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Microbial growth and enzyme kinetics in rhizosphere hotspots are modulated by soil organics and nutrients availability

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Abstract

The input of labile organics by plant roots stimulates microbial activity and therefore facilitates biochemical process rates in the rhizosphere compared to bulk soil, forming microbial hotspots. However, the extent to which the functional properties of soil microorganisms are different in the hotspots formed in soils with contrasting fertility remains unclear. We identified the hotspots related to different levels of *Zea mays* L. root architecture by zymography of leucine aminopeptidase in two soils with contrasting fertility. The hotspots localized by tiny wet-needle approach around first- and second-order roots were compared for parameters of microbial growth and enzyme kinetics. The pattern of hotspot distribution was more dispersed and the hotspot area was one order of magnitude smaller around first- versus second-order roots. The specific microbial growth rate (μ_m) and biomass of active microorganisms were soil-specific, with no difference between the hotspots and bulk soil in the fertile soil. In contrast, in the soil poor in organic matter and nutrients, 1.2-fold higher μ_m and greater growing biomass were found in the hotspots versus bulk soil. Lower enzyme affinity (1.3-2.2 times higher K_m value) of β-glucosidase and leucine aminopeptidase to the substrate was detected in the hotspots versus bulk soil, whereas only β-glucosidase showed higher potential enzyme activity (V_{max}) in the hotspots, being 1.7-2.1 times greater than that in bulk soil. Notably, the activity of C-acquiring enzyme, β-glucosidase positively correlated with the biomass of actively growing microorganisms. The fertile soil, on the whole, showed greater V_{max} and catalytic efficiency (V_{max}/K_m) and an approximately 2.5 times shorter substrate turnover time as compared to the poor

soil. Therefore, we conclude that i) the differences in microbial growth strategy between rhizosphere hotspots and bulk soil were dependent on soil fertility; ii) affinity of hydrolytic enzyme systems to substrate was mainly modulated by plant, whereas potential enzymatic activity was driven by both plant and soil quality.

Keywords: Microbial hotspots, Soil zymography, Microbial growth, Enzyme kinetics

1. Introduction

The input of root exudates and rhizodeposits, mainly easily degradable low-molecular weight organic substances, stimulates microbial growth and activity in the rhizosphere, which is defined as one of the most dynamic microbial hotspots (Kuzyakov and Blagodatskaya, 2015; Kuzyakov and Razavi, 2019). The peculiarity of the rhizosphere as a root-soil interface is that the microbial community composition is generally linked to the soil microbial community, which is determined by basic soil properties (de Ridder-Duine et al., 2005). The structure of rhizosphere community (i.e., species dominance and activity), however, is strongly modulated by the plant strategy for the nutrient acquisition, which is also dependent on basic soil properties, e.g., soil nutrition state (reviewed by Kuzyakov and Razavi, 2019). Therefore, the research question: how the difference between the hotspots and bulk soil is impacted by soil fertility, which dramatically changes soil C and nutrient status, is very relevant. This question needs to be addressed considering that microbial communities in the hotspots and bulk soil are functionally different in terms of their life strategies and enzyme kinetic properties due to different qualities and quantities of organic substrates

(Blagodatskaya et al., 2009; Hoang et al., 2016).

Microbial activity is limited by various environmental factors and especially by carbon (C) availability (Hodge et al., 2000; German et al., 2011). Microbial hotspots are formed with the input of fresh carbon sources (Hodge et al., 2000, Schimel and Weintraub, 2003). Accordingly, soil with higher quality, i.e., the availability of organic substances and nutrients, should represent higher microbial and enzyme activity. On the other hand, the relative fraction of the hotspots induced by root exudates and rhizodeposits may be lower if soil inherent substrate availability is sufficient for microbial metabolism. We hypothesize, therefore, that the differences in microbial functional parameters between the hotspots and surrounding soil will be smoothed in rich compared to the poor soil.

In the rhizosphere, root exudation and rhizodeposits stimulate the activities of extracellular enzymes (Ge et al., 2017; Ma et al., 2018), which are valuable tools for microorganisms to degrade complex polymeric organic substances for acquiring energy and nutrients from surrounding soil. However, artificially labeled fluorogenic substrates applied in soil studies for determination of extracellular enzymes activity (Marx et al., 2001) are much less than natural organic polymers. Despite it is generally assumed that fluorogenic substrates are decomposed by extracellular enzymes; this assumption still needs to be proven experimentally. Furthermore, application of sonicated soil suspension cannot distinguish the activity of enzymes released by microorganisms in response to substrate addition and earlier secreted enzymes immobilized within soil matrix (Nannipieri et al., 2018). Therefore, the cumulative activity of enzymes

presented in soil suspension is determined by this approach.

The gradients of enzyme activities as a function of distance from the root surface to the soil have been clearly related to nutrients availability (Tarafdar and Jungk, 1987; Badalucco and Nannipieri, 2007) and the spatial patterns of such gradients have been recently visualized (Sanaullah et al., 2016; Zhang et al., 2019). However, the inhomogeneous distribution of microbial hotspots along the roots has also been observed (Pausch and Kuzyakov, 2011; Razavi et al., 2016a), which might be due to soil heterogeneity (Webster, 2000; Heuvelink and Webster, 2001) or variation and distribution of exudation along the roots. Therefore, careful localization of the hotspots is necessary for precise soil sampling from microbial hotspots. Soil zymography, a novel *in situ* method, enables determining the two-dimensional spatial distribution of enzyme activities in soil (Spohn et al., 2013; Razavi et al., 2019; Heitkötter and Marschner, 2018) and localizing hotspots of various enzyme activities.

The most ecologically relevant biogeochemical processes in soils are microbially mediated, and microbial functions depend on active microbial pools in soil because only the active microorganisms drive biogeochemical processes (Blagodatsky et al., 2000; Nannipieri et al., 2003). The rate of biomass-specific respiration is 10 to 100 times greater when it is based on the active than on the total microbial pool (Salazar-Villegas et al., 2016). Additionally, the fraction of active microorganisms in the hotspots is up to 2 times higher than that in bulk soil (Blagodatskaya et al., 2014). Consequently, the simultaneous occurrence of numerous hotspots at the micro-scale level determines the microbial functions at higher scales (Blagodatsky and Smith, 2012;

Kuzyakov and Blagodatskaya, 2015). In turn, the effect of hotspots can be quantified by their relevance to functional parameters, such as respiration, microbial growth and enzyme activities (Blagodatskaya and Kuzyakov, 2013). Kinetic approaches, based on product formation, e.g., respiration, are successful for assessment of the active biomass and for relating it to basic soil processes (Blagodatsky et al., 2000). Kinetic parameters of microbial growth as well as the dominant strategy can be detected using the substrate-induced growth response (SIGR) method (Panikov, 1995). The correspondence between microbial growth and functional parameters of enzymes hydrolyzing polymeric organic compounds in soil remains to be studied in the precisely localized hotspots (Razavi et al., 2015; Ma et al., 2017).

Here, two types of soil with contrasting fertility were used to grow maize plants. The application of soil zymography enabled accurate localization of the microbial hotspots and successful collection of the micro-samples by tiny wet-needle approach. This study was designed to i) investigate the potential effect of rhizosphere hotspots on microbial growth and enzyme activities; ii) evaluate the effect of soil type on the difference in kinetic parameters between the hotspots and bulk soil. We hypothesized that 1) rhizosphere hotspots contain a high fraction of stimulated microorganisms (with a high growth rate and enzyme activity) compared to bulk soil independently of soil fertility; 2) the difference in kinetic parameters between the hotspots and bulk soil are stronger in the poor soil; 3) the enzyme activity in soil with higher fertility is higher than in the poor soil.

2. Materials and methods

2.1. Hotspot identification and sampling

Individual maize plants (*Zea mays* L., KWS, Germany) were grown in separate rhizoboxes (30 plants in total) in two soils with similar pH but contrasting texture and fertilization. Mitterfels (fertile soil) is located in the Central German Uplands. The soil type is Hyperdystric Chromic Folic Cambisol (WRB, 2015). The samples of Mitterfels soil were taken from loamy Ap horizon (Lang et al., 2017) with high C and N content. Unterlüss (poor soil) is located in Lower Saxony, Germany. The soil type is Hyperdystric Folic Cambisol. The samples taken from Ap horizon of Unterlüss sandy loam soil (WRB, 2015) were relatively barren, with respectively, 1.6, 2.0 and 4.1 times lower C, N and P content as compared with Mitterfels soil (Table 1). Further details on the sites, soil profiles and soil properties can be found in Lang et al. (2017). During growth, the rhizoboxes were kept inclined at an angle of 45° so that the roots grew at the vicinity of the lower wall of the rhizobox due to gravitropism. After cultivating maize plants for 2 weeks, soil zymography was applied to identify the spatial distribution of β-glucosidase and leucine aminopeptidase hotspots around the roots (Razavi et al., 2019).

Two types of fluorogenic substrates based on 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC) were used: 4-methylumbelliferyl-β-D-glucoside (MUF-G) for β-glucosidase; L-Leucine-7-amino-4-methylcoumarin (AMC-L) for leucine aminopeptidase. After fitting the substrate-saturated membrane to the soil surface for one hour, enzyme activity was detectable by the appearance of fluorescent

products on the membrane visible under UV light (Fig. 1a-d). The estimation of fluorescence intensity proportional to the activity of the enzyme was calibrated by the range of concentrations of corresponding products: either MUF or AMC. In order to transform zymogram images to graphical representation, digital image histograms were developed as barcharts, which showed the distribution of pixel values according to the color map. These histogram graphs show the number of pixels of the zymogram images at each 0.01 color intensity value occurring in that image. Numbers of pixels as well as area of whole image were calculated based on these histograms. All pixels with the color intensity exceeding average value (i.e., >0.75) were assigned to the hotspots for enzyme activities (Sanaullah et al., 2016).

For both soils, we found higher resolution for the hotspots identified by leucine aminopeptidase compared to β-glucosidase. In addition, we found that the hotspots around new-developed first-order roots of maize were very small in size and can be considered as dots, in comparison with large hotspot areas around second-order root. The zymography images of leucine aminopeptidase activity were treated for the hotspots sampling around first- and second-order roots, separately. For precisely localized sampling, soil particles were carefully collected using wet needle (tip 1.5 mm) of a syringe directly from the hotspots identified by zymography (Fig. 1e). No hotspots were detected at the distance exceeding 1.5 mm from the roots. About 0.1 g soil was collected from large number of hotspots and was pooled to form a composite sample for each plant replicate. Bulk soil was collected in a similar way from root-free soil.

2.2. Kinetics of substrate-induced growth response

The kinetic parameters of substrate-induced growth response were estimated by the dynamics of CO_2 emission from soil amended with glucose and nutrients (Panikov, 1995), taking advantage of the rapid automated bacterial impedance technique (RABIT) system in a climate chamber enabling to work with reduced (up to 0.5 – 1 g) soil sample size. Briefly, soil sample was incubated in a tube after solution addition with glucose (10 mg g^{-1}) and mineral salts: $(NH_4)_2SO_4$–1.9 mg g^{-1}, K_2HPO_4–2.25 mg g^{-1}, and $MgSO_4 \cdot 7H_2O$–3.8 mg g^{-1} (Blagodatsky et al., 2000). Soil water content was adjusted to 60% of water holding capacity by adding distilled water. The CO_2 production rate was measured hourly at 22 °C using RABIT.

Specific growth rate (μ_m) was determined by fitting of equation parameters to the experimental data on CO_2 evolution rate (v; Fig. 2) according to the following equation:

$$v(t) = A + B \times exp(\mu_m\, t) \quad (1)$$

where A is the initial rate of uncoupled (no growth) respiration, B is the initial rate of coupled (growth) respiration, t is the time (Panikov and Sizova, 1996; Blagodatsky et al., 2000); r_0, the so-called physiological state index of microorganisms at time zero (before substrate addition), is calculated from the ratio between A and B. The total glucose-metabolizing microbial biomass (sustaining + growing) is

$$x_0 = B \cdot \lambda \cdot Y_{CO2} / r_0 \cdot \mu_m, \quad (2)$$

Biomass yield per unit of CO_2-C (Y_{CO2}) is assumed to be constant during the experiment and equals 1.5, corresponding to a mean value of 0.6 for the microbial yield per unit of glucose-C consumed. λ= 0.9 may be accepted as a basic stoichiometric constant (Panikov and Sizova, 1996). The growing (active) microbial biomass at time

zero is given by

$$x'_0 = x_0 \cdot r_0 \quad (3)$$

The duration of the lag period (t_{lag}) was determined as the time interval between the moment of glucose addition and the moment when the increasing rate of growth-related respiration $B \times exp(\mu_m\, t)$ becomes as high as the rate of respiration uncoupled from ATP generation; it was calculated using the parameters of the approximated curve of the respiration rate of microorganisms by the equation:

$$t_{lag} = \frac{\ln\left(\frac{A}{B}\right)}{\mu m} \quad (4)$$

In addition, the kinetic approach allowed the assessment of generation time (T_g) of both actively growing and total microbial population consuming glucose. The estimation of T_g for actively growing biomass is based on specific growth rates, i.e:

$$T_g = \ln(2)/\mu_m \quad (5)$$

2.3. Enzyme kinetics

Activities of β-glucosidase and leucine aminopeptidase for the hotspots and bulk soil were measured using the same fluorogenic substrates as for zymography with seven concentrations ranging from 0-400 μmol L^{-1}. The extraction and determination were carried out according to German et al. (2011) and Razavi et al. (2015). Suspensions of 0.5 g soil (dry weight equivalent) with 50 mL deionized water were prepared using low-energy sonication (40 J s^{-1} output energy) for 2 min. Thereafter, 50 μL of soil suspension was added to 100 μL substrate solution and 50 μL of buffer [MES ($C_6H_{13}NO_4SNa_{0.5}$), (pH:6.5) for MUF substrate and TRIZMA ($C_4H_{11}NO_3$·HCl, $C_4H_{11}NO_3$), (pH:7.2) for AMC substrate] in a 96-well microplate. At 0 min, 1 h and 2 h after mixing, a

fluorescence in microplates was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, slit width of 25 nm, with a Victor3 1420-050 Multi label Counter (Perkin Elmer, USA). Enzyme activity was expressed as MUF or AMC release in nmol per g dry soil per hour (nmol g^{-1} soil h^{-1}).

The parameters of Michaelis-Menten kinetics for enzyme activities were determined using the equation:

$$V = \frac{Vmax \times [S]}{Km + [S]} \quad (6)$$

where V is the reaction rate, [S] is the substrate concentration. K_m (the substrate concentration at half-maximal rate) is related to the enzyme affinity to the substrate. V_{max} refers to decomposition rates at saturating substrate concentration.

The substrate turnover time (T_t) was calculated according to the following equation: T_t (hours) = (K_m + S) / V_{max} (Panikov et al., 1992), where S is the substrate concentration. The substrate turnover time was calculated at substrate concentration for the situations corresponding to the lack and excess of substrate, as S = K_m /10 and S = 10* K_m, respectively.

The catalytic efficiency of enzymes (K_a) was determined as K_a = V_{max} / K_m (Hoang et al., 2016). The K_a characterizes the enzyme catalytic properties and is used as an indicator to reflect the functional changes of microbial communities (Tischer et al., 2015). The higher K_a shows better catalytic properties (Moscatelli et al., 2012).

2.4. Statistical analyses

One-way analysis of variance followed by the Tukey HSD ($P < 0.05$) was used to test the effect of hotspots on microbial and enzyme kinetic parameters, e.g., specific

growth rate, V_{max}, K_m, T_t and K_a. All the statistical analyses were performed using SPSS version 22.0 for Windows (SPSS Inc. Chicago).

3. Results

3.1. Kinetics of substrate-induced growth response

Different kinetic responses of microbial growth to substrate addition between the hotspots and bulk soil were detected in low fertile soil (Fig. 2 and Table 2). Glucose and nutrient input into the poor soil induced stronger stimulation of microbial growth with a 1.2-fold higher microbial specific growth rate (μ_m) in the hotspots compared to bulk soil. In contrast, μ_m values were similar between the hotspots and bulk soil of the fertile soil. The lag time (t_{lag}) in the fertile soil was estimated as negligible, demonstrating immediate microbial growth after substrate input. Furthermore, a very high fraction of active microorganisms exceeding 10% of total biomass was observed in the fertile soil (Table 2). In contrast, a long lag time of 25.4 h was detected in bulk soil of the poor soil, accompanied by a low abundance of growing microbial biomass (Table 2). The growing microbial biomass (x'_0) was at least 4.8-fold higher in the fertile than in the poor soil. Despite the strong difference in size, no difference in growth kinetic parameters was detected between the hotspots of first- and second-order roots.

3.2. Enzyme kinetics and substrate turnover

The maximum potential enzyme activity (V_{max}) was 1.7-2.1 times higher in the hotspots than in bulk soil for β-glucosidase, whereas no difference was detected for leucine aminopeptidase (Fig. 3a). Interestingly, the difference in V_{max} of β-glucosidase between the hotspots and bulk soil showed a close dependence on the amount of

growing microbial biomass (R^2= 0.85; Fig. 4). Remarkably, V_{max} was approximately 2 times higher in the fertile than in the poor soil, whereas the K_m showed no difference between the fertile and the poor soil. Overall, the K_m values of β-glucosidase and leucine aminopeptidase were 1.3-2.2 times higher in the hotspots than in bulk soil ($P < 0.05$; Fig. 3b). Consistent with microbial growth kinetics, no difference in enzyme kinetics was detected between the hotspots of first- and second-order roots.

The turnover time for enzymes showed no difference between the hotspots and bulk soil except the leucine aminopeptidase in the poor soil in which the turnover time in the hotspots around the first-order roots was 40% longer than that in bulk soil (Fig. 5a, b). The same pattern in the turnover time was detected under a lack of substrate as under an excess of substrate. Furthermore, the turnover time was approximately 2.4-2.9 times as long in the poor as in the fertile soil. No change in the catalytic efficiency (V_{max}/K_m) was detected in the hotspots for β-glucosidase or leucine aminopeptidase (Fig. 5c). However, the catalytic efficiency was approximately 3 times higher in the fertile versus the poor soil.

4. Discussion

4.1. Microbial growth response to substrate addition

Differences in the microbial growth response to substrate addition between rhizosphere hotspots and bulk soil were soil fertility dependent and were detected only in the poor soil (Fig. 2), which partly rejects our first hypothesis that microbial hotspots always stimulate microbial growth compared with bulk soil. Equal microbial specific growth rates (μ_m) in the hotspots and bulk soil from the fertile soil might be associated

with the availability of soil organics (German et al., 2011). Due to abundant organics in soil, the energy limitation and dependence of the microbial community on labile C input by roots are weak. In contrast, substrate addition to hotspots of the poor soil induced strong stimulation of microbial growth compared to bulk soil, confirming our second hypothesis that the difference in microbial growth between the hotspots and bulk soil is stronger in the poor soil. Thus, a greater fraction of fast-growing microorganisms with r-strategy in the hotspots was selectively stimulated by the input of labile C from roots (Grayston et al., 1998; Goddard et al., 2001; Cheng, 2009; Philippot et al., 2013).

Based on the microbial respiration rate, a negligible lag time (tlag) was estimated in the fertile soil (Table 2), which was closely associated with the active microbial pool. In the fertile soil, growing microorganisms can take up the added substrate immediately for their growth (Blagodatskaya et al., 2014). In contrast, a long lag time in the bulk poor soil indicated dormancy of microbial community located far from roots and limited by labile C, when the very tiny fraction of growing microorganisms was able to maintain activity state (Blagodatskaya and Kuzyakov, 2013). In the poor bulk soil, neither inherent soil C source nor labile C input from roots could support the activation of microorganisms. Overall, the lag time showed a negative correlation with the amount of active biomass, indicating that the state of microbial activity is responsible for the duration of t_{lag} (Blagodatskaya et al., 2014). Therefore, the hotspot effect on microbial activity was not consistent among soils and was largely regulated by soil fertility.

Last, similar kinetics of the substrate-induced growth response between the hotspots associated with either first- or second-order roots suggest the same functional

groups and activity of microorganisms. Different area and distribution patterns of the hotspots do not necessarily mean functional differences, i.e., microorganisms in individual hotspots are not fully separated. The size of hotspots is governed by metabolic pathways, which strongly depend on the amount of substrate (Dippold and Kuzyakov, 2013). Therefore, different magnitudes of labile C input by first- and second-order roots would be the fundamental cause of the various shapes of rhizosphere hotspots (Pausch and Kuzyakov 2011).

4.2. Enzyme kinetics and substrate turnover

Generally, greater than doubled V_{max} values in the fertile soil versus poor soil is a result of more growing biomass in the former (Table 2). Growing microorganisms produce larger amount of active enzymes (E_0) and the V_{max} is a function of E_0 (Nannipieri and Gianfreda, 1998; Allison et al., 2010; Blagodatskaya et al., 2016).

The difference in V_{max} between the hotspots and bulk soil was specific for individual enzymes, with higher V_{max} of β-glucosidase in the hotspots compared to bulk soil (Fig. 3). This observation partly rejected our first hypothesis again. It is necessary to underline that the activity of the enzymes was used here as an example of single enzyme-mediated processes (e.g., decomposition of cellulose-like oligosaccharides or hydrolysis of amino acid residues of polypeptides), which contributed to the decomposition of soil organics along with a large number of other processes and corresponding enzymes. Despite some empirical relationships observed (Sinsabaugh et al., 2008), neither β-glucosidase nor leucine aminopeptidase can be considered as an indicator of the heterogeneous process of C- or N-cycling. It is conceptually wrong to

use the activity of single enzyme (for example, leucine aminopeptidase) as an indicator of general microbial N acquisition, which depends on the activity of many various enzymes and physiological factors conjointly (Nannipieri et al., 2018).

The higher V_{max} of β-glucosidase in the hotspots indicates that the activity of enzymes is also a function of the amount of available substrate (Allison and Vitousek, 2005). The easily available C input by roots triggers microbial activity and thus drives the fast microbial metabolism (mainly by r-strategists) on the substrate, which could favor counterbalancing the high C inputs (Kuzyakov and Blagodatskaya, 2015), resulting in the higher V_{max} in the hotspots versus bulk soil (Jones et al., 2003; Fischer et al., 2010). Supporting our interpretation, we found a good correspondence of growing microbial biomass and V_{max} of β-glucosidase (Fig. 4), indicating the strong association between microbial growth and functions (Dorodnikov et al., 2009). Thus, the fast-growing microorganisms with r-strategy in the hotspots are characterized by production of C-hydrolytic enzyme, which helps to consume the continuous input of labile C from roots (Sanaullah et al., 2016). However, the activity of the N-hydrolytic enzyme showed no significant correlation with the growing microbial biomass due to the insignificant difference in V_{max} of leucine aminopeptidase between the hotspots and bulk soil. Given the C/N ratio around and above 20 in both soils (Table 1), the microbial acquisition of N was strongly restricted by nutrient supply capacity according to stoichiometric constraints (Sinsabaugh et al., 2009). As root exudates and rhizodeposits are generally depleted in N content, the N supply capacity was even lower in the hotspots than in bulk soil, thus restricting mobilization of organic N by microorganisms (Tarafdar and

Jungk, 1987; Badalucco and Nannipieri, 2007; Kuzyakov and Xu, 2013).

In accordance with ecological principles, the K_m increased in rhizosphere hotspots compared to bulk soil (Fig. 3b), indicating decreased affinity of enzymes as an effect of root exudates and rhizodeposits (Blagodatskaya et al., 2009). It is important to note that enzyme activity determined *in situ* in soil is a cumulative action of the entire microbial community, which produced a set of isoenzymes with similar functions but different environmental optima (Nannipieri et al., 1982, Nannipieri and Gianfreda 1998). Therefore, the apparent V_{max} and K_m represent average values of kinetic constants, reflecting simultaneous activity of a suite of isoenzymes catalyzing the same reactions (Nannipieri and Gianfreda, 1998; Razavi et al., 2016a). The higher K_m values in the hotspots versus bulk soil indicated altered enzyme systems by rhizo-microbial interactions. Such changes resulted in a strong increase in V_{max} of C-acquiring β-glucosidase due to high availability of oligosaccharides in the rhizosphere hotspots; while they were insufficient to increase the V_{max} of leucine aminopeptidase due to lack of oligopeptides and other N-containing substrates, again indicating the possible restriction of V_{max} by basic soil properties.

Low enzyme affinity to substrate observed in the rhizosphere is typical for fast-growing r-strategists, showing higher K_m values (Fierer et al., 2007). The slow-growing K-strategists with enzymes of high substrate affinity are better adapted for growth on poorly available substrates, and they are uncompetitive against the r-strategists in rhizosphere hotspots (Dorodnikov et al., 2009). In fact, the decrease of substrate affinity is in line with the stoichiometric theory postulating that microbes regulate enzyme

activities in response to soil resource availability to match their nutrient requirements (Sinsabaugh and Follstad Shah, 2012). Therefore, the shift in enzyme intrinsic properties (K_m) under different substrate availability was presumably associated with changes in microbial species domination, accompanying with the expression of isoenzymes with the same function but different conformations and structures (Somero, 1978). Thus, the K_m values were independent on basic soil properties, demonstrating that enzyme affinity to substrate was mainly modulated by roots. In contrast, V_{max} was affected by both soil quality and plant-microbial interactions.

Rhizosphere hotspots contain a stimulated microbial community with a greater enzyme activity and a lower affinity for the substrate compared to bulk soil. However, no difference in the catalytic efficiency (K_a) of enzymes or turnover time of the substrate was detected between the hotspots and bulk soil due to simultaneous increases in V_{max} and K_m (Fig. 5). This finding was inconsistent with the results of Sanaullah et al. (2016) who found that higher K_a was detected in bulk soil, however, we found the same trend: both V_{max} and K_m increased in the hotspots. Thus, our study revealed strong changes in enzyme systems in the hotspots versus bulk soil (as indicated by altered activity and affinity); as a result, an increased K_m counterbalanced an increase in V_{max} resulting in similar catalytic efficiency in soil microhabitats. Furthermore, much shorter turnover time of substrate and higher catalytic efficiency in the fertile versus the poor soil suggest that microbial communities change the intrinsic properties of hydrolytic enzymes to adapt to different environments (Razavi et al., 2016b).

5. Conclusions

Microbial hotspots and bulk soil were successfully distinguished by soil zymography and were precisely sampled by tiny wet-needle approach with the goal of comparing the effects of hotspots on microbial growth and enzyme kinetic parameters. Overall, the differences in microbial growth between the hotspots and bulk soil were significant in the poor soil only, i.e., they were regulated by inherent soil substrate availability (Fig. 6). A difference in enzyme activity and affinity was detected between the hotspots and bulk soil in the fertile and the poor soils but was enzyme-specific: the difference was significant for β-glucosidase (one of enzymes involved in the decomposition of oligosaccharides), whereas it was insignificant for leucine aminopeptidase (enzyme contributing to the decomposition of proteins). In both soils, enzyme systems changed towards decreased affinity for the substrate to maintain similar catalytic efficiency in the hotspots versus bulk soil, which was the preferred microbial strategy in the tested soils.

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Figure captions

Fig. 1 Examples of maize roots grown in rhizoboxes (center) and zymographs; showing spatial distribution of enzyme activities: (a) β-glucosidase, and (b) leucine aminopeptidase in the fertile soil; (c) β-glucosidase, and (d) leucine aminopeptidase in the poor soil, and (e) the sampling scenario using wet needle.

Fig. 2 Glucose-induced respiratory responses of microbial community and their corresponding specific growth rates (μ_m; inset figures) after substrate addition into the (A) fertile and (B) poor soil. Experimental data are shown as symbols and model simulation (Equation 1) as curves. Bars show standard errors of the means (±SE). Lower-case letter indicates significant difference at a level of $P < 0.05$.

Fig. 3 V_{max} (a) and K_m (b) values of β-glucosidase, and leucine aminopeptidase in the fertile and the poor soils. Values are means of three replicates (±SE). Asterisks indicate significantly different from bulk soil. The inserts show the mean value of different samples.

Fig. 4 The relationship between the V_{max} of β-glucosidase and the growing microbial biomass ($P < 0.05$).

Fig. 5 The turnover time (a) at excess of substrate and (b) lack of substrate, and (c) the catalytic efficiency of enzymes (ratio of V_{max}/K_m) in the fertile and the poor soils. Values are means of three replicates (±SE). Asterisks indicate significantly different from bulk soil. The inserts show the mean value of different samples.

Fig. 6 Conceptual graph showing changes of microbial activities and functions in the hotspots as affected by soil fertility. Vertical and horizontal red arrow indicate increase

and no change of microbial kinetics and functions in the hotspots compared to bulk soil, respectively. Red gradient arrow indicates increasing trend, blue gradient arrow indicates decreasing trend, gray arrow indicates no change along soil fertility.

Table 1 Stand parameters at two research sites in Germany. Data taken from relevant German forest authority (Hauβmann and Lux, 1997; Lang et al., 2017).

Stand parameters	Mitterfels	Unterlüss
Locations	48°97′N, 12°87′E	52°83′N, 10°36′E
Elevation (m)	1023	115
Precipitation (mm)	1229	779
Mean annual temperature (℃)	4.9	8
Texture	Loam	Loamy sand
P_{tot} (g m^{-2})	1375	164
N_{tot} (kg m^{-2})	1.4	0.7
C_{tot} (kg m^{-2})	26	16
C/N	18.6	22.9
C/P	127	493
pH (H_2O)	3.6	3.5
Clay %	24	6
Sand %	32	19
Silt %	44	75

Table 2 Growing microbial biomass and its proportion of total biomass, lag-period and generation time of actively growing microbial community consuming substrate during incubation of soils with glucose and nutrients.

Soil	Treatment	Lag time (hours)	Microbial biomass (μg C g^{-1})		Generation Time (hours)
			Growing	% of total	
Fertile	Bulk soil	0	16.0	16.4	5.4
	Hotspots-first-order	0	27.6	17.7	5.7
	Hotspots-second-order	0.7	15.5	8.4	4.9
Poor	Bulk soil	25.4	0.2	0.2	4.7
	Hotspots-first-order	0	4.5	11.4	4.0
	Hotspots-second-order	5.44	3.2	3.6	3.8

Fig. 1

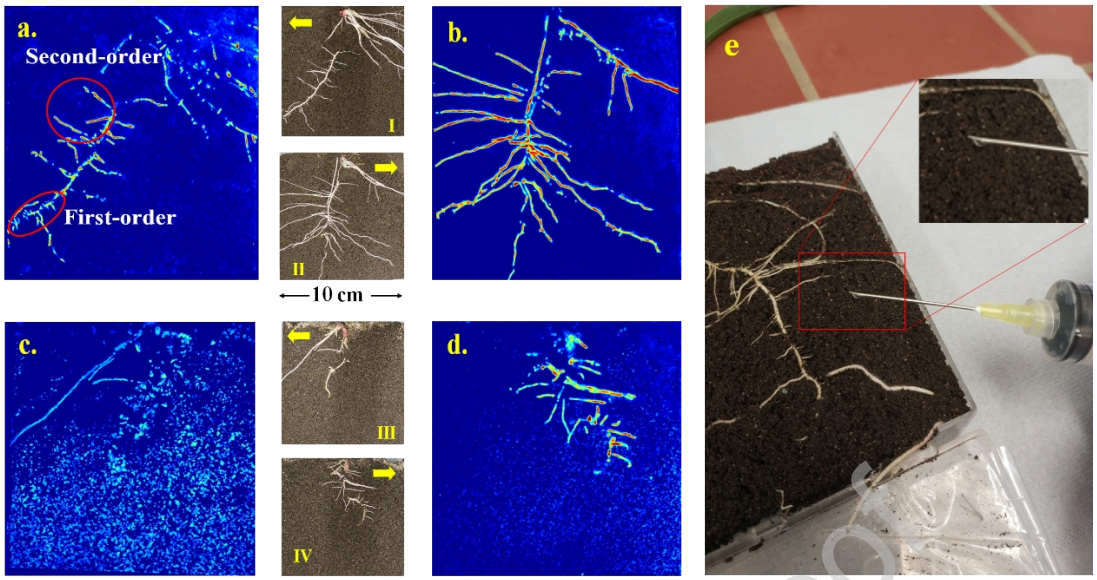


Fig. 2

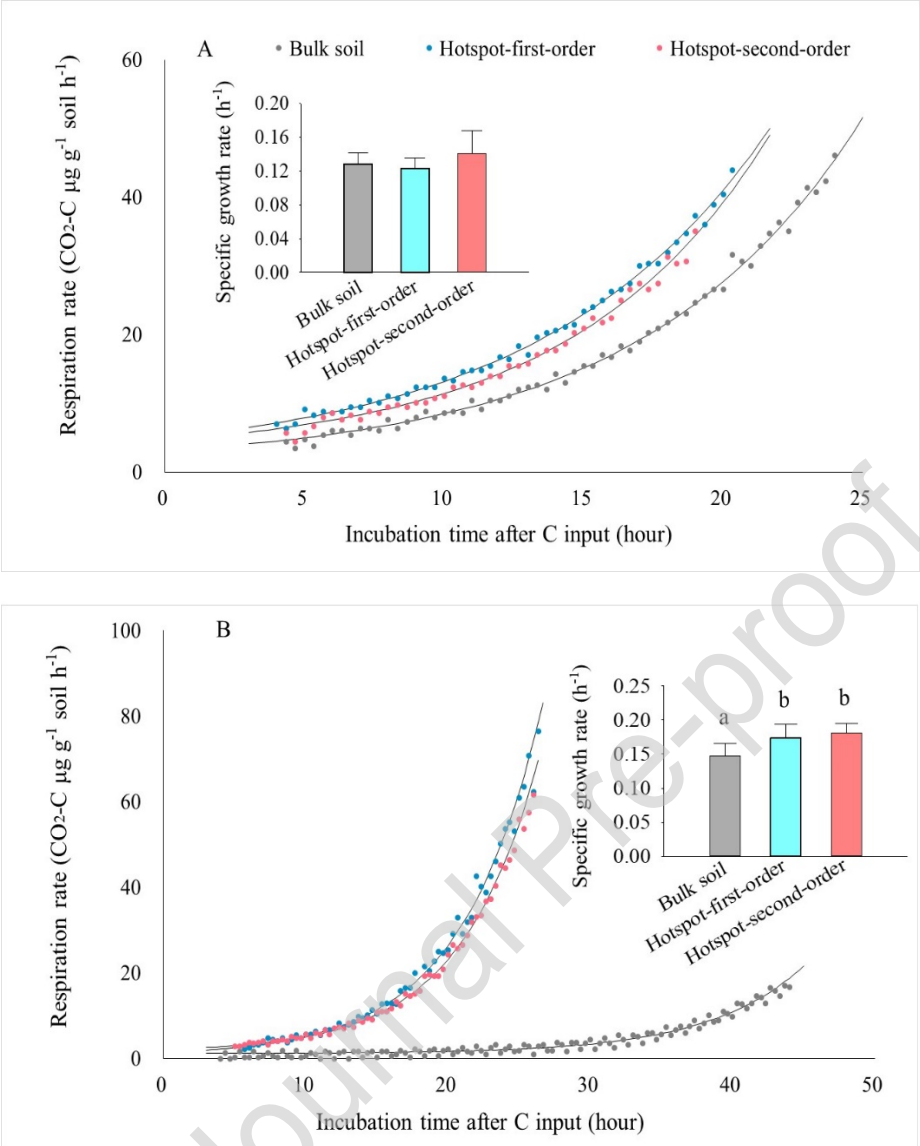


Fig. 3

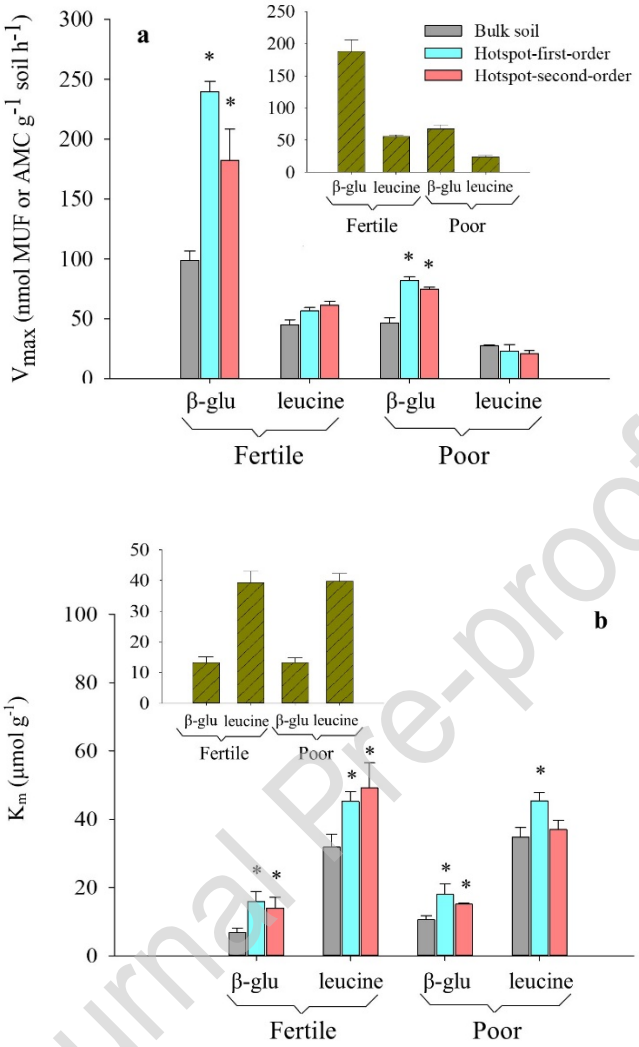


Fig. 4

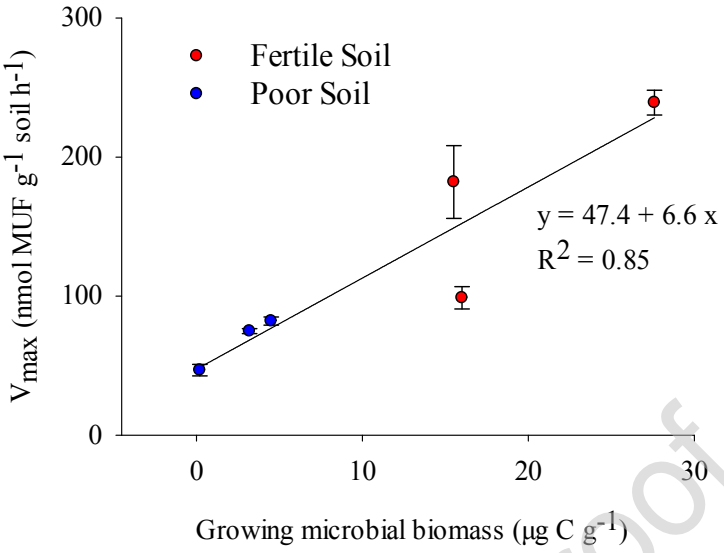


Fig. 5

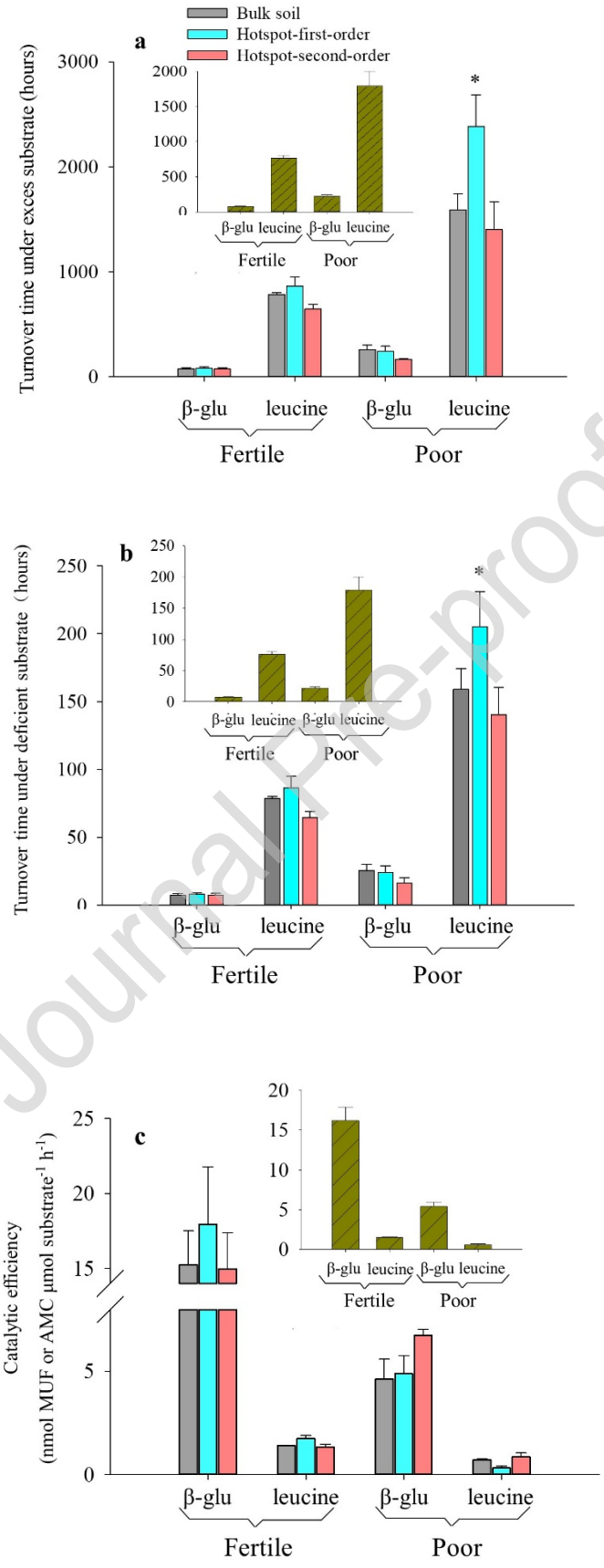
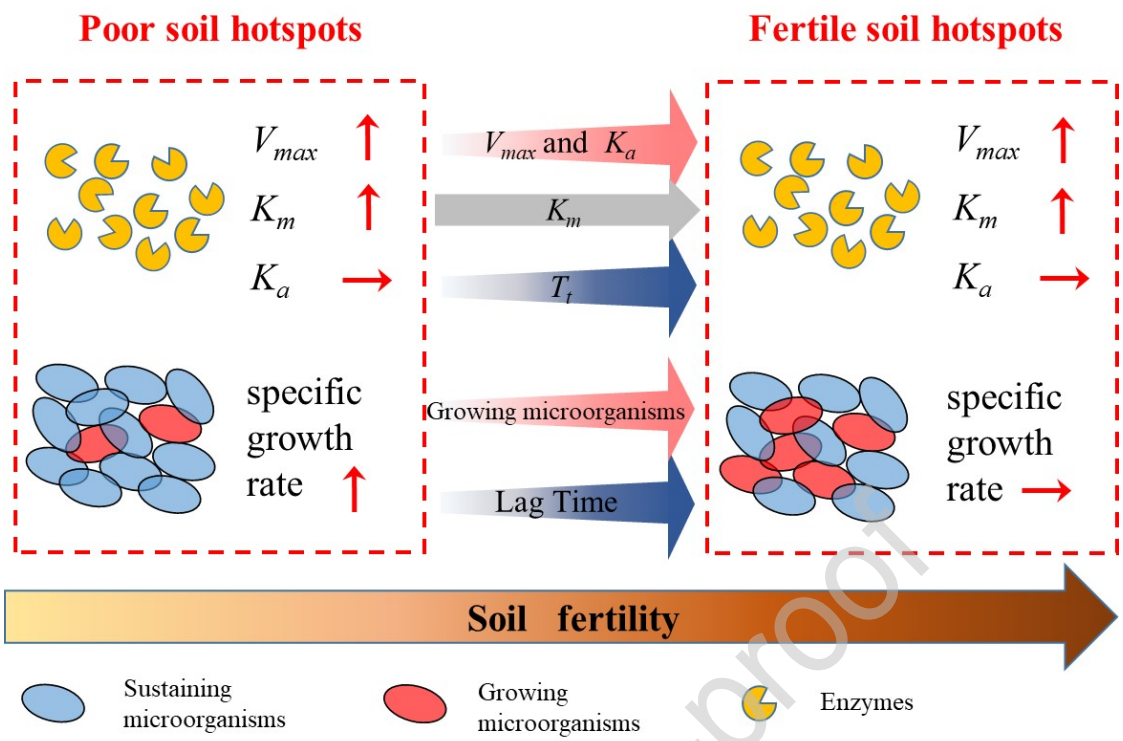


Fig. 6



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

None.

The hotspots around roots were successfully localized by tiny wet-needle approach.

Stimulation on microbial growth in the hotspots was soil-specific.

Roots modulated enzyme affinity to substrate.

The fertile soil showed greater catalytic efficiency than the poor soil.