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REGULAR ARTICLE

Spatial and temporal dynamics of hotspots of enzyme activity in soil as affected by living and dead roots—a soil zymography analysis

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Abstract
Aims Hotspots of enzyme activity in soil strongly depend on carbon inputs such as rhizodeposits and root detritus. In this study, we compare the effect of living and dead *Lupinus polyphyllus* L. roots on the small-scale distribution of cellulase, chitinase and phosphatase activity in soil.
Methods Soil zymography, a novel in situ method, was used to analyze extracellular cellulase, chitinase and phosphatase activity in the presence of i. living *L. polyphyllus* roots prior to shoot cutting and ii. dead/dying roots 10, 20 and 30 days after shoot cutting.
Results After shoot cutting, cellulase and chitinase activities increased and were highest at the root tips. The areas of high cellulase and phosphatase activity extend up to 55 mm away from the root. Moreover, we observed microhotspots of cellulose, chitinase, and phosphatase activity up to 60 mm away from the next living root. The number and activity of microhotspots of chitinase activity was maximal 10 days after shoot cutting.
Conclusions The study showed that young root detritus stimulates enzyme activities stronger than living roots. Soil zymography allowed identification of microhotspots of enzyme activity up to several cm away from living and dying roots, which most likely were caused by arbuscular mycorrhizal fungi.

Keywords Rhizosphere · Microbial hotspots · Soil microbial activity · Detritusphere · Spatial distribution · Extracellular enzymes · Synergistic effects

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Introduction

Hotspots of extracellular enzyme activity in soil strongly shape biogeochemical cycles on earth. However, little is currently known about their spatial and temporal organization and dynamics (Nunan et al. 2002; Watt et al. 2006; Marschner et al. 2012). Hotspots occupy only a very small volume of soil, yet a large portion of all element-cycling processes occurs in these microsites. Parkin (1987) showed for example that 85 % of all denitrification reactions in a 5,190 g soil core took place in a 0.08 g piece of detritus in this soil core. Thus, 85 % of all denitrification in this soil took place in less than 0.002 % of the soil mass. Hence, the analysis of the localization and the spatial and temporal dynamics of hotspots will contribute to a better understanding of biogeochemical cycles.

Hotspots of enzyme activity in soil strongly depend on the presence and activity of roots (Högberg and Read 2006), because living roots release not only enzymes but

also easily degradable rhizodeposits that can stimulate microbial activity (Hinsinger et al. 2009; Luster et al. 2009; Kuzyakov 2002). Furthermore, dead roots provide a large flux of organic carbon (C) and nitrogen (N) into soil that can strongly increase microbial activity (Poll et al. 2008; Bastian et al. 2009). Yet, little is known about how living and dead roots differ in their effects on microbial activity in soil. In this study we used a novel technique–soil zymography–to analyze and to compare the spatial and temporal distribution of the activity of three exoenzymes involved in the cycling of C, N, and phosphorus (P) in presence of living and dying roots.

Microorganisms in soils are often C limited and therefore usually remain in a dormant state (Joergensen et al. 1990; Vance and Chapin 2001; Blagodatskaya and Kuzyakov 2013). However, even trace amounts of easily degradable organic C can strongly stimulate their activity (De Nobili et al. 2001; Joergensen et al. 1990). Rhizodeposition by living roots represents a significant source of easily available organic C in soil. Rhizodeposits comprise of root cap and border cell loss, soluble organic compounds (root exudates), insoluble polymer secretion (mucilage) from living cells and gasses (Hinsinger et al. 2009; Jones et al. 2009). Due to inputs of rhizodeposits, a large population of soil biota resides in the rhizosphere (Kuzyakov 2002). Their abundance in the rhizosphere ranges from two-times higher (for Protozoa) up to more than 1000-times higher (for denitrifiers) compared to root-free soil (Westover et al. 1997). These large fluxes of rhizodeposits make the rhizosphere one of the most important hotspots of nutrients and C turnover in terrestrial ecosystems.

In his comprehensive meta-analysis, Nguyen (2003) concluded that 10- to 100-times more C is released by roots of herbaceous plants into the soil through exudation than through mucilage and border cell loss. Exudation was estimated as 0.2 to 7 % of the root dry mass per day, which corresponds to 53 to 1,855 % of the root dry mass per year (Nguyen 2003). The amount of C released into soil per year by turnover of fine roots in grasslands makes up 53 % of the root dry mass per year (Gill and Jackson 2000). Hence, quantitatively the C inputs into soil by fine root turnover and exudation can be in the same range. However, the C inputs into soil by living roots and by fine root detritus differ in their composition as well as in their dynamics. While exudates from living roots mostly consist of soluble monomers like monosaccharides and amino acids (Hinsinger et al. 2009), root detritus contains cellulose and xylan alongside the monosaccharides and amino acids in the protoplasm (Rasse et al. 2005). The temporal dimensions of the two forms of root C inputs differ as rhizodeposition is a continuous C flow that is only slightly attenuated by circadian (Kuzyakov and Cheng 2001) and seasonal cycles, while the death of roots leads to a larger but temporarily much more concentrated C input.

Due to the large amounts of C and N released by dead roots, the detritusphere, i.e. the soil influenced by the presence of plant residues, is also a hotspot of microbial activity (Bastian et al. 2009). Poll et al. (2008) reported that the microbial community in a first phase of decomposition of plant residues is dominated by bacteria; while once the easily degradable substances are metabolized, fungi become more dominant. Recently Marschner et al. (2012) compared the rhizosphere with the detritusphere. They found that the abundance of bacterial and fungal biomarkers were elevated in the first 1–2 mm away from roots and plant residues, while generally the abundance of microorganisms was larger in the vicinity of the plant residues than in the vicinity of the living roots.

In this study we used soil zymography, a novel in situ technique for determining the distribution of exoenzyme activity (Spohn et al. 2013; and Spohn and Kuzyakov 2013). The technique has two major advantages. First, it enables the mapping of the distribution of exoenzymes at a high resolution. Second, the technique is not destructive, which means that the same sample can be analyzed several times.

The objective of this study was to compare the spatial and temporal distribution of activity of three classes of enzymes that are involved in C, N, and P cycling, namely cellulases, N-acetyl-glucosaminidases (chitinases), and phosphatases in dependence of the presence of living and dying/dead roots. We hypothesized that areas of high exoenzyme activity are larger in soil in the presence of dying roots than in soil with living roots due to a temporarily concentrated, large input of organic C and N from dying roots.

Material and methods

Experimental setup

Lupinus polyphyllus L. (Kiepenkerl, Everswinkel) was grown in four rhizoboxes in loamy soil. The rhizoboxes had an inner size of 12.3 × 12.5 × 2.3 cm and were inclined

by 50° in order to make the roots grow along the lower wall of the rhizobox. The soil was taken from the Bw horizon of a Cambisol from a depth of 15–23 cm. The soil is located close to the city of Göttingen and had the following characteristics: 24.8 % sand, 55.1 % silt, 20.1 % clay, pH 5.0, 7.9 g kg^{-1}C, 0.6 g kg^{-1} N, and 70 μg g^{-1} microbial C. The soil was dried at 30 °C, passed through a 2-mm sieve and was filled into the rhizoboxes to a density of 1.3 g cm^{-3}. The water content in the rhizoboxes was adjusted to 50 % water holding capacity, and was kept stable throughout the experiment. The plants were directly sown in the rhizoboxes and were grown in a climate chamber at 20 °C with a 14 h photoperiod. After growing the plants for 18 days, the shoots were cut at the surface of the soil and the rhizoboxes were kept in the climate chamber for 30 more days at the same temperature, photoperiod and soil water content as before shoot cutting.

Soil zymography

Soil zymography enables measuring the distribution of exoenzymes in soil using membranes coated with substrate molecules that becomes fluorescent when hydrolyzed. These membranes are incubated on the soil surface protected by a gel, and are subsequently photographed under UV light that excites the fluorescent molecules. The distribution of cellulase (EC 3.2.1.4), N-acetyl-glucosaminidase (EC 3.2.1.50), and phosphatase (EC 3.1.3) activity was analyzed by soil zymography 18 days after sowing the plants directly before cutting the shoots, and 10, 20, and 30 days after cutting the shoots. Soil zymography is a novel method that allows for in situ mapping of the distribution of enzyme activity (Spohn et al. 2013) and has been enhanced by integrating fluorescent methylumberrliferyl (MUF)-substrates (Spohn and Kuzyakov 2013). MUF-cellulose, MUF-N-acetyl-glucosaminide, and MUF-phosphate (Sigma-Aldrich) were dissolved in millipore water to a concentration of 12 mM. Membrane filters of polyamide (Sartorius) with a diameter of 14.2 cm and a pore size of 0.45 μm were soaked in the solutions and were subsequently allowed to dry flat for 2–3 min at room temperature on aluminum foil. For preparation of agarose gels, 1 % agarose was dissolve at 80 °C in water. Gels were cast in systems usually used for vertical gel-electrophoresis (Biometra); they had a size of 0.1× 12.0×11.0 cm. Membranes and gels were prepared directly before analysis of enzyme activity. For the incubation, the lower side of the rhizoboxes was opened, exposing the lupine's roots. The agarose gel was attached to the soil, and the membrane was placed on top of it. Soil zymography was performed for each enzyme separately on the same rhizoboxes; first N-acetyl-glucosaminidase, second cellulase and third phosphatase activity was analyzed. This order was maintained throughout the experiment. We changed the gels before each incubation in order to control exactly the time, in which the membrane is in contact with the enzymes. It takes some time for the enzymes to diffuse through the gel to the membrane. If the gel is re-used after a first incubation, some enzymes are already in the gel. Thus, the effective incubation time for the second incubation will be longer than for the first incubation in an uncontrolled way. The incubation times of the membranes were adjusted to the enzyme activity in order to obtain images with a strong contrast. For determining N-acetyl-glucosaminidase activity, membranes were incubated for 2 h and for analyzing cellulase and phosphatase activity membranes were incubated for 3 and 1 h, respectively. After the incubation, the membrane was removed, and the gel was discarded. In contrast to Spohn and Kuzyakov (2013) we did not dry the membranes after the incubation because we found that the membranes can be kept flat more easily when they are still moist. Each membrane was placed on an epi-UV-desk (Desaga) in the dark, and exposed to light with 360 nm wavelength. A picture of the membrane was taken with a digital camera (DSC-HX10V, Sony).

A calibration line was prepared from membranes that were soaked in solutions of 4-methylumbelliferone (MUF) of different concentrations (0, 35, 70, 130, 200 μM). These calibration membranes were cut into pieces of 4 cm^2, and photographed under the UV light in the same way as the zymogram membranes. The amount of MUF on an area basis was calculated from the volume of solution taken up by the membrane, the concentration of the solution, and the size of the membrane.

Image analysis and statistics

Image processing and analysis was done using the open source software imageJ. The digital images were transformed to 8-bit, i.e. grayscale images. The matrices of the zymogram images and the images of the calibration membranes were multiplied by a factor of 6.0 in order to increase the contrast of the images. To illustrate the

results, we depicted the values of the grayscale image in color. The linear correlation between the MUF concentration and the mean of grayscale in an area of 4 cm^2 of each calibration gel was calculated using the software Origin 6.0. The zymogram images were divided into segments of 10 Gray values (in the range of 30 to 140 Gray values), and the areas of these segments were calculated as percentage of the area of the entire image. For the images depicting the N-acetyl-glucosaminidase activity also the number of areas that have a size larger than 50^2 pixels was calculated for the different segments. Since roots only occupy a very small area of the soil surface (approximately 2 %) this analysis represents largely the distribution of enzyme activity in the soil. Homogeneity

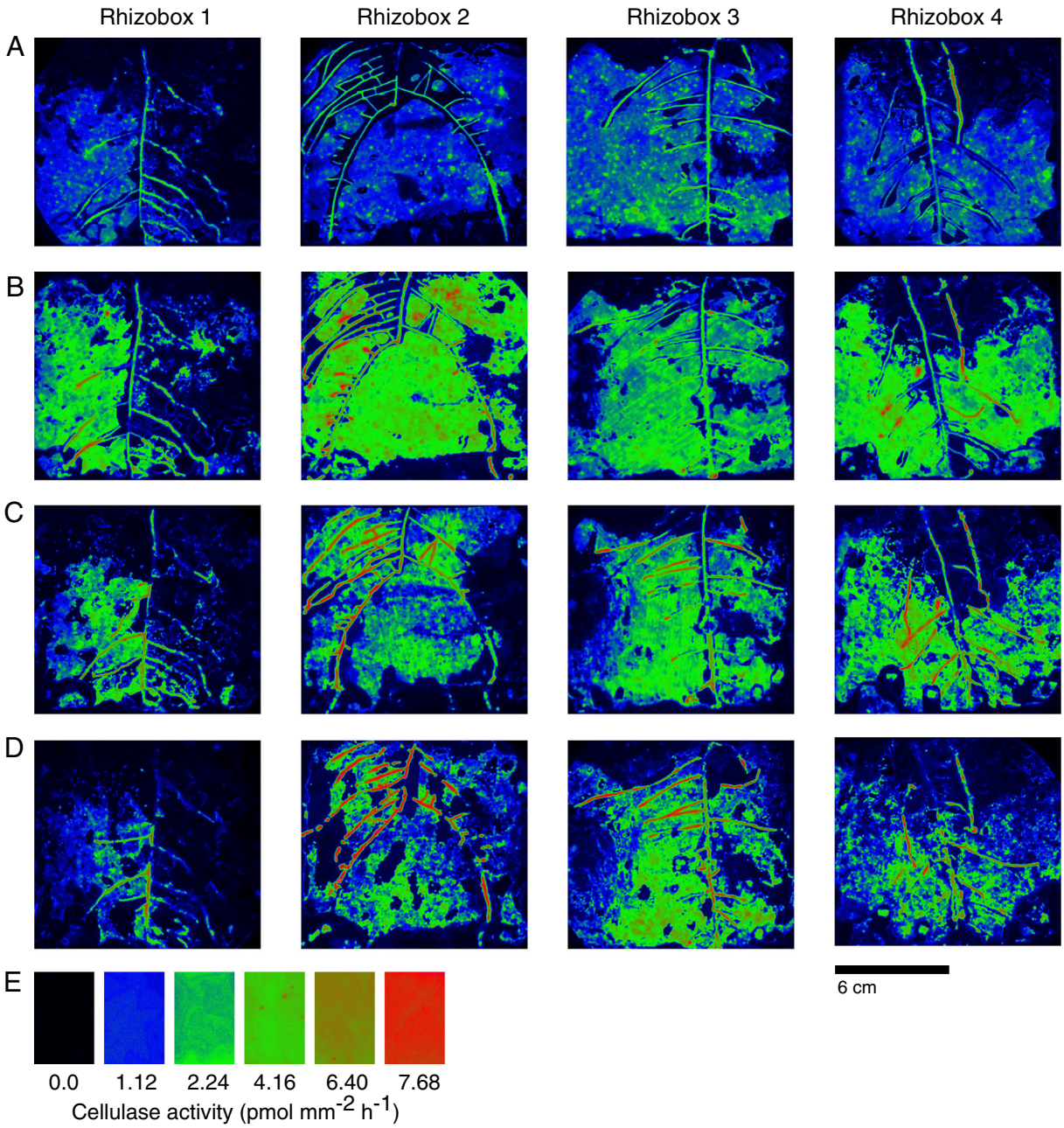

Fig. 1 Cellulase activity in four microcosms with living roots 18 days after sowing (**a**), and 10 days (**b**), 20 days (**c**) and 30 days (**d**) after cutting the shoots. The calibration line for the cellulase activity is presented at the bottom (**e**)

of variance of the sizes of the areas was tested by the Levene-test. Significance of differences between areas was tested by ANOVA followed by the Duncan-test, where $\alpha<0.05$ was considered as the threshold value for significance. Statistical analyses were performed using R environment for statistical computing (R Core Team 2013).

Results

The gray values of the calibration membranes and their MUF concentrations were linearly correlated ($R^2=0.96$, Appendix) as shown by Spohn and Kuzyakov (2013). The area of cellulase activity in soil was largest 10 days after shoot cutting and extended up to 55 mm away from the next root (Figs. 1 and 2). Twenty days after shoot cutting, the area of cellulase activity was decreased again, but not to the low extension that was found in the soil with the living roots (Fig. 2). Directly along the roots, cellulase activity increased first at the root tips after cutting (Fig. 1b), and it was increased in many parts along the lateral and tap roots 20 days after the cutting (Fig. 1c). Cellulase activity was increased by a factor of three both in the soil and at the roots 10 days after cutting compared to the cellulose activities in the soil and at the roots in the rhizoboxes with the living plants. Prior to cutting, microhotspots of high cellulase activity

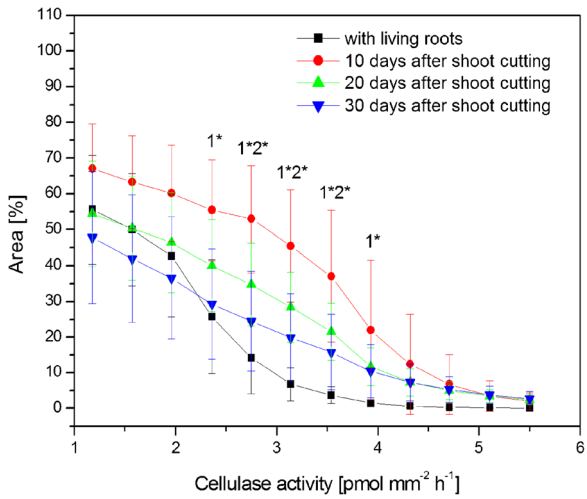

Fig. 2 Percentage of the area of cellulase activity in the total image as a function of cellulase activity. Data points depict means calculated from four independent replicates, error bars depict standard deviations. *Asterisk* indicates significant differences between the soil with the living roots and the soil with the plants 10 (1), 20 (2), and 30 (3) days after shoot cutting

with a diameter of 0.2 to 2 mm were visible in the soil up to 60 mm away from the next living root (Fig. 1a). After shoot cutting, the activity in the entire soil increased strongly, so that single microhotspots could not be identified any more (Fig. 1b, c).

After shoot cutting, N-acetyl-glucosaminidase activity directly at the root increased first at the root tips (Fig. 3b). Twenty days after shoot cutting, N-acetyl-glucosaminidase activity had increased in many parts of the root system (Fig. 3c). Ten days after shoot cutting, N-acetyl-glucosaminidase activity at the root was increased by a factor of three compared to the N-acetyl-glucosaminidase activity at the living roots (Fig. 3). However, the activity in the soil far from roots increased in the low activity range 10 days after shoot cutting (Figs. 3 and 4a). Microhotspots of high N-acetyl-glucosaminidase activity were visible up to 60 mm away from the roots at all analysis times (Fig. 3a–d). The number of the hotspots increased 10 days after cutting and decreased thereafter (Fig. 4b). However, due to large variation between rhizoboxes this trend in the number of hotspots was not statistically significant (Fig. 4b).

Phosphatase activity was most widely distributed in the soil 10 and 20 days after cutting the shoots (Figs. 5 and 6), leading to increased phosphatase activity up to 55 mm away from the closest root. It increased by a factor of two from the soil with the living roots to the soil with the dead roots 10 days after shoot cutting (Figs. 5 and 6). Thirty days after cutting, the area of phosphatase activity had decreased again (Figs. 5 and 6). Phosphatase activity directly at the roots did not change significantly during the month of observation. Microhotspots of high phosphatase activity were visible up to 60 mm away from the next living root (Fig. 5a) and in two replicate rhizoboxes 10 days after shoot cutting (Fig. 5b).

In comparison, phosphatases showed the highest maximum activity (Fig. 5e), while cellulases showed the lowest maximum activity (Fig. 1e). The largest area of activity was observed for phosphatase activity (Fig. 6) and the smallest area for N-acetyl-glucosaminidase activity (Fig. 4). Considering the living roots, cellulase activity was 9.0-times higher at the roots than in the soil, while N-acetyl-glucosaminidase and phosphatase were 12.5- and 13.9-times higher along the roots than in the soil.

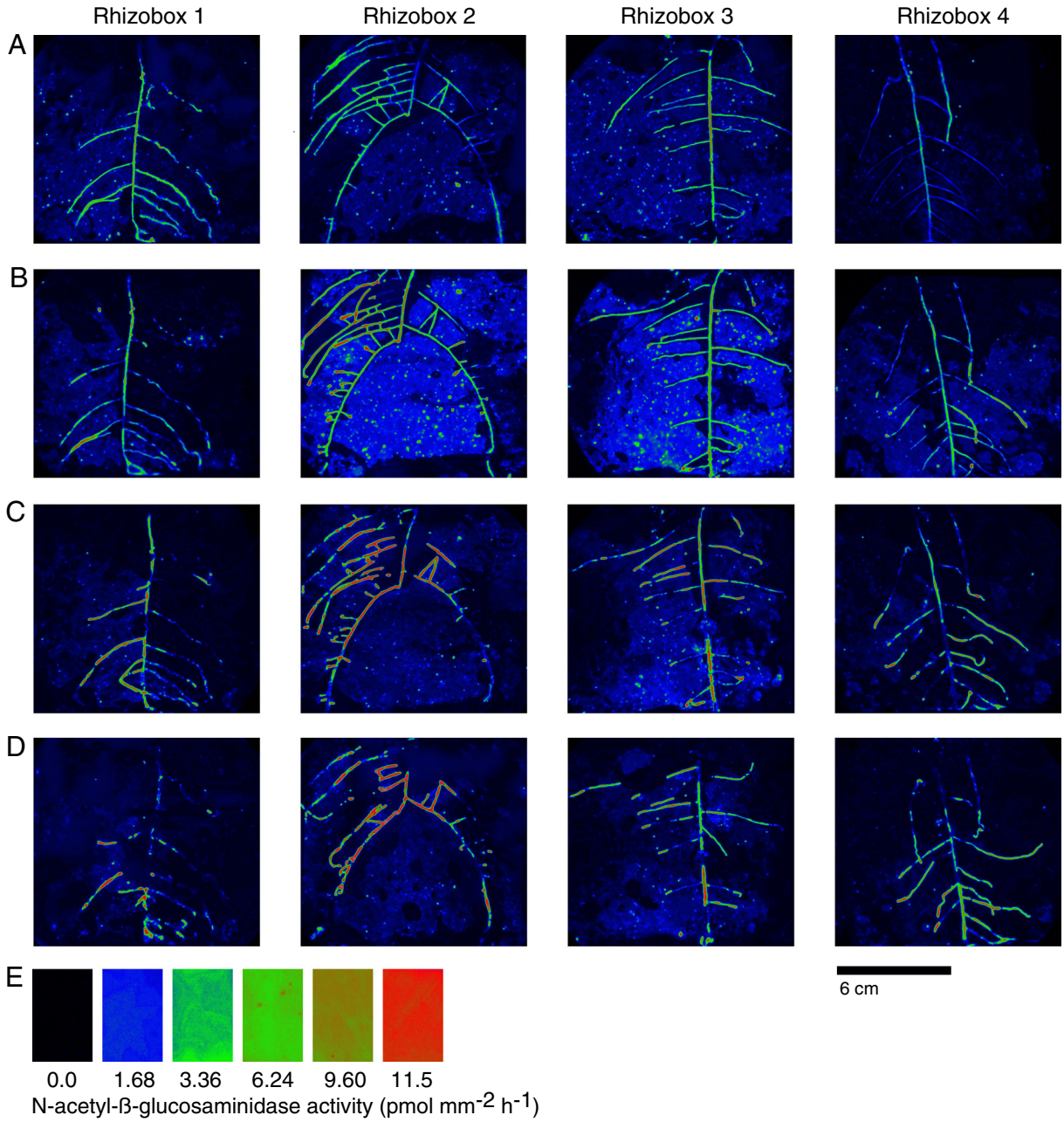

Fig. 3 N-acetyl-glucosaminidase (chitinase) activity in four microcosms with living roots 18 days after sowing (**a**), and 10 days (**b**), 20 days (**c**) and 30 days (**d**) after cutting the shoots. The calibration line for the N-acetyl-glucosaminidase activity is presented at the bottom (**e**)

Discussion

Living vs. dying roots

We found that cellulase and N-acetyl-glucosaminidase activities were higher along the roots than in the bulk soil. This is in accordance with Kandeler et al. (2002) who reported that the direct effect of the living roots on enzyme activity did not exceed a distance of 0.8 mm away from the root (Figs. 1 and 5a). Phosphatase activity was more widely distributed than the activity of cellulase and N-acetyl-glucosaminidase, which is in line

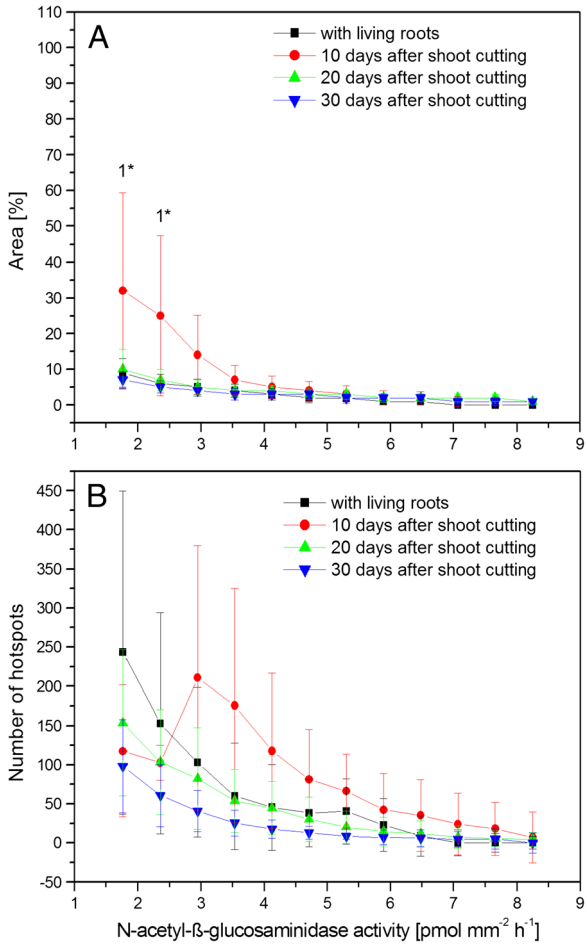

Fig. 4 Percentage of the area of N-acetyl-glucosaminidase (chitinase) activity in the total image (**a**) and number of microhotspots (**b**) as a function of N-acetyl-glucosaminidase activity. Data points depict means calculated from four independent replicates, error bars depict standard deviations. *Asterisk* indicates significant differences between the soil with the living roots and the soil with the plants 10 (1), 20 (2), and 30 (3) days after shoot cutting

with the findings of Spohn and Kuzyakov (2013) concerning alkaline phosphatase in the rhizosphere of *Lupinus albus* L. After cutting the shoots, areas of cellulase and less strongly phosphatase activities increased up to several cm away from the roots. The increase in cellulase and phosphatase activities up to 55 mm from the dying roots is surprising since previous studies found an influence of detritus only a few mm away from detritus. Kandeler et al. (1999) determined the activity of protease, xylanase and invertase in the distance from litter bags by cutting the soil in slices. They found that enzyme activities were increased in the soil at a distance of up to 11 to 13 mm away from the litter bag. In a similar study it was reported that the addition of maize litter induced distinct gradients in enzyme activities up to a distance of 3 mm (Poll et al. 2006). There might be two reasons for the larger extent of the influence of detritus in our experiment compared to the studies by Kandeler et al. (1999) and Poll et al. (2006). First, both studies added dried and rewetted maize litter to soil, while we cut the shoots of plants that were previously grown in the soil. Thus, the increased enzyme activity found in our study might not only be due to the detritus, but might also be due to increased rhizodeposition directly after cutting the shoot. This is supported by Guitian and Bardgett (2000), and Butenschoen et al. (2008) who found increased rhizodeposition of grasses and herbaceous plants following defoliation, leading to increased microbial biomass in the rhizosphere. Hence increased enzyme activities after shoot cutting might be largely due to increased exudation caused by shoot cutting.

The second reason for the differences in the distance from the piece of detritus at which the enzyme activity was increased between previous studies and the present study might be the connectivity between detritus and soil. Henriksen and Breland (2002) showed that a low connectivity between detritus and soil reduces the decomposition of detritus through delayed initial colonization by microorganisms. Since the dead roots in our experiment grew in the soil of the experiment before they were cut, they were much better connected to the soil than detritus that was placed on the soil. Hence the extent of the influence of the root detritus on enzyme activity is most likely larger in our experiment than in experiments in which detritus was placed on the soil (Kandeler et al. 1999; Poll et al. 2006) due to a higher connectivity between soil and detritus in our experiment.

We observed that cellulase and chitinase activity increased first at the root tips after shoot cutting (Figs. 1b and 3b). A strong increase of exoenzyme activity at the root tips after shoot cutting, defoliation or herbivory has not been observed so far, to our knowledge. Most likely it can be attributed to the fact that exudates are released at the root tip (Watt et al. 2006; Hinsinger et al. 2009), and therefore increased exudation caused by shoot cutting most strongly stimulates microorganisms at this microsite.

Taken together, we found larger cellulase and N-acetyl-glucosaminidase activities in the soil with dying

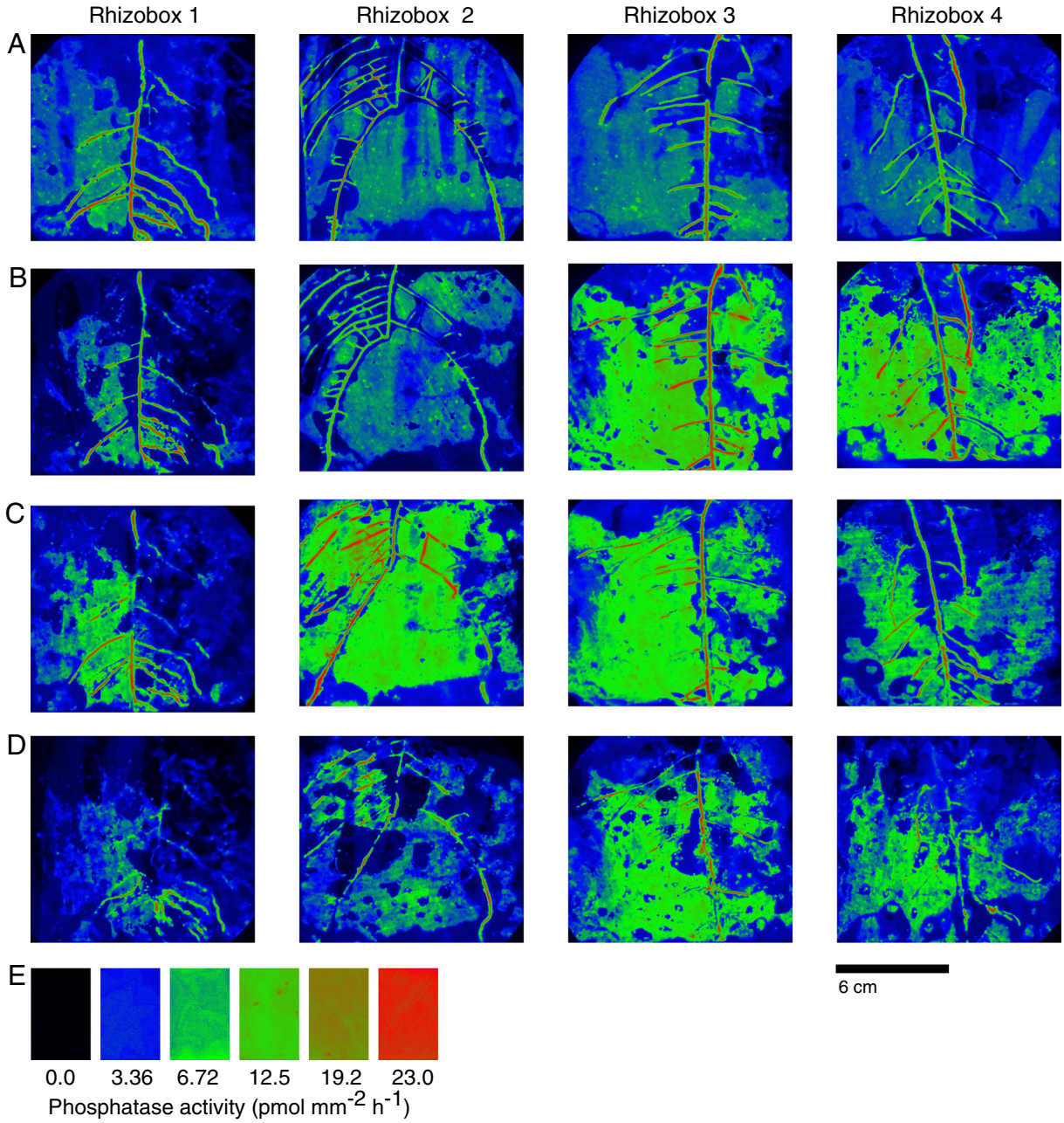

Fig. 5 Phosphatase activity in four microcosms with living roots 18 days after sowing (**a**), and 10 days (**b**), 20 days (**c**) and 30 days (**d**) after cutting the shoots. The calibration line for the phosphatase activity is presented at the bottom (**e**)

than in the soil with living roots. Furthermore, the activities of cellulase and phosphatase occupied a larger area in the soil with the dead than with the living roots. These findings are in accordance with Marschner et al. (2012) who reported higher concentrations of microbial biomass in the detritusphere than in the rhizosphere. Moreover they showed that soil microorganisms incorporated more C from the residues than from the rhizodeposits. Our results show that the detritusphere of recently died roots is larger than rhizosphere in terms of exoenzyme activities. However, the detritusphere has a very limited lifetime and after a first hot period of high enzyme activity that lasted for less than 20 days, it decreased again, at least in the soil away from the roots

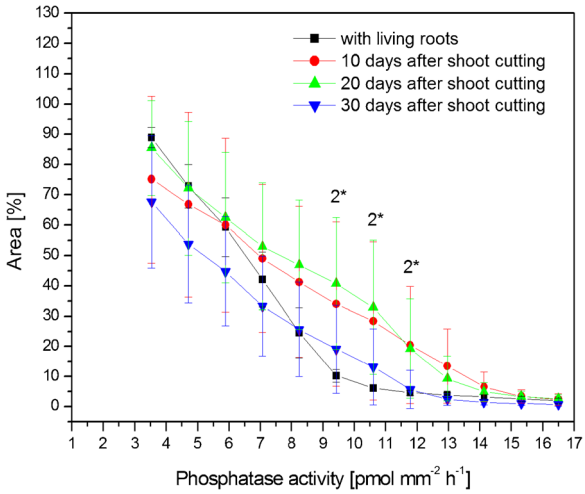

Fig. 6 Percentage of the area of phosphatase activity in the total image (A) and number of hotspots as a function of phosphatase activity. Data points depict means calculated from four independent replicates, error bars depict standard deviations. *Asterisk* indicates significant differences between the soil with the living roots and the soil with the plants 10 (1), 20 (2), and 30 (3) days after shoot cutting

(Figs. 1 and 5). Hence the effect of root detritus might level out the effect of rhizodeposition on cellulase and phosphatase activity only in soils with a very high turnover rate of fine roots and low root exudation.

Microhotspots

We found microhotspots of increased cellulase, N-acetyl-glucosaminidase (chitinase), and phosphatase activities in the soil with living roots that had a diameter of 0.2 to 2 mm (Figs. 1a, 3a, and 5a). Microhotspots of N-acetyl-glucosaminidases activity have also been observed in boreal forest soils by Dong et al. (2007) who mapped the two dimensional distribution of N-acetyl-glucosaminidase activity. Studies analyzing the small scale distribution of enzyme activity by cutting the soil into slices of down to 0.25 mm and performing a traditional enzyme assay on each slice did not find such microhotspots (Tarafdar and Jungk 1987; Kandeler et al. 1999; Kandeler et al. 2002). The most likely reason for this is that the authors mixed, i.e. homogenized the soil prior to analysis. Hence, the activities of microhotspots got strongly diluted.

The number and activity of N-acetyl-glucosaminidase hotspots increased 10 days after shoot cutting (Figs. 3 and 4b) and decreased strongly thereafter. The increase in number and activity of these hotspots due to cutting, and the strong decrease with the decomposition of the roots indicate that even though these microhotspots were up to 60 mm away from the closest root, they were influenced by the activity of the living and dying roots. We assume that these microhotspots are caused by mycorrhizal fungi that depend on C provided by the plant. The decrease in number and activity of the chitinase microhotspots can be explained by the decrease in the activity of mycorrhizal fungi due to the cessation of C supply from roots caused by shoot cutting (Leake et al. 2001; Jones et al. 2009). Phosphatase and cellulose activity was not only increased in microsites but more widely around the roots, indicating that they were not only caused by mycorrhizal fungi but also by saprotrophic fungi and bacteria.

Synergistic effects in hotspots

At sites where locally large amounts of available organic compounds enter the soil such as the rhizosphere or the detritussphere, synergistic effects between exoenzyme-producing microorganisms may further enhance microbial activity (Fig. 7). Diffusion of exoenzymes and dissolved organic matter is not a directed but a dispersive process. This means that a high proportion of exoenzymes released by a soil microorganism is lost without any benefit for it, because, first, it is unlikely that the exoenzyme meets substrate, and second, it is also unlikely that the product of the enzymatically catalyzed reaction meets the microorganism (Schimel and Weintraub 2003; Ekschmitt et al. 2005). The probability that an exoenzyme meets organic matter increases binomially with the concentrations of organic matter and of the exoenzyme. The concentration of exoenzymes increases with increasing number of microorganisms, assuming that the existence of cheaters (microorganisms that themselves do not produce exoenzymes but benefit from others (Allison 2005)) is negligible. If decomposable organic matter is not limiting, the concentration of dissolved organic compounds produced by enzymatic hydrolysis of particulate organic matter increases with exoenzyme concentration without each microorganism having to increase its exoenzyme release rate. This synergistic effect of exoenzyme producing microorganisms may help them to save energy and nitrogen and therefore, enhance microbial activity per unit of utilized substrate. The mechanism can only occur once the concentration of labile organic matter is high, which is not the case in most parts of soil. However, such a synergistic effect of exoenzyme-releasing microbes

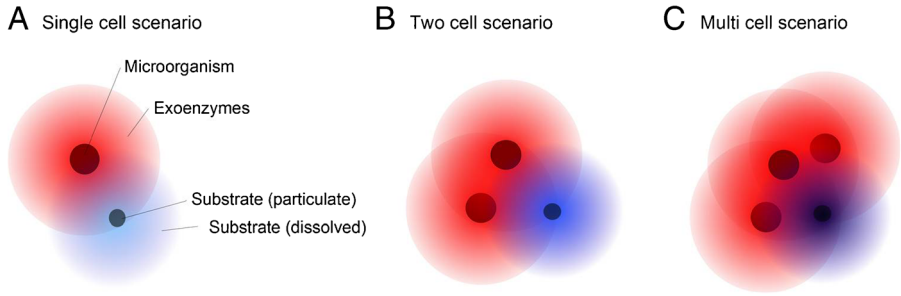

Fig. 7 Hypothesized concept of synergistic interactions of exoenzyme-producing soil microorganisms. In bulk soil, a high proportion of exoenzymes released by a soil microorganism is lost without any benefit for it (**a**). With increasing number of exoenzymes the concentration of products of enzymatically catalyzed reactions increases (as indicated by the increase in the intensity of the *blue color*, **b**). Thus, exoenzyme-producing microorganisms can benefit from each other, which might contribute to the high microbial activity at hotspots of enzyme activity (**c**)

might contribute to the formation of hotspots of element cycling at microsites with high organic matter availability. Similar synergetic interactions between microorganisms have been observed in biofilms (Davey and O'toole 2000), which can be considered as a special type of microbial hotspot at the interface of a liquid and a solid phase. Taken together, locally restricted parts of high concentrations of little-stabilized organic matter might be hotpots of element turnover not only because of high organic matter concentrations, but also because the organic matter is easily available there due to high concentrations of exoenzymes. This hypothetical concept is supported by our observation that the location of microhotspots of the three enzymes coincide (Figs. 1a, 3a, and 5a). Thus, these hotspots seem to be sites not only of high C, but also of high N, and P turnover.

Conclusions

Soil zymography allowed us to map and analyze the spatial and temporal distribution of extracellular enzyme activities in soil in the presence of living and dead roots. The short term effects of fresh root detritus on enzyme activity and its distribution in soil was larger than the effect of living roots, which can be attributed to the concentrated release of C from the dying roots. Soil zymography clearly visualized that microhotspots of enzyme activity exist several cm away from roots, which seemed to be dependent on root activity and might be caused by mycorrhizal fungi. Cellulase and phosphatase activities were increased up to 55 mm away from the next root detritus, which is a much higher extension of the detritusphere than described in previous studies. This large area of increased enzyme activity can be attributed to the fact that the detritus was formed by dying roots that were previously grown in the soil of the experiment and not by added litter as for other studies. Taken together, the soil zymography approach allowed us to gain detailed insights into the localization and the dynamics of hotspots of enzyme activity.

Appendix

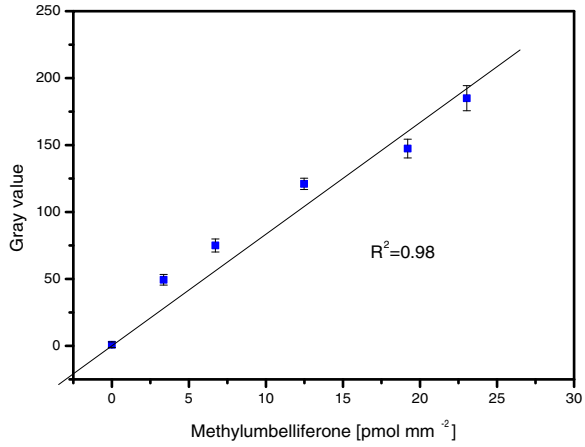

Fig. 8 Linear correlation between the gray values of the calibration membranes (Figs. 1, 3, and 5e) and their methylumbelliferone concentrations

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