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RESEARCH ARTICLE

Interactions of water and nitrogen addition on soil microbial community composition and functional diversity depending on the inter-annual precipitation in a Chinese steppe

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Abstract

Water and nitrogen are primary limiting factors in semiarid grassland ecosystems. Our knowledge is still poor regarding the interactive effects of water and N addition on soil microbial communities, although this information is crucial to reveal the mechanisms of the terrestrial ecosystem response to global changes. We addressed this problem by conducting a field experiment with a 15% surplus of the average rainfall under three levels of N addition (50, 100, and 200 kg N ha^{-1} yr^{-1}) in two consecutive years in Inner Mongolia, China. Microbial community composition and functional diversity were analyzed based on phospholipid fatty acids (PLFA) and BIOLOG techniques, respectively. The results showed that water addition did not affect the soil microbial community composition, but much more yearly precipitation generally decreased the PLFA concentration, which implied a fast response of soil microbes to changes of water condition. Soil fungi was depressed only by N addition at the high level (200 kg N ha^{-1} yr^{-1}) and without hydrologic leaching, while Gram-negative bacteria was suppressed probably by plant competition at high level N addition but with hydrologic leaching. The study found unilateral positive/negative interactions between water and N addition in affecting soil microbial community, however, climate condition (precipitation) could be a significant factor in disturbing the interactions. This study highlighted that: (1) The sustained effect of pulsed water addition was minimal on the soil microbial community composition but significant on the microbial community functional diversity and (2) the complex interaction between water and N addition on soil microbial community related to the inter-annual variation of the climate and plant response.

Keywords: water addition, nitrogen addition, phospholipid fatty acid (PLFA), BIOLOG-substrate utilization, semiarid steppe

1. Introduction

Precipitation changes and increasing nitrogen (N) deposition are two important components of ongoing global changes (Dore 2005; Gruber and Galloway 2008). It is predicted that precipitation will increase at high latitudes and decrease in most subtropical regions (IPCC 2007). N deposition is

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presently increasing in most terrestrial ecosystems, and the projected rate was estimated to be as double as the current values by 2050 (Galloway *et al.* 2004). It had exceeded the critical loads that have detrimental impacts on the ecosystems (Achermann and Bobbink 2003; Galloway *et al.* 2008). These predicted changes may in turn influence semiarid grasslands because of the limiting role of water and N in these ecosystems. On the other hand, water and N are two coupling factors in grassland ecosystems (Hooper and Johnson 1999; Niu *et al*. 2009). Firstly, the mobility and availability of N depends on water, for example, through wet N deposition and the contents of soil dissolved inorganic N (Fenn *et al.* 2003; Harpole *et al.* 2007). Secondly, soil N addition could enhance the photosynthetic capability of plant through increasing leaf chlorophyll content and the activity of Robisco, which is an important enzyme in photosynthesis (Wang *et al*. 2012; Lin *et al.* 2013), and lead to greater plant transpiration and more rapid loss of soil moisture from the rooting zone. Thirdly, N addition might increase plant productivity as mentioned above, thereby increasing canopy light interception and reducing rates of evaporation at the soil surface (Harpole *et al.* 2007). Therefore, study on the water and N synchronously is important in evaluating the real response of ecosystem to the global change.

Soil microbes are primary mediators of organic matter decomposition and nutrient cycling, and thus play a key role in maintaining function and sustainability of terrestrial ecosystems (Lou *et al.* 2011). Some studies had reported that changes of precipitation and N deposition could affect soil microbial community directly by changing the microbial living environment (Drijber *et al.* 2000; Grayston *et al.* 2004) and indirectly by influencing plants (Lü *et al.* 2011; Gutknecht *et al.* 2012). However, no consistent results had been found in different studies. It was generally believed that the microorganisms themselves had different adaptabilities to changes of environmental water condition. For example, fungi is more tolerant to dry condition than bacteria, while Gram-negative bacteria is more sensitive to soil water change (Nesci *et al.* 2004; Manzoni *et al.* 2012). However, evidences suggested that water addition could increase the relative abundances of soil fungi and Gram-negative bacteria (Bell *et al.* 2014) and resulted in greater fungi/bacteria ratios (Williams and Rice 2007). The unexpected results for fungi had been attributed to the complex relationship between various biotic and abiotic factors in soil under changed water status (Drenovsky *et al.* 2004; Williams and Rice 2007), which still needs more specific studies to figure out the exact responding mechanisms. Similar situation happened in researches about the effects of N addition on soil microbial community. It was generally reported that N addition could shift soil microbial community to a status with relatively lower proportion of fungal groups (Frey *et al.* 2004; Demoling *et al.* 2008; Zhang N *et al.* 2013). However, there also existed studies that reported positive effect (Yevdokimov *et al.* 2012) and non-effect (Rousk *et al.* 2011) of N addition on the fungal proportion in soil microbial community. Moreover, our knowledge about the interaction between water and N addition, which has more realistic significance for our global-change prediction, is still rather poor. Therefore, comprehensive studies with full consideration about effects of water addition, N addition and their interactions on the soil microbial community are in great demand.

Semiarid grassland ecosystems, one of the most extensive ecosystems in China, are notably sensitive to climate variation (Chen *et al.* 2009). Predictions made by model analyses indicate that precipitation would increase by 12–18% in the semiarid steppe in North China (IPCC 2007; Liu *et al.* 2010; Feng *et al.* 2011), and a large amount of N deposition in this region is also observed (Liu *et al.* 2011; Zhang *et al.* 2011). To address how increased precipitation and N deposition, as well as how their interactions, influence microbial communities and soil microbial C utilization profile, we conducted a field experiment in which water and N levels were manipulated to simulate the future changes of precipitation and N deposition. We hypothesized that water addition would increase the richness of bacteria comparing to fungi because of the dry condition in the experimental area, while N addition would suppress the abundance of fungi but different levels of N addition could have different influences, considering there might exist some dose effects for N addition (Sheppard *et al.* 2013; Zhang C *et al.* 2013), and the interactions between water and N will influence soil microbial community composition and functional diversity. Our objectives were to investigate: (1) the impacts of water and N additions at different levels on microbial community composition and function; (2) whether there is any interaction between water and N effects on microbial community characteristics; and (3) how the inter-annual variability of the soil microbial community responds to water and N additions.

2. Results

2.1. Soil physiochemical properties

Significant interactions among water, N addition and sampling year were observed in soil dissolved inorganic N (DIN), moisture and total soil organic carbon (TOC) (Table 1). Soil moisture and TOC were higher in 2012 than in 2011, while DIN was significantly lower in 2012 (Table 1). Water addition decreased soil DIN in 2011, and increased soil TOC in 2012. Soil moisture was improved in both of the years under water addition treatment (Table 1). However, N addition decreased soil moisture in 2012. The soil DIN increased sharply with increasing N addition in both years regardless of the water