Science of the Total Environment 639 (2018) 997–1006

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Autotrophic and symbiotic diazotrophs dominate nitrogen-fixing communities in Tibetan grassland soils

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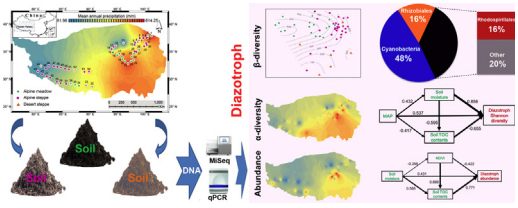
HIGHLIGHTS

- Soil diazotrophs were studied across 54 grasslands on the Tibetan Plateau.
- Cyanobacteria and Proteobacteria dominated the diazotroph communities.
- Biological crust and symbiotic nitrogen fixation may serve as vital N sources.
- Diazotroph abundance and diversity distributed according to mean annual precipitation.
- Diazotroph distribution may be mainly driven by soil moisture and nutrient contents.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 March 2018
Received in revised form 16 May 2018
Accepted 19 May 2018
Available online xxxx

Editor: J Jay Gan

Keywords:
Tibetan Plateau
Soil microbes
Biogeography
MiSeq sequencing
Biological nitrogen fixation
Grassland

ABSTRACT

Biological nitrogen fixation, conducted by soil diazotrophs, is the primary nitrogen source for natural grasslands. However, the diazotrophs in grassland soils are still far from fully investigated. Particularly, their regional-scale distribution patterns have never been systematically examined. Here, soils (0–5 cm) were sampled from 54 grasslands on the Tibetan Plateau to examine the diazotroph abundance, diversity, and community composition, as well as their distribution patterns and driving factors. The diazotroph abundance was expressed as *nifH* gene copies, measured using real-time PCR. The diversity and community composition of diazotrophs were analyzed through MiSeq sequencing of *nifH* genes. The results showed that Cyanobacteria (47.94%) and Proteobacteria (45.20%) dominated the soil diazotroph communities. Most Cyanobacteria were classified as Nostocales which are main components of biological crusts. Rhizobiales, most of which were identified as potential symbiotic diazotrophs, were also abundant in approximately half of the soil samples. The soil diazotroph abundance, diversity, and community composition followed the distribution patterns in line with mean annual precipitation. Moreover, they also showed significant correlations with prokaryotic abundance, plant biomass, vegetation cover, soil pH values, and soil nutrient contents. Among these environmental factors, the soil moisture, organic carbon, available phosphorus, and inorganic nitrogen contents could be the main drivers of diazotroph distribution due to their strong correlations with diazotroph indices. These findings suggest that autotrophic and symbiotic diazotrophs are the predominant nitrogen fixers in Tibetan grassland soils, and highlight the key roles of water and nutrient availability in determining the soil diazotroph distribution on the Tibetan Plateau.

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https://doi.org/10.1016/j.scitotenv.2018.05.238
0048-9697/

1. Introduction

Nitrogen availability predominantly limits ecosystem primary productivity globally (Lebauer and Treseder, 2008; Vitousek and Howarth, 1991). Biological nitrogen fixation (BNF) contributes 40–100 Tg nitrogen to the terrestrial ecosystem annually (Vitousek et al., 2013), being recognized as the largest source of reactive nitrogen for our planet (Cleveland et al., 1999; Galloway et al., 2004). BNF is performed by a small fraction of prokaryotes which are collectively known as diazotrophs (Gaby and Buckley, 2011; Raymond et al., 2004). Partly due to the high lateral transfer frequency of nitrogen-fixing genes (Menna and Hungria, 2011; Raymond et al., 2004), diazotrophs are highly diverse in phylogeny (Dos Santos et al., 2012; Gaby and Buckley, 2011; Zehr et al., 2003) and life strategies (e.g., autotrophic vs heterotrophic and symbiotic vs free-living). The high diversity results in a wide distribution of diazotrophs in both aquatic and terrestrial ecosystems (Gaby and Buckley, 2011; Luo et al., 2012; Munoz-Martin et al., 2014; Nelson et al., 2016). Among all the environmental media, soils have been identified to harbor the most diverse diazotrophs (Gaby and Buckley, 2011). Moreover, the abundance, diversity, and community composition of diazotrophs have been recognized as key biotic factors determining soil nitrogen-fixing capacity (Hsu and Buckley, 2009; Lindsay et al., 2010; Reed et al., 2010; Stewart et al., 2011). Therefore, determining the abundance, diversity, and community composition of soil diazotrophs can substantially contribute to the understanding of terrestrial ecosystem nitrogen cycling.

In recent decades, diazotroph abundance, diversity, and community composition have been extensively investigated employing *nifH* gene as a molecular marker (Che et al., 2017a; Che et al., 2018b; Wang et al., 2017a). A global census based on *nifH* gene revealed that Proteobacteria (α, β, and γ; 56%) and Cyanobacteria (10%) were the most abundant soil nitrogen-fixers (Gaby and Buckley, 2011). However, the soil diazotroph abundance, diversity, and community composition are highly variable across study sites, even within the same type of ecosystem (Che et al., 2017b; Tu et al., 2016; Wang et al., 2017b). Thus, illustrating soil diazotroph distribution pattern at a regional scale requires systematic and multiple-site investigations. Nevertheless, unlike the distribution patterns of marine diazotrophs or rhizobia which have been well documented (Luo et al., 2012; Wang et al., 2016; Zhang et al., 2018a), the distribution of soil diazotroph has been rarely examined across multiple sites, until recently (Shay et al., 2015; Tu et al., 2016; Wang et al., 2017b). This situation is even worse for grassland soils, in which the distribution pattern of diazotroph abundance, diversity, and community composition has never been examined at a regional scale.

Tibetan Plateau is the highest plateau in the world. Its total area is about 2.6×10^6 km^2, on which 60% is covered by natural grasslands (i.e., alpine meadow, alpine steppe, and desert steppe). Similar to other natural ecosystems, the primary productivity of the Tibetan grassland is limited by nitrogen availability (Dong et al., 2016; Mishra and Mainali, 2017; Zhang et al., 2018b). Recently, the Tibetan Plateau has been experiencing a significant increase in atmospheric nitrogen deposition (Liu et al., 2013; Lü and Tian, 2007), which may partially alleviate nitrogen limitation for plants. However, on the Tibetan Plateau, the overall nitrogen deposition rate is still much lower than those in other regions (Galloway et al., 2004; Liu et al., 2013). Moreover, anthropogenic nitrogen inputs on the plateau are almost negligible. Consequently, BNF is probably the main source of available nitrogen in this region. In the past decades, soil microbes in Tibetan soils have been extensively studied (e.g., Che et al., 2015; Deng et al., 2013; Zhang et al., 2016b). In particular, the distribution patterns of soil bacteria, archaea, fungi, and even invertebrates have been well investigated on the Tibetan Plateau (Chu et al., 2016; Shi et al., 2016; Yang et al., 2017; Zhang et al., 2016a; Zhao et al., 2017). Nevertheless, soil diazotrophs have only been examined in a few Tibetan alpine meadows (Che et al., 2017b; Wang et al., 2017b; Zhang et al., 2006), which seriously limits our understanding of the nitrogen inputs in this region. Therefore, systematically determining the distribution pattern of diazotroph in the Tibetan grassland soils can not only improve our knowledge on soil diazotroph biogeography, but also provide essential bases for understanding and managing the Tibetan grassland nitrogen cycling.

In this study, soil samples (0–5 cm) were collected from 54 grasslands on the Tibetan Plateau to investigate the abundance, diversity, and community composition of diazotrophs, and to determine their distribution patterns and main driving environmental factors. We also aimed to examine the differences in diazotroph abundance, diversity, and community compositions among alpine meadows, alpine steppes, and desert steppes.

2. Materials and methods

2.1. Study sites and soil sampling

Soils were sampled from 54 grasslands (19 alpine meadows, 29 alpine steppes, and 6 desert steppes) on the Tibetan Plateau (Fig. 1) in August 2014. As illustrated in Table S1, environmental conditions among the sampling sites differed dramatically. In general, the sampling sites were randomly selected along highways with 60–100 km intervals. The perpendicular distances between the sampling sites and the highways were >500 m to attenuate potential disturbances from traffics (Ackermann et al., 2012). At each site, we first surveyed the plant community compositions in five randomly selected quadrats. The aboveground part of each plant species was collected to determine the aboveground biomass and plant community composition. Subsequently, soil samples (0–5 cm) were collected with a 7 cm auger in each quadrat, thoroughly homogenized, and sieved to ≤2 mm. The plant roots in the remaining soils (>2 mm) were collected to measure the belowground biomass. Then, each soil sample was divided into two subsamples (200 g each). One subsample was air-dried, while another was kept at −20 °C during transportation and stored at −80 °C in the laboratory. We also recorded the elevation and coordinates at each site using GPS. In addition, mean annual temperature and precipitation (MAT and MAP; 1980–2014) were obtained from China Meteorological Data Sharing Service System (http://data.cma.cn/site/index.html). The Normalized Difference Vegetation Index (NDVI) at each site around the sampling time was collected from Moderate Resolution Imaging Spectroradiometer (MODIS).

2.2. Soil and plant characterization

Plant biomass was measured after oven drying at 65 °C for 72 h, and soil moisture contents were determined by drying at 105 °C for 24 h. Soil NH_4^+—N and NO_3^-—N contents were determined using KCl solution extraction (soil mass to solution ratio of 1:5) followed by colorimetry with an auto analyzer system (SEAL Analytical GmbH, Norderstedt, Germany). Dissolved organic carbon (DOC) contents in soils were analyzed with the same extraction method and a TOC Analyser (Liqui TOC II; Elementar Analysensysteme GmbH, Hanau, Germany). Soil available phosphorus (AP) contents were measured as depicted by Olsen (1954). Soil pH was determined with the soil mass to water ratio of 1:5, using a pH meter (STARTER 3100, Ohaus Instruments Co., Ltd., Shanghai, China). The contents of soil gravimetric moisture, NH_4^+—N, NO_3^-—N, and DOC were analyzed with field moist soils, while soil pH and available phosphorus contents were determined using air-dried soils.

2.3. Soil DNA extraction and real-time PCR

Soil DNA was extracted from 0.30 g of fresh soil using a PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). The extraction was conducted following the instructions from the manufacturer.

The copies of prokaryotic 16S rRNA and *nifH* gene were quantified using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The quantification was respectively conducted with universal