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Fertilization influences the nematode community through changing the plant community in the Tibetan Plateau

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ABSTRACT

Fertilization greatly impacts grassland ecosystems as it alters plant communities, soil fauna, and microbiota although few studies have looked at these interactions on the Tibetan plateau. Soil nematodes are ideal bioindicators for the status of below-ground ecosystems. To explore how nematode communities are impacted by the use of fertilization, we studied the top layer of soil in land that had been fertilized annually with diammonium phosphate for over 10 years. We tested effects of long term fertilization treatment at 300, 600 kg ha^{-1} yr^{-1} (F300, F600) compared to unfertilized control (F0) on soil nematode community at the 0–15 cm depth. Soil samples for analysis of nematode community were collected on September 2012 and on September 2013. We show that fertilization was associated with increased plant and microbial biomass, and decreased plant biodiversity. Fertilization increased microbial biomass C and N; grass biomass and total plant biomass, but decreased plant species richness, plant community biodiversity and the biomass of sedges, legumes and forbs. Fertilization increased nematode communities increased over the entire course of the study. We show that the amount of fertilizer used and the duration of its use significantly impacted the dynamics and composition of nematode communities. The changes in nematode community were associated with both changes in soil factors and shifts in the plant community that were caused by fertilization. According to the structural equation model (SEM), fertilization had strong effects on soil microorganisms and plant community composition, whereas plant community was the main determinant of nematode community changes. Our findings highlight the importance of soil fertilization in regulating both plant and soil fauna communities, and provide a better understanding of the responses of plants and soils to fertilization and the linkages between structure and functioning of above-ground and below-ground in the Tibetan Plateau.

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1. Introduction

The Tibetan Plateau grassland ecosystem is an important ecosystem that is currently threatened by the intensified use of chemical fertilizers in an environment already compromised by the constraints of high altitude [1]. Chemical fertilizers have been heavily used to improve grazing lands and promote livestock production over the past few decades. A clear understanding of the effects of these fertilizers on the flora and fauna of Tibetan Plateau grassland is critical for developing remediation efforts. Previous research was mostly focused on the effects of fertilization on plant communities [2,3]and on soil [3]. Although soil fauna plays an important role in ecosystem dynamics [4], only a few studies have addressed the impacts of fertilization on the fauna of Tibetan Plateau grasslands [5].

Soil nematodes represent one of the most abundant groups of soil fauna in terrestrial ecosystems, and their special characteristics make them an ideal bioindicator of the health of belowground ecosystems [6,7]. Multiple studies reported different effects of fertilization on soil nematode communities at various geographical locations. For example, a lower amount of inorganic fertilizer was linked to higher abundance and proportion of plant feeder nematodes in a sandy soil grassland ecosystem in the Netherlands [8]. Long-term fertilization has been shown to increase the abundance of herbivore and bacterivore nematodes in a tallgrass prairie

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ecosystem in the American State of Kansas [9] and to reduce the abundance of omnivores in a grass sward in British Columbia [10]. Another study conducted in a pasture in New Zealand showed that 3-year use of fertilization increased the abundance of total and herbivorous nematodes, while it decreased the abundance of fungivores and predators [11]. Given the demonstrated variability of fertilizer effects, and due to the uniqueness of Tibetan alpine meadow, we suspect that intensive fertilization effects in the latter may be different from other locations. Therefore, a study addressing the effects of inorganic fertilizers in the Tibetan alpine meadow on the dynamics of local belowground nematode communities is warranted.

Apart from the question of how fertilization affects nematode communities, it is also unknown whether the effects of fertilization on soil nematode communities operate via altering soil microbial communities. There is no doubt that soil microbiota impact the survival of nematodes via direct and indirect means. Apart from providing a direct food source for some nematodes and causing disease in others, microorganisms alter their environment by degrading complex organic matter, forming biofilms, and altering soil pH [12–14]. In general, an increase in microbial abundance is likely to stimulate the populations of nematodes that feed on them [13]. In particular, fertilization could stimulate opportunistic bacterial-feeding nematodes (so-called *r* strategists) in soil [11,15]. On the other hand, shifts in plant community composition towards plant species that support some nematode trophic groups could account for changes in nematode community abundance [16]. The effect of plant communities on nematodes is influenced by the net primary production (NPP), that is the quantity and quality of resources it returns to the soil as plant litter or root exudates [8,16,17]. Multiple studies focused on the effects of NPP on nematode communities. For example, in monoculture plots of each of eight grass and forb species, De Deyn et al. (2004) found little relationship between plant shoot mass and abundance of soil nematodes [18]. In New Zealand, Schon et al. (2010) found a positive correlation between average nematode abundance and NPP [16]. These studies, however, addressed the effects of fertilization, plant communities, and soil microbes on soil nematode communities individually. In the current study, we address the interconnection between these factors and their net impact on nematode communities. We collected plant and soil microorganism data from a long term fertilization field study and assessed the relative importance of alternative causal pathways by structural equation modeling (SEM). The SEM can quantify the relative strength of relationships among variables in complex systems and tests the hypothesized causal relationships among the variables [19].

The specific goals of our study are: (1) to examine the effects of fertilization on nematode communities in Tibetan alpine meadows and (2) to confirm the main determinants of effects of fertilization on nematode communities. We will be addressing three hypotheses: (1) fertilization increases nematode community abundance, (2) fertilization reduces the biodiversity of both plant and nematode communities, and (3) fertilization affects soil nematode communities through altering plant communities.

2. Materials and methods

2.1. Site description and experimental design

This study was carried out at the Walaka experimental site of the Research Station of Alpine Meadow and Wetland Ecosystems of Lanzhou University, located on the eastern Tibetan Plateau of China (35°58′N, 101°53′E; 3500 m above sea level). The local climate is characterized by strong solar radiation with short, cool summers and long, cold winters. The area has more than 270 frost days and 2580 h of sunshine per year. During the past 35 years, the mean annual temperature and precipitation have been 1.2 °C (range from 11.7 °C in July to 10 °C in January) and 620 mm per year, respectively. The native vegetation consists mainly of Arctic alpine and Chinese Himalayan plants, and is dominated by *Kobresia setchwanensis* and some grasses, such as *Elymus. nutans*. The experimental site (*c.* 8 ha) had been overgrazed in the past, but it has been fenced and been only grazed in the winter and early spring (October to April) since 2001.

The long-term fertilization experiment was established on a flat field in the Walaka experimental site on March 2002. Fifteen 6×10 m^2 plots—composed of three fertilization levels with five replicates each—were distributed using a randomized block design. Each plot was separated from others by a 1-m buffer strip. The fertilization treatment was generated with different amounts of $(NH_4)_2HPO_4$ fertilizer applied annually from 2002 at the beginning of the growing season (usually in the middle of May). Fertilizer applications of 0, 300, 600 kg ha^{-1} yr^{-1} are hereafter referred to as F0, F300, F600, respectively. Fertilizer was broadcasted evenly in each plot. The corresponding N and P inputs of the three fertilization levels are as follows: F0, control; F300, 64 kg N and 70 kg P ha^{-1} yr^{-1}; F600, 127 kg N and 141 kg P ha^{-1} yr^{-1}.

2.2. Sampling procedure

Soil samples for analysis of soil physicochemical properties and soil microbial carbon and nitrogen biomass were collected on September 2013. Six soil cores (diameter, 3.8 cm; depth, 25 cm) were taken randomly from each plot, mixed adequately as one sample. Each composite soil sample was placed into a plastic bag, mixed thoroughly, and stored refrigerated at 4 °C. Approximately one half of each sample was retained for analysis of soil pH, soil organic carbon (C) and total N concentrations, soil available P and N concentrations and microbial biomass C and N. The remained of each sample was used for extraction of nematodes.

The aboveground vegetation was sampled in two 0.25 m^2 quadrats in each plot on September 2013. Species richness and abundance (based on the number of individuals per species; we regarded a ramet as an individual for the clonal species) were estimated, and individual plants in each quadrat were clipped to the soil surface. All shoot samples were dried at 80 °C for 48 h, and weighed.

2.3. Nematodes extraction and identification

Soil samples for analysis of nematode community were collected on September 2012 and on September 2013. Experiment induced changes in Tibetan plateau grassland soil ecosystem mainly happen in the top 15-cm soil depth, hence, soil samplings for analysis of nematode communities were taken in this horizon [4]. The composite soil samples were stored at 4 °C for 1 day before nematode extraction. Soil samples were hand-mixed and 50 g portions were removed for nematode extraction. Nematodes were extracted for 48 h by using the modified Baermann wet funnel. All nematodes were identified when the nematodes were fewer than 150 individuals per sample. In samples containing more than 150 individuals per sample, the first 150 individuals encountered were identified to genus. Nematode taxa were classified into five trophic groups (Plant feeders, fungivores, bacterivores, predators, and omnivores) using a method modified from that published by Yeates et al. (1993) [20]. In order to use nematode as bioindicators, they were allocated to colonizer-persister (c-p) classes following a protocol designed by Bongers (1990) [21].

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