Soil Biology and Biochemistry 121 (2018) 67–76

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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

Microbial stoichiometric flexibility regulates rice straw mineralization and its priming effect in paddy soil

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ARTICLE INFO

Keywords:
Element stoichiometry
Extracellular enzyme activity
Soil microbial biomass
Soil organic matter turnover
Priming effect
Structural equation models

ABSTRACT

Nitrogen (N) and phosphorus (P) availability plays a crucial role in carbon (C) cycling in terrestrial ecosystems. However, the C:N:P stoichiometric regulation of microbial mineralization of plant residues and its impact on the soil priming effect (PE), measured as CO_2 and CH_4 emission, in paddy soils remain unclear. In this study, the effect of soil C:N:P stoichiometry (regulated by the application of N and P fertilizers) on the mineralization of ^{13}C-labelled rice straw and the subsequent PE was investigated in a 100-day incubation experiment in flooded paddy soil. N and P additions increased straw mineralization by approximately 25% and 10%, respectively. Additions of both N and P led to higher CO_2 efflux, but lower CH_4 emission. With an increase in the ratios of DOC:NH_4^+-N, DOC:Olsen P, and microbial biomass C:N, $^{13}CO_2$ efflux increased exponentially to a maximum. Compared with sole straw addition, exclusive N addition led to a weaker PE for CO_2 emission, whereas exclusive P addition induced a stronger PE for CO_2 emission. In contrast, CH_4 emitted from native soil organic matter (SOM) was reduced by 7.4% and 46.1% following P and NP application, respectively. Structural equation models suggest that available N had dominant and direct positive effects, whereas microbial biomass stoichiometry mainly exerted negative indirect effects on PE. The stoichiometry of soil enzyme activity directly down-regulated CH_4 emission from SOM. Microbes obviously regulate soil C turnover via stoichiometric flexibility to maintain an elemental stoichiometric balance between resources and microbial requirements. The addition of straw in combination with N and P fertilization in paddy soils could therefore meet microbial stoichiometric requirements and regulate microbial activity and extracellular enzyme production, resulting in co-metabolism of fresh C and native SOM.

1. Introduction

Paddy fields cover an area of approximately 165 million ha worldwide and have a considerable potential for expanded carbon (C) sequestration (Lal, 2004; Conrad et al., 2012; Ge et al., 2012). Plant residues are a major source of soil organic matter (SOM) input (Nguyen, 2003; Wu, 2011), and the addition of such 'fresh' organic matter to the soil might change the mineralization of native SOM, which is commonly referred to as the priming effect (PE) (Kuzyakov et al., 2000; Yuan et al., 2014; Zhu et al., 2016). This process has been widely investigated in upland ecosystems (Chen et al., 2014; Qiao et al., 2016); however, there have been relatively few studies that have linked this effect with nutrient supply and nutrient stoichiometric control in paddy soils.

Nutrient supply critically affects soil C turnover (Chen et al., 2014; Dimassi et al., 2014; Fisk et al., 2015) by modifying microbial composition and activities (Neff et al., 2002; Luo et al., 2013). Being one of the most important nutrients, nitrogen (N) often limits crop growth and controls soil C turnover. The effects of direction and magnitude of N addition on the mineralization of SOM vary widely (Chen et al., 2014; Fisk et al., 2015). With sufficient C supply, an increasing N availability meets the microbial C:N stoichiometric requirement and stimulates

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https://doi.org/10.1016/j.soilbio.2018.03.003
Received 20 October 2017; Received in revised form 2 March 2018; Accepted 3 March 2018

microbial activity, thereby accelerating SOM mineralization (Cleveland and Liptzin, 2007; Qiu et al., 2016). Additionally, phosphorus (P) plays an important role, as it is necessary for ATP production and can limit microbial growth. It, therefore, regulates microbial activity and metabolic rates, which have inevitable feedback effects on soil C turnover (Mooshammer et al., 2011; Liu et al., 2013; Vogt et al., 1980). Hartman and Richardson (2013) reported that C and N limits biosynthesis, whereas P limits total microbial metabolism. Several studies have shown an increase in heterotrophic respiration after P addition, mostly in P-limited soils (Cleveland et al., 2002; Craine et al., 2007; Liu et al., 2013). Poeplau et al. (2016) reported a significant net decrease in SOM stocks after long-term P fertilization when compared with that in unfertilized control soils. However, it remains unclear how nutrient availability interacts with soil C dynamics. Understanding the underlying mechanisms is vital for incorporating nutrient cycling into SOM turnover (Reed et al., 2011; Chen et al., 2014).

Nutrient availability and C:N:P stoichiometric ratios are critical in the regulation of added substrate decomposition, SOM mineralization, and nutrient transformation (Mooshammer et al., 2011; Sinsabaugh and Shah, 2012; Creamer et al., 2014). The effects of nutrient availability on soil C mineralization have been explained using the basic stoichiometric mineralization theory, which predicts that microbial activity is driven by microbial demand for resources, with an optimal C:N:P ratio of 60:7:1 (Cleveland and Liptzin, 2007; Mooshammer et al., 2011; Sistla and Schimel, 2012; Creamer et al., 2014). However, when returning rice straw with a high ratio of C to nutrients, the available N or P in the soil is often not sufficient to meet the demands of microbes in paddy soil, which consequently inhibits the microbial mineralization of native SOM (negative PE) (Li et al., 2012). Nutrient limitation may lead to a shift in the microbes from r-to K-strategists, the latter of which are considered to be able to decompose more stable SOM for organic N or P acquisition, and thus induce a higher mineralization of SOM (positive PE) (Blagodatskaya et al., 2009; Luo et al., 2013; Chen et al., 2014). Moreover, in response to nutrient deficiency, the microbial community composition can be altered, e.g. with respect to the fungal to bacterial ratios (Li et al., 2015) or microbial diversity in general (Lin et al., 2012; Wei et al., 2017).

In order to meet their stoichiometric requirements and adjust to their resources, microorganisms release extracellular enzymes to mine C, N, and P from native SOM (Sinsabaugh and Shah, 2012; Mooshammer et al., 2014). Extracellular enzyme activities, therefore, effectively reflect the functions of decomposer communities, depending on metabolic requirements and nutrient availability (Adamczyk et al., 2014; Yang and Zhu, 2015; Cui et al., 2018). According to Sistla and Schimel (2012), and Mooshammer et al. (2014), these interactions are in dynamic balance, in which microorganisms respond to resource changes to adjust to shifting soil elemental ratios, in conjunction with other environmental fluctuations, i.e., 'stoichiometric flexibility'. It is, therefore, important to clarify the stoichiometry of soil nutrient elements and their interactions with residue mineralization to better understand the PE mechanisms in paddy soils.

The aim of the present study was to clarify the effect of soil C:N:P stoichiometry on straw mineralization and its PE in paddy soil. The stoichiometry of N and P was regulated by the application of fertilizers. We hypothesized that (i) the application of no or a single element fertilizer (N or P) might cause resource imbalance, which will inhibit microbial activity and SOM mineralization, and (ii) the combined addition of N and P fertilizers might meet microbial stoichiometric requirements, increase microbial activities, and, therefore, promote SOM mineralization. To test these hypotheses, the effects of soil C:N:P elemental stoichiometry and enzyme activity stoichiometry on straw mineralization and its PE were determined on the basis of CO_2 and CH_4 emission from native SOM.

2. Materials and methods

2.1. Study site and soil sampling

The experimental rice field is located at the Changsha Research Station for Agricultural and Environmental Monitoring, Hunan, China (113°19′52″E, 28°33′04″N; 80 m above sea level). It is characterized by a subtropical climate, with a mean annual temperature and rainfall of 17.5 °C and 1300 mm, respectively. The soil of this area is derived from highly weathered granite and is classified as a typical Stagnic Anthrosol (Eutric, Siltic, Gleyic) (Gong et al., 2007). Field-moist soils were collected from the plough layer (0–20 cm) and sieved < 4 mm to remove visible plant residues. The soil contained 13.1 g kg^{-1} organic C, 78.7 mg kg^{-1} dissolved organic C, 1.4 g kg^{-1} total N, 18.0 mg kg^{-1} available N, 0.3 g kg^{-1} total P, and 3.7 mg kg^{-1} Olsen P, and had a pH of 6.2 at a soil:water ratio (w:v) of 1:2.5.

2.2. Production of ^{13}C-labelled rice straw

Rice cultivation and continuous $^{13}CO_2$ labelling were performed according to Ge et al. (2012), with some modifications. A total of 10 pots were filled with 1 kg dry soil and were each planted with three 30-day-old rice seedlings (*Oryza sativa* L. 'Zhongzao 39'). All pots were watered every 2–3 days until harvest, to maintain a water depth of 2–3 cm from the soil surface. Weeds were removed manually. For ^{13}C labelling, the 10 pots were transferred to an automatically controlled gas-tight growth chamber (length, 110 cm; width, 250 cm; and height, 180 cm) and exposed to $^{13}CO_2$ fumigation during the vegetative growth period for 30 d. The CO_2 concentrations of the growth chambers were measured using an infrared analyser (Shsen-QZD; Qingdao, China) and maintained at 360–380 μL L^{-1}. The $^{13}CO_2$ was generated by acidifying $Na_2{}^{13}CO_3$ (1.0 M, 99 atom% ^{13}C; Cambridge Isotope Laboratories, Tewksbury, MA, USA) with H_2SO_4 (0.5 M) in beakers that were placed inside the growth chambers. During the labelling period, $^{13}CO_2$ was only released when CO_2 concentrations fell below 360 L L^{-1}. At CO_2 concentrations > 380 μL L^{-1}, the gas flow was diverted and passed through CO_2 traps (NaOH solution). An air-conditioning system was used to control the temperature inside the chambers to within 1 °C of the ambient temperature. Two fans continuously circulated air in the growth chambers. All the rice plants were destructively sampled after 30 d of $^{13}CO_2$ labelling. Rice straw was cut at the base, washed with deionized water, dried at 60 °C for 48 h, and then cut into < 5 mm pieces. The C content of the rice straw was determined by dry combustion by using an elemental analyser (vario MAX; Elementar Analysensysteme GmbH, Hanau, Germany). The stable C isotope composition was analysed using an isotope ratio mass spectrometer coupled with an elemental analyser (FLASH 2000; Thermo Fisher Scientific, Waltham, MA, USA). Finally, a 4.5 atom% enrichment of ^{13}C-labelled rice straw was obtained.

2.3. Soil incubation

This experiment consisted of four treatments with three replications in a completely randomized design. The treatments were as follows: (1) paddy soil with no additions (Control); (2) paddy soil supplemented with straw (Straw); (2) paddy soil supplemented with straw and N fertilizer (Straw + N); (3) paddy soil supplemented with straw and P fertilizer (Straw + P); (4) paddy soil supplemented with straw, N fertilizer, and P fertilizer (Straw + NP). Paddy soil supplemented with unlabelled straw was used to determine the natural abundance of ^{13}C.

A total of 150 g of pre-incubation soil (equivalent to 100 g dry soil) was placed in 500 mL serum bottles. The ^{13}C-labelled rice straw was applied at a rate of 2.5 g C kg^{-1} dry soil. The soil samples were homogeneously mixed with straw, and then mineral N (NH_4Cl) and/or P (NaH_2PO_4) fertilizers were applied to the soil at a dose of 90 mg NH_4Cl-N kg^{-1} dry soil and 30 mg NaH_2PO_4-P kg^{-1} dry soil,