The counteractive effects of nitrogen addition and watering on soil bacterial communities in a steppe ecosystem

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ABSTRACT

In many grassland ecosystems, mowing, nitrogen (N) addition, and watering are carried out to improve plant productivity for the development of livestock husbandry. However, as a result of human activities, N deposition rate is predicted to increase and precipitation is expected to change. These environmental changes affect biodiversity and ecosystem functioning, and most of the previous studies have primarily focused on their effects on macro-organisms, neglecting the responses of soil microbial communities. In this study, we examined the changes in three community attributes (abundance, richness, and composition) of the entire bacterial kingdom and 16 dominant bacterial phyla/classes in response to mowing, N addition, watering, and their combinations, by conducting a 5-year experiment in a steppe ecosystem in Inner Mongolia, China. Overall, various bacterial groups responded differentially to these experimental treatments, with Acidobacteria, Proteobacteria, Alphaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria being the most sensitive. Furthermore, the treatments produced different effects, with N addition and watering having much greater effects than mowing. Addition of N primarily affected these bacterial groups by reducing soil pH, while watering affected the bacterial groups by elevating both soil water content and soil pH. As N addition and watering had contrasting effects on soil pH, they also produced counteractive effects on many bacterial groups. The counteractive effects of N addition and watering suggest that the combination of these two treatments or the concurrence of N deposition and increased precipitation may be helpful for the maintenance of soil bacterial diversity and their ecosystem functions.

1. Introduction

The semiarid temperate steppe in northern China is an important part of the Eurasian grassland biome. Adoption of strategies such as mowing, nitrogen (N) addition and watering has been suggested to improve plant productivity in this area (Bai et al., 2010; Yang et al., 2012). It has been predicted that N deposition rate and precipitation will increase in this area in the future (IPCC, 2007; Wan et al., 2009; Bai et al., 2010; Sun and Ding, 2010; Niu et al., 2011). Water and N are the two key factors that limit plant productivity in arid and semiarid grassland ecosystems, and the changes in the levels of these two factors profoundly influence the biodiversity and ecosystem functioning (Vitousek et al., 1997; Yang et al., 2011; Xu et al., 2012). Previous studies have primarily focused on the influences of these two factors on the higher organisms and their associated ecosystem functions (Bai et al., 2010; Zhang et al., 2011; Yang et al., 2012). Although soil microbial communities are among the most abundant and diverse groups of organisms on Earth and are responsible for numerous key ecosystem processes (Torsvik et al., 2002; Fierer et al., 2009; Zhou et al., 2012), their response to these environmental changes and, especially, their combinations, has not been comprehensively explored.

Mowing, N addition, and watering may affect soil microbial communities through different mechanisms. Mowing removes much of the plant biomass from an ecosystem, and thus, its main effect is reduction in the amount of energy resource (or carbon resource) available to soil microorganisms (Kowalchuk et al., 2002; Berg and Smalla, 2009). N addition may have dual effects on soil microbial communities. On the one hand, it increases the soil inorganic labile N (NH_4^+-N and NO_3^--N) content, which is favorable to soil microbial communities. On the other hand, it may decrease soil pH, which is unfavorable to soil microorganisms, because soil

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pH is the most important ecological factor structuring soil bacterial communities (Fierer and Jackson, 2006; Rousk et al., 2010; Zhang et al., 2011; Zhang and Han, 2012). Watering may affect soil microbial communities through multiple routes: It may reduce soil water limitation and stimulate microbial growth, promote microbial passive dispersion and lead to the increase in competitive intensity (Zhou et al., 2002), and elevate soil pH and further affect soil microbial communities (Zhang et al., 2013). Besides, mowing, N addition, and watering may have combined effects on soil microbial communities. Most importantly, as N addition and watering may have contrasting effects on soil pH (Zhang et al., 2013), they may further have counteractive effects on soil microbial communities, and their combined use may be helpful in the maintenance of microbial diversity and ecosystem functions. Therefore, there is an urgent need to study the effects of mowing, N addition, watering, and their combinations on soil microbial communities and to elucidate the underlying mechanisms.

Although the influences of these environmental changes on soil microbial communities and their associated functions have received much attention in the past several decades, only the responses of some microbial functional groups (e.g. ammonia-oxidizing bacteria) and the entire soil microbial/bacterial community as a whole have often been studied (Horz et al., 2004; Martiny et al., 2011; Zhang et al., 2011; Zhang and Han, 2012). In particular, changes in the community attributes (e.g. diversity and composition), enzyme activities, and functional indices (e.g. N cycling rates) have often been detected, which have sometimes been found to be interrelated (Cusack et al., 2011). For example, it was noted that in temperate hardwood and pine forests, N deposition decreased the bacterial/fungal biomass ratio as well as the fungal lignin-degrading enzyme activity, which might have decreased the litter decomposition rates and altered the N cycling rates (Frey et al., 2004). Soil microbial communities comprise diverse taxonomic groups (e.g. different phyla/classes) with different genetic, physiological and ecological traits (Fierer et al., 2007; Lennon et al., 2012). However, the responses of various taxonomic groups to these environmental changes have not yet been investigated comprehensively owing to technological limitation (Schloss and Handelsman, 2005). For example, the traditional PCR-dependent cloning and sequencing technology allows acquiring only tens or hundreds of gene sequences from a sample, and thus is very labor-intensive for the simultaneous evaluation of the diversity of various bacterial phyla/classes. Fortunately, the current metagenomic approaches that exploit next-generation sequencing technologies can acquire millions or even billions of DNA sequences in a run, thus enabling rapid, accurate, and simultaneous survey of microbial communities in hundreds or thousands of samples (Hamady et al., 2008; Rousk et al., 2010).

To comprehensively examine the response of various soil bacterial taxonomic groups to the environmental changes in the steppe ecosystem in northern China, a long-term field manipulative experiment of mowing, N addition, and watering with eight treatments (control, mowing, N addition, watering, mowing and N addition, mowing and watering, N addition and watering, and simultaneous mowing, N addition and watering) was conducted. The pyrosequence technology targeting bacterial 16S rRNA gene was used to measure the diversity and composition of soil bacterial communities (Hamady et al., 2008). The specific questions of this study were as follows: (1) Whether and how the abundance, richness, and composition of the entire soil bacterial kingdom and the dominant phyla/classes are affected by mowing, N addition, watering, and their combinations? (2) What are the mechanisms of these treatments altering soil microbial communities? (3) Are there any counteractive effects of N addition and watering on soil bacterial communities?

2. Materials and methods

2.1. Study site and experimental design

This study was part of a long-term experiment conducted at the Duolun Restoration Ecology Station of Institute of Botany, Chinese Academy of Sciences, approximately 30 km from Duolun County (42°02′N, 116°17′E), Inner Mongolia Autonomous Region of China. Our field studies did not involve endangered or protected species, so no specific permissions were required for the location/activity. The experimental site was a typical temperate zone characterized by a semiarid continental monsoon climate. Mean annual temperature was 2.1 °C with monthly mean temperature ranging from 18.9 °C in July to −17.5 °C in January. Mean annual precipitation was about 385.5 mm with 80% precipitation occurred from June to September. Soil was chestnut soil (Chinese classification), corresponding to Calcis-orthic Aridisol in the US Soil Taxonomy classification, with sand, silt, and clay being 62.7%, 20.3%, and 17.0%, respectively. Mean soil bulk density was 1.31 g/cm^3. This temperate steppe was dominated by perennials, including *Stipa krylovii*, *Artemisia frigida*, *Potentilla acaulis*, *Cleistogenes squarrosa*, *Allium bidentatum*, and *Agropyron cristatum*.

This experiment commenced from 2005 and the effects of mowing, N addition, watering, and their combinations were investigated. There were four replicates for each of the eight treatments. Within a 199 m × 265 m area, eight 60 m × 92 m primary plots were set-up with a 5-m-wide buffer zone among plots (Fig. S1). Four primary plots were randomly assigned to the mowing treatment and the other four were controls. Aboveground plants were mowed with a lawn mower on 20 August every year, leaving only 10 cm of stubble. Within each of the eight primary plots, two 28 m × 44 m secondary plots were set-up (Fig. S1). The two secondary plots were randomly assigned to N addition and control. N was added in the form of urea in 2005 and NH_4NO_3 in 2006−2010 at a rate of 10 g N m^{-2} y^{-1}, conducting on a rainy day in the middle of July every year. In fact, the actual N deposition rate was less than 1.5 g N m^{-2} yr^{-1} in this area (Ti et al., 2012), so the total N deposition rate for the N addition treatments was less than 11.5 g N m^{-2} yr^{-1}, mimicking the projected rates for this region in the future (Bai et al., 2010). Within each secondary plot, two 10 m × 15 m third-level plots were established (Fig. S1), with one third-level plot being un-watered and the other being watered during summer (July and August) in 2005−2010. Within each watered three-level plot, six sprinklers were evenly arranged into two rows with 5 m between rows and 5 m between adjacent sprinklers. Each sprinkler covered a circular area with a diameter of 3 m; therefore, the six sprinklers covered the 10 m × 15 m third-level plot. Fifteen millimeters of water was applied weekly; thus, in the increased precipitation treatment, about 120 mm water was added each year, which is approximately 30% of the annual mean precipitation at the study site.

2.2. Sampling and measurement of soil physicochemical indices, bacterial abundance and community structure

Soil samples were taken on 22 August of 2010. Four soil cores (10 cm deep, 3.5 cm diameter) were collected from each three-level plot at random and thoroughly mixed. The soil physicochemical indices had been measured and described elsewhere (Zhang et al., 2013). Briefly, soil total carbon (TC) content was not significantly changed by mowing, N addition, watering and their combinations (three-way analysis of variance (ANOVA), $P > 0.05$). On the other hand, the soil inorganic labile N content was significantly increased by N addition, but the extent of increase was small under the combined treatment of N addition and watering. Furthermore, the