Henan, China in 2011. Because soil microfauna are under the influence of both resource availability and microenvironment, we hypothesized that: 1) water addition would promote the abundance and diversity of nematode community, 2) nitrogen addition would result in a high abundant but a “simpler” community (lower diversity and shorter food chain length), and 3) there might be significant interactive effects of water and N additions on the abundance of soil nematodes.

## 2. Materials and methods

### 2.1. Study site and experimental design

The study was conducted at the Experimental Station of Global Change Ecology, Henan University (34°49′16.84″N, 114°17′56.76″E), Kaifeng, Henan. Mean annual precipitation and temperature are 625 mm and 14.4 °C, respectively. Interannual fluctuations of precipitation in the local area varied from −21% to +21% around the long-term average over the past 60 years (1953–2013, data from Chinese Meteorological Administration). The experimental site was originally a cultivated land with wheat and corn rotation until 2000, and then the land was allowed to return to its natural successional stage as an old-field. In 2006, some *Populus* seeds were planted at random and the sparse seedlings were removed one year before the experiment establishment. The dominant plant species in the old-field grassland include *Cynodon dactylon, Gaura parviflora,Conyza canadensis, Glycine soja, Melilotoides ruthenica*, and *Lespedeza bicolor*.

The soil in the local area is sandy loam (FAO classification system) which is conducive to more evaporation, combined with the shallow groundwater, slow surface runoff and high salinity, the soil pH (8.66) is strongly alkaline even though the rainfall in the region is high.

Henan province is an intensively managed agricultural region and economically developed area. According to a previous report, the mean annual N deposition rate in this area is 47 kg N $ha^{-1}yr^{-1}$, and at some hotspots, higher than 80 kg N $ha^{-1}$ $yr^{-1}$ (Zhang et al., 2011).

A completely randomized design was used with two factors (water and N) and 4 treatments: control (C), water addition (W), N addition (N), and water plus N additions (WN) with 6 replicates for each treatment. From April to October, 30% of additional water was added to the W and WN plots after each rainfall event (> 10 mm). The level of N addition rate in this experiment is 10 g N $m^{-2}$ $yr^{-1}$ according to the maximum values in other studies in northern China, which found N effects on species composition and ecosystem production saturate at N addition rates of about 10.5 g N $m^{-2}$ $yr^{-1}$ (Bai et al., 2010b; Li et al., 2013). From May to September, 2 g N $m^{-2}$ in the form of $NH_4NO_3$ was applied to the N and WN plots on the first rainfall date of each month.

### 2.2. Soil sampling

Soil samples were collected on July 8, September 8, December 12, 2013, and March 31, 2014. Four cores were randomly taken in each plot with a soil auger (5 cm in diameter and 10 cm in depth), and mixed carefully to obtain a total of 24 composite samples. Stones, larger roots and macro-arthropods were excluded by hand. Each soil sample was divided into two aliquots. The first one was used to analyze soil physicochemical properties including soil water content (WC), soil organic carbon (SOC), soil total N (TN), soil pH, $NO_3^-$-N, and $NH_4^+$-N concentration. The other one was used to extract and identify soil nematodes.

### 2.3. Soil physicochemical properties and plant measurements

Soil moisture at 10 cm-depth was measured six times per month at 5-day intervals using a time-domain reflectometer (TDR 200, Spectrum Scientific Inc., USA). Soil temperature (ST) at 10 cm depth was determined using a thermocouple probe. Soil pH was measured in a 1: 2.5 soil −distilled $H_2O$ suspension using a glass electrode (Sartorius PB-10). SOC and TN were analyzed using an element analyzer (Vario MACRO cube, Elementar INC., Germany). The concentrations of nitrate and ammonium were measured using a Smart Chem 200 Discrete Auto Analyzer (AMS Systea, Italy). Briefly, 10 g fresh soil was extracted with 50 mL 2 M KCL solution and then put on shaker for 60 min. The extracts were analyzed for [$NH_4^+$] and [$NO_3^-$] (Wei et al., 2012).

One permanent quadrat (1 × 1 $m^2$) was established in each plot. A 1 × 1 $m^2$ frame with one hundred equally distributed grids (10 × 10 $cm^2$) was put above the canopy in each quadrat to estimate plant coverage. Plant species richness was recorded as the number of plant species in the quadrat. The sum of all plant individuals in the quadrat was considered as an estimated of plant community density. All standing litter and living aboveground biomass of one 0.5 × 1 $m^2$ quadrat were clipped in each plot at peak biomass in September 2013, oven-dried at 65 °C to constant weights, and then added together as aboveground net primary production (ANPP). Roots were sampled to 20 cm depth by taking 4 soil cores with a 5-cm diameter soil augur. After cleaning and drying, constant weight was used to calculate root biomass.

### 2.4. Methods of extraction and identification of soil nematode

Nematodes were extracted from 50 g soil using a modified Baermann method (Ruess, 1995). After an extraction of 48 h, nematodes were preserved in 4% formaldehyde, counted, and then adjusted to 100 g dry soil. Subsequently, 100 individuals were identified to genus level using a microscope (Nikon, E200), and assigned to the following trophic groups: bacterivores (Ba), fungivores (Fu), plant parasites (PP), and omnivores- predators (Om). Nematode genera were also assigned to colonizer-persister (c-p) groups (*cp1- cp5*) according to Bongers (1999).

Nematode data were also used to calculate Maturity Index (MI) (Bongers, 1990), Channel Index (CI), Enrichment Index (EI) and Structure Index (SI) (Ferris et al., 2001). Lower and higher values of MI indicate disturbed and stable nematode communities, respectively. CI was calculated to evaluate the relative functional intensity of soil decomposition pathways. A low CI indicates the dominance of the bacterial channel, while a high CI refers the dominance of the fungal channel. A high EI suggests a resource-enriched soil ecosystem. A high SI indicates a complex and stable food web.