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## Variations in the patterns of soil organic carbon mineralization and microbial communities in response to exogenous application of rice straw and calcium carbonate

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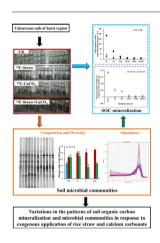
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#### HIGHLIGHTS

#### Different patterns of <sup>14</sup>C-rice straw and Ca<sup>14</sup>CO<sub>3</sub> addition on positive priming effects of SOC mineralization.

- Inorganic C is involved in soil C cycling with the participation of soil microbial communities.
- Bacterial and fungal communities were sensitive to available organic subtrates and soil pH, respectively.
- Soil MBC, pH, and bacterial diversity were closely correlated with SOC mineralization.

#### GRAPHICAL ABSTRACT



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#### ABSTRACT

The addition of exogenous inorganic carbon (CaCO<sub>3</sub>) and organic carbon has an important influence on soil organic carbon (SOC) mineralization in karst soil, but the microbial mechanisms underlying the SOC priming effect are poorly understood.

We conducted a 100-day incubation experiment involving four treatments of the calcareous soil in southwestern China's karst region: control, <sup>14</sup>C-labeled rice straw addition, <sup>14</sup>C-labeled CaCO<sub>3</sub> addition, and a combination of <sup>14</sup>C-labeled rice straw and CaCO<sub>3</sub>. Changes in soil microbial communities were characterized using denaturing gradient gel electrophoresis with polymerase chain reaction (PCR-DGGE) and real-time quantitative PCR (q-PCR). Both <sup>14</sup>C-rice straw and Ca<sup>14</sup>CO<sub>3</sub> addition stimulated SOC mineralization, suggesting that organic and inorganic C affected SOC stability. Addition of straw alone had no significant effect on bacterial diversity; however, when the straw was added in combination with calcium carbonate, it had an inhibitory effect on bacterial and fungal diversity. At the beginning of the experimental period, exogenous additives increased bacterial abundance, although at the end of the 100-day incubation bacterial community abundance had gradually declined.

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Soil organic carbon mineralization PCR-DGGE

Incubation time, exogenous input, and their interaction significantly affected SOC mineralization (in terms of priming and the cumulative amount of mineralization), microbial biomass carbon (MBC), and microbial community abundance and diversity. Moreover, the key factors influencing SOC mineralization were MBC, bacterial diversity, and soil pH. Overall, these findings support the view that inorganic C is involved in soil C turnover with the participation of soil microbial communities, promoting soil C cycling in the karst region.

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#### 1. Introduction

Soil organic carbon (SOC) plays an important role in carbon cycling under global climate change because only small changes in SOC would bring about large fluctuations in atmospheric CO<sub>2</sub> concentration (Post et al., 1990; Asner et al., 2004). Priming effects are intense responses of SOC mineralization to exogenous matter, which is very critical for SOC cycling. Extensive research has focused on the priming effects of low-quality plant materials (e.g., rice straw, wheat, and maize) (Luo et al., 2011), and addition of high-quality small molecules (e.g., fructose, alanine, oxalic acid, roots exudates) (Hamer and Marschner, 2005). However, the addition of organic C has been shown to have equivocal effects on priming direction, with positive (Fontaine et al., 2007; Zhang and Wang, 2012), negative (Hamer and Marschner, 2005), and no priming (Fang et al., 2015; Weng et al., 2015) effects being reported. Generally, positive priming effects are induced due to microbial growth and an accompanying increase in enzyme production, whereas negative priming effects are due to the toxicity of the substrates and preferential uptake of C-rich substrates by microorganisms (Kuzyakov et al., 2000).

As SOC mineralization driver, the microbial communities are increasingly being recognized to have an important control over SOC mineralization (Blagodatsky et al., 2010; Strickland et al., 2009; Fontaine et al., 2011). Indeed, studies have reported that in response to environmental factors, microbial communities determine the magnitude and direction of variations in soil C stocks (Billings and Ziegler, 2008; Carney et al., 2007). Furthermore, SOC mineralization is mediated by many factors, including temperature (Osanai et al., 2015), soil moisture (Mi et al., 2015), SOC content and its quality (Six et al., 2002), soil pH (Rousk et al., 2009), soil texture (Côtéa et al., 2000), etc. Above all, the changes in soil microorganisms owing to land-use change (Sun et al., 2013) and agricultural measures (Kaisermann et al., 2013) play an influential role in mediating changes in SOC mineralization. For example, a study in Saskatchewan, Canada, found that liming with the addition of Ca(OH)<sub>2</sub> increased SOC mineralization and soil respiration by up to 67% (Curtin et al., 1998).

As the world's largest carbon reservoir, carbonate rocks contain approximately  $6.1 \times 10^{16}$  tons of carbon, which represents 1694 times and  $1.1 \times 10^5$  times the carbon content of oceans and vegetation, respectively (Houghton and Woodwell, 1989). Carbonate rocks occupy a global area of approximately 22 million km<sup>2</sup>, a quarter of which is in China (Yuan, 1997). Carbonate weathering, controlled by CaCO<sub>3</sub>-H<sub>2</sub>O-CO<sub>2</sub> interactions, produces the world's largest carbon sink (Houghton and Woodwell, 1989), which also actively affects the global C cycle through karstification (Yuan, 1993, 1997). In this regard, Bertrand et al. (2007) found that soil respiration would be overestimated by 35% if the contributions of soil inorganic carbon were ignored. Another means of karstification is SOC mineralization (Jiang et al., 2013). Thus, the interaction between soil inorganic (CaCO<sub>3</sub>) and organic C directly affects the C cycle in karst soil. A special property of karst soil is its high CaCO<sub>3</sub> content compared to zonal soil, and the relationship between CaCO<sub>3</sub> and organic C directly affects the C cycle in soil ecosystem in karst region (Jacques, 2003). However, we currently know little about the mechanisms underlying the relationships between the priming of SOC mineralization and variations in microbial community structure and composition (Hu et al., 2012).

In this study, we hypothesized that the addition of organic matter or CaCO<sub>3</sub> could significantly affect SOC mineralization and soil microbial

communities, but with different patterns and mechanisms in this particular soil. We performed an experiment in which typical karst soil was supplemented with exogenous <sup>14</sup>C-CaCO<sub>3</sub> and <sup>14</sup>C-labeled rice straw. Specifically, the study aims were to (1) explore the effects of exogenous <sup>14</sup>C-CaCO<sub>3</sub> and <sup>14</sup>C-rice straw on SOC mineralization and the priming effect, (2) investigate the shifts in soil microbial communities (bacteria and fungi) in response to exogenous CaCO<sub>3</sub> and rice straw addition, and (3) explore the key factors controlling SOC mineralization and the priming effects in calcareous soils. The results will deepen our understanding of the microbial mechanisms underlying soil C cycling.

#### 2. Material and methods

#### 2.1. Study site and soil sampling

Soil samples were collected at the end of April 2009 in Huangjiang County (25°9'876''N, 108°3'47''E), in the Guangxi Autonomous Region of southwestern China. A subtropical mountainous monsoon climate dominates the area, with an annual precipitation of 1389 mm and a mean annual temperature of 18.5 °C. Shrubs comprise the primary vegetation in the area.

Soils on dolomite limestone were sampled in four replicates from each replicate plot in the Mulun National Nature Reserve. Eight soil cores, each with a diameter of 10 cm, were collected from a depth of 0-15 cm using a soil auger, and then mixed to form one composite sample. The soil samples (sieved to <2 mm) were divided into two portions. One was stored at  $4^{\circ}$ C for the microbial biomass carbon (MBC) determination and an incubation experiment, and the second portion was airdried for the analysis of physiochemical properties (shown in Table 1).

#### 2.2. Preparation of <sup>14</sup>C-labeled rice straw and Ca<sup>14</sup>CO<sub>3</sub>

We grew rice for 60 days in a closed transparent chamber under a  $^{14}\text{C}$ -labeled CO $_2$  atmosphere. The  $^{14}\text{C}$ -labeled CO $_2$  was produced by reacting 500 mL of 1 mol·L $^{-1}$  NaH $^{14}\text{CO}_3$  (approximately 5.55  $\times$  10 $^8$  disintegrations per minute [DPM]) with 500 mL of 2.5 mol·L $^{-1}$  HCl twice per week, with a reaction time of 12 h controlled by a valve. After harvest, the rice straw was dried at 60 °C, weighed and ground to <0.5 mm, and then used as the organic substrate.  $^{14}\text{C}$  was uniformly distributed throughout the rice straw. The radioactivity of the  $^{14}\text{C}$ -labeled rice straw was 0.87 Bq·µg $^{-1}$ C (C, 39.67%; N, 2.18%; C/N, 18.23).

The unlabeled and high DPM-labeled NaHCO $_3$  (each at a concentration of 1 mol·L $^{-1}$ ) were prepared by adding powdered Ca(OH) $_2$  to a large plastic bucket containing 1 L of mixed NaHCO $_3$ /NaH $^4$ CO $_3$  solution, while stirring continuously with a glass rod for a few minutes to ensure even mixing.

Installed the suction filter device of glass dish and rubber hose, opened the vacuum chestnut power switch, poured the completely reaction solution into the device. After a few minutes, 1 mol CaCO $_3$ /Ca $^{14}$ CO $_3$  was produced, then, used the glass rod to pour out it to a big iron pan covered by plastic film, drying in the laboratory, standby. The above operations were performed in the isotopic laboratory.

#### 2.3. Experimental design

The experimental soil was pre-incubated for 7 days, and then four treatments with four replicates were used: (i) control (CK) of untreated

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