Geoderma 313 (2018) 17–24

Contents lists available at ScienceDirect

Geoderma

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

Influence of soil bacteria and carbonic anhydrase on karstification intensity and regulatory factors in a typical karst area

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ARTICLE INFO

Handling Editor: Junhong Bai

Keywords:
Field soil column experiment
Karstification intensity (KaI)
Soil microbial community
Carbonic anhydrase (CA)
High-throughput sequencing
Regulatory factors

ABSTRACT

Sequestration of atmospheric CO_2 is attracting considerable attention due to global warming. Karstification significantly affects the sequestration of atmospheric CO_2. Microorganisms and carbonic anhydrase (CA) can promote karstification, but the actual contribution of microorganisms and CA to karstification intensity (KaI) under natural conditions remains unclear. In this study, the influence of soil microorganisms and CA on KaI and main regulatory factors was investigated through field soil column dissolution experiments. Guilin Yaji Karst Experimental Site was used as the typical karst area, and Silai clastic rock area was the control area. Results showed that surface soil in various geomorphological positions in the karst area presented KaI in the following order: saddle > slope > depression. Addition of composite CA-producing microbial inoculants or composite inhibitors regulated CA activity in surface soil, thereby influencing KaI. Illumina high-throughput sequencing results indicated that the soil dominant bacterial phyla were generally the same in various experimental groups and plots, and the bacterial abundance in surface soil was regulated by additives. Addition of composite microbial inoculants increased the total bacterial abundance of surface soil, whereas addition of composite inhibitors reduced the total bacterial abundance. The ratio of CA-producing bacterial abundance to total bacterial abundance was the largest at saddle in the karst area, in corresponding to the highest KaI. This finding suggested the significant role of CA-producing bacteria in promoting KaI. Correlation analysis revealed that the main soil regulatory factors of KaI were soil pH, total hydrogen, total organic carbon, and exchangeable calcium.

1. Introduction

Global warming has been confirmed by air temperature observation data worldwide, and it is one of the most serious challenges confronting mankind (ICSU, 2010). Carbon dioxide, a main greenhouse gas that participates in the carbon cycle and contributes to global warming, has drawn considerable attention (Booth et al., 2012). In the global carbon cycle, when the carbon emitted into the atmosphere is higher than the carbon fixed in ecosystems, a carbon source occurs; otherwise, a carbon sink is formed (Fang and Guo, 2007). However, there is an imbalance between carbon source and carbon sink, which leading to a large amount of missing sink (Gifford, 1994). Chinese karst scholars reported a large amount of atmospheric CO_2 sink occurs in the dissolution of carbonate rocks in karst processes, which may be an important contribution to the missing sink (Jiang et al., 2013; Liu and Dreybrodt, 2015; Yuan, 1997).

Karstification refers to the process in which carbonate rocks are deposited or dissolved in the carbon cycle and associated calcium and water cycles (Yuan and Zhang, 2008). However, the carbon sink effect caused by karstification is not widely recognized by researchers. On the one hand, as a geological action in medium- and long-term scales, karstification was believed to exert a minimal and negligible effect on the absorption and emission of atmospheric CO_2 (Zhang, 2011a). On the other hand, the process of CO_2 absorption and conversion to HCO_3^- was thought to be unstable, thus, karstification was suggested to be a carbon transfer process rather than a carbon sequestration process (Curl, 2012). With regard to the rate of karstification, an increasing number of studies reported that microbes and carbonic anhydrase (CA) can significantly accelerate karstification (Li et al., 2005a; Li et al., 2007; Li et al., 2009; Lian et al., 2011). However, current studies on the effects of microbes, CA, and other biological factors on karstification are based on laboratory simulation experiments (Shen et al., 2014; Shen et al., 2017). Some fundamental questions still remain, including the effects of microbes and CA on karstification intensity (KaI) under natural karst soil conditions.

Studies on bacterial diversity and abundance in the karst area are

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http://dx.doi.org/10.1016/j.geoderma.2017.10.016
Received 13 March 2017; Received in revised form 6 October 2017; Accepted 16 October 2017

generally based on traditional culture-based methods. Because most soil microorganisms are unculturable, the accuracy of these methods is limited (Pace, 1997). High-throughput sequencing, or the next-generation sequencing technology, can simultaneously determine DNA sequences from hundreds of thousands to millions, which considerably improves the rate of sequencing and guarantees high accuracy (Schuster, 2008). High-throughput sequencing has been widely used in biological fields (Von Rein et al., 2016; Zwirglmaier et al., 2015).

To clarify the aforementioned question, we investigated the influence of soil microorganisms and CA on KaI under natural karst conditions through field soil column dissolution experiments. The main soil regulatory factors, such as soil pH, total hydrogen (TH), total organic carbon (TOC), and exchangeable calcium, etc., which may affect KaI, were also analyzed. Guilin Yaji Karst Experimental Site was used as the typical karst area, and Silai clastic rock area was the control area. The bacterial diversity and abundance of surface soil in different geomorphological positions and experimental groups were investigated using Illumina high-throughput sequencing and real-time quantitative PCR. The relationship between the abundance of CA-producing bacteria and KaI was also discussed. This study is the first to analyze the influence of soil microorganisms and CA on KaI under natural karst conditions, and results provide direct evidences to support the hypothesis that soil CA-producing microorganisms and CA have considerable practical contribution to KaI.

2. Materials and methods

2.1. Study area

The study area included the Yaji Karst Experimental Site and Silai clasolite control area in Guilin, China.

The Yaji Karst Experimental Site is located near Yaji village, and it is 8 km to the southeastern suburb of Guilin City, Guangxi Province. The site is a typical subtropical karst mountainous area. The annual mean temperature is 18.8 °C, and the annual average rainfall is 1915 mm; > 85% of rainfall occurs from April to August (He et al., 2000). Rongxian limestone of Devonian age is the karst strata. The soil coverage rate is 30%, which is mainly brown limestone soil that belongs to Calcareous Udic Luvisols according to Soil Taxonomy(Gong, 1999). The vegetation cover is 60%–80%, which mainly consists of secondary shrubs that show calciphilous and drought tolerance. *Vitex negundo* is the main species found in vegetation in saddle that presents a soil thickness of 30–50 cm. *V. negundo* and *Loropetalum chinense* are the main species found in vegetation at slope whose soil thickness ranges from 70 cm to 150 cm. *Pterolobium punctatum* and *Broussonetia papyrifera* are the main species found in vegetation at depression whose soil thickness is in the range of 300–500 cm (Jin et al., 2013).

The Silai clasolite area is located near Maocun village, and it is about 30 km to the southeast of Guilin City, China. This site has a humid and rainy subtropical monsoon climate. The area also experiences a hot and humid summer and significantly reduced rainfall in autumn. The rainfall is affected by southeast monsoon, and it is unevenly distributed. Silai clastic rock area is a non-karst area, and the soil mainly consists of acidic red soil with a depth of > 1 m. The vegetation in the study area is mainly evergreen broad-leaf forest composed of *Iteachinensis*, *Castaneahenryi*, *Schimasuperba*, *Castanopsis fargesii*, and a few *Miscanthus* species (Cao et al., 2011).

2.2. Field soil column experiments

To study the influence of soil microorganisms and CA on KaI under natural karst conditions, we designed a field soil column experimental device. The main component of the device is a PVC pipe with a length of 600 mm and a diameter of 75 mm. A 3 mm-thick plexiglass sieve plate, in which 96 of 2 mm meshes were distributed evenly, was fixed in the bottom of pipe. The sieve plate was 50 mm away from the bottom of the pipe to ensure that the tested soil did not contact the natural soil to avoid interference.

Four experimental plots were chosen to embed the soil columns: saddle, slope and depression in Yaji Karst Experimental Site, and Silai clasolite control area in Guilin, China.

A vertical pit with a depth of 600 mm and a certain distance from the nearby vegetation was dug in each experimental plot. Part of excavated soil was packed into each soil column after removal of stones, plant roots, and fallen leaves. Standard limestone tablets with a thickness of 4 mm and a diameter of 40 mm were embedded at 5 (surface), 20 (middle), and 40 cm (bottom) from the top of soil column. Each soil column was inserted into the pit vertically, and a certain distance among columns was maintained.

Buried soil columns in four plots (saddle (Y), slope (P), and depression (W) in the Yaji Karst Experimental Site, as well as the Silai non-karst area (F)) were established with five experimental groups, i.e. five kinds of treatments. The control group (K) contained nonexogenous substances in addition to the original soil. The composite-reinforced groups (MR1 and MR2) were added with typical CA-producing microbial inoculants, including bacteria (Li et al., 2013), fungi, and actinomycetes, which were all screened from karst areas, to surface soil of the corresponding columns. The microbial inoculant concentration was 10^6 ind./ml, 1 and 3 ml of each microbial inoculant were added for the composite-reinforced low concentration group (MR1) and composite-reinforced high concentration group (MR2), respectively. The composite inhibition groups (CI1 and CI2) were added with freeze-dried activated carbon particles containing microbial inhibitors and CA inhibitor acetazolamide to surface soil of the corresponding columns, respectively. Approximately 300 g of activated carbon was adsorbed with 1 g of ampicillin, kanamycin, and chloramphenicol, respectively. The additive amounts of activated carbon and acetazolamide were 20 and 0.2 g in the composite inhibition low concentration group (CI1) and 60 and 0.6 g in the composite inhibition high concentration group (CI2), respectively. Each experimental group was supplemented with the above composite microbial inoculants or inhibitors every 3 months. Two replicates were applied to each experimental group.

Soil columns were dug up after being embedded in soil for a full year. Limestone tablets and nearby soil samples were collected. Samples were brought to the laboratory in an ice box. Part of each soil sample was conserved at 4 °C to analyze CA activity and physicochemical properties. Another portion of the samples was conserved at − 80 °C to extract soil microbial DNA.

2.3. Determination of karstification intensity

Karstification intensity (KaI) was determined according to a modified method from Zhang (2011b). Standard limestone tablets were weighed before they were buried in the field soil column. The tablets were taken out after being buried for a certain time. After ultrasonic cleaning, the tablets were dried at 105 °C overnight and then weighed again.

The annual dissolution amount per unit area of a standard limestone tablet was calculated according to the following equation:

$$E = (W1 - W2) \times 1000 \times T^{-1} \times 365 \times S^{-1}$$

where E is the annual dissolution amount per unit area (mg/cm^2·a), W1 is the initial weight of tablet (g), W2 is the weight of tablet after being buried for a certain time (g), T is the buried time (d), and S is the surface area of tablet (cm^2).

2.4. Determination of soil carbonic anhydrase activity

Soil CA activity was determined from the rate of CO_2 hydration by following the changes in pH (Li et al., 2005a). Values are the means of triplicate samples.