



Short Communication

Nitrogen deposition combined with elevated precipitation is conducive to maintaining the stability of the soil fungal diversity on the *Stipa baicalensis* steppe

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ABSTRACT

Changes in nitrogen (N) deposition and precipitation can strongly influence grassland ecology. However, the combined effects of N and water addition on soil fungal genetic diversity, and especially the underlying influencing factors, are not well understood. We investigated the response of soil fungal α diversity to experimental N and water addition in a northern Chinese steppe. We found that N addition alone exceeding N100 (100 kg N·ha⁻¹·yr⁻¹) significantly increased soil fungal α diversity. However, when water was added, soil fungal diversity did not significantly change following N enrichment. Structural equation modelling (SEM) results indicated that N addition increased soil fungal α diversity, while N addition combined with irrigation maintains soil fungal diversity that would otherwise be enhanced by N addition. This results from reversing the correlation between soil fungal diversity and plants\soil physicochemical properties. Our results provide evidence that the effects of N deposition regarding soil fungal diversity might be overestimated if we ignore variations in water availability.

Soil fungal communities are functionally diverse groups (Paungfoo-Lonhienne et al., 2015) that mediate many ecological processes and influence plant growth and soil health (Klaubauf et al., 2010). It has been widely reported that increased nitrogen (N) deposition and climate change (i.e. altered precipitation) contributed to shifts in the soil fungal community (Mitchell et al., 2010; Jumpponen and Jones, 2014; Cassman et al., 2016), which can indicate the sensitivity of soil carbon cycles and ecosystem functions to climate change (Hawkes et al., 2011). The average annual atmospheric N deposition in China increased 0.41 kg N·ha⁻¹ between 1980 and 2010 (Liu et al., 2013a). Previous studies have shown that N addition decreases fungal biomass (Demoling et al., 2008; Contosta et al., 2015), reduces the relative abundance of some fungal phyla (Mueller et al., 2014; Leff et al., 2015) and reduces fungal biodiversity (Zhou et al., 2016). Precipitation change is one of the most important phenomena associated with global climate change (Liu et al., 2013b) and it directly affects the soil water balance and determines the availability of water to plants and microorganisms (Sorensen et al., 2013). Water addition directly affects the soil fungal diversity (Hawkes et al., 2011) and can modify the effects of N on soil microbes (Zhang et al., 2015). There have been several studies

addressing the combined effects of N and water addition on soil bacteria (Bi et al., 2012; Zhang et al., 2014), soil fungal biomass (Ma et al., 2016), soil fungal functional diversity (Zhang et al., 2015) and Arbuscular Mycorrhizal (AM) fungi (Li et al., 2015). However, there are still no reports on the combined effects of N addition and precipitation changes on soil fungal genetic diversity.

Here, we conducted a factorial field experiment in a semi-arid grassland on the intact *Stipa baicalensis* (*S. baicalensis* Roshev) (Sun and Guo, 1987) steppe in Hulun Buir of northern China (119°42'E, 48°30'N). The *Stipa baicalensis* steppe is endemic in Central Asian steppe region. The soil is dark chestnut (Chinese classification), which is one of the main types of soil in the Inner Mongolia Autonomous Region, China. Since 2010, N was added at eight levels: 0 (denoted as CK), 15, 30, 50, 100, 150, 200, and 300 kg N·ha⁻¹ yr⁻¹ (in addition to atmospheric N deposition), which are referred to as N0, N15, N30, N50, N100, N150, N200, and N300, respectively. Our N15 (15 kg N·ha⁻¹·yr⁻¹) treatment is the real N input in the study region, while the N30-300 were designed to simulate the potential path for this region, as N deposition has been increasing (Liu et al., 2013a,b). N was added in the form of ammonium nitrate (NH₄NO₃). In this area,

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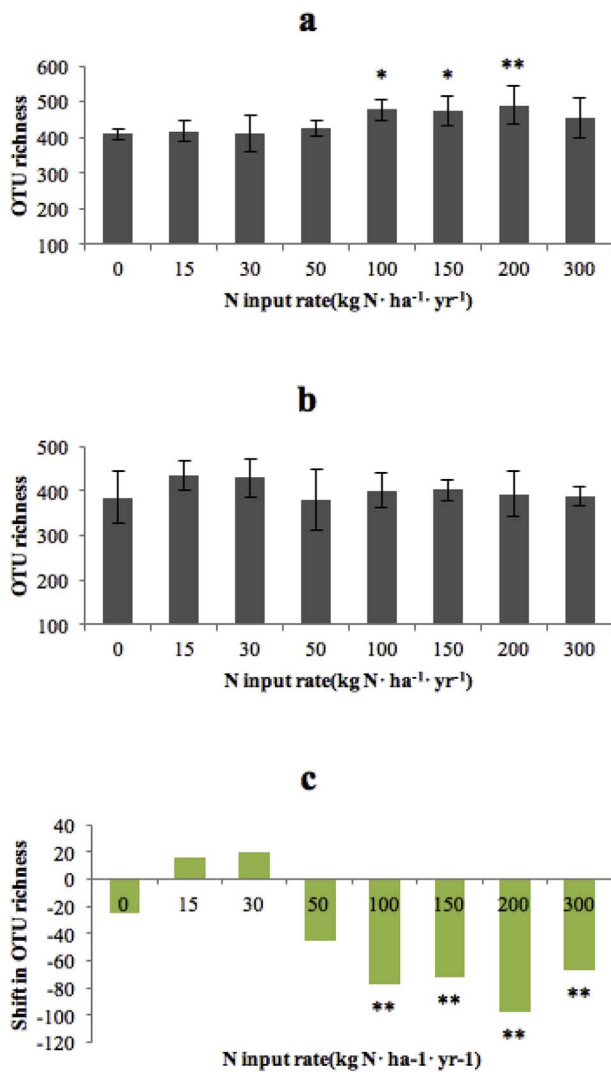


Fig. 1. OTU richness of soil fungi under (a) normal precipitation and (b) irrigation. (c) Shifts in OTU richness induced by irrigation at identical N levels. * and ** denote $P < 0.05$ and $P < 0.01$, respectively. Error bars indicate the standard deviation of six replicates per treatment.

precipitation has increased steadily since 1959. Historical observations show that the average annual precipitation in the *Stipa baicalensis* steppe in 1987–2014 was 6.4% higher than that in 1959–1986. The average rainfall is 327.4 mm (1959–2010). We adopted two irrigation regimes - no irrigation (only N addition) and 100 mm of added water, equivalent to almost 30% enhanced annual rainfall. Each treatment was replicated six times in 96 plots of 8 m × 8 m each with 5 m isolation belt, with a total experimental area of approximately 1.4 ha. Plant sampling was performed on the 15th and 16th August in 2013, and more detailed information on plants is found in Zhang et al. (2017). We also sampled soil in the 96 plots after plants were collected. In each plot, 10–15 mixed soil subsamples were collected at a depth of 0–20 cm, with 6 replicates per treatment with a mass of 2 kg soil into aseptic self sealing bags. Each soil sample was passed through a 2 mm sieve and then sealed in an aseptic self sealing bag, placed in an ice box and returned to the laboratory for analysis of total organic carbon-TOC, total nitrogen-TN, NO₃⁻-N, NH₄⁺-N and pH. The other soil was stored at -20 °C for soil DNA extraction.

Fragments of the ITS1-ITS2 region were amplified via PCR (Mackelprang et al., 2011) and sequenced using the Illumina MiSeq platform. Detailed analysis of data is provided in the supplementary material. The raw sequence data were submitted to the National Center

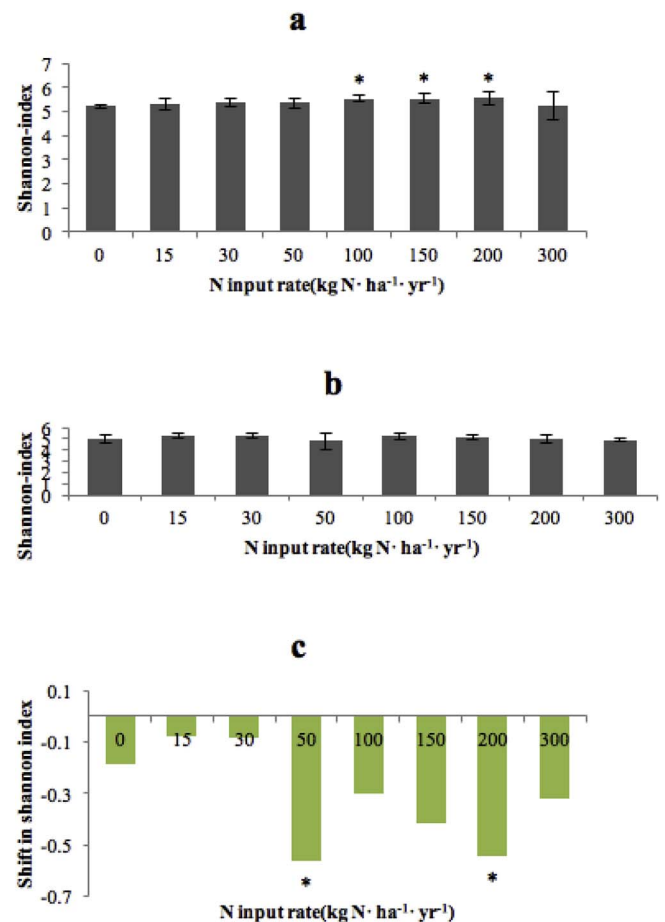


Fig. 2. Shannon diversity index of soil fungi under (a) normal precipitation and (b) irrigation. (c) Shifts in the Shannon index induced by irrigation at identical N levels. * and ** denote $P < 0.05$ and $P < 0.01$, respectively. Error bars indicate the standard deviation of six replicates per treatment.

for Biotechnology Information Sequence Reads Archive under accession No. SRP078091. One-way ANOVA was used to evaluate the effects of the N and water addition on soil fungal α diversity. All analyses were performed with SAS v9.2 (SAS Institute Inc., Cary, NC, USA). Structural equation modelling (SEM) was performed to investigate the integrated responses of the overall plant-soil-microbe system (Wei et al., 2013). The original data applied in the SEM could be found in Table S1 of the supplementary material. We hypothesized that soil fungal diversity would be reduced or enhanced under N fertilization; however, the effects of N fertilization could be altered by additional water availability.

N addition alone ($> 100 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$) significantly ($P < 0.05$) increased fungal OTU richness and the Shannon index at N100-N200 (Figs. 1a and 2a), and the Chao1 index at N100-N300 (Fig. 3a). Mueller et al. (2014) also found that N addition increased fungal richness. However, when water was added, these three indexes did not significantly change following N enrichment (Figs. 1b, 2b and 3b). At identical N levels, irrigation modified the effects of N addition on fungal diversity (Figs. 1c, 2c and 3c). Previous studies also reported that the soil fungal communities are resilient to predicted environmental changes, i. e. precipitation and nitrogen fertilization (Jumpponen and Jones, 2014; Li et al., 2015). The impacts of climate change on soil fungal diversity might be difficult to predict from single-factor experiments (Eisenhauer et al., 2012).

Structural equation modelling (SEM), an advanced and robust multivariate statistical method, allows hypothesis testing of complex path-relation networks (Grace et al., 2007). Wei et al. (2013) has employed SEM to explore the complicated relationship in plant-soil-

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