# The diversity and co-occurrence patterns of diazotrophs in the steppes of Inner Mongolia

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## ARTICLE INFO

## ABSTRACT

Soil diazotrophs possess the function of fixing atmospheric $N_2$ into biologically available ammonium in terrestrial ecosystems. However, there are limited studies into how diazotrophic communities change when grassland environments differ. In this study, we elucidated the diversity and interactions of diazotrophic communities in three steppe types of Inner Mongolia, China, including non-desert steppe (MT), desert steppe (D) and sandy steppe (S). We expected to improve the predictions of diazotrophic responses to grassland degradation at time gradients based on their discrepancies among steppe types along spatial gradients. Steppe types affected the alpha- and beta-diversity of diazotrophic communities, which were mainly controlled by plant biomass and soil pH, respectively. Diazotrophic communities were strongly aggregated with their respective steppe environments, but dissimilar under different types according to Bray-Curtis distance. In addition, network analysis showed that the network structure of diazotrophs from the D was relatively simpler than the other two types (MT and S). More exclusionary relationships in diazotrophic communities occurred in the D and S steppes than in the MT steppe. The species shared across the steppes changed their interactions with other species to adapt to different habitats. Moreover, different steppes had distinct key species to underpin the whole network. The disturbances or extinctions of these key species may lead to the instability of the diazotrophic co-occurrence network. Overall, we speculate that if grassland degradation happens, the change of grassland microenvironment may mediate the differences in the diversity and co-occurrence patterns of diazotrophs, allowing them to adapt to environmental changes.

## 1. Introduction

Nitrogen input and availability are key factors regulating the organization and metabolism of terrestrial biomes (Boring et al. 1988). However, nitrogen limitation results in insufficient availability of N for primary production or other productive activities (Vitousek and Howarth 1991). Nitrogen-fixing organisms are responsible for the biological fixation of atmospheric nitrogen and the process is the major pathway for nitrogen inputs to the terrestrial ecosystem (Galloway et al. 2004). Therefore, elucidating the diversity and interactions of nitrogen-fixing communities is invaluable for understanding soil N supply processes. The *nifH* gene, which encodes the iron protein subunit of nitrogenase, is a common biomarker for studying nitrogen-fixing communities (Zehr et al. 2003).

Today, climate change and anthropogenic stress frequently affect grassland ecosystems, often leading to habitat transformation (Porter et al. 2013). Habitat type and quality are important factors in maintaining biodiversity and stability of ecosystem structure and function (Chen et al. 2013; Li et al. 2014; Li et al. 2016; Wang et al. 2015; Zhou et al. 2016). Inner Mongolian steppe is located in arid and semi-arid region of China, where the steppe ecosystem is especially sensitive to natural and anthropogenic disturbances (Wang et al. 2017; Zhao et al. 2014). In recent decades, the observed rainfall has decreased in the Inner Mongolia grassland and this region has become warmer, reflecting a trend of increasing aridity during this time (Li et al. 2012; Xue 1970). Directional changes in aridity as a result of global warming may maintain current trends towards grassland degradation and desertification in sensitive steppe regions. Recent studies have reported that about 70% of this region is now considered degraded due to this climate variation or human activities (e.g. prolonged overgrazing, poor farmland management and over-lumbering) (Hilker et al. 2014).

Previous studies show that the desertification of a habitat may lead to losses of biological diversity, changes of species composition, or disappearances of sensitive organisms (Zhao et al. 2014). Moreover,

**Table 1**
Environmental parameters in three steppe types.

| Sample sites | MT | | D | | S | |
|---|---|---|---|---|---|---|
| | HL | XL | XS | XZ | EE | TL |
| Longitude (E) | 120°12′E | 116°64′E | 112°70′E | 111°78′E | 110°19′E | 120°71′E |
| Latitude (N) | 49°35′N | 43°58′N | 42°80′N | 41°70′N | 39°50′N | 42°94′N |
| Altitude (m) | 669.60 | 1263.60 | 1112.20 | 1473.80 | 1293.80 | 358.40 |
| PC (%) | 92.00 | 70.00 | 28.00 | 46.00 | 45.00 | 73.00 |
| PR | 8.80 | 7.00 | 4.20 | 7.40 | 4.60 | 4.40 |
| MAT (°C) | -1.44 | 1.36 | 4.33 | 3.53 | 6.66 | 6.83 |
| MAP (mm) | 383.00 | 334.00 | 198.00 | 221.00 | 414.00 | 399.00 |
| PDW (g) | 60.00 ± 14.68c | 15.85 ± 5.54a | 8.80 ± 4.17a | 6.00 ± 1.61a | 17.05 ± 12.04a | 31.50 ± 13.99b |
| pH | 7.17 ± 0.24a | 7.50 ± 0.21b | 8.02 ± 0.06c | 8.39 ± 0.14d | 8.24 ± 0.13d | 8.47 ± 0.20d |
| Conductivity (μS/cm) | 38.68 ± 5.18ab | 23.06 ± 12.75a | 76.84 ± 22.84de | 86.34 ± 18.19e | 50.04 ± 16.49bc | 63.70 ± 9.07 cd |
| TOC (%) | 4.24 ± 0.41c | 1.17 ± 0.64b | 0.52 ± 0.04a | 1.11 ± 0.07b | 0.40 ± 0.20a | 0.51 ± 0.19a |
| TN (%) | 0.41 ± 0.05e | 0.17 ± 0.03d | 0.07 ± 0.02b | 0.11 ± 0.01c | 0.03 ± 0.01a | 0.05 ± 0.02ab |
| $NH_4^+$-N (mg/kg) | 10.98 ± 3.15c | 6.25 ± 1.45b | 2.46 ± 1.18a | 5.05 ± 2.15ab | 5.19 ± 1.81ab | 3.55 ± 3.10ab |
| $NO_3^-$-N (mg/kg) | 2.06 ± 0.95a | 1.42 ± 0.19a | 1.80 ± 0.89a | 3.94 ± 1.66b | 3.96 ± 1.63b | 3.58 ± 0.99b |
| MBC (mg/kg) | 780.93 ± 272.8c | 571.38 ± 194.15bc | 314.25 ± 96.75a | 400.97 ± 127.56ab | 328.74 ± 140.54ab | 344.80 ± 154.78ab |
| MBN (mg/kg) | 98.31 ± 34.10a | 78.19 ± 44.32a | 50.10 ± 18.05a | 55.76 ± 15.52a | 57.12 ± 36.37a | 76.16 ± 42.66a |

Values are means with standard deviations ($n = 5$). ANOVA was used to test the differences between steppe types. Different letters (a,b,c) represent significant differences ($p < 0.05$). PC: Plant coverage; PR: Plant richness; PDW: Plant dry weight (50 cm x 50 cm); MAT: Mean average temperature; MAP: Mean average precipitation; TOC: Total organic carbon; TN: Total nitrogen; MBC: Microbial biomass carbon; MBN: Microbial biomass nitrogen.

such environmental changes or habitat transformations play dominant roles in microbial community assembly. Numerous studies indicate that most microorganisms are not cosmopolitan among habitat types (Lozupone and Knight 2007; Wang et al. 2013a). In terrestrial systems, diazotrophic community size and composition can vary according to soil environments (Izquierdo and Nüsslein 2006), soil types (Collavino et al. 2014), soil use history (Izquierdo and Nüsslein 2015), soil management (Ashok et al. 2006) and vegetation (Junier et al. 2009; Mao et al. 2013). Walker et al. (2008) report that the site is the dominant factor differentiating diazotrophic communities, reflecting the variation in plant community composition, soil characteristics, moisture and temperature that combine to create the unique characteristics of each site. However, little attention has been paid to the variabilities of diazotrophic communities among various steppes in the Inner Mongolia grassland.

Natural ecosystem represents a complex network of potential interactions among microbes, such as predation, competition and mutualisms (Faust and Raes 2012). Previous studies have examined the complex interactions among microbial species and the correlations between microbial modules and environmental factors based on network analysis, which shed light on how particular microbes occur together in a system and how they vary with environmental conditions (Fuhrman 2009; Steele et al. 2011). For example, a previous study shows that *nifH*-containing microorganisms exhibit more complex positive interactions under elevated carbon dioxide (Zhou et al. 2010). Under environmental change conditions, the responses of co-occurring assemblages of organisms to the fluctuations can indicate which conditions the organisms prefer or avoid (Fuhrman 2009). As a whole, comparing different networks will allow exploration of the resilience and resistance of different communities in response to environmental changes (Albert et al. 2000; Montoya et al. 2006; Steele et al. 2011).

However, it remains unclear how the diversity and interactions of nitrogen-fixing communities respond to different steppe types in Inner Mongolia grassland. Therefore, we selected non-desert steppe (MT), desert steppe (D) and sandy steppe (S) in this region to investigate the influences of steppe types on diazotrophs, and provided a theoretical basis for maintaining the grassland ecosystem N cycling balance and stabilization. This study addressed the following key questions: (1) What are the differences in the compositions and diversity of diazotrophs among three steppes? Are diazotrophs sensitive to the changes of steppe types? (2) How do diazotrophs interact in different habitats?

How do the co-occurrence patterns of diazotrophs change when in face of different habitats? According to the comparative analysis in this study, we intended to provide important information about the diversity and interactions of $N_2$-fixing communities across different steppe types along spatial gradient, and to predict the responses of diazotrophs to grassland degradation at time gradient in the Inner Mongolia.

## 2. Materials and methods

### 2.1. Site descriptions

The research sites at Inner Mongolia (97°12′ E ~ 126°04′ E, 37°24′ N ~ 53°23′N, elevation varies from 353 m to 1476 m) is located in temperate climate zone of northern China (Fig.S1). Mean annual precipitation is diminishing from east to west (from 414 to 198 mm). Therefore, the area is divided into five climatic regions from the northeast to southwest according to aridity: humid, semi-humid, semi-arid, arid, and extreme-arid (Fan et al. 2009). To compare the responses of diazotrophs to non-desert steppe (MT), desert steppe (D) and sandy steppe (S), six distinct sites were investigated in this study: Hulun Buir (HL), Tongliao (TL), Xilingol League (XL), Xisu Banner (XS), Siziwang Banner (SZ) and Eerduosi (EE) along the climate gradient. Non-desert sampling sites was located at meadow and typical steppes (HL and XL), where average vegetation coverage was about 81%. *Stipa baicalensis*, *Leymus chinense* and *Stipa grandis* and other herbaceous species are the dominant plants in non-desert steppe. Desert steppe (XS and SZ) and sandy steppe (TL and EE) have lower average vegetation coverage (37% and 59%, respectively) than non-desert steppe, at which soils are susceptible to be eroded by wind leading to the decrease in soil qualities. Their dominated vegetation type is shortgrass or shrub. Soil samples were collected from the upper 10 cm after removing the plant litter on the surface. Each site contained five plots (1 m × 1 m), and five soil cores in each plot were collected and mixed. Thus, a total of 30 soil samples were collected. Roots and stones in soils were removed with 2 mm sieve, and then one part was stored at 4 °C for soil property measurements and the other part at − 80 °C for total DNA extraction. The detailed information about environmental variations was found in Table 1and Table S1. In the field sampling, plant richness (PR), plant coverage (PC) and plant dry weight (PDW) were investigated based on routine survey approaches.