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Metagenomic and ^{13}C tracing evidence for autotrophic atmospheric carbon absorption in a semiarid desert

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ABSTRACT

Atmospheric carbon dioxide (CO_2) absorption by desert soils has received increasing interests in recent years; however, the underlying physical and chemical mechanisms are not commonly acceptable. Here, we hypothesised that autotrophic carbon fixation of soil microbes contributes to this process. To test this postulate, we investigated the genomic and biochemical potential of autotrophic carbon fixation and traced atmospheric autotrophic carbon absorption using metagenomics and $^{13}CO_2$ labelling approaches in the Mu Us Desert in northern China. More than 30000 genes involved in the six carbon fixation pathways (approximately 2% of the assembled metagenomes, in relative abundance) were found in the metagenome of the desert soil, and the relative abundance for genes encoding for the reductive citrate cycle was the highest among the six pathways. The main autotrophic microbes employing the six pathways belong to *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Thaumarchaeota*, *Nitrospirae*, *Planctomycetes*, and *Bacteroidetes*, respectively. $^{13}CO_2$ labelling revealed that the contents of microbially incorporated soil organic carbon (^{13}C-SOC) and dissolved organic carbon were 0.572–1.45 mg kg^{-1} and 0.290–0.914 mg kg^{-1}, respectively. Further, the ^{13}C-SOC correlated with the relative abundance of genes of the total six pathways, reductive citrate cycle, 3-hydroxypropionate bi-cycle, and reductive acetyl-CoA pathway. Another *in situ* labelling experiment showed a significant increase in $\delta^{13}C$ of SOC, and the incorporated carbon (^{13}C) in SOC accounted for 3.85% of total atmospheric carbon absorption. These results showed that desert soil microbes containing genetic potential for autotrophic carbon fixation spread over a broad taxonomic range, and incorporated atmospheric carbon into organic components, which contributed to atmospheric carbon absorption. Although more research is required to accurately evaluate the portions of autotrophic carbon in the amount of atmospheric carbon absorption, the biotransformation of carbon from the atmosphere to soil via autotrophic carbon fixation represents a microbial pathway for persistent atmospheric CO_2 absorption in desert soils, and further implicates an important carbon biochemical cycle for carbon accumulation in oligotrophic desert soils.

1. Introduction

'Where does the carbon go?' is the first simple, yet powerful, guiding question in climate research (Marotzke et al., 2017). With an increase of anthropogenic carbon emission in the environment, many studies have been focusing on the identification of the 'missing sink' (Tans et al., 1990; Houghton, 2007), and drylands have been considered as systems that are potentially involved in this carbon process (Stone, 2008; Serrano-Ortiz et al., 2010). Atmospheric carbon dioxide (CO_2) can be absorbed by soils in various desert ecosystems (Yates et al., 2013; Liu et al., 2015); however, this direct uptake is not completely supported by abiotic mechanisms (Ma et al., 2013; Liu et al., 2015; Fa et al., 2016). Several recent studies have indicated that the process of autotrophic CO_2 fixation occurs in drylands (Lynch et al., 2014; Li et al., 2017; Yang et al., 2017) and can be an important microbial pathway for the fixation of atmospheric carbon in soils for persistent CO_2 absorption (Ji et al., 2017). However, whether autotrophic carbon fixation actually occurs and contributes to CO_2 absorption in semiarid desert ecosystems has not yet been adequately investigated. Thus, understanding atmospheric CO_2 absorption through autotrophic carbon fixation in drylands is of importance for understanding carbon sequestration from the atmosphere.

Autotrophic carbon fixation accounts for crucial components of the total carbon fixation in oceans, wetlands, paddy soils, and vents (Selesi

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et al., 2005; Yuan et al., 2012; Nowak et al., 2015; Beulig et al., 2016; Lynn et al., 2017), and hence significantly contributes to carbon sequestration. For example, autotrophic carbon fixation is responsible for nearly 40% of the annual CO_2 fixation in oceans and 4–10% of the total carbon fixation in wetlands (Cannon et al., 2001; Stanley et al., 2003). In addition, in some oligotrophic environments such as caves, marine sediments, and the Precambrian continental crust, autotrophic carbon fixation can occur as a primary production strategy with potentially novel nutrient cycling processes such as sulfate reduction (Magnabosco et al., 2016), nitrogen metabolism (Tetu et al., 2013), and iron and hydrogen metabolism (Wrighton et al., 2012; Emerson et al., 2016). Previous studies revealed that autotrophic microbes assimilate inorganic carbon into organics employing the following six major pathways: reductive pentose phosphate cycle (Calvin cycle), reductive citrate cycle (rTCA cycle), hydroxypropionate-hydroxybutylate cycle (3-HP/4-HB cycle), dicarboxylate-hydroxybutyrate cycle (DC/4-HB cycle), 3-hydroxypropionate bi-cycle (3-HP cycle), and reductive acetyl-CoA pathway (WL pathway; Berg, 2011; Bar-Even et al., 2012).

The primary carbon fixation pathways vary under different conditions. For example, the Calvin cycle is the predominant pathway utilised by microbes in nutrient-rich conditions, such as paddy soils, upland soils, grasslands, and forests (Yuan et al., 2012; Lynn et al., 2017). In contrast, autotrophic microbes assimilate inorganic carbon mainly through the energy-conserving rTCA cycle and WL pathway in some oligotrophic environments such as caves, marine sediments, and the Precambrian continental crust (Ortiz et al., 2014; Magnabosco et al., 2016; Wang and Sun, 2016). These studies suggest that nutrition conditions affect the primary carbon fixation pathways owing to the different energy demands of the pathways. In addition, redox conditions impact the utilisation of the autotrophic carbon fixation pathway. More energy is required to assimilate inorganic carbon under oxidized and aerobic conditions compared with reducing and anaerobic environments (McCollom and Amend, 2005; Hügler and Sievert, 2011). Thus, carbon fixation pathways (rTCA cycle, DC/4-HB cycle, and WL pathway) that contain oxygen-sensitive enzymes and thus are used by anaerobic or microaerophilic microbes require remarkably less energy for synthesis compared with pathways (Calvin cycle, 3-HP/4-HB cycle, and 3-HP cycle) that also function under aerobic habitats (Berg et al., 2010). Moreover, some other factors, including active carbon species and microbial distributions, influence the activation of specific carbon fixation pathways (Berg, 2011). Although several studies have observed the genomic potential of carbon fixation via the Calvin cycle (Lynch et al., 2014; Ji et al., 2017) in desert soils, it remains unclear whether other alternative autotrophic pathways, contributing to carbon fixation (Hügler and Sievert, 2011), occurs in desert soils.

Further, although autotrophic microbes have been detected in desert soils (Lynch et al., 2014; Ji et al., 2017), sufficient evidence of whether they effectively assimilate inorganic carbon and contribute to atmospheric CO_2 absorption is still lacking. These findings represent an important gap in our knowledge of atmospheric carbon absorption via autotrophic carbon fixation. Therefore, investigating the genomic and biochemical potential of autotrophic carbon fixation and the capacity of atmospheric carbon absorption by this process in detail in desert ecosystems is essential. We hypothesised that autotrophic microbes assimilate CO_2 by one of the six carbon fixation pathways that are also found in desert soils. Further, we hypothesised that microbial atmospheric CO_2 assimilation contributes to soil organic carbon (SOC) formation in these soils. To test these hypotheses, we used shotgun metagenomics and $^{13}CO_2$ labelling approaches under laboratory conditions to determine the genomic and biochemical potential of autotrophic carbon fixation performed by desert soil microbes. Next, we performed an *in situ* labelling experiment to confirm the process of autotrophic atmospheric carbon absorption.

2. Materials and methods

2.1. Site description

The research site is located on the south-western edge of the Mu Us Desert, north of Yanchi County, Ningxia Provence, China (37°42′N, 107°13′E; 1509 m above sea level). The region has a typical temperate continental climate, with 275 mm mean annual precipitation that mainly falls in August and September, and 2014 mm annual potential evaporation. The mean annual temperature is 7.60 °C, and the frost-free period lasts for about 128 days. The main soil type is Aripsamment. The vegetation is dominated by *Artemisia ordosica* Krasch, *Salix psammophila* C. Wang & Chang Y. Yang, and *Leymus secalinus* (Georgi) Tzvelev.

2.2. Sample collection

In August 2016, three 100 m × 100 m sampling sites with relatively uniform vegetation were selected from an area that had been fenced for ten years. Three 20 m × 20 m sampling plots were randomly selected in each of the fenced sampling sites, separated by a distance of 20 m from each other. Soil samples were collected from each sampling plot. The plant litter on the soil surface was removed before sample collection. Representative soil samples were obtained and impact of soil heterogeneity on the reliability of data was avoided, by randomly collecting twelve soil cores from each sampling plot from the depth range of 0–20 cm using a sterilised soil auger 2.5 cm in diameter. The twelve soil cores were mixed into one composite sample that was sieved through a 2 mm mesh to remove plant residue and roots, and then divided into four subsamples. One subsample was stored immediately at −78.5 °C on dry ice for metagenomic analysis. A second sample was incubated for $^{13}CO_2$ labelling in the laboratory. The third was kept at 4 °C in a refrigerator for the assessment of soil microbial biomass carbon (MBC) and nitrogen (MBN), soil dissolved organic carbon (DOC), soil water content (SWC), and extractable NO_3^-–N and NH_4^+–N. The final sample was air-dried for the analysis of SOC, total nitrogen (TN), pH, and soil particle size.

2.3. Determination of soil physicochemical properties

MBC and MBN were determined using a fumigation extraction method (Wu et al., 1990). DOC was measured as described by Li et al. (2015a). SWC was determined by oven-drying at 105 °C to a constant weight. NH_4^+–N and NO_3^-–N were measured colorimetrically using a Lachat autoanalyzer (Fierer and Schimel, 2002). SOC was measured using the potassium dichromate oxidation method (Walkley and Black, 1934), and TN was measured using the micro-Kjeldahl method using a Kjeldahl Apparatus Nitrogen Analyser (FOSS2200). Soil pH was measured one time from a soil:water (*w:v*, 1:2.5) mixture using a pH meter (FE20; Mettler Toledo, Switzerland), and the accuracy and resolution were ± 0.01 pH and 0.01 pH. Soil particle size was determined using a Mastersizer 2000 particle size analyser (Malvern Instruments, Malvern, England) and classified based on clay (< 0.002 mm), silt (0.002–0.05 mm), and sand (0.05–2.00 mm) standards developed by the U.S. Department of Agriculture.

2.4. Metagenomic shotgun sequencing, assembly, and annotation

For each sample, total microbial community DNA was isolated from 1.0 g of soil using the MoBio Power Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to manufacturer's instructions. The DNA sample was tested using three methods: (1) DNA degradation degree and potential contamination were monitored on 1% agarose gels; (2) DNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA); and (3) DNA concentration was measured using Qubit® dsDNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). After DNA sample testing, a total of 1 μg of