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Warming increases hotspot areas of enzyme activity and shortens the duration of hot moments in the root-detritusphere

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ABSTRACT

Temperature effects on enzyme kinetics and on the spatial distribution of microbial hotspots are important because they are crucial to soil organic matter decomposition. We used soil zymography (*in situ* method for the two dimensional quantification of enzyme activities) to study the spatial distributions of enzymes responsible for P (phosphatase), C (cellobiohydrolase) and N (leucine-aminopeptidase) cycles in the rhizosphere (living roots of maize) and root-detritusphere (7 and 14 days after cutting shoots). Soil zymography was coupled with enzyme kinetics to test temperature effects (10, 20, 30 and 40 °C) on the dynamics and localization of these three enzymes in the root-detritusphere. The percentage area of enzyme activity hotspots was 1.9–7.9 times larger and their extension was broader in the root-detritusphere compared to rhizosphere. From 10 to 30 °C, the hotspot areas enlarged by a factor of 2–24 and V_{max} increased by 1.5–6.6 times; both, however, decreased at 40 °C. For the first time, we found a close positive correlation between V_{max} and the areas of enzyme activity hotspots, indicating that maximum reaction rate is coupled with hotspot formation. The substrate turnover time at 30 °C were 1.7–6.7-fold faster than at 10 °C. The K_m of cellobiohydrolase and phosphatase significantly increased at 30 and 40 °C, indicating low affinity between enzyme and substrate at warm temperatures. We conclude that soil warming (at least up to 30 °C) increases hotspot areas of enzyme activity and the maximum reaction rate (V_{max}) in the root-detritusphere. This, in turn, leads to faster substrate exhaustion and shortens the duration of hot moments.

1. Introduction

The rhizosphere is one of the most dynamic habitats on Earth (Hinsinger et al., 2009) because living plants stimulate microbial and enzyme activity (Parkin, 1993; Asmar et al., 1994) by releasing labile carbon and other rhizodeposits (Jones et al., 2009). Enzymes excreted by both plants and microbes are the main mediators of organic matter decomposition (Nannipieri et al., 2007; Sinsabaugh et al., 2008). The root-detritusphere (soil around dying and dead roots) also forms the hotspots of microbial and enzyme activity because carbon inputs through the dying root after harvest releases highly polymers as well as low molecular weight organics (Kögel-Knabner, 2002; Bastian et al., 2009). The rhizosphere and root-detritusphere are both considered to be hotspots of enzyme activity (Kuzyakov and Blagodatskaya, 2015); nonetheless, they differ in the composition of their substances (Kögel-Knabner, 2002; Jones et al., 2004) and in nutrient exudation dynamics (Bastian et al., 2009; Poll et al., 2010). Whereas living roots release abundant readily available monomers such as monosaccharides and amino acids (Hinsinger et al., 2009), root detritus mainly contains macromolecular compounds such as cellulose and xylan (Rahn et al., 1999). Moreover, while rhizodepositions represent a continuous flow of substances during plant growth (Kuzyakov and Domanski, 2000), the death of roots is a temporally concentrated C input (Spohn and Kuzyakov, 2014). Due to the concentrated input of available organics from dead roots, it is generally accepted that microorganisms are more abundant (Marschner et al., 2012) and that the hotspots' areas of enzyme activity are larger in the root-

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detritusphere than in the rhizosphere (Spohn and Kuzyakov, 2014).

Enzyme activities in soil are controlled by abiotic factors (temperature, water potential, pH, soil texture) and biotic factors (enzyme synthesis and secretion) (Burns et al., 2013). Among abiotic factors, the temperature sensitivity of enzyme activity has received considerable interest because of its potential feedback to climate change (Davidson and Janssens, 2006). Temperature directly affects enzyme activity by changing the conformational flexibility of enzymes, indirectly by causing shifts in the microbial community (Bárcenas-Moreno et al., 2009; Rousk et al., 2012).

Both microbial and enzyme activities increase with temperature (Davidson and Janssens, 2006; Steinweg et al., 2008). Thus, soil warming increases the breakdown and assimilation of organic matter, enhancing microbial growth and enzyme synthesis (Davidson and Janssens, 2006). Nonetheless, long-time experiments showed that warming initially stimulated soil respiration, microbial biomass and enzyme activity, but the effect diminished over time - a phenomenon frequently termed acclimation (Allison and Treseder, 2008; Frey et al., 2008). This can be attributed to faster depletion of easily accessible organic matter and changes in carbon use efficiency at warm temperatures (Kirschbaum, 2004; Eliasson et al., 2005). The depletion of substrate further results in microorganism starvation (Bradford et al., 2008) and enzyme pool reduction (Wallenstein et al., 2010). The decrease of carbon use efficiency lead to more C to waste with increasing temperature (Ågren, 2010). Therefore, the duration of hot moments - (periods of high microbial and enzyme activities) (Kuzyakov and Blagodatskaya, 2015) are shorter at high temperatures.

Substrate-dependent enzyme activity is described by the Michaelis-Menten function (Michaelis and Menten, 1913). Both parameters of the Michaelis-Menten equation-V_{max} (maximum reaction rate) and K_m (half-saturation constant indicating the affinity of enzyme to substrate) - are temperature sensitive (Davidson and Janssens, 2006) and usually increase with temperature (Stone et al., 2012; Baldrian et al., 2013). It remains unresolved, whether temperature affects the temporal and spatial distribution of enzyme activity hotspots. It is imperative to measure the spatial distribution of enzyme activity as affected by temperature in order to reveal complex interactions between microorganisms, enzymes, and SOM decomposition (Wallenstein and Weintraub, 2008). Especially in the rhizosphere and root-detritusphere, due to the high heterogeneity, the substrate availability varies in time and space (Ekschmitt et al., 2005).

The recently developed imaging technique termed zymography (Spohn et al., 2013) offers an opportunity to analyze the two-dimensional spatial distribution of enzyme activity in soil (Vandooren et al., 2013; Spohn and Kuzyakov, 2014). Combining soil zymography with enzyme kinetics enabled relating the distribution of hotspots to enzyme catalytic properties in bulk soil, in the rhizosphere (Sanaullah et al., 2016) as well as in biopores (Hoang et al., 2016). For the first time, zymography was coupled with Michaelis-Menten kinetics in the root-detritusphere to test temperature effects (10, 20, 30 and 40 °C) on the dynamics and spatial distribution of enzyme activity. Cellobiohydrolase, leucine-aminopeptidase and acid-phosphatase (involved in C, N, and P cycling, respectively) were used to study the kinetic parameters (V_{max} and K_m) and to localize enzyme activities in the rhizosphere and root-detritusphere. We hypothesized that 1) due to the concentrated input of substrate in the root-detritusphere, the areas of enzyme activities hotspots are larger in the root-detritusphere than in the rhizosphere, 2) the hotspot areas of enzyme activates in the root-detritusphere response faster but the duration of hot moment is shorter at warm temperatures than at cold temperatures, 3) catalytic properties (K_m and V_{max}) respond positively to increasing temperature.

2. Material and methods

2.1. Sample preparation

The soil was collected from the top 10 cm of the Ap horizon of an arable loamy Haplic Luvisol located on a terrace plain of the Leine River north-west of Göttingen, Germany. The soil had the following physiochemical properties: 7% sand, 87% silt, 6% clay, pH 6.5, organic carbon 12.6 g C kg^{-1}, total nitrogen 1.3 g N kg^{-1} (Kramer et al., 2012; Pausch et al., 2013). The soil was passed through a 2 mm sieve before the experiments.

Maize (*Zea mays* L.) seeds were germinated on filter paper for 72 h. Sixteen pre-germinated maize seedlings were selected. One seedling was planted in a depth of 5 mm in each rhizobox, which was filled with soil to a final density of 1.4 g cm^3. The rhizoboxes had an inner size of 12.3 × 12.5 × 2.3 cm. During 2 weeks of growth, the rhizoboxes were kept inclined at an angle of 45° so that the roots grew near the lower wall of the rhizobox. The rhizoboxes were kept in a climate chamber with a controlled temperature of 20 ± 1 °C and a daily light period of 14 h with a photosynthetically active radiation intensity of 300 μmol m^{-2} s^{-1}. During the growth period, the soil water content was maintained at 60% of the water holding capacity by irrigating the soil from the bottom with distilled water. Soil water content was kept constant during the experiments. After growing the plants for 2 weeks, the shoots were cut at the surface of the soil and the rhizoboxes were incubated at 10, 20, 30 and 40 °C, for 14 more days, i.e., 4 rhizoboxes (replicates) at each temperature.

2.2. Soil zymography

Direct soil zymography (Sanaullah et al., 2016) was applied after cultivating the plants for 2 weeks (at a climate chamber temperature of 20 ± 1 °C), before the cutting shoots (living roots - rhizosphere), as well as 7 and 14 days after the cutting shoots (root - detritusphere) (for samples kept at 10, 20, 30 and 40 °C). Enzyme activities were visualized using membranes saturated with 4-methylumbelliferone (MUF)-substrates and 7-amino-4-methylcoumarin (AMC)-substrates. The MUF and AMC become fluorescent when substrates are enzymatically hydrolyzed by a specific enzyme (Spohn et al., 2013). Cellobiohydrolase was detected by 4-methylumbelliferyl-β-D-cellobioside, phosphatase by 4-methylumbelliferyl-phosphate, and leucine-aminopeptidase by L-leucine-7-amido-4-methylcoumarin hydrochloride (Koch et al., 2007; Razavi et al., 2015). Each substrate was dissolved to a concentration of 12 mM in universal buffers (MES buffer for MUF substrate and TRIZMA buffer for AMC substrate. All substrates and chemicals were purchased from Sigma Aldrich (Germany). Polyamide membrane filters (Tao Yuan, China) with a diameter of 20 cm and a pore size of 0.45 μm were cut into sizes adjusted for the rhizobox. The cut membranes were saturated with the substrates for each enzyme. The rhizoboxes were opened from the lower, rooted side and the saturated membranes were applied directly to the soil surface. Soil zymography was performed for each enzyme separately on the same rhizobox; firstly acid-phosphatase, secondly cellobiohydrolase and thirdly leucine-aminopeptidase activity were measured. This order was maintained throughout the experiments. After incubation for 1 h at the given temperature, the membranes were carefully lifted off the soil surface and any attached soil particles were gently removed using tweezers (Razavi et al., 2016b). The time span between each measurement was 30 min. Based on the preliminary test we considered the residue of fluorescence of the previous enzyme to be negligible. The membranes were placed under ultraviolet (UV) illumination with an excitation wavelength of 355 nm in a dark room. The camera (EOS