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Desert and steppe soils exhibit lower autotrophic microbial abundance but higher atmospheric CO_2 fixation capacity than meadow soils

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ABSTRACT

CO_2-fixing by soil autotrophic microbes is as important as by plants in semi-arid and arid ecosystems, such as the Tibetan Plateau grassland. CO_2-fixing microbial community characteristics, capacity and their driving environmental factors remain unclear. Here we investigated the autotrophic microbial community in grassland surface soils on the Tibetan Plateau using molecular methods targeting the large subunit gene (*cbbL*) of ribulose-1, 5-bisphosphate carboxylase/oxygenase. The CO_2 fixation capacity was assessed by the $^{13}CO_2$ probing method. The results showed that soil autotrophic microbial abundance substantially increased from desert, steppe to meadow. The autotrophic abundance significantly increased with enhancing mean annual precipitation (MAP), soil ammonium concentration and aboveground plant biomass (APB). Forms IAB and IC autotrophic microbial communities strongly varied with grassland types. Variation partitioning analysis revealed that the structure variations were mainly explained by MAP and aridity, which explained 4.2% and 2.6% for the IAB community, and 7.6% and 8.5% for the IC community. Desert and steppe soils exhibited significantly higher atmospheric $^{13}CO_2$ fixation rate than meadow soils (29 versus 18 mg kg^{-1}soil d^{-1}). The $^{13}CO_2$ fixation rate negatively correlated with APB and soil ammonium concentration, demonstrating the substantially important role of autotrophic microbes in oligotrophic soils. Form IAB autotrophs were phylogenetically affiliated with *Cyanobacteria*. Form IC autotrophs were affiliated with *Rhizobiales* and *Actinobacteria*, the former gradually increased and the latter decreased from desert, steppe to meadow. Our findings offer new insight into the importance of MAP in driving soil autotrophic microbial community and highlight microbial roles in carbon cycling in dryland ecosystems.

1. Introduction

In semi-arid and arid ecosystems, soil autotrophs can be as important as plants in carbon (C) fixation (Su et al., 2013; Wohlfahrt et al., 2008). The dryland ecosystems occur globally and represent ~41% of the Earth's land surface area (Ferrenberg et al., 2015). It is roughly estimated that up to 4.9 Pg C is fixed per year by soil autotrophs in global soils (Yuan et al., 2012a,2012b). Soil autotrophic microbes contribute substantially to C fixation in diverse terrestrial ecosystems (Miltner et al., 2004; Selesi et al., 2005; Tolli and King, 2005; Hart et al., 2013). They even play a more dominant role in C fixation in plant-constrained ecosystems with stressful conditions, such as cold desert (Thomas, 2005; Novis et al., 2007; Namsaraev et al., 2010; Ferrenberg et al., 2015), hot desert (Zaady et al., 2000; Gunnigle et al., 2017), temperate desert (Rajeev et al., 2013), and deglaciated soils (Strauss et al., 2012; Liu et al., 2016). Many soil autotrophs are diazotrophs, such as *Nostocales* and *Rhizobiales,* and are associated with nitrogen cycling in terrestrial ecosystems (Steven et al., 2012; Che et al., 2018). They thus conduct biological nitrogen fixation and are a primary nitrogen source for semi-arid and arid ecosystems (Belnap, 2003).

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While attention has been paid to the CO_2 fixation capacity by soil autotrophs, the key environmental factors driving soil autotrophic microbial community and CO_2 fixation capacity remain largely unexplored. Few existing studies have shown that water availability is usually regarded as the main factor driving many ecological processes in arid and semi-arid ecosystems, including CO_2 fixation (Schwinning and Sala, 2004; Maestre et al., 2015; Chen et al., 2016). Water dramatically activates soil net CO_2 fixation rate in semi-arid (Schwinning and Sala, 2004; Zhang et al., 2013) and Antarctic ecosystems (Novis et al., 2007; Strauss et al., 2012). The water activation effect is not sustained, and usually regains in hours (Sponseller, 2007; Heisler-White et al., 2008). Compared to the few studies in natural dryland ecosystems, autotrophic microbial communities in agricultural soils have received more attention. Agricultural management practices, such as tillage, land use change and fertilization, play important roles in driving autotrophic microbial communities and their CO_2 fixation capacity (Yuan et al., 2013, 2015; Ge et al., 2016).

We hypothesized that autotrophic microbes are abundant in cold desert ecosystems, typified by the grassland soils of Tibetan Plateau, and their community is driven by a series of environmental factors. This study aimed to characterize the soil autotrophic communities and compare their CO_2 fixation capacities in three grassland types on the Tibetan Plateau using quantitative PCR, sequencing of *cbbL* genes and stable isotope methods. The Tibetan Plateau covers an area of 2.5 million km^2, of which 64% is alpine grassland ecosystems (Ma et al., 2017). The plateau is characterized by harsh conditions, such as permanent drought, low temperatures and high ultraviolet incidence. Our previous studies demonstrated that autotrophic microbes are abundant in barren deglaciated soils (Liu et al., 2016) and meadow soils (Guo et al., 2015) on the Tibetan Plateau. The autotrophic microbial community was characterized by targeting the large subunit (encoded by *cbbL* gene) of form I ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO). Form I RubisCO are divided into four subtypes (IA, IB, IC, and ID) (Tabita et al., 2008). The *cbbL* genes have been widely used as phylogenetic biomarkers to assess autotrophic microbial diversity in various habitats, such as waters and soils (Kong et al., 2012; Ge et al., 2013).

2. Materials and methods

2.1. Study area and soil sampling

The study region is located between 31 and 33°N latitude and 79 and 93°E longitude, with a 2, 000 km geographical scale, at an altitude above 4400 m a.s.l. (above sea level). Tibetan Plateau is climatically controlled by monsoons in Summer and 90% of precipitation occurs from July to September (Yang et al., 2014). The annual precipitation gradually decreases from the southeast meadow area (~800 mm) to the northwest steppe and desert areas (ranging from 400 to 60 mm) (Chen et al., 2016). The plant community is dominated by *Stipa breviflora* and *S. purpurea* in desert grassland and steppe ecosystems, and *Kobresia pygmaea* and *K. humilis* in meadow ecosystem (Wu, 1995). Meadow soils contain less lithoidal particles and are more mature than those in the steppe and desert grasslands on the Tibetan Plateau (Zhang et al., 2016). Mean annual precipitation (MAP) and mean annual temperature (MAT) were calculated for the period from 2003 to 2012, using meteorological data recorded at 33 meteorological stations (China meteorological Data Sharing Service System; http://cdc.cma.gov.cn/) across the Tibetan Plateau.

Surface soils (0–1 cm) were collected from a total of 19 sampling sites, covering desert (4 sites), steppe (11 sites) and meadow (4 sites) grassland in July of 2015 (Fig. S1 and Table S2). Five quadrats (1 m × 1 m) were randomly collected at each sampling site, and five surface soils (0–1 cm) were sampled at each quadrat and thoroughly mixed. Soil samples passed through a 2.0 mm sieve to remove roots and stones, and were immediately transported to the laboratory on ice bags. Soil samples for DNA extraction were stored at −80 °C, samples for CO_2 fixation capacity assay were stored at 4 °C, and the rest for physicochemical analyses were air-dried. Root samples and above ground plant biomass were also collected at each sampling site with five replicates to serve as environmental factors driving soil autotrophs. Root samples at each quadrat were randomly collected from five soil cores (2.5 cm diameter × 10 cm depth). The roots were carefully collected by washing soil cores in running water using a 0.5 mm sieve to remove soils and stones. The roots were then oven-dried in paper bags (65 °C for 24 h) for biomass measurements. The aboveground plants were clipped and stored in paper bags and were also oven-dried for biomass measurements.

2.2. Soil physicochemical factor analysis

Soil physicochemical factor were measured using standard methods (Tan, 2005). Soil pH was determined using a soil to water ratio of 1:2.5 by a pH meter (Sartorius PB-10, Germany). Soil nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were measured in 2 M KCl (soil/solution, 1:5) using Smartchem200 Discrete Auto Analyzer (Alliance, France). Soil total carbon (TC) and total organic carbon (TOC) were measured in solid using a TOC analyzer (TOC-VCPH, Shimadzu, Japan).

2.3. $^{13}CO_2$ fixation capacity assessment

Soils stored at 4 °C were used for microcosm incubation within one month of collection. Surface soils were incubated for 9 days with the addition of 5% (v/v) $^{13}CO_2$ (Long et al., 2015). Soils were adjusted to a constant moisture (40%) and then incubated for 48 h before adding $^{13}CO_2$. For each soil sample, 10 g was added into a 120 ml transparent serum bottle sealed with a rubber stopper and aluminum cap. The $^{13}CO_2$ (99 atom %, Sigma-Aldrich Co., MO, USA) was injected into the sealed bottle, and incubated at 20 °C for 12 h in darkness and at 24 °C for 12 h with 180 μ mol m^{-2} s^{-1} artificial light. To maintain aerobic conditions, fresh air was flushed into the bottles every 48 h and then the atmospheric $^{13}CO_2$ was re-injected in as described above.

After the incubation, soil samples were immediately freeze-dried and stored at −80 °C. The $\delta^{13}C$ values of the original and incubated soils were determined using DeltaPlus XP isotope ratio mass spectrometer (IRMS) and a gas chromatography/combustion/isotope ratio mass spectrometry system (GC-C-IRMS) (Thermo Fisher Scientific, CA, USA). Before $\delta^{13}C$ analysis, soils were passed through a 0.074 mm sieve and freeze-dried after being treated with 2 M HCl for 24 h. The $^{13}CO_2$ fixation capacity was calculated according to the ^{13}C atom% of soil before and after incubation (Long et al., 2015). The stable isotope abundance was expressed as δ-values (‰) relative to the standard PDB, which was calculated as:

$$\delta^{13}C = [(R_{sample}/R_{standard})\text{-}1] \times 10^3$$

Where R is the ratio of ^{13}C–^{12}C, $R_{standard}$ is 0.0112372.

^{13}C atom% was calculated as:

$$F = R/(R+1)$$

The CO_2 fixing amount (T) was calculated as:

$$T = TOC^*(Fa\text{-}Fb)$$

Where Fa and Fb were soil ^{13}C atom% value before and after incubation.

2.4. Real-time quantitative PCR

Form IAB, IC, and ID *cbbL* genes were quantified using primer sets IAB-F/IAB-R (Selesi et al., 2005), IC-F/IC-R (Alfreider et al., 2009) and ID-F/ID-R (Paul et al., 2000), respectively, on a LightCycler 480II thermocycler (Roche, Switzerland). PCR reactions were performed in a