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Stability and dynamics of enzyme activity patterns in the rice rhizosphere: Effects of plant growth and temperature

Tida Ge [a, b], Xiaomeng Wei [a, b], Bahar S. Razavi [c, *], Zhenke Zhu [a, b], Yajun Hu [a, b], Yakov Kuzyakov [a, c], Davey L. Jones [a, d], Jinshui Wu [a, b]

[a] Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan 410125, China
[b] Changsha Research Station for Agricultural and Environmental Monitoring, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan 410125, China
[c] Department of Agricultural Soil Science, University of Göttingen, 37077 Göttingen, Germany
[d] School of Environment, Natural Resources & Geography, Bangor University, Gwynedd LL57 2UW, Wales, UK

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ABSTRACT

The rhizosphere is the most dynamic hotspot of microbial activity in the soil. Despite these dynamics, the spatial pattern of many rhizosphere properties may remain stable because they are continuously reproduced in the changing environment. Low substrate concentration can strongly reduce the rate response of an enzymatic reaction subjected to increased temperature and is recognized as a canceling effect on enzyme temperature sensitivity. Carbon input from rhizodeposits affects C availability in the rhizosphere, and thus the enzyme activities responsible for organic matter decomposition, and their temperature sensitivities, upset the dynamics and stability of biochemical processes in the rhizosphere. However, it is unclear whether a canceling effect occurs in the rhizosphere. We studied temperature effects on chitinase and phosphatase during rice (*Oryza sativa* L.) growth at 18 and 25 °C. The spatial distribution of enzyme activities was imaged using soil zymography and showed that the overall activities of these enzymes increased with temperature but decreased with rice growth. The temporal dynamics of hotspot areas were enzyme-specific. During growing days 14–30, hotspot areas decreased from 2-2.5% to 0.3–0.5% for chitinase, but increased from 2% to 6–7% for phosphatase. The distribution pattern of both enzymes shifted from being dispersed throughout the soil to being associated with the roots. For the first time, we showed the extent of rhizosphere enzyme activity in paddy soil and demonstrated that it is temporally stationary and independent of temperature. However, the temperature sensitivity of enzyme activities declined radically (Q_{10}~1.3–1.4) at the root surface compared to that of bulk soil (Q_{10} ~1). We conclude that the spatio-temporal pattern of rhizosphere enzymatic hotspots is mainly affected by plant growth. High temperature sensitivity ($Q_{10} > 1$) at the root-soil interface for the tested enzymes revealed that warming will lead to faster nutrient mobilization in the rhizosphere than in root-free soil.

1. Introduction

The rhizosphere—the volume of soil affected by plant roots—is one of the most dynamic spheres in the biosphere. The spatial distribution of the rhizosphere is a dynamic function of the soil matrix and plant properties, including root development and morphology, microbial colonization, nutrient uptake, root exudation and rhizodeposition (Dazzo and Gantner, 2012; Neumann and Romheld, 2002). Rhizodeposits include lost root cap and border cells, dead and lysed root cells, lost gasses, passively and actively released solutes (root exudates), and gelatinous material from the surface of roots (mucigel) (Curl and Truelove, 1986; Hinsinger et al., 2009; Jones et al., 2009).

Rhizodeposits play a crucial role for rhizosphere processes: stimulates microbial activity (Hinsinger et al., 2009; Kuzyakov and Domanski, 2000) and the production of enzymes (Asmar et al., 1994) and, thus, nutrient availability and soil organic matter (SOM) decomposition (Cheng and Coleman, 1990). However, the higher enzyme activity of the rhizosphere than of root-free soil depends not only on microbial activity, but also on the direct

* Corresponding author.
E-mail address: brazavi@gwdg.de (B.S. Razavi).

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release of enzymes by roots or by lysis of root cells (Jones et al., 2009). On average, plants release 20% of photosynthesized carbon (C) through their roots in the form of high- and low-molecular weight organic compounds (Badri and Vivanco, 2009; Fischer et al., 2010; Hinsinger et al., 2006). The exact amount and quality of root exudates of an individual plant strongly depend on photosynthetic activity (Siczek and Lipiec, 2016) and root development (Aulakh et al., 2001), which are largely controlled by plant age. The temporal dynamics of enzyme activities in soil are affected by the quality and quantity of root exudates during each growth stage (Ren et al., 2016; Zhang et al., 2014).

While the interactions between plants and microorganisms are recognized as the major biotic factors influencing enzyme activities, abiotic factors such as temperature, water potential, pH, and soil texture are also important controls (Burns et al., 2013). Among these factors, temperature sensitivity of enzyme activity has recently received considerable interest because of its potential feedback to climate change (Davidson et al., 2006). Temperature sensitivity is commonly presented as the Q_{10} index, the factor by which reaction rate is multiplied when temperature increases by 10 °C (Birgander et al., 2013). When compared to respiration rates commonly assumed to have Q_{10} values of 2–3, enzyme activities are less temperature sensitive, with Q_{10} values < 2 (Browman and Tabatabai, 1978; Koch et al., 2007; Tabatabai, 1994). High temperatures generally increase enzyme activity (Nottingham et al., 2016; Razavi et al., 2017; Stone et al., 2012). However, the temperature sensitivity of enzymatic reactions also associates with variable factors, including temperature range, substrate supply, desiccation stress, etc. (Davidson et al., 2006). Higher temperatures commonly generate lower Q_{10} values (Razavi et al., 2016a; Tjoelker et al., 2001). Besides, when substrate concentration is low, a canceling effect (the absence or strong reduction of a response of an enzyme to temperature) decreases the expected enzymatic reaction rate (Berry and Raison, 1981; Davidson et al., 2006; Razavi et al., 2015). When Q_{10} of catalytic reactions is ~1, the reaction is restricted by temperature sensitivity of a bottle-neck process that accesses available substrate, e.g., during soil organic matter decomposition, by decomposition of recalcitrant or stabilized SOM (Ågren and Wetterstedt, 2007). When substrate diffusion varies with temperature, the temperature sensitivity of enzyme activity will be further affected (Davidson et al., 2006) Accordingly, the canceling effect can be an important phenomenon controlling the 'actual' temperature sensitivity of organic material decomposition in soils (Razavi et al., 2015; Von Lützow and Kögel-Knabner, 2009) and nutrient mobilization. Thus the strength of the canceling effect is affected by substrate availability and this can vary during plant growth. Despite theoretical predictions (Davidson et al., 2006), and experimental evidence (Blagodatskaya et al., 2016; Razavi et al., 2015), there is still a lack of data on the occurrence of canceling as dependent on temperature and substrate amount in the rhizosphere—the sphere with a high concentration of labile compounds.

In situ visualization of the spatio-temporal distribution of enzyme activity in critical spheres, such as the rhizosphere, and how it is affected by temperature is required to reveal complex interactions between microorganisms, enzymes, and SOM decomposition (Wallenstein and Weintraub, 2008). However, it is still an unsolved question whether and how temperature affects the dynamics and localization of enzymatic hotspots in the rhizosphere. The recently modified soil enzyme activity imaging technique—so called direct soil zymography (Razavi et al., 2016b; Sanaullah et al., 2016)—offers an opportunity to analyze the two-dimensional spatial distribution of enzymes in soil (Vandooren et al., 2013). Direct soil zymography enables the mapping of enzyme activity at the soil surface (Sanaullah et al., 2016), in biopores (Hoang et al., 2016b) the rhizosphere (Razavi et al., 2016b) and the detritusphere (Liu et al., 2017; Ma et al., 2017). Here for the first time, we quantitatively imaged the impact of temperature on the spatio-temporal distribution of enzyme activities in the rhizosphere during rice growth—one of the most important food crops in China. Our study aimed to illustrate: 1) how the spatial distribution of soil enzyme activities is affected by temperature and 2) how the impact of temperature varies with plant growth stage. We hypothesized that 1) high temperature, root development, and plant growth will increase enzymatic activity and hotspot area; 2) such an increase in hotspot area is enzyme dependent; and 3) there is no canceling effect in the rhizosphere of rice (*Oryza sativa* L.), which is an important agricultural crop for food production. Thus, we studied the spatio-temporal distribution of enzymes involved in P and N cycles and crucial for improving nutrient availability. Phosphatase that catalyzes the degradation of phosphorous-containing organic compounds (Asmar et al., 1994; Eivazi and Tabatabai, 1988) and *N*-acetylglucosamine (e.g., chitinase), which accomplishes the decomposition of chitin to a low molecular weight chitooligomer (German et al., 2011; Hoang et al., 2016a), at two temperatures (18 and 25 °C) after 14 and 30 days.

2. Materials and methods

2.1. Sample preparation

Hydragric Anthrosol (Gong et al., 2007) developed from a granite parent material after very long, intensive subtropical weathering was collected from a rice field (113°19′52″E, 28°33′04″N, 80 m above the sea level) located at the Changsha Research Station for Agricultural and Environmental Monitoring, Hunan Province, China. Soil samples were collected from the Ap horizon (15% water content) and sieved (<4 mm) to remove coarse plant residues. The soil texture was 10.4% clay, 76.5% silt, and 13.1% sand. We grew 16 rice plants (*Oryza sativa* L. 'Zhongzao 39'), each in a separate rhizobox with inner dimensions of 20.5 × 13.4 × 5.2 cm. The rhizoboxes were placed horizontally with one side open and then soil was slowly and continuously poured into the rhizoboxes through a 2 mm sieve to achieve uniform soil packing and to avoid soil layering. The open side was then closed, the samples were turned vertically, and they were gently shaken to achieve stable soil packing. The seeds were germinated on filter paper for 72 h. Then one seedling was planted in each rhizobox at a depth of 5 mm.

During 30 days of growth, the rhizoboxes were kept inclined at an angle of 45° so that the roots grew along the lower wall of the rhizoboxes. All samples were kept in climate-controlled chambers which were regulated by automatic temperature control system with a deviation of ±1 °C, set to 18 or 25 °C and a daily light period of 16 h with 300 μmol m^{-2} s^{-1} of photosynthetically active radiation intensity. The water level was maintained throughout the rice growing season at 2–3 cm above the soil surface to simulate the water condition in most paddy fields before zymography analysis.

2.2. Direct soil zymography

After cultivating rice for 14 and 30 days, direct soil zymography was applied as an *in situ* technique to study the spatial distribution of enzyme activity around the roots. We followed the protocol optimized by Razavi et al. (2016b). Visualization of enzyme activities involved using membranes saturated with 4-methylumbelliferone (MUF)-substrates, which become fluorescent when enzymatically hydrolyzed by a specific enzyme. 4-Methylumbelliferyl-*N*-Acetyl-α-D-glucosaminide (MUF-*N*-Ac) was used as substrate to detect *N*-acetyl-glucosaminidase (chitinase) activity; phosphatase activity was detected using 4-methylumbelliferyl-phosphate (MUF-Phos). Each of these