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Differential seasonal effects of water addition and nitrogen fertilization on microbial biomass and diversity in a temperate desert

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ABSTRACT

We investigated seasonal changes of soil microbial respiration (MR), biomass carbon (MBC) and nitrogen (MBN) and bacterial and fungal community composition in response to manipulative water addition and N fertilization over three years in the Gurbantunggut Desert, Northwestern China. Water addition significantly interacted with season to affect MR, MBC and MBN, with significant promotions of MR by 59.4–107.7%, MBC by 43.1–43.5% and MBN by 56.3–91.4% in summer and autumn, and no impacts in spring and winter. Water addition increased bacterial biomass in summer and autumn, bacterial and fungal diversity and abundance in summer, and altered microbial community composition by increasing the relative abundance of *Proteobacteria* phylum and decreasing *Ascomycota* phylum. Nitrogen fertilization increased microbial biomass by 14.0% and fungal biomass by 30.0% in winter, and altered microbial community composition in winter and spring by increasing the relative abundance of *Ascomycota* phylum and decreasing *Actinobacteria* phylum. Our results indicate the high sensitivity of soil microbial communities to water and nitrogen availability in a temperate desert. The projected increase in precipitation and N deposition in the desert may induce corresponding changes in ecological processes that involve microbes. The results also highlight the importance of seasonal effects on the responses of soil microbial community, which should be considered in ecosystem models.

1. Introduction

Soil microbial communities are impacted by various facets of global change, including variable precipitation and increasing nitrogen (N) deposition (Bell et al., 2008, 2010, 2014; Clark et al., 2009), therefore, understanding how microbial communities respond to long-term changes in water and N availability is critical to predict ecosystem responses and contributions to carbon (C) and N cycles (Bell et al., 2008, 2014; Eilers et al., 2010; Gutknecht et al., 2012). However, we are currently not clear how microbial communities respond to increasing precipitation and N deposition in situ across variable environments in deserts (Makhalanyane et al., 2015; Steinberger et al., 1999; Treseder, 2008; Van Horn et al., 2014).

Soil moisture regulates substrate diffusion rates for microbial utilization and cellular hydration (Harris, 1981; Manzoni et al., 2012). In previous studies, additions of N have increased N availability, decreased soil pH and ion concentration, sometimes causing toxicity to soil microbes (Vitousek et al., 1997), and enhance plant growth (LeBauer and Treseder, 2008; Lu et al., 2011). Compared to N depositon, water availability is a primary driver for changes in microbial rRNA (Bell et al., 2008; Clark et al., 2009) or lipid (Bachar et al., 2010; Steinberger et al., 1999) community structure, and microbial functions, including microbial respiration (Bell et al., 2008; Cregger et al., 2012). However, using water availability to predict soil microbial responses to simultaneous changes in precipitation and N deposition would be complicated by other seasonal fluctuations, primarily temperature and precipitation, as well as concomitant effects on vegetation productivity and community composition (Bachar et al., 2010; Bell et al., 2008; Bell et al., 2010).

Seasonal fluctuations in temperature and precipitation exert profound influences on soil microbial community composition and functions, such as N mineralization, NH_4^+ and NO_3^- cycling (Parker and Schimel, 2011), and SOM mineralizaiton (Jia et al., 2014). Generally, due to the pulse patterns of water and biogeochemical cycles in deserts (Austin et al., 2004), microbial biomass and activities in dry seasons may be more responsive to increasing water availability than in wet seasons, and N fertilization in dry season may be slight due to the high N volatilization, low substrate diffusion and microbial activities (Bell

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et al., 2010; Gallardo and Schlesinger, 1995; Zhang et al., 2015). Therefore, the respones of the soil microbial community to water addition and N fertilization are season-dependent, and the response can be delayed from days to weeks (Bell et al., 2010; Cruz-Martinez et al., 2009; Slaughter et al., 2015).

Responses of the soil microbial community composition and respiration to increasing precipitaiton depend largely on the antecedent soil moisture in drylands. Bell et al. (Bell et al., 2014) reported no variaiton in microbial (16S rRNA) communitiy structure in response to a 25% increase in seasonal rainfall in the first two years, but the community showed higher microbial biomass in years 3–5 in Chihuahuan Desert grasslands. In a four-year experiment, soil bacterial and archaea communities estimated using 16S rRNA gene microarrays showed no difference between rainfall addition and control in a grassland (Cruz-Martinez et al., 2009). The temporal-dependent responses of soil microbial communities to climate change parameters may be tightly related with available carbon pool and the critical ratios of soil nutrients which greatly determine microbial growth and nutrient use efficiency (Bradford et al., 2008). Microbial community structure estimated using phospholipid fatty acid analysis has shown similar patterns between water addition and ambient precipitation treatments in many arid and semiarid ecosystems (Cruz-Martinez et al., 2009; Gutknecht et al., 2012; Zhang et al., 2013), while altered bacterial community using 16S rRNA under water addition has also been observed (Cregger et al., 2012; Van Horn et al., 2014). These studies were primarily conducted in the hot season of summer, which ignored seasonal variations in temperature and precipitation as well as concomitant variations in vegetation effects on microbial responses.

To fill this gap in knowledge and contribute to the understanding of soil microbial responses to increasing precipitation and N deposition in desert ecosystems, we used a 3-yr multi-factor field experiment in a temperate desert in Central Asia. Our objective was to determine how soil microbial communities respond to water addition and N fertilization at seasonal and year around temporal scales using a factorial combination of water addition (+ 30% of annual precipitation) and N fertilization (50 kg ha^{-1} yr^{-1}). Given the temperate location and distinct seasonality of the climate (cold, wet winters and hot, dry summers), we hypothesized that the effects of water addition on soil microbial communities would be stronger in hot and dry seasons than in cold and wet seasons, N fertilization effects on soil microbial communities would depend on soil moisture, with stronger effects in wetter soils.

2. Materials and methods

2.1. Study site description

The study site was in the vicinity of the southeastern fringe of the Gurbantunggut Desert, northwestern China (44°17′N, 87°56′E and 475 m a.s.l.). This region has an arid temperate continental climate, with a hot, dry summer and cold, wet winter. The annual mean temperature is 6.6 °C, with mean temperature of 25.6 °C in the dry and hot season of July and − 19.4 °C in the wet and cold season of January. The annual mean precipitation is 160 mm, of which 60% to 70% is distributed in the plant growth season from late April to early September. Soils are desert solonetz, with aeolian sandy soil at the top (0–100 cm). The shrubs are primarily *Haloxylon ammodendron*, *Haloxylon persicum*, and *Tamarix ramosissima*, with coverage of ca. 30%. The herbaceous layer is composed of spring ephemerals and spring-summer annuals, with a coverage reaching 40% at growth peak (Huang et al., 2015). The dominant spring ephemerals include *Erodium oxyrrhynchum*, *Alyssum linifolium*, *Schismus arabicus*, and *Lactuca undulata*. Spring ephemerals account for > 85% of the herbal biomass. Spring-summer annuals are primarily composed of *Salsola subcrassa*, *Ceratocarpus arenarius*, *Seriphidiam santolinum*, and *Agriophyllum squarrosum*.

2.2. Experimental design and sample collection

The experiment used a completely randomized block design with four treatments - control (C), water addition (W), N fertilization (N), and water addition plus N fertilization (WN), with each treatment replicated six times. A total of 24 plots were arranged in a 4 × 6 matrix, each plot was 10 × 10 m, with a 10 m wide buffer zone between adjacent plots. In the W and WN treatments, water was added equivalent to 30% of the annual precipitation, in accordance with predictions for northern China over the next 30 years from 2001 to 2030 (Liu et al., 2010). The 30% extra precipitation was collected using “precipitation collection pans”. The pans were constructed from galvanized iron sheets, with an area of 1.9 × 1 m, a total of 18 pans were installed in each plot. Each pan was erected at a slight angle, and the intercepted precipitation was collected in a bucket which was buried in the soil. Immediately after a rainfall event, the collected rain was evenly sprayed onto the plots during the late afternoon to prevent excessive evaporation. Snow fallen in the pan was also evenly added to the corresponding plot before snowmelt. In the N and WN treatments, N was applied in liquid form: 1667-g NH_4NO_3 was diluted in 15l distilled water and evenly sprayed onto the corresponding plots in early April and mid-July. The same amount of distilled water (15l) was added in the C and W treatments. N fertilization (corresponding to 50 kg ha^{-1} yr^{-1}) was based upon the real mean airborne N deposition rate (36 kg ha^{-1} yr^{-1}) registered in this region in 1996–2006 (He et al., 2007). The experiment was arranged in September 2010, and water and N treatments were applied every year since the beginning of 2011.

After two years of experimental treatment, five soil cores (5 cm in diameter, 5 cm in depth) were collected in each plot on 15 November in 2012 (winter), 22 March (spring), 10 May (summer), and 20 August (autumn) in 2013, respectively. After removing plant roots, stones and debris using a 2-mm sieve, soil samples were mixed and packed into a portable refrigerated box and transported to the laboratory for soil and microbial properties measurements. For microbial respiration and microbial biomass measurements, soil samples from each plot were measured and each treatment was replicated six times; for microbial composition measurement, soils in two adjacent plots were further mixed as one sample, and each treatment was replicated three times. After sample collection, soil moisture (0–5 cm) was measured immediately using time domain reflectometry (Diviner-2000, Sentek Pty Ltd., Balmain, Australia) on 10 May and 20 August. Because surface soil was frozen at sampling on 15 November and 22 March, soil mass water content was measured by weighting the wet and dry mass of soil, and then transformed to soil volumetric water content (SVWC). Soil inorganic N (In-N) was measured by extraction with 2 M KCl and measured with the Auto Analyzer 3 (AA3, BRAN-LUEBBE Ltd., Hamburger, Germany). Dissolved organic carbon (DOC) and dissolved nitrogen (DN) was extracted by adding 50 ml of 0.5 M K_2SO_4 to subsamples of 12.5 g homogenized soil and agitating it on an orbital shaker at 120 rpm for 1 h. The filtrate was analyzed using a TOC analyzer (multi N/C 3100, Jena, Germany). Air temperature and precipitation were monitored hourly by an automatic meteorological station (Campbell Science Equipment, Logan, UT, USA). A thermocouple (microlab lite, Fourier Technologies Ltd., Israel) was placed in each plot to record soil surface temperature hourly throughout the experiment.

2.3. Microbial respiration measurements

Microbial respiration (MR) was measured as CO_2 evolution of fresh soil samples at 60% of water-holding capacity and incubated in sealed containers for 72 h at 25 °C. The CO_2 efflux from the soil was determined by the alkali absorption method (Page et al., 1982). Before incubation, 5 ml of 50 mM NaOH solution was injected into the connecting tube to absorb CO_2 released from the soil, and the respired carbon was determined by titrating the residual OH^- with a