Applied Soil Ecology 108 (2016) 128–135

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Opposing effects of nitrogen and water addition on soil bacterial and fungal communities in the Inner Mongolia steppe: A field experiment

Hai-kun Ma[a,b,**], Guang-ying Bai[a], Yang Sun[a], Olga Kostenko[b], Xiang Zhu[c], Shan Lin[d], Wei-bin Ruan[a,*], Nian-xi Zhao[a], T. Martijn Bezemer[b]

[a] College of Life Sciences, Nankai University, Tianjin 300071, People's Republic of China
[b] Netherlands Institute of Ecology (NIOO-KNAW), Department of Terrestrial Ecology, P.O. Box 50, 6700 AB, Wageningen, The Netherlands
[c] Department of Statistics, The University of Chicago, Chicago, IL 60637, USA
[d] College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, People's Republic of China

ARTICLE INFO

Article history:
Received 28 September 2014
Received in revised form 2 August 2016
Accepted 3 August 2016
Available online xxx

Keywords:
Soil microbial community
Steppe
Irrigation
Fertilization
PLFA

ABSTRACT

Grasslands are important ecosystems and make up 40% of the terrestrial ecosystems worldwide. The Inner Mongolia steppe is the main grassland region of China, and nitrogen (N) and water availability are two important factors that limit the productivity of these grasslands. We tested how N and water addition influence the composition of the microbial community in the soil using PLFA, and soil physical and chemical properties in two semiarid grassland sites in Inner Mongolia during two consecutive years. In both sites, a split-plot design was employed with two water treatments (natural precipitation, stimulated wet year precipitation) and three N treatments (0 kg N ha^{-1}, 25 kg N ha^{-1}, 50 kg N ha^{-1}). Water addition greatly increased soil fungi and decreased bacteria while N had opposite effects. Water addition resulted in a significant increase in soil pH and electric conductivity. N addition did not lead to consistent changes in soil characteristics. Multivariate analysis showed that PLFA composition varied between all treatments but was mainly influenced by water addition. This study provides insight into how climatic changes such as alternations in rainfall and N deposition shape the soil microbial communities in Inner Mongolia steppes.

1. Introduction

Grasslands make up approximately 40% of the terrestrial land surface and grassland ecosystems are affected by human induced climatic changes (Wang and Zhou, 2012). Carbon (C) sequestration by vegetation and soil can be an important mean of mitigating increasing CO_2 concentrations in atmosphere (Batjes, 1998), and temperate grassland ecosystems may be an important C sink (Frank, 2002). Even though N is the major element in the atmosphere, N availability is one of the main factors that limits plant growth and most grassland ecosystems are N limited (Vitousek and Howarth, 1991; Bai et al., 2008). N-enrichment in grasslands often increases aboveground primary production and causes a decline in plant species richness (Tilman, 1987; Clark and Tilman, 2008; Pierik et al., 2011).

N-Enrichment can also affect the abundance of soil-dwelling organisms such as bacteria and fungi (Bardgett et al., 1999), and can result in changes in the activity or composition of soil microbes (Fierer et al., 2012). A meta-analysis of 82 N addition studies showed that on average microbial biomass declined by 15% by N fertilization and that the strength of the effects was positively related to the amount of N added and the duration of the experiment (Treseder, 2008). The effects of N addition are closely linked to carbon dynamics and mineralization in the soil (Sinsabaugh et al., 2005; Waldrop and Zak, 2006), which in turn influence soil microbes, as the soil microbial community is sensitive to soil carbon availability (Drenovsky et al., 2004).

Soil water plays an important role in the activity of the soil microbial community. It influences N-mineralization mediated by microbes (Araya et al., 2013). Also, soil water plays a key role in the transportation of nutrients, in cellular metabolism and serves as a medium for bacterial mobility (Drenovsky et al., 2004). Changes in soil water, therefore, can cause changes in the physiology and structure of the soil microbial community (Paul et al., 2003). A

* Corresponding author.
** Corresponding author at: College of Life Sciences, Nankai University, Tianjin 300071, People's Republic of China.
E-mail addresses: M.Haikun@nioo.knaw.nl (H.-k. Ma), ruanweibin2004@hotmail.com (W.-b. Ruan).

http://dx.doi.org/10.1016/j.apsoil.2016.08.008
0929-1393/

study in temperate grassland in the UK, for example, showed that changes in soil water content resulted in differences in soil bacterial and fungal phospholipid fatty acid (PLFA) patterns (Bardgett et al., 1999). Soil water availability can also impact substrate availability and soil properties, which in turn, impact the soil microbial composition and activity (Han et al., 2007). Soil microbes are important for below ground N availability, and many processes that are driven by soil microbes such as the decomposition of soil organic matter, mineralization and biological N fixation, are affected by N and water availability (Inglett et al., 2011). Hence, water and N availability interactively affect the soil microbial community and grassland ecosystem functioning.

In this study, we examined the effects of N and water addition on the soil microbial community of the Inner Mongolia steppe ecosystem, which is a preeminent part of the Eurasian continent (Chen et al., 2011). The net primary productivity of these steppe ecosystems is largely determined by N and water availability (Chen et al., 2011). We determined the microbial community and soil abiotic characteristics in a long-term field experiment with water and N addition treatments, with an identical set-up at two sites. Other studies conducted at the same site that examined the effects of N and water addition on these steppe ecosystems, have focused on net primary productivity (Li et al., 2011), plant species composition (Chen et al., 2011), and how tradeoffs between N and water-usage-efficiency affect dominant plant species of the semiarid steppe of Inner Mongolia (Gong et al., 2011). How the soil microbial community responds to water and N addition in the Inner Mongolia Steppe, and what part of the soil microbial community is influenced by these treatments has not received much attention so far (Bi et al., 2012). In this study, we use phospholipid fatty acid analysis (PLFA) to examine how three levels of N addition (0, 25 and, 50 kg N ha^{-1}) in plots with and without water addition influence soil microbial communities. PLFA analysis has been used in many studies as a reliable method to assess the structure of the soil microbial community (Green and Scow, 2000; Bardgett and Walker, 2004; Bååth and Anderson, 2003).

The objectives of our study were to determine how soil microbial communities change in response to N and water addition. We hypothesized that N will positively influence bacteria and have a negative effect on fungi, and that water addition will have the opposite effect. We also determined the effects of water and N addition on a range of abiotic soil properties, and analyzed how soil microbial community composition in the experimental plots was related to soil abiotic factors.

2. Materials and methods

2.1. 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, P.R. China. The average annual precipitation is 343 mm, 80% of which falls during the months May to September. The soil type is classified as Calcic Chernozems.

2.2. Experimental design

Two experimental sites fenced in 2005 were used in this study. The dominant plant species are perennial bunch grasses, perennial rhizome grasses, perennial forbs, and shrubs. The experimental design has been reported previously (Gong et al., 2011). Briefly, the experiment was set-up using a two-factorial split-plot design. The main plots have two water supply levels (natural precipitation and simulated wet year precipitation) divided into four blocks at each site. The subplots have three N addition levels (unfertilized control, 25 kg N ha^{-1} and 50 kg N ha^{-1}, N0, N25 and N50 respectively) with four replicates, one replicate of each N-level per block. Each subplot was 5×8 m in size. Main plots and subplots were separated by 3 m and 0.8 m walkways respectively. The same experimental design was established at two sites. Both experimental areas are 0.2 ha in size and have similar vegetation composition but the level of grazing prior to the start of the experiment was higher at Site 2 than at Site 1 (Li et al., 2011). The sites are 3 km apart. The experiment started in 2005, and annually since 2005 the vegetation was cut at 3 cm above the ground manually and the biomass removed.

2.3. N Application and irrigation

To make sure that each plot received the same amount of fertilizer, granular urea NH_2CONH_2 (1.5 mm diameter, Luxi Company, China) was mixed with air-dried and fine-sieved (<2 mm) soil particles at a ratio of 1:10 and then spread manually on May 15th each year.

The simulated wet year's amount of precipitation was 431 mm based on the long-term rainfall data (1982–2003) obtained from the meteorological station at IMGERS. To simulate the amount and distribution of the wet year precipitation from May to September, additional irrigation was applied at 10-day intervals 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 average in the same period of the predicted wet year simulation, no additional irrigation was applied during this 10-day interval and the amount of irrigation during the following 10-day interval would be adjusted accordingly.

2.4. Soil sampling

Soil samples were collected on August 18th 2011 and August 20th, 2012. In each plot, three cores were randomly taken with an auger (3.5 cm diameter) at 0–15 cm depth. Soil samples were mixed up evenly in a plastic bag in order to get a composite sample from each plot of about 400 g. Ten gram subsample of soil was immediately sieved (425 μm) and kept separately in an ice box filled with ice (Schnecker et al., 2012) and was transported to Nankai University, Tianjin, P.R. China within 1 day, and then stored at −20 °C for PLFA analysis. The other subsample of soil was stored at 4 °C. About 150 g subsample of soil was used to analyze soil total nitrogen (TN), soil total carbon (TC), electrical conductivity (EC), soil pH and soil moisture content.

2.5. Soil physicochemical measurements

Soil pH was measured in a 1:2 soil–distilled H_2O suspension with a glass electrode (Sartorius PB-10). Soil EC was determined using a Conductivity Meter (DDS-307A) using 4 g air-dried soil and 20 g deionized water. Soil total carbon (TC) of samples collected in 2011 was analyzed using the Potassium dichromate oxidation method, soil total nitrogen (TN) was analyzed using a Semi-micro kjeldahl method (Bao, 2000) and samples collected in 2012 were analyzed using an elemental analyzer (Elemantar Vario EL cube). Soil moisture was measured by oven-drying a 10 g subsample of soil at 105 °C until constant weight.

2.6. PLFA analysis

The biomass and composition of the soil microbial community were assessed by analyzing the PLFA composition of the soil, using the method outlined in Schutter and Dick (2000). Briefly, 50 ml