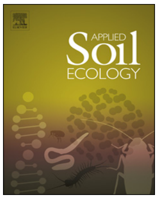
Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

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



Impacts of long-term nitrogen addition, watering and mowing on ammonia oxidizers, denitrifiers and plant communities in a temperate steppe

Cui-Jing Zhang[a,b], Zhong-Ling Yang[c], Ju-Pei Shen[a,b], Yi-Fei Sun[a,b], Jun-Tao Wang[a], Hong-Yan Han[c], Shi-Qiang Wan[c], Li-Mei Zhang[a,b,*], Ji-Zheng He[a,b,d]

[a] State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] State Key Laboratory of Cotton Biology, Key Laboratory of Plant Stress Biology, College of Life Sciences, Henan University, Kaifeng, Henan 475004, China
[d] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010, Australia

ARTICLE INFO

Keywords:
Global changes
Ammonia oxidizers
Denitrifiers
Community structure
Plant biomass

ABSTRACT

Global changes in nitrogen (N) deposition, precipitation patterns and land use could have an impact on the biogeochemical N cycle mediated by microorganisms. Microbial responses to global changes have been generally evaluated in short-term studies, while the long-term effects are not well understood. In this study, the interactive effects of multiple global change factors on N-cycling microorganisms and their interactions with plants were investigated in a 9-year field experiment with N addition, watering and mowing in a temperate steppe in northern China. Quantitative PCR, terminal restriction fragment length polymorphism (T-RFLP) and clone library were used to analyze the abundance and community structure of ammonia oxidizers and denitrifiers, respectively. Bacterial 16S rRNA gene abundance significantly increased under mowing, but the functional gene abundances including *amoA*, *nirS*, *nirK* and *nosZ* genes were not affected. Watering significantly affected the ammonia-oxidizing archaea (AOA) community and N addition showed a strong effect on soil ammonia-oxidizing bacteria (AOB) and *nosZ*-containing denitrifier community structure. No significant interactive effects of mowing, N addition and watering on communities of soil ammonia oxidizers and denitrifiers were observed. Structure equation models suggested that plant biomass and community were significantly correlated with microbial communities and activities. These findings suggest that there are complex interactions between plants and soil microorganisms in grassland ecosystems.

1. Introduction

The temperate steppe in Inner Mongolia is an integral component of Eurasian grasslands. The soil has low moisture and nutrient availability, and the ecosystem is sensitive to global changes in land use, nitrogen (N) deposition and precipitation patterns (Bai et al., 2010; Niu et al., 2010). N deposition increased sevenfold in 2010 relative to 1850 and it is predicted that the N deposition rate will continue to increase in East Asia (Wang et al., 2017b). Precipitation in this area is expected to experience a small increase (~1%) before 2040 and a large increase (~9%) during the period of 2040–2100 (Sun and Ding, 2009). Previous studies have investigated the responses of aboveground plants to global changes, while microbial responses and plant-soil interactions in the context of global changes are largely unknown (Osanai et al., 2017).

Nitrification and denitrification driven by soil microorganisms are two important processes in N cycling. Ammonia oxidation, conversion of ammonia (NH_3) into nitrite (NO_2^-), is the first and rate-limiting step of nitrification and is mainly mediated by autotrophic ammonia-oxidizing bacteria (AOB) and archaea (AOA). Marker gene *amoA*, which encodes the α-subunit of the ammonia monooxygenase, is used to study ammonia oxidation. Comammox – a novel complete ammonia oxidizer within the *Nitrospira* genus – has been discovered recently, which can convert ammonia directly to nitrate (Hu and He, 2017). Denitrification, reduction of nitrate (NO_3^-) into NO_2^-, nitric oxide (NO), nitrous oxide (N_2O) and nitrogen (N_2), is mediated by a variety of microorganisms including bacteria, archaea and fungi domains (Levy-Booth et al., 2014; Philippot et al., 2007). Functional genes for denitrification (*nirK, nirS* and *nosZ*) are used to link denitrifying microbial groups to ecosystem processes. Alteration of the N-cycling microbial community could result in serious environmental consequences such as nitrate leaching and release of the greenhouse gas N_2O (Levy-Booth et al., 2014).

As a representative factor of global changes, N deposition has dramatic impacts on biodiversity and ecosystem functions, including

---

[*] Corresponding author at: State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China.
E-mail address: zhanglm@rcees.ac.cn (L.-M. Zhang).

https://doi.org/10.1016/j.apsoil.2018.06.017
Received 9 March 2018; Accepted 26 June 2018

Please cite this article as: Zhang, C.-J., Applied Soil Ecology (2018), https://doi.org/10.1016/j.apsoil.2018.06.017

decreasing plant diversity, and altering microbial community composition (Clark and Tilman, 2008; Fornara and Tilman, 2012; Yang et al., 2012). Though N deposition in a N limited ecosystem like a semiarid steppe could increase soil inorganic N content and alleviate N limitation, long-term N deposition could exert a potential risk to soil fertility and ecosystem functions by causing soil acidification (Högberg et al., 2006; Liu et al., 2012b). Generally, substrate content and soil pH are the main factors influencing the abundance and community structure of ammonia oxidizers and denitrifiers, and different microbial groups respond to substrate and soil pH changes differently due to their separated niche (Hamonts et al., 2013; Shen et al., 2011; Zhang et al., 2012a). It has been reported that the abundance of AOA was constant while the abundance of AOB increased in response to N addition in grassland ecosystems (Chen et al., 2013b; Lipson et al., 2005; Shen et al., 2008). Both abundance and community structure of denitrifiers were significantly affected by N addition (Hamonts et al., 2013).

Water availability is another limiting factor of plant productivity in the semiarid steppe. Watering was applied to simulate increased precipitation in many field experiments (Chen et al., 2008; Gutknecht et al., 2012). Watering could mitigate the limitation of water availability in semiarid ecosystems, which is beneficial for plant and microbial growth (Bauweraerts et al., 2013; Bell et al., 2008). It has been reported that the abundance of AOA and AOB was enhanced by watering (Hu et al., 2015; Ma et al., 2012). Watering might also increase soil pH, which can be offset by reduction effects of N addition on soil pH (Zhang et al., 2013). Previous studies revealed that the interactive effect of watering and N addition on soil bacteria and ecosystem functions is not simply additive, and watering could mitigate the negative effects of N addition on soil bacterial diversity (Chen et al., 2013a; Zhang et al., 2014).

Land use changes induced by human activities in grasslands, such as mowing and overgrazing, could also alter ecosystem services and cause severe degradation and desertification (Cao et al., 2004). Long-term mowing, removing of above-ground plant biomass, could reduce the soil carbon resource and significantly influence the soil microbial abundance and community structure (Aleklett et al., 2015; Baumann et al., 2012; DuPont et al., 2010; Han et al., 2012). Soil organic carbon is used as an energy source in denitrification (Xu et al., 2008). The effect of mowing on soil carbon might interact with that of N addition since mowing decreases soil carbon, while N addition increases N supply and enhances plant nutrient uptake, amplifying the limitation of carbon availability (Reich et al., 2006). Overall, although the single effects of N addition, watering, mowing on microbial biomass and activities have been widely investigated (Cao et al., 2004; Fu et al., 2012; Liu et al., 2012a; Tian et al., 2013), there is a knowledge gap in the interactive effects of the three global change factors on soil ammonia oxidizers and denitrifiers, especially in long-term experiments.

Furthermore, soil microorganisms play an important role in maintaining plant productivity and diversity (van der Heijden et al., 2008). However, the interaction and feedback between plants and soil microorganisms in the context of global changes are largely unknown (Compant et al., 2010). Statistical analysis approaches can correlate aboveground plant biomass with the belowground microbial community. In order to explore the responses of N-cycling microorganisms to the long-term practices of mowing, N addition and watering, and their interactions with plants, a field experiment in the temperate steppe in Inner Mongolia has been conducted since April 2005. Our objectives were (1) to investigate the influences of 9 years of N addition, increased precipitation and mowing on communities of ammonia oxidizers and denitrifiers; (2) to assess the plant-microbe interaction under future climate scenarios.

2. Materials and methods

2.1. Study site and experimental design

The study site was located at Duolun Restoration Ecology Station near Duolun County (42°02′N, 116°17′E, and 1324 m a.s.l.), Inner Mongolia, China. This area has a continental monsoon climate with long-term mean annual precipitation (MAP) and mean annual temperature (MAT) being approximately 385 mm and 2.1 °C, respectively. Eighty percent of precipitation occurred between June and September and monthly mean temperature ranged from −17.5 °C in January to 18.9 °C in July. Soil was classified as chestnut (Chinese classification) or Calcis-orthic Aridisol according to the US Soil Taxonomy classification. Soil was composed of 62.8% of sand, 20.3% of silt and 16.9% of clay. Soil organic carbon content was 16.1 g kg^{-1}. The main vegetation contained the grasses *Stipa krylovii*, *Agropyron cristattum* and *Cleistogenes squarrosa*. This grassland was overgrazed during the last century and had been protected by fencing since 2001.

The field experiment was established in 2005 as a part of Global Change Multifactor Experiment as described previously (Niu et al., 2010; Zhang et al., 2014). In this study, we focused on the treatments of control (C), mowing (M), N addition (N), summer watering (W) and their combinations (i.e. NW, MW, MN and MNW). Four replicates of each treatment were set up, resulting in a total of 32 plots. Eight 60 m × 92 m primary plots were set up in this 199 m × 265 m area, four of which were assigned to the mowing treatment and the other four were the control. Mowing was conducted using a lawn mower annually in August, leaving 10 cm of stubble. Two 28 m × 44 m secondary plots (i.e. N addition and control) were set up within each of the eight primary plots. The N addition treatment received 100 kg N ha^{-1} $year^{-1}$ fertilizer in the middle of July, and N fertilizer was applied as urea in 2005 and then NH_4NO_3 since 2006. Two 10 m × 15 m third-level plots (i.e. watering and control) were established within each secondary plot. The watering treatment received irrigation with 15 mm water weekly in July and August since its setting-up; thus, about 120 mm water was added each year, corresponding to 30% precipitation at the study site. Irrigation was applied with six sprinklers evenly distributed in two rows and each sprinkler covered a circular area with a diameter of 3 m.

Soil samples were collected by mixing three soil cores (7.5 cm diameter) randomly from the surface soil (0–20 cm) and the sub-surface soil (20–50 cm) of each plot in May 2014. All the 64 samples were transported to the laboratory in three days on ice. Soil samples were divided into two parts after passing through a 2 mm sieve: one part was stored at 4 °C for analysis of soil chemical properties and microbial activities, and the other part was stored at −80 °C for molecular analysis.

2.2. Soil chemical properties and microbial activities

Soil moisture was measured after drying for 12 h at 105 °C. Soil pH was determined at a ratio of soil: water = 1 g: 2.5 ml with a Delta pH-meter (Mettler-Toledo Instruments, Columbus, OH, USA). Soil total carbon (TC) and total nitrogen (TN) were measured using the Dumas method with an Elemental Analyzer (Vario EL III, Elementar, Germany). Soil organic matter was measured using the $K_2Cr_2O_7$ oxidation–reduction colorimetric method. Soil NH_4^+-N and NO_3^--N were extracted with 1 M KCl (soil: 1 M KCl = 1 g: 5 ml) and determined by a Continuous Flow Analyzer (SAN++, Skalar, the Netherlands (Li et al., 2014)).

Potential nitrification rate (PNR) was determined by the method of chlorate inhibition (Kurola et al., 2005). In brief, 5.0 g of fresh soil and 20 ml of phosphate buffer solution (PBS) (NaCl 8 g L^{-1}, KCl 0.2 g L^{-1},