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Substrate quality affects kinetics and catalytic efficiency of exo-enzymes in rhizosphere and detritusphere

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ABSTRACT

Microbial and enzyme functioning depends on the quality of substrates, which strongly differ in bare soil and in the hotspots of microbial activity such as the rhizosphere and detritusphere. We established a field experiment to determine the effects of contrasting substrate quality, namely, soil organic matter, maize shoot litter (detritusphere) and maize rhizodeposits (rhizosphere) on microorganisms and their extracellular enzymes in an arable soil. Kinetic parameters (V_{max} and K_m) of four hydrolytic extracellular enzymes: β-cellobiohydrolase, β-glucosidase, acid phosphate and β-xylosidase were analyzed in 0–10 and 10–20 cm to elucidate the effects of substrate content on substrate affinity and catalytic efficiency (V_{max}/K_m). Living roots increased microbial biomass by 179% and microbial respiration by 100% compared to fallow soil. Lower enzyme affinities to substrates (e.g. 93% for β-glucosidase) in rooted soil pointed to the domination of r-strategists, which are favored in the decomposition of labile organics common in the rhizosphere. No differences in catalytic properties of cellulolytic enzymes were detected between bulk and litter-treated soil, indicating the recalcitrance of organics in both treatments. The rhizosphere and detritusphere effects on enzyme kinetics were negligible in 10–20 cm, except β-glucosidase. The reduction of K_m of all enzymes in 10–20 cm versus the upper 10 cm indicated increasing substrate affinity with depth. Nonetheless, the catalytic efficiency increased from 0 to 10 to 10–20 cm (e.g. up to 420% for acid phosphatase), reflecting changes in properties and functioning of enzymatic systems. This pointed to a shift towards a more K-selected microbial community with higher affinity and more efficient substrate utilization. It also indicated the microbial adaptation to decreasing substrate contents with depth by altered enzyme functioning. Overall, the catalytic properties of cellulolytic enzymes were much more strongly affected by plants (substrate quality in the rhizosphere and detritusphere compared to bare fallow) than by depth (substrate content).

1. Introduction

Microbial community composition in soils is governed by substrate quantity, quality and input regularity. Microhabitats with high substrate input but contrasting quality such as rhizosphere and detritusphere differ in their dominating microbial species (Kandeler et al., 2001; Marschner et al., 2004, 2012; Kuzyakov and Blagodatskaya, 2015). As a result of the differences in microbial key players, the decomposition pathways of organic compounds are significantly different in the rhizosphere and detritusphere.

In the rhizosphere, low molecular weight organic substrates, such as root released exudates, lysates and mucilage may accelerate microbial growth (Neumann and Römheld, 2007). The microbes become more active and thereby, produce more intracellular and extracellular enzymes compared to bare fallow (Burns, 1978, 1982). Hence, rhizodeposition directly couples plant and microbial activities in the root channel. Besides the regular input of rhizodeposits, root-litter is abundant as a substrate source for microbial decomposition. However, the small differences in C availability due to the root-litter do not affect the impact of root-exudation on soil organic matter decomposition (De Graaff et al., 2014).

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The detritusphere is characterized by high concentrations of easily degradable C sources, particularly at the early stages of residue decomposition (Bastian et al., 2009; Poll et al., 2010). As a result, the remaining substrates in the detritusphere are mainly polymeric plant residues of low degradability, for example cellulose and hemicelluloses (Nannipieri et al., 2012). Besides fast microbial uptake, the diffusion of soluble C and advective transport is frequently responsible for the depletion of the water-soluble C compounds (Gregorich et al., 2003; Poll et al., 2008). The recalcitrant plant-originated compounds require cascades of enzymes causing slower decomposition (Theuerl and Buscot, 2010). Their mineralization involves the action of several cellulases (e.g. β-cellobiohydrolase, β-glucosidase) to produce oligomeric cellobiose and to further degrade it to monomeric glucan (Nannipieri et al., 2012). The β-xylosidase is an exo-cellular enzyme involved in the degradation of the major polymeric constituents of plant litter by degrading the hemicellulose xylan (linear polysaccharide β-1,4-xylan) into its readily available compounds xylose and other carbohydrates (Sinsabaugh and Moorhead, 1994).

The quantity and quality of plant litter inputs to the soil (both above- and belowground) influences substrate availability for microbes. This may control microbial community structure, and alter enzyme systems (Paul and Clark, 1996; Horwath, 2007). It remains unclear which factor – substrate quantity or quality – is mainly responsible for the catalytic properties of enzymes hydrolyzing plant organics.

We compared the rooted and the litter-treated soil to a bare fallow soil, suggesting lower microbial biomass and microbial respiration due to lower C availability for the litter-treated and the fallow soil. Easily available substrates, such as root exudates, are quickly consumed by microorganisms with enzymes of low substrate affinity (typical for fast-growing r-strategists), reflecting higher K_m values (MacArthur and Wilson, 1967; Fierer et al., 2007). The slow-growing K-strategists with enzymes of high substrate affinity (lower K_m) are better adapted for growth on poorly degradable substrates (e.g. on the litter channel) (Blagodatskaya et al., 2009; Dorodnikov et al., 2009). Therefore, in hotspots with contrasting substrate quality, the shift in species domination may result in production of iso-enzymes, i.e. enzymes with the same function but different catalytic properties (Khalili et al., 2011) reflected in the enzyme kinetics (Marx et al., 2001). Furthermore, hydrolytic exo-enzymes in contrasting locations in the soil (i.e. immobilized vs. free) may change intrinsic enzyme properties, such as K_m values (Paulson and Kurtz, 1970; Rao et al., 1996).

There is a lack of studies comparing kinetic parameters of enzymes in hotspots of microbial activity such as the rhizosphere and detritusphere. Therefore, we measured the substrate affinity (K_m) and catalytic efficiency (V_{max}/K_m) (Gianfreda et al., 1995; Moscatelli et al., 2012) of 4 extracellular enzymes (β-cellobiohydrolase, β-glucosidase, acid phosphatase, β-xylosidase) in the vicinity of living roots of maize (rhizosphere) and maize litter (detritusphere).

According to evolutionary-economic principles the microbial communities allocate resources to enzyme production in relation to substrate availability and growth requirements to reduce costs and maximize their resource returns (Allison and Vitousek, 2005). The metabolic energy required for protein synthesis and excretion, as well as the C and nutrient content of the enzymes themselves are considered as costs of enzyme production in soils. The resource benefits of enzyme production can be invested in reproduction effort of microorganisms (Allison et al., 2011). Available forms of N and P are suggested to suppress the production of N- and P-acquiring enzymes and stimulate the microbial allocation to C-degrading enzymes (Sinsabaugh and Moorhead, 1994; Allison et al., 2011).

We hypothesized, that the kinetic parameters (V_{max}, K_m) of hydrolytic enzymes are different in microbial communities formed in soil hotspots as compared with bulk soil. To prove the effects of the substrate contents, the soil was sampled from 0 to 10 and 10–20 cm depths reflecting similar quality but lower input of substrate with depth. We further hypothesized that decreasing substrate content with depth increases the substrate affinity and catalytic efficiency.

To test these hypotheses we determined the parameters of microbial respiration (Anderson and Domsch, 1985; Cheng and Coleman, 1989; Anderson and Joergensen, 1997) and of enzyme kinetics (Sinsabaugh, 2010; Nannipieri et al., 2012), as indicators of organic C mineralization and substrate-specific utilization (Kourtev et al., 2002). This was done in the rhizosphere, detritusphere and soil from a bare fallow. This is the first study, combining such general microbial activity indicators as respiration with specific indicators as the kinetics of extracellular hydrolytic enzymes. This enables elucidating the effect of two contrasting C sources – rhizodeposits and plant litter – on the functioning of microbial communities under field conditions.

2. Materials and methods

2.1. Study site and sampling

This study was conducted at an experimental agricultural site in the north-west of Göttingen (Lower-Saxony), Germany (51°33′N, 9°53′E; 158m NN). The area has a temperate climate with a long-term annual mean precipitation of 645 mm and an air temperature of 8.7 °C. The dominant soil types are loamy haplic -Luvisols, partly with slight stagnic properties (Table 1).

In spring 2012, 12 experimental plots (5 × 5 m) were established in the field and separated from each other by buffer stripes of 2 m and 6 m in row and inter-row, respectively. Three treatments, – rooted, litter amended and fallow soil – were set up on the experimental plots, with 4 replicates each.

All experimental plots were tilled with a chisel plough to a depth of 10 cm (tillage date: 12th of April 2012). For the rooted treatment, hybrid maize (*Zea mays L.*, Codisco/TMTD 98% Satec) was sown on 4 plots at a density of 12 plants m^{-2} (sowing date: 16th of April 2012) N fertilizers (ammonium nitrate urea solution: 110 kg N ha^{-1} and NP fertilizer (diammonium phosphate: 110 kg N ha^{-1}) were amended to all treatments, shortly before and after sowing the maize. For the litter treatment 4 plots received 0.8 kg m^{-2} dry maize residues with a C -content of about 44%. Litter application took place in 10 cm soil depth in early June at the start of the crop growth period to ensure the same conditions for the herbivore and detritivore community in the soil. In addition 4 plots remained unplanted as a fallow control. All treatments were kept free from vegetation by manually removing weeds. The obtained differences in the enzyme systems between 0–10 cm and 10–20 cm were thus established within a relatively short period. The shading level represented a mean leaf area index of plants during the vegetation period to accomplish comparable environmental conditions between the plots.

In July, the soil was sampled at 0–10 cm and 10–20 cm on each plot. Soil sub-samples from each plot and depths were dried at 105 °C (24 h) to determine the soil moisture content. The water contents of the sampled soil ranged from 28% for fallow to 25% for the rooted soil, which was significantly lower than the fallow control ($P < 0.001$). All soil samples were frozen at −18 °C until the analyses. Prior to the analysis the soil samples were thawed at 4 °C. After thawing the soil samples were sieved (<2 mm) and fine roots and other plant debris were carefully removed with tweezers and the soil was pre-conditioned at 22 °C for 72 h. Afterwards, the

Table 1
Soil properties (±SEM) of the loamy haplic Luvisoil determined before the start of the experiment (Kramer et al., 2012; Pausch et al., 2013). Significant differences are indicated by letters ($P < 0.05$).

Horizon	Depth [m]	Texture clay/silt/sand [% (w/w)]	pH [$CaCl_2$]	SEM	Bulk density [g cm^{-3}]	SEM	C_{org} [g kg^{-1}]	SEM	N_{total} [g kg^{-1}]	SEM	C/N
Ap1	0–0.25	7.0/87.2/5.8	6.0a	0.1	1.38a	0.0	12.4a	0.4	1.3 ± 0.0a	0.0	9.8a
Ap2	0.25–0.37	7.1/87.8/5.0	6.2a	0.1	1.61b	0.0	6.9b	1.2	0.8 ± 0.1b	0.1	9.2a
Btw1	0.37–0.65	7.1/87.7/5.1	6.6b	0.1	1.55c	0.0	3.3c	0.5	0.4 ± 0.0c	0.0	8.9ab
Btw2	>0.65	6.8/88.4/4.8	7.0c	0.1	1.68 b	0.0	1.8c	1.8	0.3 ± 0.0c	0.0	6.9b

moisture contents of the soil samples were adjusted to 60% of water holding capacity (WHC) for analyses. No significant differences were detected in pH, C_t, or N_t contents of rooted, litter-treated and fallow soil.

2.2. Analyses

The experiments were conducted with the 4 plot replicates for each treatment. Enzyme activities, microbial biomass and CO_2 data were expressed as means ± standard errors of means (±SEM).

2.2.1. Soil microbial biomass

Soil microbial biomass C (C_{mic}) was estimated using the chloroform fumigation extraction (CFE) method described by Brookes et al. (1985) and Vance et al. (1987). Non-fumigated, moist soil (7.5 g) was extracted with 30 ml of 0.05 M K_2SO_4 for 1 h (Bruulsema and Duxbury, 1996) by overhead shaking (40 rev min^{-1}). The same amount of soils was fumigated with ethanol-free chloroform (80 ml) first and then extracted in the same way. The fumigation was carried out in desiccators at room temperature for 24 h. The soil suspension of the fumigated and the non-fumigated samples was centrifuged for 10 min at approx. 2500 × g. Afterwards, the supernatant was filtered through Rotilabo-rondfilters (type 15A, Carl Roth GmbH & Co.KG). The organic C-content of the K_2SO_4 extracts was measured using a multi N/C analyzer (multi N/C analyzer 2100 S, Analytik Jena).

Microbial biomass C and microbial biomass N were calculated by dividing the microbial C or N flush (E_C; E_N), i.e. the difference between extracted C or N from fumigated and non-fumigated soil samples, with a k_{EC} or k_{EN} factor of 0.45 (Wu et al., 1990; Joergensen, 1996).

2.2.2. Basal and substrate-induced microbial respiration

Microbial respiration was determined by substrate-induced respiration (SIR) based on CO_2 efflux after adding glucose and mineral salts (Anderson and Domsch, 1985; Anderson and Joergensen, 1997). The SIR method was conducted in a climate chamber (22 °C). Thereby, 23 g (dry weight) of each soil sample was incubated in flasks for 4 h after addition of the substrate. The amended substrate mixture contained glucose (10 mg g^{-1}), talcum (20 mg g^{-1}) and mineral salts, i.e. 1.9 mg g^{-1} $(NH_4)SO_4$, 2.25 mg g^{-1} K_2HPO_4 and 3.8 mg g^{-1} $MgSO_4$–$7H_2O$ (Blagodatsky et al., 2000). Gas samples (15 ml) were taken hourly and the CO_2 concentrations were analyzed by gas chromatography (GC 6000 VEGA series 2, Carlo Erba instruments, UK). The basal respiration (BR) was measured in the same way as the SIR without any substrate amendment and a sampling time interval of 2 h.

To obtain CO_2 flux rates, the slopes of hourly measured CO_2 concentrations were corrected by the specific gas flux and multiplied with the headspace volume (1098 cm^3). We then related the CO_2 fluxes to the soil carbon content and incubation duration. The metabolic quotient (q_{CO2}) indirectly reflects the microbial maintenance expenses, availability and efficiency of microbial substrate utilization and was determined by the ratio of BR to C_{mic} (Anderson and Domsch, 1990).

2.2.3. Enzyme assays

We used fluorogenic methylumbelliferone-based (MU) substrates to measure the enzyme activities of β-cellobiohydrolase, β-glucosidase, acid phosphates and β-xylosidase were measured (Marx et al., 2001). The following 4-Methylumbelliferone derivatives were used as substrates: EC 3.2.1.21, 4-MU-β-D-glucoside; EC 3.2.2.27, 4-MU-β-D-xylopyranoside; EC 3.2.1.91, 4-MU-β-D-cellobioside; EC 3.2.1.30, 4-MU-phosphate. Half a gram of moist soil was added in 50 ml sterile water in autoclaved jars and was dispersed by an ultrasonic disaggregator (50 J s^{-1} for 120 s (De Cesare et al., 2000). Aliquots of 50 µl were withdrawn and dispensed in 96-well microplates (Brand pureGrade, black) while stirring the suspension. In addition to four field replicates we used three analytical replicates for each soil sample and each substrate. Fifty microliter of 0.1 M 2-(N-morpholino) ethanesulfonic acid (MES) (pH 6.1) was used as buffer (German et al., 2011). The substrates were pre-solved in 300 µl Dimethyl-sulfoxide (DMSO) and were further diluted by MES to 1 mM a working solution. Finally, 100 µl of series concentrations of substrate solutions (20, 40, 60, 80, 100, 200, 400 µmol substrate g $soil^{-1}$) were added to the wells. Plates were kept at 21 °C, agitated and measured fluorometrically (excitation 360 nm; emission 450 nm) after 1 h, 2 h, and 3 h incubation with an automated fluorometric plate-reader (Wallac 1420, Perkin Elmer, Turku, Finland).

The substrate-dependent rate of reaction (v) mediated by hydrolytic enzymes, followed Michaelis–Menten kinetics (Marx et al., 2001, 2005; Sinsabaugh, 2010; Nannipieri et al., 2012).

$$v = (V_{max} \times [S])/(K_m + [S]) \quad (1)$$

Plotting the initial velocity of reaction (v) against increasing concentrations of substrate ([S]) yields a rectangular hyperbola. Based on experimental data, the calculation enables characterizing the specific enzyme–substrate reaction by 2 kinetic parameters: 1) V_{max}, the maximal velocity of enzyme catalysis that theoretically is attained when the enzyme has been saturated by an infinite concentration of substrate, and 2) K_m, the Michaelis constant, which is numerically equal to the concentration of substrate for the half-maximal velocity (Marx et al., 2005). V_{max} is responsible for decomposition rates at saturating substrate concentrations; the K_m reflects the enzyme affinity to the substrate. We calculated the catalytic efficiency factor (catalytic efficiency/specificity constant/performance constant), known as the ratio between V_{max} and K_m (Gianfreda et al., 1995; Koshland, 2002; Moscatelli et al., 2012). The catalytic efficiency reflects the total enzyme catalytic process combining enzyme–substrate complex dissociation (V_{max}) and the rate of enzyme–substrate complex formation (K_m) (Cornish-Bowden, 1995; Koshland, 2002).

Significant effects of soil treatments were assessed by ANOVA at $P < 0.05$. The parameters of the equation were fitted by minimizing the least-square sum using GraphPad Version 6 software (Prism, USA). The three analytical replicates of enzyme activity curves were used for each of four replicated soil samples at two depths (0–10 and 10–20 cm). Parameter optimization was restricted to the applied model equation as indicated by maximum values of statistic criteria:

r^2, the fraction of total variation explained by the model defined as the ratio of model weighted sum of squares to total weighted sum of squares. Outliers were identified by the ROUT method, based on the False Discovery Rate (FDR), where Q was specified, which was the maximum desired FDR (Motulsky and Brown, 2006).

3. Results

3.1. Microbial biomass C and N, extractable organic C and extractable N

Living roots strongly increased microbial C and N (Fig. 1a, b). C_{mic} and N_{mic} contents in the upper 10 cm of the rooted soil were 178% and 222% higher than the fallow treatment. The average microbial biomass C content in fallow soil was 128 (±46) μg C_{mic} g^{-1} soil, and the microbial biomass N was 20 (±5) μg N_{mic} g^{-1}. The litter application did not affect C_{mic} and N_{mic} compared to the fallow. Living roots increased microbial biomass C and N only in the upper 10 cm, whereas below 10 cm the root effect was negligible.

Furthermore, the salt-extractable organic C (EOC) and salt-extractable N (EN) contents doubled in the rooted soil compared with the litter-amended plots, indicating abundant easily available organics in the upper 10 cm (Fig. 1c,d). The EOC and EN contents increased through planting compared to litter-amended and fallow soil solely in the upper 10 cm. Rhizodeposition increased C_{mic}, N_{mic}, EOC and EN for rooted soil compared to litter-amended and fallow soil only in the first 10 cm.

3.2. Basal respiration and substrate-induced respiration

The basal respiration (BR) (80 ± 20 μg CO_2–C g^{-1} C_{org} h^{-1}) of rooted soil in the upper layer was twice as high relative to litter-amended and fallow soil (Fig. 2a). In 10–20 cm, BR of the planted soil showed significantly ($P < 0.05$) reduced rates compared to the upper 10 cm.

The largest SIR values were measured for rooted soil in 0–10 cm. A 2-fold greater CO_2 production rate (0.66 ± 0.07 mg CO_2–C mg^{-1} g^{-1} C_{org} h^{-1}) was determined for rooted compared to litter-amended and fallow soil (Fig. 2b). The effect of planting disappeared in the 10–20 cm layer and showed comparable BR and SIR values for the litter-amended and fallow soil. For litter-amended soil the BR:SIR ratio was lower than for rooted soil, especially in 10–20 cm (Fig. 2c). The decomposition of easily available organics in the rhizosphere clearly increased microbial respiration (BR and SIR) relative to litter-treated and fallow soil in the surface layer.

3.3. Enzyme kinetics

Living plants strongly stimulated the β-glucosidase activity in the upper 10 cm resulting in the highest maximal reaction rate (V_{max}) compared to litter-amended and fallow soil (Fig. 3a; Table 2). The V_{max} of β-glucosidase and β-cellobiohydrolase increased 2-fold for rooted soil in the upper layer at substrate saturation compared to fallow soil (Fig. 3a,b). This indicates high production of glycolytic enzymes by the microbes. The rates of β-glucosidase, acid phosphatase and β-cellobiohydrolase reduced for rooted soil ($P < 0.05$) from 0 to 10 to 10–20 cm depth (Fig. 3a,b,c).

For the rooted soil we determined about 2- and 3-fold higher K_m values for β-glucosidase and β-cellobiohydrolase, respectively, compared to that of the fallow control in 0–10 cm. This reflects a lower affinity to the substrate. The K_m decreased with depth for all

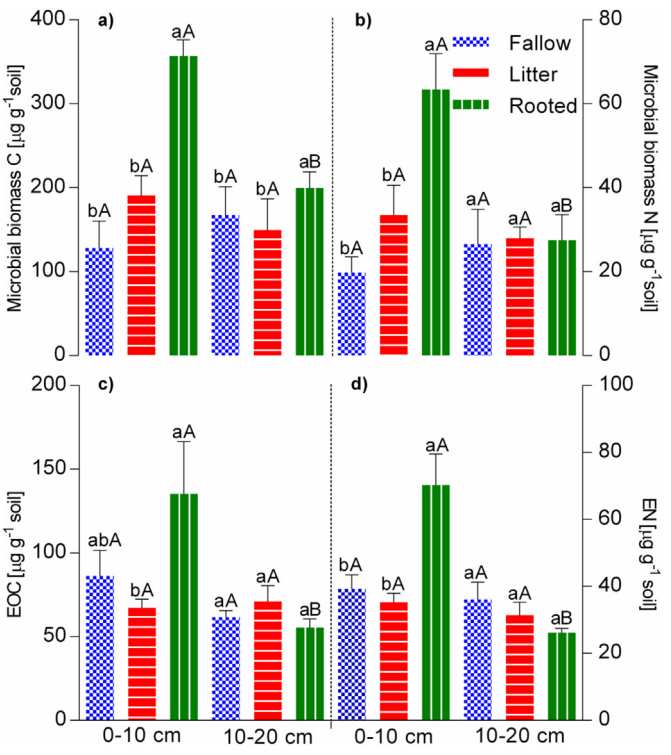

Fig. 1. a) Microbial biomass C (C_{mic}), b) extractable organic carbon (EOC), c) microbial biomass N (N_{mic}) and d) extractable nitrogen (EN) (±SEM) for fallow, litter-amended and rooted soils at two depths (0–10 and 10–20 cm). Significant treatment effects are assessed by ANOVA ($P < 0.05$) and indicated by different lower-case letters. Capital letters denote significant soil treatment effects with depth.

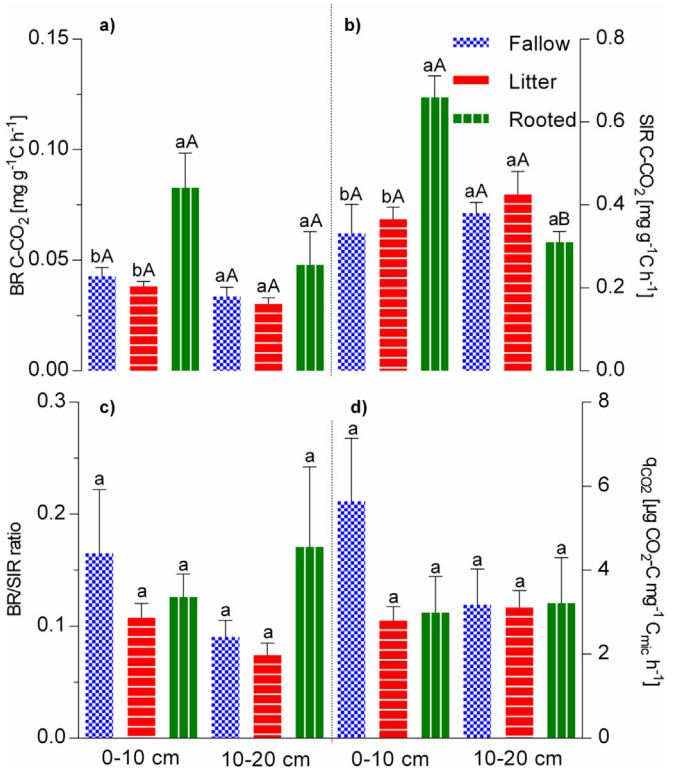

Fig. 2. a) Basal respiration (BR), b) substrate-induced respiration (SIR), c) respiratory quotient (BR/SIR ratio) and d) metabolic quotient (q_{CO2}) (±SEM) for fallow, litter-amended and rooted soils at two depths (0–10 and 10–20 cm). Significant treatment effects are assessed by ANOVA ($P < 0.05$) and indicated by different lower-case letters. Capital letters denote significant soil treatment effects with depth.

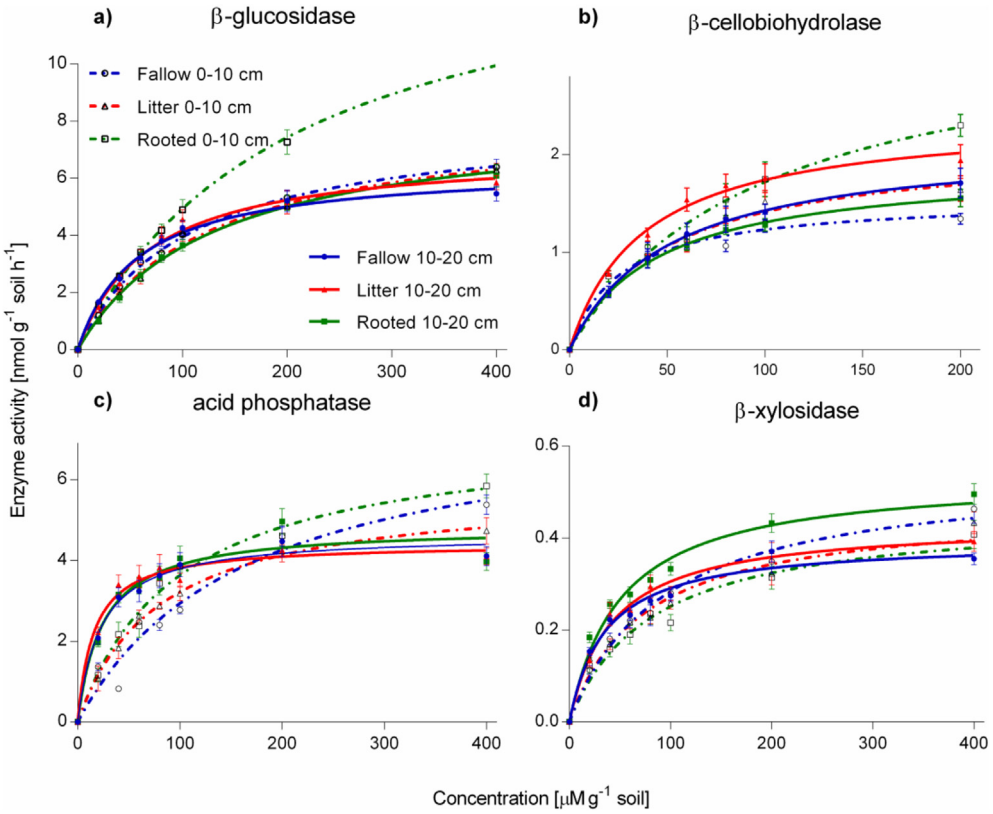

Fig. 3. Enzyme kinetics (±SEM): a) β-glucosidase, b) β-cellobiohydrolase, c) acid phosphatase and d) β-xylosidase. The blue color indicates the fallow, litter-amended and rooted soils. Statistics are given in Table 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

analyzed enzymes except for β-cellobiohydrolase where K_m increased in the deeper soil layer of the fallow soil (Table 2). For acid phosphatase we recorded a 6-fold reduction of K_m from 0 to 10 cm to the 10–20 cm depth (fallow soil, Table 2). The consistent decrease of K_m with depth indicated the reduction of substrates in deeper the soil layer.

The catalytic efficiency (V_{max}/K_m) of acid phosphatase was higher in rhizosphere and detritusphere compared to fallow soil in the upper 10 cm, with significantly higher enzyme affinity to the substrate (Table 2). Furthermore the V_{max}/K_m ratio increased with depth for β-glucosidase, acid phosphatase and β-xylosidase, and was maximal for acid phosphatase.

The two-way ANOVA for V_{max} and K_m with treatment (substrate quality) and depth (substrate content) as main factors revealed that the effects of both roots and depth were enzyme-specific (Fig. 4). The strongest effect for substrate quality was revealed for V_{max} of β-glucosidase, β-cellobiohydrolase and acid phosphatase, explaining 44, 22 and 11% of variation, respectively. The β-cellobiohydrolase and β-xylosidase were also strongly affected by substrate quality and depth interactions. For K_m of β-glucosidase, 48% of the

Table 2
Kinetic parameters (V_{max} and K_m; ±SEM) of extracellular hydrolytic enzymes at 0–10 cm and 10–20 cm for fallow, litter-amended and rooted soils. Lower-case letters indicate significant differences ($P < 0.05$) of V_{max} and K_m by ANOVA with post-hoc Tukey multiple comparison of different treatments at same depth, whereas capital letters indicate the comparison with depth.

Depth [cm]	Treatment	β-cellobiohydrolase					β-glucosidase				
		V_{max} [nmol g^{-1} h^{-1}]	SEM	K_m [μmol g^{-1}]	SEM	V_{max}/K_m	V_{max} [nmol g^{-1} h^{-1}]	SEM	K_m [μmol g^{-1}]	SEM	V_{max}/K_m
0–10	Fallow	1.6b	0.1	26.1b	4.9	0.06	8.1b	0.3	105.9b	9.0	0.08
	Litter	2.1b	0.1	49.3b	8.2	0.04	8.3b	0.4	124.9bA	13.5	0.07
	Rooted	3.4aA	0.4	97.3aA	22.8	0.03	15.0aA	0.8	204.5aA	21.5	0.07
10–20	Fallow	2.2	0.2	50.7	11.9	0.04	6.4b	0.3	57.3b	6.5	0.11
	Litter	2.4	0.2	37.8	7.8	0.06	7.0b	0.4	68.5bB	9.9	0.10
	Rooted	1.9B	0.1	44.7B	6.2	0.04	8.2aB	0.5	129.0aB	14.8	0.06
Depth [cm]	Treatment	Acid phosphatase					β-xylosidase				
		V_{max} [nmol g^{-1} h^{-1}]	SEM	K_m [μmol g^{-1}]	SEM	V_{max}/K_m	V_{max} [nmol g^{-1} h^{-1}]	SEM	K_m [μmol g^{-1}]	SEM	V_{max}/K_m
0–10	Fallow	7.7aA	0.4	164.2aA	19.3	0.05	0.54A	0.03	92.6A	13.7	0.01
	Litter	5.8bA	0.3	79.5bA	12.0	0.07	0.46	0.03	71.9	10.2	0.01
	Rooted	7.2acA	0.4	98.5bA	13.8	0.07	0.45	0.04	81.5	15.5	0.01
10–20	Fallow	4.6B	0.2	22.5B	4.8	0.21	0.39B	0.01	35.4B	4.1	0.01
	Litter	4.4B	0.2	15.8B	3.3	0.28	0.43	0.02	42.1	6.8	0.01
	Rooted	4.8B	0.2	24.3B	4.7	0.20	0.54	0.02	50.6	5.7	0.01

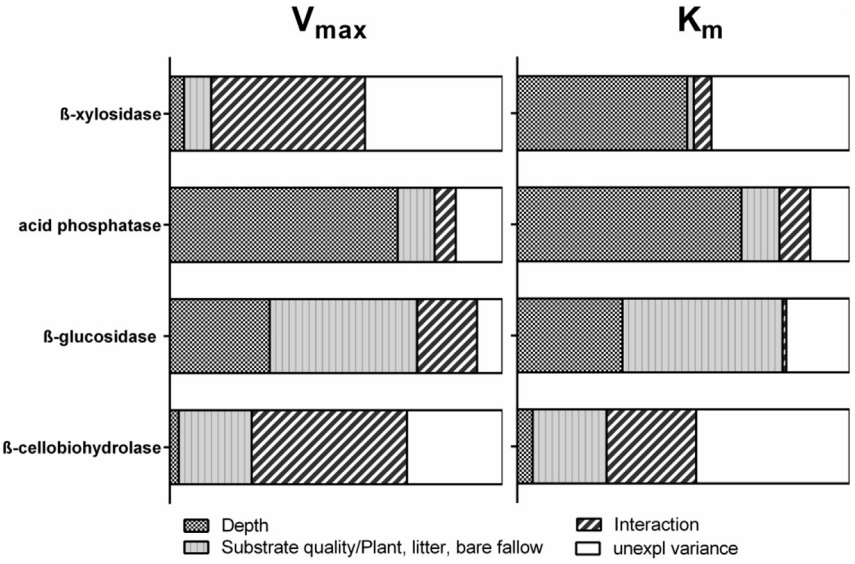

Fig. 4. Contribution of the two factors substrate quality and substrate content and their interactions (two depths: 0–10 and 10–20 cm) for variation of V_{max} and K_m. Results of two-way ANOVA.

variation could be explained by the substrate quality factor (Fig. 4). Overall, the cellulolytic enzymes were more strongly affected by substrate quality, whereas for phosphatase and xylanase the depth effect was most important.

The enzyme activities for β-glucosidase and β-cellobiohydrolase were greater in rooted plots relative to litter-treated and fallow ones. Moreover, all measured enzyme affinities to the substrates increased strongly from 0 to 10 cm to the 10–20 cm depth, indicating a shift in enzymatic systems.

4. Discussion

4.1. Effects of roots

We determined that the strong effects of living plants in the upper 10 cm yielded 179, 222, 100 and 100% higher values for C_{mic}, N_{mic}, BR, and SIR, respectively, compared to the fallow soil (Figs. 1 and 2). This indicates that microbial proliferation was strongly related to the quantity and quality of substrates available for growth. Meta-analysis of microbial biomass and respiration data from various studies showed 62% and 80% higher values in rhizosphere soil compared to bulk soil, respectively (Finzi et al., 2015). For rooted soil we found a 44% reduction of microbial biomass content from 0 to 10 to 10–20 cm, reflecting the role of root exudates. Maize roots are concentrated in the upper soil layers (Amos and Walters, 2006). At our field site about 50% of the roots were allocated to the upper 10 cm (Pausch et al., 2013). Since rhizodeposition is positively correlated to root biomass (Van der Krift et al., 2001), the decreasing root biomass led to lower rhizodeposition with depth (Pausch et al., 2013). Accordingly, EOC, N_{mic}, EN contents and microbial respiration were reduced from 0 to 10 to 10–20 cm for rooted soil by 59, 57 and 63%, respectively. This reduction corresponded with the decrease in the relative amounts of water-soluble C with increasing distance to wheat roots (Merbach et al., 1999; Hafner et al., 2014). Remarkably, EN was lowest for rooted soil compared with litter-amended soil and fallow soil in 10–20 cm. We suggest that a reduced rhizodeposition in 10–20 cm – and thus less EOC and EN – promotes substrate competition between microbes and plants (Fontaine et al., 2003; Kuzyakov and Xu, 2013; Blagodatskaya et al., 2014b). The Michaelis constant (K_m) doubled for β-glucosidase and tripled for β-cellobiohydrolase in the rhizosphere compared to fallow soil, indicating lower enzyme affinity to the substrate. Easily available substrates such as glucose (as a component of root exudates) stimulated fast-growing r-strategists in the upper 10 cm (Pianka, 1970; Blagodatskaya et al., 2009). This agrees with the increased SIR (Fig. 2).

The great reduction of K_m for acid phosphatase, β-glucosidase and β-xylosidase with depth (Table 2, Fig. 3) means high rates of reaction already present at very low substrate concentrations (Davidson and Janssens, 2006; Davidson et al., 2006). Remarkably, all tested enzymes reflected this strong effect of decreasing substrate content with depth. This confirms that the activities of extracellular enzymes are mainly a function of the amount of available substrate (Kandeler et al., 1999) and of the microbial biomass present to potentially synthesize them (Geisseler and Horwath, 2009). Due to reduced rhizodeposition in the lower layer, the strong competition for easily degradable C sources favors the K-selected microbes, which are reported to be more competitive under resource limitation even in the rhizosphere (Blagodatskaya et al., 2014b). Accordingly, we determined that the catalytic efficiency of all measured hydrolytic enzymes (except β-cellobiohydrolase, fallow soil; β-glucosidase, rooted soil) increased from 0 to 10 to 10–20 cm, again indicating a shift to K-selected microbes (Table 2, Fig. 3). Therefore, a shift in microbial strategy towards higher substrate affinities of enzyme systems suggested a change in substrate content (Blagodatskaya et al., 2009). Such physiological adaptations of microorganisms to substrate content and quality are more important for efficient substrate utilization than the microbial community structure (Stone et al., 2014). Extracellular enzyme systems adapted to the altered substrate supply resulted in a change of catalytic efficiency and in a corresponding shift in the functional structure of the microbial community. Thus, a lower catalytic efficiency indicated the dominance of zymogenous microbial communities (r-strategists) in 0–10 cm depth as compared with 10–20 cm depth, where the K-strategists relatively dominated (Table 2) (Panikov, 1995; Blagodatskaya et al., 2009).

High variations in the unexplained variance of the measured enzymes (Fig. 4), reflected highly enzyme-specific determining

factors. Increased probability of explained variance for β-glucosidase and acid phosphatase pointed to strong impacts of quantity and quality of the substrate.

4.2. Effects of litter

C_{mic}, EN, EOC, BR and SIR values were similar for the litter-amended and fallow soil (Figs. 1 and 2). This further confirmed that total C_{mic} does not change after litter addition (Potthoff et al., 2008). Basal and substrate-induced respiration indicated a more efficient C mineralization for the detritusphere than the rhizosphere, suggesting a lower microbial turnover in the detritusphere. Empirical studies of N mineralization have agreed upon a threshold of litter C:N ratio (e.g., 20–40) below which microbial growth will not be N limited. As such, microbes may shift the equilibrium production of enzymes to favor C-acquiring ones (Tian and Shi, 2014). The β-cellobiohydrolase activity, however, was highest in the litter-amended soil in 10–20 cm compared to rooted and fallow plots (Fig. 3). This can be due to the quality of the amended maize leaves, which mostly consist of nonlignified primary cell walls, thus making the cellulose and hemicellulose less resistant to enzymes. In contrast, the abundant root-litter in the maize planted treatment, which is rich in secondary cell walls (Amin et al., 2014). The litter C:N ratios frequently show a negative relation to cellulose and β-glucosidase activities (Leitner et al., 2012; Tian and Shi, 2014). This is in line with the comparatively low C:N ratio (21.5) of the used maize litter (Potthoff et al., 2005, 2008) and the increased response of β-cellobiohydrolase activity (Blagodatskaya et al., 2014a). Thus, litter C:N is a good indicator for the total amount, but not for the dynamics of soil enzyme activity (Tian and Shi, 2014). Nonetheless, the reduced substrate affinity (Table 2) for litter-amended soil throughout all tested extracellular enzymes were in line with a lower BR:SIR ratio, especially in 10–20 cm. This points to a shift in substrate availability and thus to changes in the efficiency of C and N utilization.

When comparing the litter-treated with rooted soil, the microbial community develops according to substrate quality and regularity of the input. Therefore, slow-growing microorganisms with more efficient metabolism are usually developed on low available plant residues as compared with easily decomposable root exudates. Thus, the C-cycling hydrolytic exo-enzymes demonstrated slower decomposition rates in litter amended soil, but similar or higher catalytic efficiencies compared to rooted soil. This may reflect a lower waste metabolism of microorganisms in plant litter-treated soil.

5. Conclusions

The β-glucosidase, β-cellobiohydrolase and acid phosphatase were strongly affected by substrate quality, which differed in the rhizosphere and detritusphere of maize. Thus, the contrasting substrate quality of living roots and shoot litter created hotspots for the microorganisms, which produced extracellular enzymes for their distinct needs. A pronounced effect of roots was determined in the upper 10 cm caused by rhizodeposition, which maintained an increased microbial biomass C and N, EOC, EN, microbial respiration as well as enzyme activities in the rhizosphere compared to the detritusphere and bare fallow. This effect disappeared in 10–20 cm due to lower contents of easily available substrates, reflecting a lower root biomass. A clear increase of enzyme affinity in 10–20 cm compared to the first 10 cm, pointed a shift towards a more K-selected microbial community.

We conclude that the availability of C and nutrients in the soil clearly affected the metabolic respiratory response as well as the efficiency of enzymes mediating the catalytic reaction, especially in the presence of roots. Substrates with different availability (e.g. root exudates, plant residues) changed functional properties of the soil microbial community and induced a shift in enzymatic systems. These changes are crucial for microorganisms to benefit from the costs of energy investments, caused by a stronger competition for resources.

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