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Spatial patterns of enzyme activities in the rhizosphere: Effects of root hairs and root radius

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ABSTRACT

The importance of root hairs and root radius for exudation and nutrient acquisition by plants is known mainly from nutrient solution studies. The *in situ* effects of root hairs and root radius on the spatial distribution of enzyme activity in the rhizosphere of various plants are unknown. Four plants with contrasting root morphology (maize, wheat, lentil and lupine) were chosen to test the effects of root hairs and root radius on the spatial distribution of β-glucosidase, cellobiohydrolase, leucine aminopeptidase and acid phosphatase. We combined zymography with enzyme kinetics to evaluate the effects of root hairs on the rhizosphere extent and on substrate turnover. The extent of enzyme activity in the rhizosphere of four plants ranged from 0.55 to 2 mm. The extent of β-glucosidase was 1.5 times broader (1.2 mm versus 0.8 mm) and the substrate turnover was 2-fold faster around wheat root regions with hairs than hairless locations. The rhizosphere extent relative to root radius and the enzyme activity per root surface area were plant and enzyme specific: the rhizosphere extent was 1.5–2 times broader and the enzyme activity was 2–8-fold higher in wheat (with thin roots and long root hairs) compared to maize, lentil and lupine. The rhizosphere extent of acid phosphatase (1.1–2.0 mm) was 1.5–2-fold broader than that of other enzymes (0.5–1.0 mm). For the first time, we showed that the rhizosphere extent relative to root radius was 20–100% broader and enzyme activity per surface area was 4–7-fold higher around thin roots (wheat) than around thick roots (maize). Moreover, the rhizosphere extent relative to root radius was 10–30% broader and enzyme activity per root area was 2–7 times higher around roots with long and dense hairs (lupine) than around roots with short and sparse hairs (lentil). We conclude that root hairs and root radius shape the rhizosphere: root hairs contributed mainly to the rhizosphere extent, while root radius more strongly affected the enzyme activity per root surface area.

1. Introduction

Plant roots exude a very broad range of compounds into the soils (Jones et al., 2009), stimulating microbial and enzyme activities (Parkin, 1993; Asmar et al., 1994) and forming one of the most chemically, physically and biologically complex spheres with very intensive interactions – the rhizosphere (Bertin et al., 2003). The rhizosphere is one of the most important enzymatic hotspots (Kuzyakov and Blagodatskaya, 2015), where extracellular enzymes are produced by both microorganisms and living roots. Moreover, a considerable amount of intracellular enzymes is continuously released into the rhizosphere by lysis and damage of root and microbial cells (Bais et al., 2004).

The production and spatial distribution of enzymes in soils are a dynamic function of microbial and root properties, including microbial physiology (Henry et al., 2005; Allison and Treseder, 2008), root morphology, root exudation and rhizodeposition (Kuzyakov, 2002). Microorganisms synthesize and release enzymes largely as a nutrient acquisition strategy and roots may express more enzymes when nutrients are scarce (Harder and Dijkhuizen, 1983). The release of labile rhizodeposits varies spatially and temporally and depends on plant physiology and root morphology (Nguyen, 2003). Furthermore, the

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quality and composition of exudates vary between plant species, cultivars, over plant development and even along the root segments (Aulakh et al., 2001; Badri and Vivanco, 2009). Accordingly, the enzymes decomposing various components of rhizodeposits and SOM change in quantity and quality over time and between plant species. Thus, enzyme activity, reflecting microbial and roots activities, is also heterogeneously distributed in soil (Grierson and Comerford, 2000; Wallenstein and Weintraub, 2008).

Nutrients are very often limited in soils (Hodge, 2004), and this limitation is extremely strong in the rhizosphere because microorganisms and plants compete for the same nutrients (Kuzyakov and Xu, 2013). Plants use root morphological strategies to overcome nutrient limitation, such as the development of the roots with large surface area and long length (Jungk, 2001; Ma et al., 2001). Among these, root hairs, the tubular-shaped outgrowths from root epidermal cells (Peterson and Farquhar, 1996), strongly increase the root surface area, and play important roles in nutrient and water acquisition as well as in the interactions with microbes (Gilroy and Jones, 2000). Apart from these functions, root hairs are also essential in modulating the properties and composition of the rhizosphere through exudation, and, in some species, exudates are apparently produced solely by root hairs (Czarnota et al., 2003; Datta et al., 2011). Root hairs have a short lifespan (at most a few days), and dead root hairs therefore released abundant C into the soil (Nguyen, 2003). These large amounts of labile carbon and other rhizodeposits released by root hairs stimulate microbial activity (Parkin, 1993; Asmar et al., 1994) and further influences enzyme dynamics such as accelerated substrate turnover. Moreover, root hairs actively participate in the interactions between plants and nitrogen-fixing microorganisms and symbiotic mycorrhizal fungi by providing nutrients, hormones and signaling molecules (Peterson and Farquhar, 1996; Libault et al., 2010). Root hairs, however, vary highly in number, length, density and longevity, depending on both the genetic potential of plants and on environmental conditions (Jungk, 2001). It is widely accepted that exudation rates and nutrient acquisition capacities are positively correlated with root hair length and density (Yan et al., 2004).

Root radius is another root morphology parameter that influences exudation and nutrient acquisition. Thin and thick roots have distinctive nutrient absorption strategies (Kong et al., 2016). Exudation quantity and nutrient absorption ability are proportional to the root radius (Lambers et al., 2006). Therefore, the spatial pattern and dynamics of enzyme activities might be influenced by root hairs and root radius, dependent on root exudation, rhizodeposition and root hair morphology.

The role of root hairs and root radius and their interactive effects on the spatial distribution of enzymes and their *in situ* activity are completely unknown. Specifically, the effects of root hairs on the rhizosphere extent of enzyme activity and on the enzyme mediated turnover of various substrates in the rhizosphere remain unclear. The rhizosphere extent of enzyme activities can be estimated by a novel approach: zymography, which enables visualizing the spatial distribution of enzyme activities in the soil and rhizosphere (Spohn et al., 2013; Sanaullah et al., 2016). The estimation of enzyme-mediated substrate turnover (T_t) can be calculated based on enzyme kinetics considering the parameters V_{max} (maximum reaction rate) and K_m (half-saturation constant) in the Michaelis-Menten equation (Michaelis and Menten, 1913; Tischer et al., 2015).

We used zymography to visualize the spatial distributions of β-glucosidase, cellobiohydrolase, leucine aminopeptidase and acid phosphatase, which are responsible for C, N, and P cycles in the rhizosphere of four plants with contrasting root morphology. We combined zymography with enzyme kinetics to test the effect of root hairs on the spatial distribution of the rhizosphere and on substrate turnover. Maize (*Zea mays*) and wheat (*Triticum aestivum*) are gramineous plants with fibrous root system, with contrasting root morphologies: while both have long root hairs, maize roots are thicker than wheat roots. Lentil (*Lens culinaris*) and lupine (*Lupinus polyphyllus*) are members of the *Fabaceae*; both have a tap-root system and are nitrogen-fixing legume crops (Dinkelaker et al., 1989; Tovar, 1996). Both also have contrasting root morphology: the roots of lentil are thin with short hairs, whereas the roots of lupine are thicker with longer root hairs. All selected plants are key agricultural crops for food and fodder production and can be grown on a broad range of soils. We hypothesized that 1) due to intensive root exudation by root hairs, the rhizosphere extent of enzyme activities is broader in root regions with hairs than without hairs, 2) root hairs influence enzyme dynamics and lead to accelerated substrate turnover in the rhizosphere, and 3) the spatial pattern of enzyme activity is plant species- and enzyme-specific and depends on root morphology (root hairs and root radius).

2. Material and methods

2.1. Soil and plant 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 Gottingen, Germany. The soil was then passed through a sieve with a mesh radius of 2 mm. The soil had the following 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 rhizoboxes with inner dimensions of 21.2 × 10.8 × 3.3 cm were filled with soil to a final density of 1.2 g cm^3.

Maize (*Zea mays*), wheat (*Triticum aestivum*), lupine (*Lupinus polyphyllus*) and lentil (*Lens culinaris*) seeds were germinated on filter paper for 72 h and thereafter one seedling was planted in a depth of 5 mm in each rhizobox. Each species had 6 replications in separate rhizoboxes. The rhizoboxes were kept in a climate chamber at a controlled temperature of 20 ± 1 °C and a daily light period of 14 h with a photosynthetically active radiation intensity of 250 µmol m^{-2} s^{-1}. During the growth period, the rhizoboxes were kept inclined at an angle of 45° so that the roots grew along the lower wall of the rhizobox. The rhizoboxes were irrigated with distilled water to maintain water content at 60% of the water holding capacity.

2.2. Direct soil zymography

When plants were one week old, direct zymography (Sanaullah et al., 2016) was used to visualize the activity of four enzymes in the rhizosphere. Thin polyamide membrane filters (Tao Yuan, China) with a size of 20 × 10.8 cm and a pore size of 0.45 mm were saturated with the following substrates: 1) 4-methylumbelliferyl-β-D-glucoside to detect β-glucosidase activity, 2) 4-methylumbelliferyl-β-D-cellobioside to detect cellobiohydrolase activity, 3) 4-methylumbelliferyl-phosphate to detect acid phosphatase activity, and 4) L-leucine-7-amido-4-methylcoumarin hydrochloride to detect leucine-aminopeptidase activity (Koch et al., 2007; Razavi et al., 2015). Each of these substrates was dissolved to a concentration of 12 mM in buffers, MES buffer for 4-methylumbelliferyl (MUF) based substrate and TRIZMA buffer for 7-amido-4-methylcoumarin (AMC) based substrate. All substrates and chemicals were purchased from Sigma Aldrich (Germany). Under UV-light, the MUF and AMC become fluorescent when the respective specific enzyme hydrolyzes the substrate. 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