Soil Biology & Biochemistry 96 (2016) 229–237

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Rhizosphere shape of lentil and maize: Spatial distribution of enzyme activities

Bahar S. Razavi [a, *], Mohsen Zarebanadkouki [b], Evgenia Blagodatskaya [c, d], Yakov Kuzyakov [a, c]

[a] Department of Agricultural Soil Science, University of Göttingen, Göttingen, Germany
[b] Division of Soil Hydrology, University of Göttingen, Göttingen, Germany
[c] Department of Soil Science of Temperate Ecosystems, University of Göttingen, Göttingen, Germany
[d] Institute of Physicochemical and Biological Problems in Soil Science, Pushchino, Russia

ARTICLE INFO

Article history:
Received 7 January 2016
Received in revised form
23 February 2016
Accepted 29 February 2016
Available online xxx

Keywords:
Rhizosphere extent
Spatial pattern
Enzyme distribution
Soil zymography
Lentil and maize

ABSTRACT

The rhizosphere, the small soil volume that surrounds and is influenced by plant roots, is one of the most dynamic biological interfaces on Earth. Enzymes, produced by both roots and microorganisms, are the main biological drivers of SOM decomposition. *In situ* soil zymography was applied to test hypotheses that 1) the spatial pattern of rhizosphere activity is enzyme-specific and 2) the distribution of enzyme activity along the roots is dependent on root system and plant species. Lentil (*Lens culinaris*) and maize (*Zea mays* L.), two species with contrasting root physiology, were chosen to test their effects on spatial distribution of activities of β-glucosidase, cellobiohydrolase, leucine-aminopeptidase and phosphatase.

The extent of the rhizosphere for each enzyme and plant species was estimated as a function of distance from the root. For the first time, we demonstrated plant-specific patterns of exoenzyme distribution: these were uniform along the lentil roots, whereas in the rhizosphere of maize, the enzyme activities were higher at the apical or proximal root parts. We conclude that the shape and extent of the rhizosphere for enzyme activities is plant species specific and varies due to different rhizosphere processes (e.g. root exudation) and functions (e.g. nutrient mobilization abilities). The extension of enzyme activity into the rhizosphere soil was minimal (1 mm) for enzymes responsible for the C cycle and maximal (3.5 mm) for enzymes of the phosphorus cycle. This should be considered in assessments and modeling of rhizosphere extension and the corresponding effects on soil properties and functions.

1. Introduction

The rhizosphere, as a soil volume surrounding living roots, represents one of the most dynamic habitats and interfaces on Earth (Hinsinger et al., 2009; Kuzyakov and Blagodatskaya, 2015). The rhizosphere effect is typically most intense at the root surface (termed the rhizoplane) and extends several millimeters out into the soil (Dazzo and Gantner, 2012). The spatial distribution of the rhizosphere is a dynamic function of the soil matrix and plant properties, including root morphology, microbial colonization, nutrient uptake, root exudation and rhizodeposition (Neumann and Römheld, 2002; Dazzo and Gantner, 2012). Activity of microorganisms in the rhizosphere is strongly affected by root exudates and other rhizodeposits (Parkin, 1993; Högberg and Read, 2006; Oburger et al., 2014). Plants release about one third of their photosynthetic products in the form of rhizodeposits into the soil (Kuzyakov et al., 2003) providing the basis for the establishment of plant-microbial interactions (Bais et al., 2006). Rhizodeposits include root cap and border cell loss, death and lysis of root cells, gaseous losses, passive and active release of solutes (root exudates) and gelatinous material at the surface of roots (mucigel) (Curl and Truelove, 1986; Hinsinger et al., 2009; Jones et al., 2009). Root exudation stimulates microbial activity (Kuzyakov and Domanski, 2000; Hinsinger et al., 2009), production of extracellular enzymes (Asmar et al., 1994) and, thus, 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 release of enzymes by roots or by lysis of root cells (Jones et al., 2009; Marinari et al., 2014).

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

http://dx.doi.org/10.1016/j.soilbio.2016.02.020
0038-0717/

The plant plays an important role in selecting, enriching and stimulating the functional groups of microorganisms depending on its root physiology and exudate constituents (Asmar et al., 1994; Fontaine et al., 2007; Blagodatskaya et al., 2009). Thus, root exudates affect microbial community composition, and their corresponding ability to utilize various C and nutrient sources (Kuzyakov, 2002; Frank and Groffman, 2009). Microbial diversity differs between the rhizospheres of plant species (Kowalchuk et al., 2002; Valentinuzzi et al., 2015), cultivars (Averill and Finzi, 2013) or even along the roots (Schmidt and Eickhorst, 2014) and over the course of root development (Remenant et al., 2009; Philippot et al., 2013; Schmidt and Eickhorst, 2014). Similarly, exoenzyme activity is a function of the morphological and physiological attributes of microbial and plant species and root type (Grierson and Adams, 2000). Enzymes, produced by both roots and microbes, are the main biological drivers of SOM decomposition (Nannipieri et al., 2007). Enzyme activity in the rhizosphere reflects plant-microbial interactions and is a sensitive indicator for changes in microbial community composition, activity and function (Baldrian, 2009; Nannipieri et al., 2012).

The exoenzyme activities of plant species may vary, depending on root morphology, rhizodeposition, and interactions with microorganisms (Grierson and Adams, 2000). However, a clear understanding of the variation and distribution along and around the roots still is lacking. Furthermore, it is not clear whether the enzyme activities follow the patterns of root exudation (mainly concentrated at the root tips) (Pausch and Kuzyakov, 2011), or rhizodeposition along the root (Neumann and Römheld, 2000), or whether it is mainly dependent on the nutrient uptake strategy of the plant. For the latter, both 1) nutrient acquisition solely at the root tip and 2) along the whole root length have been proposed (Schnepf et al., 2008; Hinsinger et al., 2011). Such specific patterns have not yet been analyzed or discussed for the spatial distributions of enzymes in the rhizosphere.

Due to complex microbial community structures and diversity, the evaluation of enzyme activities in the rhizosphere requires consideration of the spatial variability along and radially outward from the roots (Pinton et al., 2001). This calls for studies on the spatial distribution of rhizosphere enzymes in undisturbed samples (Mackie et al., 2014; Kuzyakov and Blagodatskaya, 2015). The spatial distributions of enzyme activities in soil have been investigated by destructive methods for different root zones and root proximities (Tarafdar and Jungk, 1987; Kandeler et al., 2002). However, these approaches only provide one-dimensional distributions (Tarafdar and Jungk, 1987; Gahoonia and Nielsen, 1991; Marinari et al., 2014). Consequently, our knowledge about rhizosphere enzyme activities remains limited. The development of *in situ* and non-invasive techniques for measurement of root enzyme activities could alleviate these difficulties. Visual approaches and advanced analytical tools such as functional gene probes (Naseby and Lynch, 1998), histochemical techniques (Shaykh and Roberts, 1974; Gahan, 1984; Joner et al., 2000), electron microscopy of soil sections (Ladd et al., 1996), nano-sensors (Rodríguez-Lorenzo et al., 2012), root window-based approaches (Dinkelaker et al., 1997; Grierson and Comerford, 2000; Dong et al., 2007), and zymography (Spohn et al., 2013a) have opened new avenues to reveal the origin, location and distribution of enzyme activities in soil.

Zymography, a non-destructive *in situ* technique for two-dimensional imaging, now offers an opportunity for visualization of enzyme activities -spatial and temporal- in soil and in the rhizosphere (Spohn and Kuzyakov, 2013, 2014; Vandooren et al., 2013). We applied *in situ* soil zymography by placing substrate-saturated membranes in direct contact with roots and soil (Dinkelaker et al., 1997; Grierson and Comerford, 2000; Dong et al., 2007). We used this technique to test the hypothesis that spatial patterns of activity of various enzymes vary along the root and depend on the plant species. To cover a broad range of functions, we studied the spatial distribution of enzymes involved in decomposing soil organic materials: cellulose (e.g. β-glucosidase and cellobiohydrolase which are commonly measured as enzymes responsible for consecutive stages of cellulose degradation); proteins (e.g. leucine aminopeptidase, which hydrolysis L-peptide bonds) and phosphorous-containing organic compounds (e.g. acid phosphatase, which catalyzes the hydrolysis of organic P compounds to phosphate esters), (Eivazi and Tabatabai, 1988; Asmar et al., 1994). Lentil (*Lens culinaris*) and maize (*Zea mays* L.), species with contrasting physiology and root morphology, were chosen to test their effects on enzyme activity distribution. The lentil, a member of the Fabaceae, was selected as a plant with a tap-root system and is a nitrogen-fixing legume crop (Erskin et al., 2009, 2011). Maize was selected because of its fibrous root system and is an important non-legume crop. Both plants are very important agricultural crops for food and fodder production and can be grown on a broad range of soils.

We aimed at quantitative imaging of enzyme activities in soil as a function of distance along and outward from the root to clarify 1) whether spatial distributions of enzyme activity show enzyme-specific patterns along the root, 2) whether enzyme activity is associated mainly with root tips, and 3) to estimate the extent of the rhizosphere for each enzyme and plant species as a radial distance from the root.

2. Materials and methods

2.1. Sample preparation

Soil samples were taken from the top 10 cm of the Ap horizon of an arable loamy Haplic Luvisol, located on a terrace plain of the river Leine in the north-west of Göttingen, Germany. The soil consisted of 7% sand, 87% silt, 6% clay, with a bulk density of 1.4 g cm^{-3}, a water content of 30% at field capacity, a pH of 6.5, total carbon of 12.6 g C kg^{-1}, and total nitrogen of 1.3 g N kg^{-1} (Kramer et al., 2012; Pausch et al., 2013).

We grew sixteen maize (*Z. mays*) and sixteen lentil (*L. culinaris*) plants, each in a separate rhizobox with inner dimensions of 12.3 × 12.5 × 2.3 cm. The rhizoboxes were placed horizontally with one side open (like a door) and then soil was slowly and continuously poured into the rhizoboxes through a 2 mm sieve to achieve a 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 a stable soil packing (Carminati, 2013). Maize and lentil seeds were germinated on filter paper for 72 h. Then one seedling was planted in each rhizobox at a depth of 5 mm. During 3 weeks of growth, the rhizoboxes were kept inclined at an angle of 50° so that the roots grew at the vicinity of the lower wall of the rhizobox due to gravitropis. The samples were kept in a climate chamber with a controlled temperature of 20 ± 1 °C and a daily light period of 16 h with 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.

2.2. Soil zymography and imaging procedure

After cultivating maize and lentil plants for 3 weeks, zymography was applied as an *in situ* technique to study the spatial distribution of exoenzymes around the roots. We followed the protocol proposed by Spohn and Kuzyakov (2013) with slight modifications, and in combination with the root-window approach (Dong et al., 2007). Visualization of enzyme activities consisted of using membranes saturated with 4-methylumbelliferone (MUF)-

substrates (Spohn and Kuzyakov, 2013) and 7-amino-4-methylcoumarin (AMC)-substrates. The substrates become fluorescent when enzymaticaly hydrolyzed by a specific enzyme (Dong et al., 2007). 4-Methylumbelliferyl-β-D-glucoside (MUF-G) was used as substrate to detect β-glucosidase activity; cellobiohydrolase was detected by 4-methylumbelliferyl-β-D-cellobioside (MUF-C); 4-methylumbelliferyl-phosphate (MUF-P) to detect phosphatase activity; and L-leucine-7-amido-4-methylcoumarin hydrochloride (AMC-L) for leucine aminopeptidase activity. Each of these substrates was separately dissolved to a concentration of 12 mM in buffer (MES ($C_6H_{13}NO_4SNa_{0.5}$) buffer for MUF substrate and TRIZMA ($C_4H_{11}NO_3$•HCl, $C_4H_{11}NO_3$) buffer for AMC substrate (Koch et al., 2007), Sigma–Aldrich, Germany). Polyamide membrane filters (Tao Yuan, China) with a diameter of 20 cm and a pore size of 0.45 μm were saturated with the substrates for each enzyme. The membranes were cut into sizes adjusted for the rhizobox. The rhizoboxes were opened from the lower, rooted side and the saturated membranes were applied directly to the soil surface (Grierson and Comerford, 2000; Dong et al., 2007). After incubation for 1 h, the membranes were carefully lifted off the soil surface and any attached soil particles were gently removed using tweezers. One hour of incubation time was selected based on preliminary experiments and previous studies (Dong et al., 2007; Spohn and Kuzyakov, 2014).

In previous studies, the saturated membrane was protected by filter paper (Dong et al., 2007) or by a 1 mm gel plate (Spohn and Kuzyakov, 2013, 2014). Here, the membrane with substrates was in direct contact with the soil surface (Grierson and Comerford, 2000). Direct contact with soil particles and roots (1) reduces the necessary incubation time for the membrane on the soil surface, since the enzyme does not have to diffuse through the gel layer to reach the membrane; (2) enables standardization of the incubation time for all the enzymes; (3) reduces the risk of underestimating enzyme activity due to retention of enzymes in the gel or filter paper (Spohn and Kuzyakov, 2014) (Fig. S1); (4) improves the contrast of images by avoiding diffusion within the gel or filter paper. However, direct application of the membrane to the soil may induce quenching of fluorescence in the membranes. We tested the quenching effect of soil particles by 60-min application of membranes saturated with a series of MUF and AMC concentrations to the soil surface. The results showed that quenching for this soil (loamy Haplic Luvisol at 60% of water holding capacity) was negligible (Fig. S2).

After incubation, the membranes were placed under ultraviolet (UV) illumination with an excitation wavelength of 355 nm and an emission wavelength of 460 nm, in a light-proof room. To maintain constant conditions for all samples, the distance between the UV light resource, the camera (SX10IS, Canon) and the samples was fixed. A fixed position of UV light source, camera and samples was important for the further comparison and quantification of images. To correct for variations of the light intensity over the image area, we collected background images from uncoated membrane as well as background images without any membrane (Menon et al., 2007). The scaled black flat field similar in all images was considered as a background (reference object) during whole image processing.

To quantify the zymogram images, a standard calibration that relates the activities of various enzymes to the gray-value of zymogram fluorescence (i.e. of the saturated membrane) is required. The calibration function was obtained by zymography of

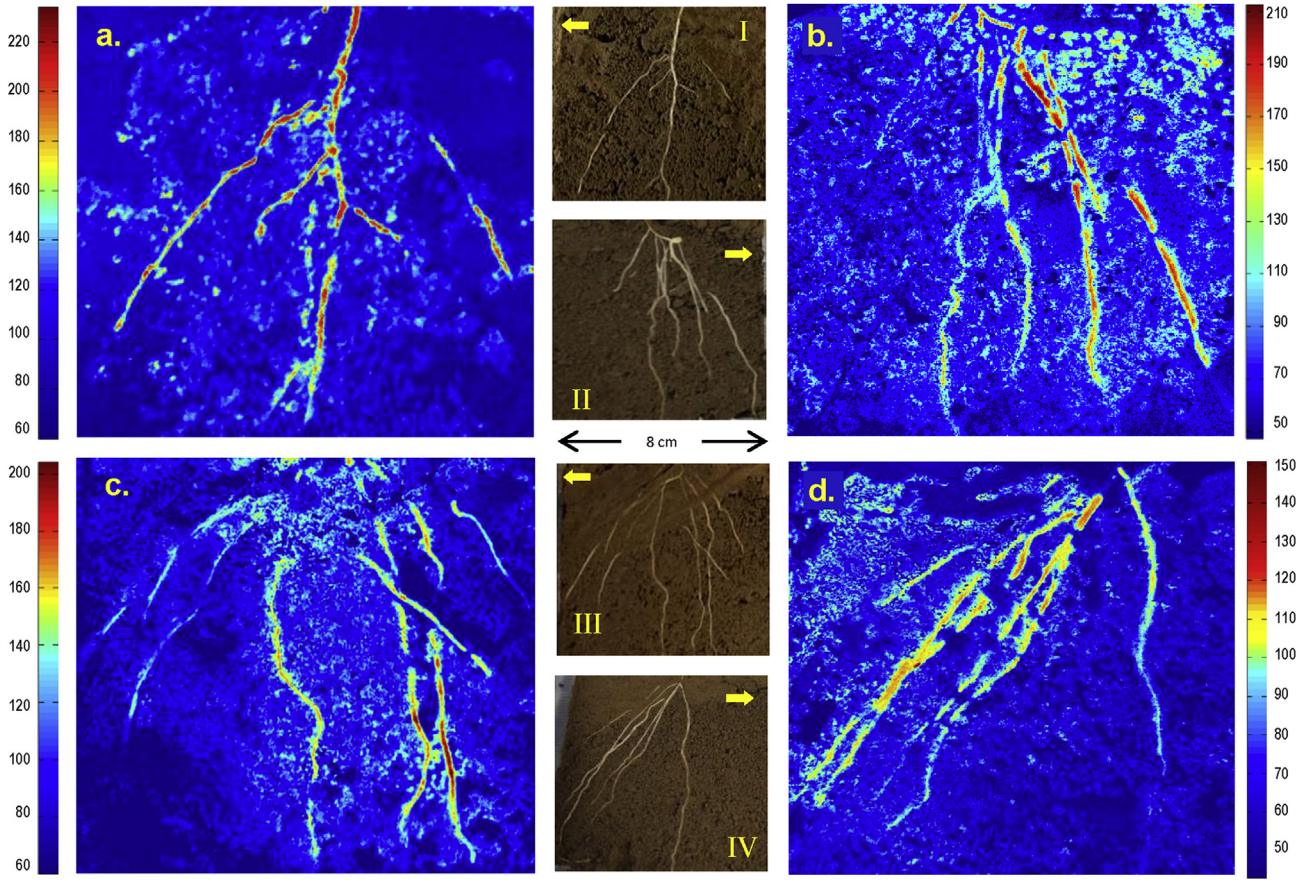

Fig. 1. Examples of lentil roots grown in rhizoboxes (center) and zymographs (left and right); showing spatial distribution of enzyme activities: a. leucine aminopeptidase, b. acid phosphatase, c. β-glucosidase and d. cellobiohydrolase. Side color maps are proportional to the enzyme activities (pmol cm^{-2} h^{-1}).

4 cm^2 membranes soaked in a solution of MUF or AMC – the fluorescent tag attached to each substrate proxy – with concentrations of 0.01, 0.2, 0.5, 1, 2, 4, 6, 10 mM. The amount of MUF and AMC on an area basis was calculated from the solution volume taken up by the membrane and its size. The membranes used for calibration were imaged under UV light and analyzed in the same way as for the samples.

2.3. Image processing and analysis

Image processing consisted of 5 steps: 1) transformation of projected signal (fluorescence) on the images to grayvalues, 2) background adjustment, 3) root segmentation, 4) root skeletonization and 5) conversion of grayvalues to enzyme activity.

Fluorescence on the zymograms under UV light shows the areas in which the substrate has been enzymatically degraded. The intensity of fluorescence is proportional to the activity of the enzyme. To get quantitative information, we processed the zymograms using the image processing toolbox in Matlab. Zymograms were transformed to 16-bit grayscale images as matrices and corrected for light variations and camera noise (Menon et al., 2007; Zarebanadkouki et al., 2012). Then, all the zymograms were referenced based on the grayvalue received from a reference object embedded in all the zymograms. We used the grayvalue obtained from the blank sides of the sample as the referencing point. After referencing the zymograms, we calculated an average background grayvalue through the zymograms of calibration lines at concentration of zero and subtracted this value from all the zymograms. Note that the same filters were applied to all of the images, including both zymograms of the roots and the calibration base line.

The resulting images were used for further analysis: The roots were segmented easily as they were distinguishable from the surrounding soil due to remarkable contrast between the soil and roots. To calculate enzyme activity as a function of distance along the root, we selected the roots that were not overlapping and were entirely visible at the soil surface. A threshold method in Matlab was used to detect the boundaries of the roots (Chaudhuri et al., 1989; Hoover et al., 2000). The images were then skeletonized with a thinning algorithm (Lam et al., 1992). The segmented roots, their length and radius were calculated using the Euclidean distance map function in Matlab (Menon et al., 2007; Zarebanadkouki and Carminati, 2014).

The pixel-wise grayvalues in the zymograms were converted to enzyme activity using the calibration function (Fig. S3). For this, the grayvalues of the calibration function were correlated with their substrate concentration and enzyme activity by fitting with the linear correlation of STATISTICA (Fig. S3) (Spohn and Kuzyakov, 2014). Then, we masked the selected roots for further analysis by multiplying the zymogram to the mask obtained from root segmentation. This enabled us to calculate average enzyme activity as a function of distance from the root tip or root center for each individual root. A four-parameter logistic curve was fitted to enzyme activity as a function of distance from the root tip for each plant species, using the same form of equation for both plants:

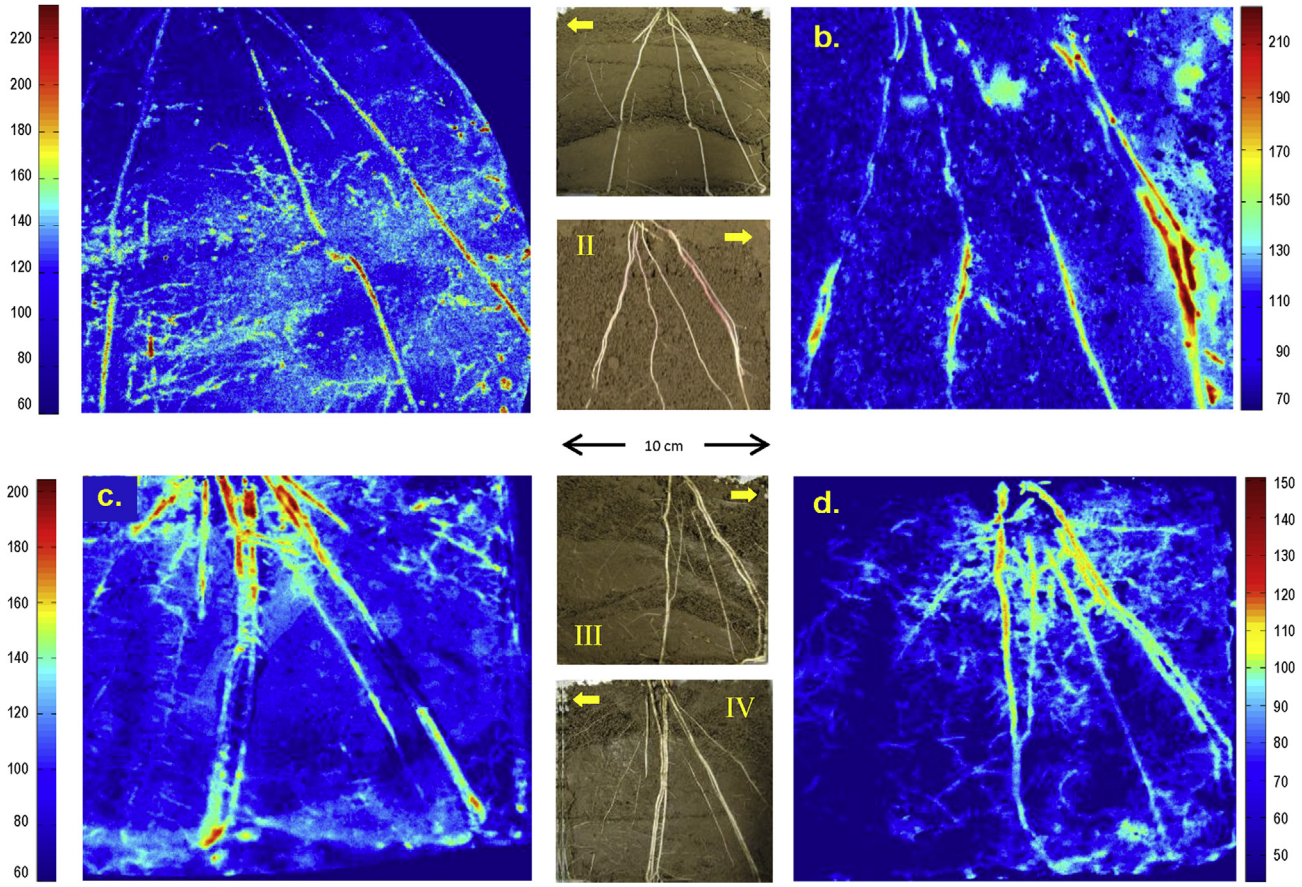

Fig. 2. Examples of maize roots grown in rhizoboxes (center) and zymographs (left and right); showing spatial distribution of enzyme activities: a. leucine aminopeptidase, b. acid phosphatase, c. β-glucosidase and d. cellobiohydrolase. The visibility of roots in zymographs depends on their growing direction along the lower wall of the rhizobox. Side color maps are proportional to the enzyme activities (pmol cm^{-2} h^{-1}).

$$y = \min + \frac{(\max - \min)}{1 + (x/EC)^{-Hillslope}} \quad (1)$$

where, (min and max) are minimum and maximum asymptote (the lowest and the highest activity), (x) is the independent value, EC and Hillslope respectively are the point of inflection (the point on the S shaped curve halfway between min and max) and Hill's slope of the curve (which reflects the steepness of the curve at point EC), in the STATISTICA environment (Table. S1). The criteria were an equation which gives highest correlation with obtained results and could better describe the observed pattern.

3. Results

Both lentil and maize plants grew well in the rhizoboxes (Figs. 1 and 2(a–d)). Maize roots penetrated the rhizoboxes rapidly and in some cases roots had reached the edges of the rhizoboxes at early growth stages (roots varied in length from 4 to 13 cm and average radius of 0.50 cm). In contrast, lentil roots developed slowly, were shorter, and did not penetrate the entire surface of the rhizoboxes (roots length varied from 2 to 10 cm and average radius of 0.45 cm).

3.1. Distribution of enzyme activities along the roots

The zymograms of individual plants are presented to illustrate their enzyme activity distributions along and outward from the root (Figs. 1 and 2). Thereafter, the statistical analysis of the replicates is summarized for 5 selected roots (Figs. 3 and 4).

Zymography revealed specific patterns of exoenzyme distribution as a function of distance from the root tips of two tested plants (Figs. 1 and 2). The distribution of enzyme activity along the lentil roots was uniform and homogenous (Fig. 1). Such a uniform distribution was consistent for enzyme activities as a function of distance from the lentil root tips of all replicate (Fig. 3). The activity was lower at the root tip, (from 0 to 1 mm), and increased thereafter up to 2–3 mm from the tip, and did not change significantly further along the roots (Fig. 3).

The distributions of enzyme activities along the maize root (Fig. 2) differed from those for lentil. Along an individual maize root, the activities of enzymes were higher at the apical and proximal parts of the roots (Figs. 2 and 4). Enzyme activity was low at the border of the tip, increased thereafter and slightly decreased along the root until relatively stable. Hence, highest activity was located at the 2 cm apical part of maize roots (root tip). Remarkably, such heterogeneity was enzyme specific: i. High activity at the root

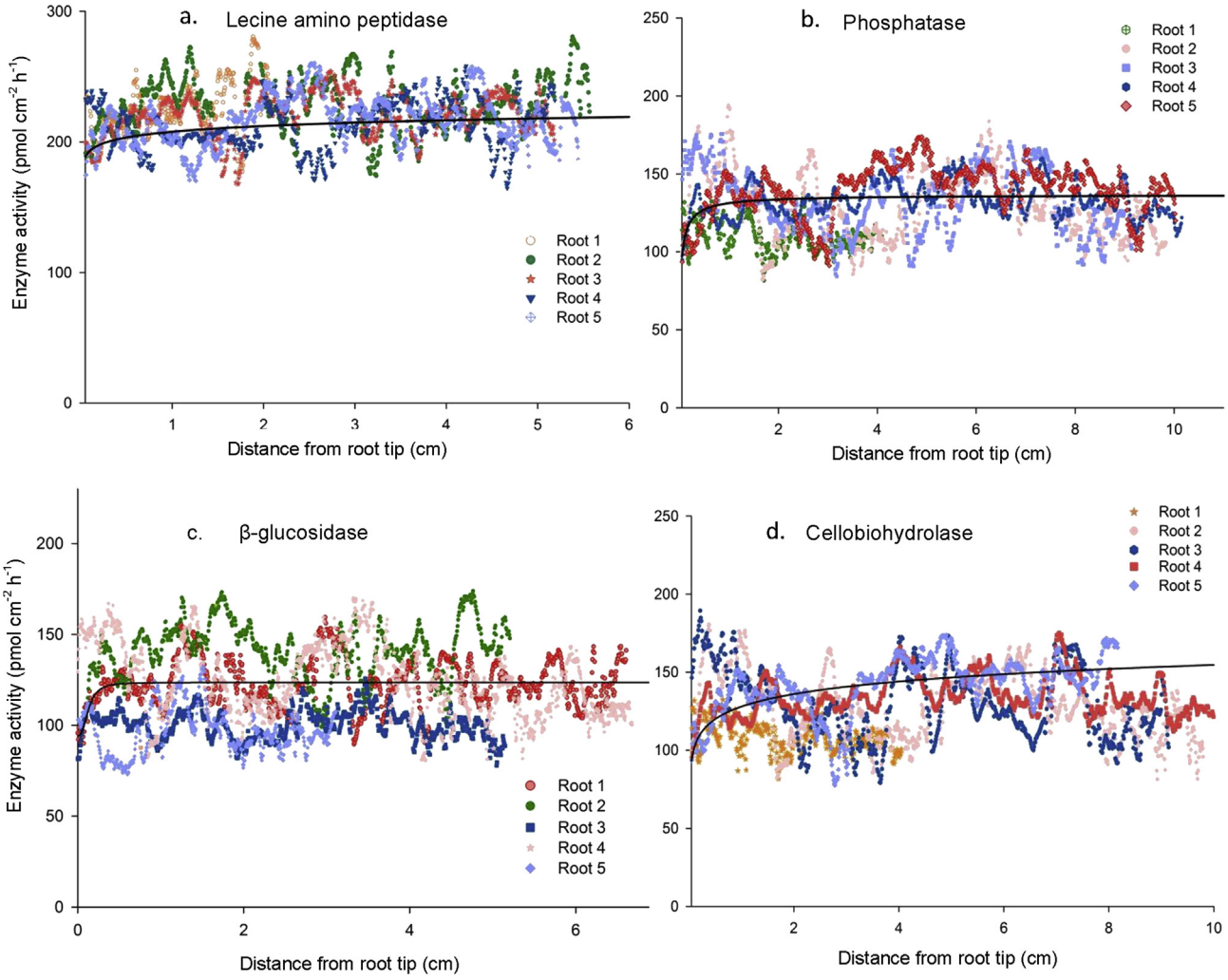

Fig. 3. The distribution of enzyme activities, a. leucine aminopeptidase, b. acid phosphatase, c. β-glucosidase and d. cellobiohydrolase, along the lentil roots. Values obtained from analysis of five individual roots as replicates. Black curves present fitting of enzyme activity as a function of distance from root tip by non-linear regression. Dotted arrow on the zymogram shows direction.

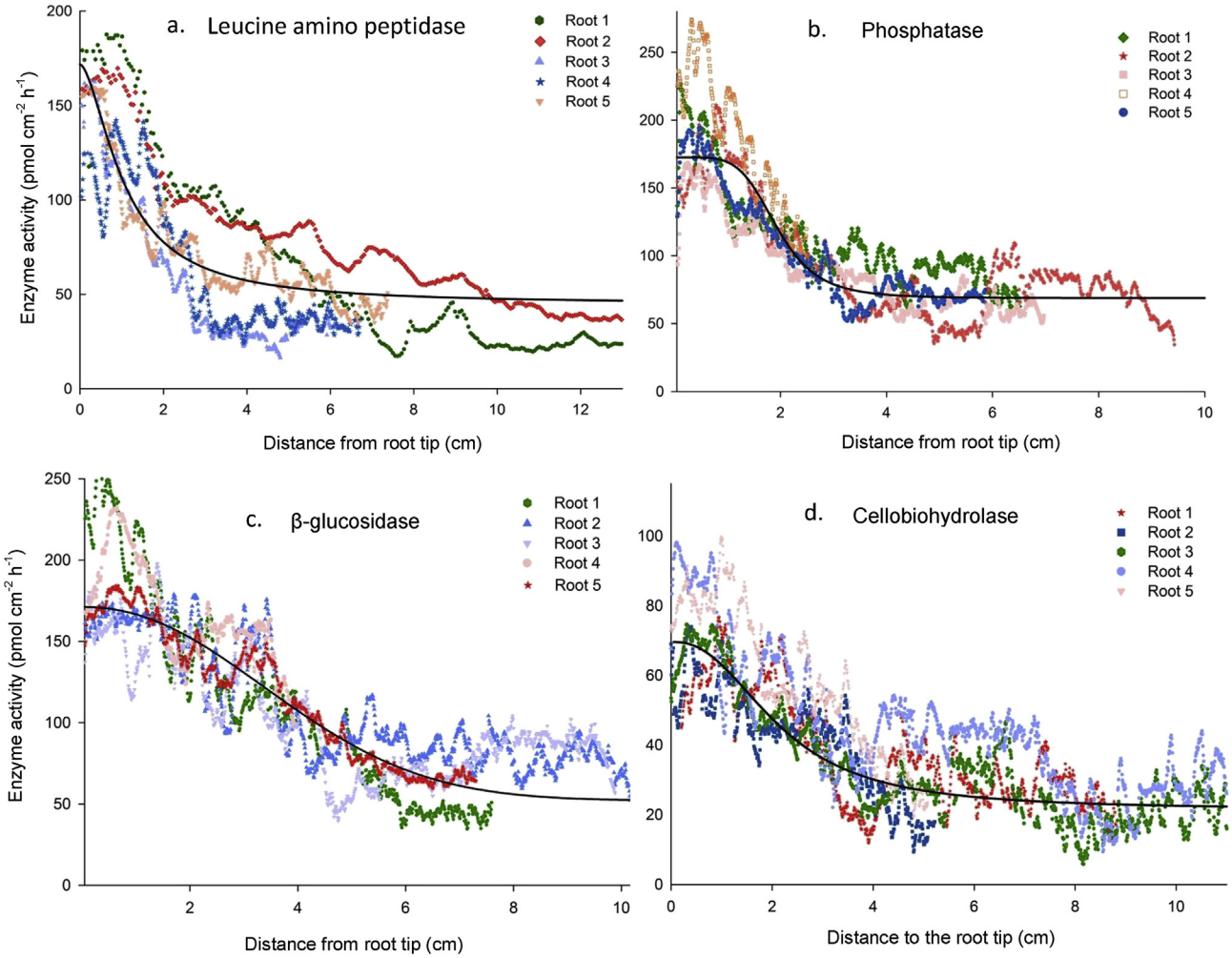

Fig. 4. The distribution of enzyme activities, a. leucine aminopeptidase, b. acid phosphatase, c. β-glucosidase and d. cellobiohydrolase, along the maize roots was not uniform. This observation was consistent in five root samples. Black curves present fitting of enzyme activity as a function of distance from root tip by non-linear regression. Dotted arrow on the zymogram shows direction. Note that the (x) axis of plot (d) is distance to the root tip.

tip was a common pattern for acid phosphatase and leucine aminopeptidase. ii. High activity at both proximal and apical parts of the root was measured for C-cycle enzymes.

Thus, the spatial pattern of enzyme activity did not change strongly along the lentil roots. In contrast, the distribution of enzyme activity around maize roots not only varied along the root length, but also demonstrated enzyme-specific patterns.

3.2. Rhizosphere extension of enzyme activities: distribution around the roots

The zymography images revealed remarkable detail on the spatial distribution of enzyme activity along and outward from the roots. This showed that the extension of the rhizosphere was plant- and enzyme-specific (Fig. 5). Acid phosphatase activity distribution was broader (2.5–3.5 mm) compared with the other three enzymes. The extent of the rhizosphere for leucine aminopeptidase varied from 1.5 to 2.5 mm and the narrowest extent was observed for β-glucosidase and cellobiohydrolase (1–1.5 mm). Rhizosphere extension was enzyme specific: For instance acid phosphatase activity had a biphasic pattern: close to the root it reduced gradually (0–1.5 mm) but the decrease accelerated rapidly with greater distance (1.5–3.5 mm) until levelling off. In contrast, β-glucosidase and cellobiohydrolase decreased rapidly from 0 to 1.5 mm and thereafter slowed down. Remarkably, the observed patterns of enzyme rhizosphere extension were root-length independent (selected root lengths varied from 5 to 13 cm) (Figs. 3 and 4).

4. Discussion

The 2D-images revealed that exoenzyme activity was mainly associated with the rhizoplane and rhizosphere. This high activity is primarily attributed to the inputs of easily degradable organic compounds from the roots and resulting stimulation of microorganisms (Kuzyakov and Domanski, 2000), and the direct release of enzymes by roots (Asmar et al., 1994; Marinari et al., 2014).

4.1. Distribution of enzyme activities along the roots

Our results consistently supported the hypothesis that the spatial patterns of enzyme activities along the root are plant species specific (Fig. 6). However, the hypothesis that enzyme activity is mainly associated with root tips was supported only for maize. We observed continuous distribution of all tested exoenzymes along the lentil roots. Such homogeneity suggests that the distribution of enzyme activity along the lentil root follows the patterns of

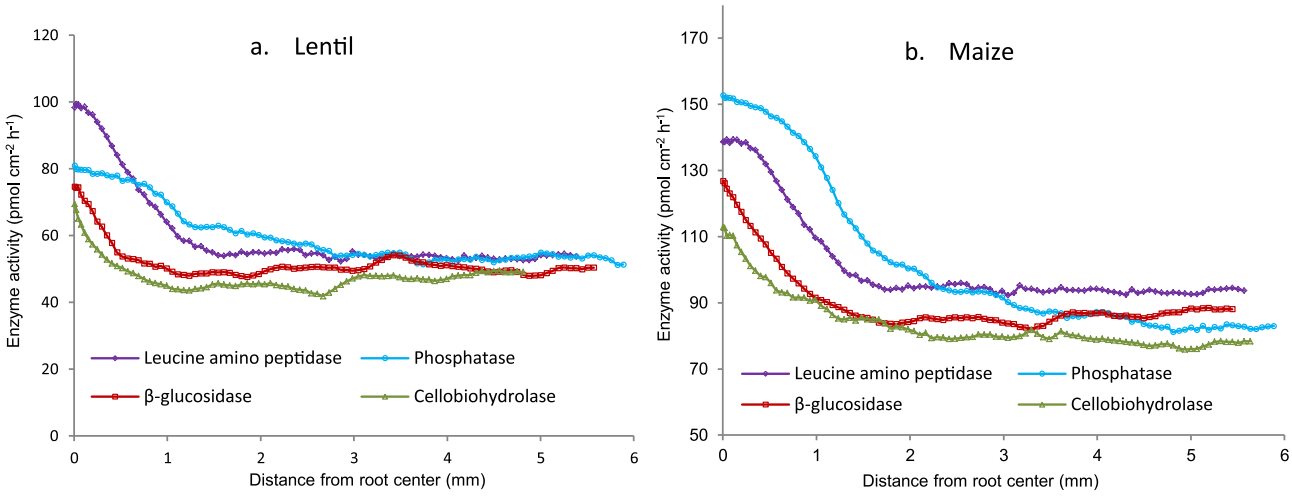

Fig. 5. The profile of enzyme activity distribution as a function of distance from the root center, a. lentil, b. maize, to the surrounding soil. Each line refers to the mean values of five sampled roots. Error bars are omitted to improve visualization. Please note that the (x) axis is in mm.

rhizodeposition along the root (Neumann and Römheld, 2000, 2002). A continuous distribution of exoenzymes along lentil roots is connected with the nutrient acquisition strategy along the whole root (Clarkson, 1991; Schnepf et al., 2008; Hinsinger et al., 2011). A similar homogenous spatial pattern is evident in previously reported results for acid phosphatase, cellobiohydrolase and N-acetyl-glucosaminidase along the roots of another leguminous plant (*Lupinus polyphyllus* L.), (Fig. 3 in Spohn and Kuzyakov, 2013, Figs. 1, 3 and 5 in Spohn and Kuzyakov, 2014). Consequently, based on this and previous studies, we conclude that enzyme distribution along roots of legumes is homogeneous, probably because of feeding microorganisms (mainly rhizobia) fixing N_2 (Spehn et al., 2000). Because the rhizobia colonization can occur anywhere along the legume root, the roots should maintain an attractive rhizosphere environment for potential symbionts (Vance and Heichel, 1991).

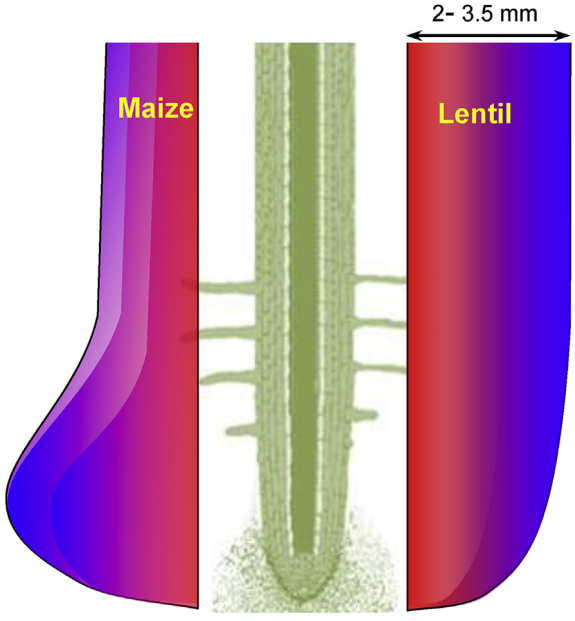

Fig. 6. General pattern of distribution of enzyme activity along the roots of lentil and maize. High enzyme activity is focused at root tips for maize. Relatively uniformly distributed enzyme activity along the root for lentil.

In contrast, exoenzyme activities along the maize roots were not constant. High activity of acid phosphatase and leucine aminopeptidase at the root apex confirms the common concept that root exudation is confined to the root tip (Nannipieri et al., 2007; Pajares et al., 2010; Pausch and Kuzyakov, 2011), and therefore increases microbial activities (Kuzyakov and Domanski, 2000; Hinsinger et al., 2009; Jones et al., 2009) and production of extracellular enzymes there (Asmar et al., 1994).

The enzymes involved in carbohydrate decomposition revealed higher activity focused at both the apical and proximal parts of the maize roots (Figs. 2 and 4). The region with lateral roots is particularly rich in organic materials because the secondary roots, in forcing their way through the cortical tissue of primary roots, cause considerable tissue damage (Neumann and Römheld, 2002). Consequently, the damaged cells and their nutritionally rich cytoplasmic contents leak out (Neumann and Römheld, 2002). Importantly, changes in substrate concentration affect coincidence of substrate and exoenzymes. By increase of substrate concentration chance of exoenzyme will increase to meet substrates.

The plant- and enzyme-specific distribution patterns of exoenzymes in the rhizoplane and rhizosphere of lentil and maize could be connected to the rhizodeposition and root exudate quality and quantity (Lynch and Whipps, 1990; Lupwayi et al., 1998; Hertenberger et al., 2002), which vary between plant species (Pajares et al., 2010) and location along the root (Lupwayi et al., 1998; Yang and Crowley, 2000; Hertenberger et al., 2002). Remarkably, even the liberation of sloughed–off root cells is a genetically controlled process and differs between plant species (Tscherko et al., 2004; el Zahar et al., 2008). Accordingly, the pattern obtained for maize is mainly related to processes ongoing at root tips, i.e. root exudation (Pausch and Kuzyakov, 2011) and mucilage release (Ahmed et al., 2015). The observed pattern for lentil is related to functions such as rhizodeposition (Neumann and Römheld, 2000), microbial colonization (Foster, 1986), pH (Göttlein et al., 1999), water uptake (Gahoonia and Nielsen, 1991; Zarebanadkouki et al., 2013) and release of protons and organic acids (Hinsinger et al., 2009), which occur along the root.

4.2. Rhizosphere extension of enzyme activities

In contrast to plant-specific patterns obtained along the roots, both plants demonstrated similar radial patterns around the roots (Fig. 5). However, the extent of rhizosphere varied between

enzymes: acid phosphatase extension (2.5–3.5 mm) was broader compared to the other three enzymes. This is in agreement with previous estimations of around 2–4 mm for acid phosphatase (Tarafdar and Jungk, 1987; Kandeler et al., 1999, 2002). Nonetheless, destructive approaches (e.g., slicing the soil and traditional enzyme assays) did not reveal the enzyme-specific two-dimensional distribution patterns of activity in the rhizosphere. We assume that mixing and homogenizing of the soil, commonly done prior to conventional analyses, masks the specifics of enzyme distribution.

The wide distribution of acid phosphatase is mainly due to the origin of this common enzyme, which can be produced by both plants and microorganisms (Dick and Tabatabai, 1984; Blagodatskaya and Kuzyakov, 2008; Nannipieri et al., 2012). Additionally, distribution and production of exoenzymes are affected by the plants and microorganisms demand for nutrients (Frank and Groffman, 2009). P is an essential nutrient (Schachtman et al., 1998; Tischer et al., 2015) and a component of key molecules such as nucleic acids and phospholipids, and is involved in controlling key enzyme reactions (Wardle, 1992).

The gradient of enzyme activities from the root surface to the rhizosphere varied between the enzymes. Acid phosphatase demonstrated a biphasic gradient for both plants, possibly because of the ability of the root to modulate soil acidity, increasing the pH values in surrounding soil by up to 1–2 units (Faget et al., 2013). As acid phosphatase is much more active at low pH, its high activity could be associated with the pH distribution around the root. This is supported by broad extension of acid phosphatase activity around the roots explained by influence of root exudates (Spohn et al., 2013b) usually abundant by organic acids (Jones et al., 2003; Lambers et al., 2006) and release of H^+ ions having much faster diffusion compared to organic compounds. Such an explanation, however, requires experimental confirmation by simultaneous determination of the pH along with enzyme activity, e.g. by optode techniques (Blossfeld, 2013; Rudolph et al., 2013).

In contrast, β-glucosidase and cellobiohydrolase (specific enzymes to degrade cellulose), showed the narrowest extent for both lentil and maize rhizospheres (1–1.5 mm) and a steep gradient in activity. This is associated with the distribution of polymeric and oligomeric components of rhizodeposits. However, investigation of a variety of further species with consideration of other effects (e.g. water content, temperature and nutrient availability) is called for.

Overall, for the first time, we visualized the enzyme-specific distribution patterns in soil and in the rhizosphere of different plants with contrasting root physiology. The shape and extent of the rhizosphere for enzyme activities varies with “super-active” sites at the growing root tip and proximal parts. Depending on the tested enzyme, the rhizosphere extension varied from 1 to 3.5 mm. In conclusion, the rhizosphere shape is plant- and enzyme-specific and reflects the soil volume, from which roots and associated microorganisms mobilize nutrients and utilize carbon.

Acknowledgment

The authors thank Susann Enzmann for laboratory assistance and Dirk Böttger for support with zymography equipment installation. We gratefully acknowledge the German Academic Exchange (DAAD) for supporting BSR. Contribution of EB was supported by Russian Scientific Foundation (Project No. 14-14-00625). This study was supported by the German Research Foundation (DFG) within the project: KU 1184/26-1.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.02.020.

References

Ahmed, M.A., Holz, M., Woche, S.K., Bachmann, J., Carminati, A., 2015. Effect of soil drying on mucilage exudation and its water repellency: a new method to collect mucilage. J. Plant Nutri. Soil Sci. 178, 821–824.
Asmar, F., Eiland, F., Nielsen, N.E., 1994. Effect of extracellular enzyme activities on solubilization rate of soil organic nitrogen. Biol. Fertility Soils 17, 32–38.
Averill, C., Finzi, A., 2013. Reprint of “Plant regulation of microbial enzyme production in situ”. Soil Biol. Biochem. 56, 49–52.
Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu. Rev. Plant Biol. 57, 233–266.
Baldrian, P., 2009. Microbial enzyme-catalyzed processes in soils and their analysis. Plant Soil Environ. 55, 370–378.
Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol. Fertility Soils 45, 115–131.
Blagodatskaya, E., Blagodatsky, S., Anderson, T.H., Kuzyakov, Y., 2009. Contrasting effects of glucose, living roots and maize straw on microbial growth kinetics and substrate availability in soil. Eur. J. Soil Sci. 60, 186–197.
Blossfeld, S., 2013. Light for the dark side of plant life: planar optodes visualizing rhizosphere processes. Plant Soil 369, 29–32.
Carminati, A., 2013. Rhizosphere wettability decreases with root age: a problem or a strategy to increase water uptake of young roots? Front. Plant Sci. 4.
Chaudhuri, S., Chatterjee, S., Katz, N., Nelson, M., Goldbaum, M., 1989. Detection of blood vessels in retinal images using two-dimensional matched filters. IEEE Trans. Med. Imaging 8, 263–269.
Cheng, W., Coleman, D.C., 1990. Effect of living roots on soil organic matter decomposition. Soil Biol. Biochem. 22, 781–787.
Clarkson, D.T., 1991. Root structure and sites of ion uptake. In: Waisel, Y., Eshel, A., Kafkafi, U. (Eds.), Plant Roots – the Hidden Half. Marcel Dekker, Inc., New York, USA, pp. 417–453.
Curl, E.,A., Truelove, B., 1986. Root Exudates. The Rhizosphere. Springer Berlin Heidelberg, pp. 55–92.
Dazzo, F.B., Gantner, S., 2012. The rhizosphere. In: Schmidt, T.M., Schaechter, M. (Eds.), Topics in Ecological and Environmental Microbiology. Academic Press.
Dick, W.A., Tabatabai, M.A., 1984. Kinetic parameters of phosphatases in soils and organic waste materials. Soil Sci. 137, 7.
Dinkelaker, B., Hengeler, C., Neumann, G., Eltrop, L., Marschner, H., 1997. Root exudates and mobilization of nutrients. In: Rennenberg, H., Eschrich, W., Ziegler, H. (Eds.), Trees — Contributions to Modern Tree Physiology. Backhuys Publishers, Leiden, The Netherlands, p. 441.
Dong, S., Brooks, D., Jones, M.D., Grayston, S.J., 2007. A method for linking in situ activities of hydrolytic enzymes to associated organisms in forest soils. Soil Biol. Biochem. 39, 2414–2419.
Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606.
el Zahar, H.F., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., et al., 2008. Plant host habitat and root exudates shape soil bacterial community structure. ISME J. 2, 1221–1230.
Erskine, W., Muehlbauer, F., Sarker, A., Sharma, B., 2009. The Lentil: Botany, Production and Uses. CAB International, Wallingford, UK, p. 457.
Erskine, W., Sarker, A., Kumar, S., 2011. Crops that feed the world 3. Investing in lentil improvement toward a food secure world. Food Secur. 3, 127–139.
Faget, M., Blossfeld, S., von Gillhaussen, P., Schurr, U., Temperton, V.M., 2013. Disentangling who is who during rhizosphere acidification in root interactions: combining fluorescence with optode techniques. Front. Plant Sci. 4, 392.
Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–281.
Foster, R.C., 1986. The ultrastructure of the rhizoplane and rhizosphere. Annu. Rev. Phytopathol. 24, 211–234.
Frank, D.A., Groffman, P.M., 2009. Plant rhizospheric N processes: what we don't know and why we should care. Ecology 90, 1512–1519.
Gahan, P.B., 1984. Plant Histochemistry and Cytochemistry. Academic Press, London, p. 241.
Gahoonia, T.S., Nielsen, N.E., 1991. A method to study rhizosphere processes in thin soil layers of different proximity to roots. Plant Soil 135, 143–146.
Göttlein, A., Heim, A., Matzner, E., 1999. Mobilization of aluminium in the rhizosphere soil solution of growing tree roots in an acidic soil. Plant Soil 211, 41–49.
Grierson, P.F., Adams, M.A., 2000. Plant species affect acid phosphatase, ergosterol and microbial P in a Jarrah (*Eucalyptus marginata* Donn ex Sm.) forest in southwestern Australia. Soil Biol. Biochem. 32, 1817–1827.
Grierson, P.F., Comerford, N.B., 2000. Non-destructive measurement of acid phosphatase activity in the rhizosphere using nitrocellulose membranes and image analysis. Plant Soil 218, 49–57.
Hertenberger, G., Zampach, P., Bachmann, G., 2002. Plant species affect the concentration of free sugars and free amino acids in different types of soil. J. Plant Nutr. Soil Sci. 165, 557–565.
Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321, 117–152.
Hinsinger, P., Brauman, A., Devau, N., Gérard, F., Jourdan, C., Laclau, J.P., Le Cadre, E., Jaillard, B., Plassard, C., 2011. Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail? Plant Soil 348, 29–61.

Högberg, P., Read, D.J., 2006. Towards a more plant physiological perspective on soil ecology. Trends Ecol. Evol. 21, 548–554.
Hoover, A., Kouznetsova, V., Goldbaum, M., 2000. Locating blood vessels in retinal images by piecewise threshold probing of a matched filter response. IEEE Trans. Med. Imaging 19, 203–210.
Joner, E.J., Van Aarle, I.M., Vosatka, M., 2000. Phosphatase activity of extra-radical arbuscular mycorrhizal hyphae: a review. Plant Soil 226, 199–210.
Jones, D.L., Dennis, P.G., Owen, A.G., Van Hees, P.A.W., 2003. Organic acid behavior in soils –misconceptions and knowledge gaps. Plant Soil 248, 31–41.
Jones, D., Nguyen, C., Finlay, D.R., 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant Soil 321, 5–33.
Kandeler, E., Luxhøi, J., Tscherko, D., Magid, J., 1999. Xylanase, invertase and protease at the soil–litter interface of a loamy sand. Soil Biol. Biochem. 31, 1171–1179.
Kandeler, E., Marschner, P., Tscherko, D., Gahoonia, T.S., Nielsen, N.E., 2002. Microbial community composition and functional diversity in the rhizosphere of maize. Plant Soil 238, 301–312.
Koch, O., Tscherko, D., Kandeler, E., 2007. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils: temperature sensitivity in alpine soils. Global Biogeochem. Cycles 21.
Kowalchuk, G.A., Buma, D.S., de Boer, W., Klinkhamer, P.G., van Veen, J.A., 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie van Leeuwenhoek 81, 509–520.
Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., et al., 2012. Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems. Pedobiologia 55, 111–119.
Kuzyakov, Y., 2002. Review: factors affecting rhizosphere priming effects. J. Plant Nutr. Soil Sci. 165, 382.
Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: concept and review. Soil Biol. Biochem. 83, 184–199.
Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. J. Plant Nutr. Soil Sci. 163, 421–431.
Kuzyakov, Y., Raskatov, A., Kaupenjohann, M., 2003. Turnover and distribution of root exudates of *Zea mays*. Plant Soil 254, 317–327.
Ladd, J., Ralph, N., Foster, C., Nannipieri, P., Oades, J.M., 1996. Soil structure and biological activity. Soil Biochem. 9, 23.
Lam, L., Lee, S.W., Suen, C.Y., 1992. Thinning methodologies: a comprehensive survey. IEEE Trans.Pattern Anal. Mach. Intell. 14, 869–885.
Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S.J., Veneklaas, E.J., 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Ann. Bottani 98, 693–713.
Lupwayi, N.Z., Rice, W.A., Clayton, G.W., 1998. Soil microbial diversity and commuinty structure under wheat as influenced by tillage and crop rotation. Soil Biol. Biochem. 30, 1733–1741.
Lynch, J.M., Whipps, J.M., 1990. Substrate flow in the rhizosphere. Plant Soil 129, 1–10.
Mackie, K.A., Schmidt, H.P., Müller, T., Kandeler, E., 2014. Cover crops influence soil microorganisms and phytoextraction of copper from a moderately contaminated vineyard. Sci. Total Environ. 500, 34–43.
Marinari, S., Moscatelli, C., Grego, S., 2014. Enzymes at plant-soil interface. In: Gianfreda, L., Rao, M.A. (Eds.), Enzymes in Agricultural Sciences. OMICS Group eBooks, USA, pp. 94–109.
Menon, M., Robinson, B., Oswald, S.E., Kaestner, A., Abbaspour, K.C., Lehmann, E., Schulin, R., 2007. Visualization of root growth in heterogeneously contaminated soil using neutron radiography. Eur. J. Soil Sci. 58, 802–810.
Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., Valori, F., 2007. Microbial diversity and microbial activity in the rhizosphere. Ciencia del suelo 25, 89–97.
Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M.C., Marinari, S., 2012. Soil enzymology: classical and molecular approaches. Biol. Fertility Soils 48, 743.
Naseby, D.C., Lynch, J.M., 1998. Impact of wild type and genetically-modified Pseudomonas fluorescens on soil enzyme activities and microbial population structure in the rhizosphere of pea. Mol. Ecol. 7, 617–625.
Neumann, G., Römheld, V., 2000. The release of root exudates as affected by the plant's physiological status. In: Pinton, R., Varanini, Z., Nannipieri, P. (Eds.), The Rhizosphere: Biochemistry and Organic Substances at the Soil–plant Interface. Dekker, New York, pp. 41–93.
Neumann, G., Römheld, V., 2002. Root-induced changes in the ability of nutrients in the rhizosphere. In: Waisel, Y., Eshel, A., Kafkafi, U. (Eds.), Plant Roots–the Hidden Half. Dekker, M., Inc., New York, pp. 617–649.
Oburger, E., Gruber, B., Schindlegger, Y., Schenkeveld, W.D.C., Hann, S., Kraemer, S.M., Wenzel, W.W., Puschenreiter, M., 2014. Root exudation of phytosiderophores from soil-grown wheat. New Phytologist 203, 1161–1174.
Pajares, S., Gallardo, J.F., Masciandaro, G., Ceccanti, B., Etchevers, J.D., 2010. Enzyme activity as an indicator of soil quality changes in degraded cultivated Acrisols in the Mexican trans-volcanic belt. Land Degrad. Dev. 22, 373–381.
Parkin, T.B., 1993. Spatial variability of microbial processes in soil – a review. J. Environ. Qual. 22, 409–417.
Pausch, J., Kuzyakov, Y., 2011. Photoassimilate allocation and dynamics of hotspots in roots visualized by 14C phosphor imaging. J. Plant Nutr. Soil Sci. 174, 12–19.
Pausch, J., Tian, J., Riederer, M., Kuzyakov, Y., 2013. Estimation of rhizodeposition at field scale: upscaling of a 14C labeling study. Plant Soil 364, 273–285.
Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., 2013. Going back to the roots: the microbial ecology of the rhizosphere. Nature Rev. Microbiol. 11, 789–799.
Pinton, R., Varanini, Z., Nannipieri, P., 2001. The rhizosphere as a site of biochemical interactions among soil components, plants, and microorganisms. In: Pinton, R., Varanini, Z., Nannipieri, P. (Eds.), The Rhizosphere: Biochemistry and Organic Substances in the Soil-plant Interface. Marcel Dekker, New York, pp. 1–17.
Remenant, B., Grundmann, G.L., Jocteur-Monrozier, L., 2009. From the microscale to the habitat: assessment of soil bacterial community structure as shown by soil structure directed sampling. Soil Biol. Biochem. 41, 29–36.
Rodríguez-Lorenzo, L., de La Rica, R., Álvarez-Puebla, R.A., Liz-Marzán, L.M., Stevens, M.M., 2012. Plasmonic nanosensors with inverse sensitivity by means of enzyme-guided crystal growth. Nature Mater. 11, 604–607.
Rudolph, N., Voss, S., Moradi, A.B., Nagl, S., Oswald, S.E., 2013. Spatio-temporal mapping of local soil pH changes induced by roots of lupin and soft-rush. Plant Soil 369, 669–680.
Schachtman, D.P., Reid, R.J., Ayling, S.M., 1998. Phosphorus uptake by plants: from soil to cell. Plant Physiol. 116, 447–453.
Schmidt, H., Eickhorst, T., 2014. Detection and quantification of native microbial populations on soil-grown rice roots by catalyzed reporter deposition-fluorescence in situ hybridization. FEMS Microbiol. Ecol. 87, 390–402.
Schnepf, A., Roose, T., Schweiger, P., 2008. Impact of growth and uptake patterns of arbuscular mycorrhizal fungi on plant phosphorus uptake—a modelling study. Plant Soil 312, 85–99.
Shaykh, M.M., Roberts, L.W., 1974. A histochemichal study of phosphatases in root apical meristems. Ann. Bot. 38, 165–174.
Spehn, E.M., Joshi, J., Schmid, B., Alphei, J., Körner, C., 2000. Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant Soil 224, 217–230.
Spohn, M., Kuzyakov, Y., 2013. Distribution of microbial-and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation–Coupling soil zymography with 14 C imaging. Soil Biol. Biochem. 67, 106.
Spohn, M., Kuzyakov, Y., 2014. Spatial and temporal dynamics of hotspots of enzyme activity as affected by living and dead roots – a soil zymography analysis. Plant Soil 79, 67–77.
Spohn, M., Carminati, A., Kuzyakov, Y., 2013a. Soil zymography – a novel in situ method for mapping distribution of enzyme activity in soil. Soil Biol. Biochem. 58, 275.
Spohn, M., Ermak, A., Kuzyakov, Y., 2013b. Microbial gross organic phosphorus mineralization can be stimulated by root exudates – a 33P isotopic dilution study. Soil Biol. Biochem. 65, 254–263.
Tarafdar, J.C., Jungk, A., 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol. Fertility Soils 3, 199–204.
Tischer, A., Blagodatskaya, E., Hamer, U., 2015. Microbial community structure and resource availability drive the catalytic efficiency of soil enzymes under land-use change conditions. Soil Biol. Biochem. 89, 226–237.
Tscherko, D., Hammesfahr, U., Claude, M.M., Kandeler, E., 2004. Soil Biol. Biochem. 1685–1698.
Valentinuzzi, F., Cesco, S., Tomasi, N., Mimmo, T., 2015. Influence of different trap solutions on the determination of root exudates in *Lupinus albus* L. Biol. Fertility Soils 51, 757–765.
Vance, C.P., Heichel, G.H., 1991. Carbon in N2 fixation: limitation or exquisite adaptation. Annu. Rev. Plant Biol. 42, 373–390.
Vandooren, J., Geurts, N., Martens, E., Van den Steen, P.E., Opdenakker, G., 2013. Zymography methods for visualizing hydrolytic enzymes. Nature Methods 10, 211–220.
Wardle, D.A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol. Rev. 67, 321–358.
Yang, C.H., Crowley, D.E., 2000. Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Appl. Environ. Microbiol. 66, 335–351.
Zarebanadkouki, M., Carminati, A., 2014. Reduced root water uptake after drying and rewetting. J. Plant Nutr. Soil Sci. 177, 227–236.
Zarebanadkouki, M., Kim, Y.X., Moradi, A.B., Vogel, H.J., Kaestner, A., Carminati, A., 2012. Quantification and modeling of local root water uptake using neutron radiography and deuterated water. Vadose Zone J. 11.
Zarebanadkouki, M., Kim, Y.X., Carminati, A., 2013. Where do roots take up water? Neutron radiography of water flow into the roots of transpiring plants growing in soil. New Phytologist 199, 1034–1044.