Biol Fertil Soils (2016) 52:1105–1119
DOI 10.1007/s00374-016-1148-y

ORIGINAL PAPER

Hotspots of microbial activity induced by earthworm burrows, old root channels, and their combination in subsoil

Duyen T. T. Hoang[1,4] · **Johanna Pausch**[1] · **Bahar S. Razavi**[2] · **Irina Kuzyakova**[3] · **Callum C. Banfield**[1] · **Yakov Kuzyakov**[1,2]

Received: 20 June 2016 / Revised: 27 August 2016 / Accepted: 12 September 2016 / Published online: 26 September 2016

Abstract Biopores are pores or voids in soil produced by roots, by earthworms, or by the occupation of earthworms in root pores, which are considered important microbial hotspots, especially in subsoil. We hypothesized that earthworms (*Lumbricus terrestris* L.) exert stronger effects on microbial activities than decaying plant roots (of *Cichorium intybus* L.) in the subsoil because of the addition of pre-digested organic material. We tested this hypothesis by analyzing microbial biomass (C_{mic}), total organic C (C_{org}), and activities of eight enzymes (cellobiohydrolase, β-glucosidase, xylanase, acid phosphomonoesterase, leucine aminopeptidase, tyrosine aminopeptidase, chitotriosidase, and N-acetylglucosaminidase) down to 105-cm depth. The C_{mic} increase was associated with a two- to threefold increase of C_{org} content in biopores as compared to bulk soil. The highest percentage of C_{mic}-to-C_{org} (3.7 to 7.3 %) in the drilosphere demonstrated the enhancement of microbial efficiency for organic matter decomposition by earthworms. The availability of organic matter in biopores increased the activities of C- and N-targeting enzymes by 1.2–11.3 times, but reduced acid phosphomonoesterase activity by 10–40 % in biopores versus bulk soil. Introducing earthworms in root biopores caused 1.5–1.8 times higher microbial biomass and 1.2–1.9 times increased enzyme activities compared to the sole effect of earthworms. Soil depth showed a strong effect on the drilosphere, but only slight effects on the biochemical properties of root biopores and bulk soil. In conclusion, biopores are important microbial hotspots of C, N, and P transformations in subsoil. Earthworms exerted stronger effects on biochemical properties of biopores than decaying roots.

Keywords Earthworms · Biopores · Enzyme activities · Catalytic efficiency · Microbial activities · Subsoil processes

Introduction

Microbial activity is the most important indicator of soil health (van Bruggen and Semenov 2000; Kibblewhite et al. 2007) due to its high sensitivity to changes of environmental conditions (Anderson and Domsch 1989) and land use (Matson et al. 1997; Mganga et al. 2015). Microbial activity is affected by many factors, including labile C availability (C) and energy supply (Blagodatskaya and Anderson 1999; Schimel and Weintraub 2003), microbial community composition (Goberna et al. 2005), and soil aggregate (Miller and Dick 1995). Microorganisms are heterogeneously distributed in the soil. There are regions with faster/higher microbial process rates and more intensive interactions compared to the surrounding soil. These regions are termed microbial hotspots (Kuzyakov and Blagodatskaya 2015). Hotspots occur when substrate availability for microbes is high. Biopores, for example, are important hotspots with high microbial process rates (Kuzyakov 2010; Hoang et al. 2016). They are defined as tubular soil macropores (Nakamoto 2000) or voids

Electronic supplementary material The online version of this article (doi:10.1007/s00374-016-1148-y) contains supplementary material, which is available to authorized users.

✉ Duyen T. T. Hoang
dhoangt1@gwdg.de; duyenhoang42@gmail.com

[1] Department of Soil Science of Temperate Ecosystems, Georg-August University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

[2] Department of Agricultural Soil Science, Georg-August University of Göttingen, Göttingen, Germany

[3] Department of Ecoinformatics, Biometrics and Forest Growth, Georg-August University of Göttingen, Göttingen, Germany

[4] Department of Soil Science, Forestry University, Hanoi, Vietnam

(Kautz 2014) formed (1) by roots (the pores remaining after root decay) (detritusphere), (2) by earthworms (drilosphere), or (3) by continuous colonization of old root channels by earthworms (detritus-drilosphere). Root litter addition and earthworm burrows strongly increase microbial biomass in soils (Sanaullah et al. 2011). Subsoil biopores can become crucial for nutrient acquisition by plants because nutrients and organic C may be depleted in arable topsoil after long-term cultivation and litter removal from the field (Kautz et al. 2013). Biopores affect soil physical conditions by increasing air and water circulation in subsoil horizons (Uteau et al. 2013; Kautz 2014). Moreover, they also change soil biochemical properties by altering microbial biomass and the activities of enzymes involved in cycles of carbon (C), nitrogen (N), phosphorus (P), and other nutrients (Stehouwer et al. 1993; Tiunov and Scheu 2000; Jégou et al. 2001). Most biopore investigations, however, have focused on topsoil layers (Menichetti et al. 2014). The roles of subsoil biopores for plant growth are still underestimated despite their importance.

Microbial activities are controlled by C and N availabilities (Blagodatsky et al. 1998; Hodge et al. 2000), which depend on soil organic matter decomposition. Organic C and total N contents decrease with soil depth in the bulk soil (Salomé et al. 2010). This makes biopores an important source of nutrients and C in subsoil layers. Several studies (Don et al. 2008; Sanaullah et al. 2011; Bamminger et al. 2014) have documented an increase in N and C stocks within burrow walls compared to the bulk soil. Organic P compounds such as phytate, phospholipids, and nucleic acids are hydrolysed to inorganic P by phosphatases, which are released by phosphate-solubilizing bacteria, for example, *Pseudomonas*, *Bacillus*, and *Rhizobium* (Rodríguez and Fraga 1999; Nannipieri et al. 2011). Earthworms increase the solubilization of P in soil by decreasing pH, desorbing P from soil particles, and by generally accelerating microbial activities (Barois and Lavelle 1986; Lopez-Hernandez et al. 1993).

Organic matter lability, C and N availability, and lifetime of biopores of various origins may specifically regulate the microbial community composition and activity (Kuzyakov and Blagodatskaya 2015). The aim of this study was to measure microbial activities in different biopore types (old root channels colonized or not colonized by earthworms, mixed-origin pores by both old root channels and earthworms) through microbial biomass, basal respiration, and enzyme activities. It was hypothesized that (i) the combined effect of decomposed roots and short-term incubation with epi-anecic earthworms (*Lumbricus terrestris* L.) boosts microbial activities more than mere earthworm presence or root decay and (ii) earthworm presence causes higher microbial biomass, higher basal respiration, and higher enzyme activities involved in C, N, and P cycles compared to the bulk soil.

To test these hypotheses, soil was sampled from pore walls made by chicory roots (*Cichorium intybus* L.), earthworms, and the introduction of earthworms into chicory root pores. We analyzed total organic C (C_{org}), microbial biomass (C_{mic}), basal respiration (CO_2), and the activities of enzymes involved in the decomposition of soil organic compounds. Despite enzyme activity is a sensitive indicator of changes in soil organic matter, it remains unclear whether the microbial mechanisms differ in these hotspots and in bulk soil. This may be revealed by enzyme kinetics parameters (Nannipieri and Gianfreda 1998; Pathan et al. 2015). They describe the catalytic activity and enzyme substrate affinity (Nannipieri et al. 1988, 1996) and the probable distribution of enzyme activity in the biopores (Nannipieri et al. 2012; Uksa et al. 2015). Therefore, the kinetics of eight enzymes were measured including the following: cellobiohydrolase (CBH) and β-glucosidase (BGL), which are commonly measured as enzymes responsible for consecutive stages of cellulose degradation (German et al. 2011); xylanase (XYL), which is responsible for breaking down hemicelluloses (German et al. 2011); and acid phosphomonoesterase (PHO), which hydrolyzes (mono) ester bonds of organic P to phosphate under acidic conditions (Eivazi and Tabatabai 1977; Malcolm 1983). Activities of leucine aminopeptidase (LAP) and tyrosine aminopeptidase (TYR) were analyzed to assess the hydrolysis of leucine and tyrosine residues from the amino-termini of protein or peptide substrates (Rawlings et al. 2006). Activities of chitotriosidase (CHI), which is involved in the first step of chitin degradation, and N-acetylglucosaminidase (NAG-chitinase), which accomplishes the decomposition of chitin to lower molecular weight chitooligomers (Hamid et al. 2013), were determined. The subsoil was divided into two depth intervals: 45–75 and 75–105 cm because (i) the decomposition rate of fresh roots decreases linearly with soil depth down to 100 cm (Gill and Burke 2002), and (ii) the total soil C stock as well as the stability of root-derived C and of root-derived N are different at different depths (Goberna et al. 2005). The choice of soil depths was also based on the study by Perkons et al. (2014), who found the largest difference of chicory root diameters between 45–65 cm and deeper horizons.

Materials and methods

Soil sampling and preparation

The research station is located at the Campus Klein-Altendorf (50° 37′ N, 6° 59′ E) south-west of Bonn, Germany. The soil is a Haplic Luvisol (WRB). Detailed soil properties are given in Vetterlein et al. (2013).

Twenty-five biopores of each type were investigated. (1) Chicory (*Cichorium intybus* L.), characterized by a taproot system, was grown for three consecutive years from 2010 to 2012. In September 2012, the topsoil (0–45 cm) was removed from 3 plots (2.1 × 1.5 m), and the positions of 50 fresh chicory roots were mapped on transparent foil (plastic map) placed

against the subsoil. Afterwards, plastic tubes, as artificial epi-earthworm burrows, were installed from the subsoil (starting in 45-cm depth) to the soil surface at the half number of positions of chicory roots (25 pores) before filling up the topsoil. The plots were kept under fallow until September 2014 so that chicory roots decayed and created root pores (Root-B). (2) In spring 2014, half of the root pores were introduced with earthworms (*Lumbricus terrestris* L.) to create mixed origin pores (WRoot-B). Epi-earthworms were fed with ryegrass residue (*Lolium perenne* L.) placed on the soil surface. At sampling, in September 2014, epi-earthworms were removed with the octet method (Thielemann 1986) from the mixed origin pores. (3) Additionally, native earthworms were expelled from the soil by the octet method. Biopores (diameter ≥ 2 mm) containing large mature earthworms were selected as earthworm pores (Worm-B). These biopores were compared with the bulk soil sampled from the respective depths.

After removing the topsoil in September 2014, biopore positions were re-identified based on previously mapped positions. Biopore material (root pores, mixed origin pores, and epi-earthworm pores) was sampled from four plots at two soil depths 45–75 and 75–105 cm. The biopores were opened from one side with a small sharp knife, and soil material from the pore wall was carefully sampled with micro-spoons. Soil samples were taken 2 mm around the pore because this layer is highly affected by epi-earthworms and root decomposition (Tiunov and Scheu 2000; Don et al. 2008). In addition, bulk soil (Bulk-S) was taken at random positions unaffected by biopores (>2-cm distance) from 45–75- and 75–105-cm depth at the four plots. The samples were stored at ~5 °C until analyses. Prior to the analyses, coarse organic matter (free roots and plant debris) was carefully removed with tweezers.

Total organic C and total N content analysis, basal respiration, and microbial biomass C

Air-dried soil samples (40–50 mg) were ground to powder in a ball mill and weighed into tin capsules (IVA, Meerbusch, Germany). The capsules plus sample were loaded on a FLASH 2000 CHNS/O Elemental Analyser (Thermo Fisher Scientific, Cambridge, UK) coupled with ConFlo III interface and Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) to measure total organic C (TOC) and total N (TN) contents.

Soil moisture was adjusted to ~0.2 g water g^{-1} soil dry weight and controlled daily by weighing. The soil was incubated at room temperature (22 °C ± 1) for 2 days (Blagodatskaya et al. 2011). To measure basal respiration (BR) and microbial biomass C, an amount equivalent to 1 g of oven-dry soil was incubated at 20 °C in 12-mL septum-capped vials; six blanks (empty vials) were prepared as controls. An incubation temperature of 20 °C was chosen because Creamer et al. (2014) identified the greatest stability of the initial respiration rate at 20 °C.

Immediately after adding the soil to the vials, they were closed air-tight, flushed with ambient air, and CO_2 concentrations were measured for the first time (time 0) with a gas chromatography electron capture detector (GC 6000 Vega Series 2, Carlo Erba). Following each measurement, all the samples were again flushed with air, incubated at 20 °C, and measured with GC every 24 h for the first 3 days (time 1, 2, and 3). Thereafter, all the vials were equally supplemented with 100-μL glucose solution containing 6 mg glucose (Anderson and Domsch 1978; West and Sparling 1986; Lin and Brookes 1999) to determine soil microbial biomass (μg C_{mic} g^{-1} soil dry mass) by the substrate-induced respiration (SIR) method. Microbial biomass C was calculated based on the maximum respiratory response to glucose amendment. The water content was adjusted to 70 % (WHC) with the glucose solution to attain the highest CO_2 evolution within 2–6 h of incubation (Ritz and Wheatley 1989). After adding glucose, all the vessels including blanks were immediately flushed with air, incubated at 20 °C for 2 h (Lin and Brookes 1999), and directly measured by GC. We used the same incubation times as reported by Lin and Brookes (1999) due to similarities in pH, TOC, and TN values with our soil (Kautz et al. 2013). The CO_2 evolution (ppm) was calculated by subtracting the CO_2 concentrations of blanks from that of the samples.

Enzyme activities and kinetics

The activity of the eight enzymes targeting C, N, and P containing organic compounds was measured by fluorometric microplate assays of 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC) (Marx et al. 2005). According to Razavi et al. (2015), suspensions of 0.5 g soil (dry weight equivalent) with 50 mL sterilized water were prepared. Fifty microliters of soil suspension were added to 100-μL substrate solution and 50 μL of MES ($C_6H_{13}NO_4SNa_{0.5}$) buffer (pH 6.5) for MUF ($C_{10}H_8O_3$) substrate and TRIZMA ($C_4H_{11}NO_3$•HCl, $C_4H_{11}NO_3$) buffer (pH 7.2) for AMC ($C_{10}H_9NO_2$) substrate in a 96-well microplate (Koch et al. 2007). The time intervals of fluorescence measurement (after 30 min, 1 h, and 2 h) were kept similar for all the enzymes and treatments. We determined enzyme activities in a range of substrate concentrations (0, 10, 20, 30, 40, 50, 100, and 200 μmol g^{-1} soil). The calibration solutions were prepared using soil suspension (50 μL) and MUF or AMC to obtain a series of concentrations of 0 1.2 mM (Razavi et al. 2015). Fluorescence was measured using a Victor 1420–050 multilabel counter (PerkinElmer, USA) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. Enzyme activities (V_{max}) were calculated as released MUF

or AMC in nmol per g dry soil per hour (Marx et al. 2005), and substrate affinity (K_m) was expressed in μmol g^{-1}.

Calculations

Basal respiration and microbial biomass C Basal respiration (μg C g^{-1} h^{-1}) was calculated based on the CO_2 volume per soil dry weight and incubation time in relation with headspace volume of the sealed flask containing the soil sample, incubation temperature, and air pressure (Orchard and Cook 1983). The CO_2 concentrations of blank vials and the initial CO_2 concentration of soil vials (time 0) were subtracted from the corresponding CO_2 concentration of sample vials at time intervals (time 1, 2, and 3) to calculate cumulative CO_2 evolution (Creamer et al. 2014).

Applying the ideal gas equation, CO_2 respiration in the SIR experiments was expressed in μg C g^{-1} h^{-1}. Microbial biomass C (μg C g $soil^{-1}$) was calculated based on the equation by Anderson and Domsch (1978):

$$x = 40.4*y + 0.37, \quad (1)$$

where y is the maximum initial rate of respiration (CO_2 evolution 2 h after adding glucose). The C_{mic}-to-C_{org} was calculated as a percentage of C_{org} expressed by total $C_{mic.}$ The microbial metabolic quotient (qCO_2) was calculated by dividing initial respiration (BR) by C_{mic} and expressed as μg CO_2-C mg^{-1} C_{mic} h^{-1} (Anderson and Domsch 1989).

Enzyme kinetics The Michaelis-Menten equation was applied to calculate the K_m and V_{max} for each enzyme:

$$V = \frac{V_{max}[S]}{K_m + S}, \quad (2)$$

where V is the rate of substrate converted in nmol g^{-1} dry soil h^{-1}, V_{max} is the maximum rate of enzyme activity, and K_m is the saturation constant of each enzyme defined by the substrate amount at which reaction velocity is equal to the half-maximum rate. Both V_{max} and K_m values were determined by the nonlinear regression routine of OriginPro 8.5 (OriginLab, MA, USA).

Statistics All results are given as means and standard errors (SE). Normality and homogeneity of variance were checked in groups using Shapiro-Wilk's W test and Levene tests, respectively. Following this step, the significance of differences among four soil groups (Root-B, Worm-B, WRoot-B, and Bulk-S) and two soil depths was tested by two-way ANOVA, with subsequent multiple comparisons by the Turkey HSD test where p values <0.05 indicated the significance. All statistical analyses were performed using STATISTICA 12 (StatSoft Inc., USA).

A constrained ordination in reduced space using redundancy analysis (RDA) was performed to explore the relationship between enzyme kinetic parameters and soil properties, as well as between biopore types and bulk soil, and between the two depths. To achieve the assumption of normality, the rank-based inverse normal transformation (INT) of all variables was performed:

$$Y_i = \Phi^{-1}\left(\frac{r_i}{N + 1}\right), \quad (3)$$

where Y_i is the transformed variable, r_i is the ordinary rank of the i case among N observations, and Φ^{-1} is the standard normal quantile function (van der Waerden 1952). The RDA analysis was conducted using the web-based GUide to STatistical Analysis in Microbial Ecology (GUSTA ME; Buttigieg and Ramette 2014). For the RDA, the V_{max} of the eight investigated enzymes were considered response variables, and C_{org}, C_{mic}, and qCO_2 were considered explanatory variables. The overall significance of the RDA model was determined based on a permutation test (999 permutations) ($p < 0.05$). Object focused type I scaling was used to interpret the relationship between sampling sites and variables. Response-variable focused type II scaling was used to interpret the relationship between response variables and explanatory variables.

Results

Total organic C and microbial biomass C

The C_{org} content in the mixed origin pores (WRoot-B) and earthworm pores (Worm-B) ranged from 10.4 to 11.4 mg g^{-1} soil, whereas lower C_{org} values of ~9 and 3–4 mg g^{-1} soil were measured for the root pores (Root-B) and bulk soil (Bulk-S), respectively (Fig. 1). The higher C_{org} content of epi-earthworm versus root pores ($p < 0.05$) was only found in the upper layer due to the higher variability of this parameter at lower depth. The absence of interactions between biopore origins and soil depths ($p = 0.24$) shows that biopore origin ($p < 0.001$) affected C_{org} irrespective of soil depth ($p = 0.2$) (two-way ANOVA).

Soil depth did not affect the total N content (Fig. 1). The Worm-B and WRoot-B showed similar total N content (~1 mg g^{-1} soil), which were double those of the Bulk-S at both soil depths. The WRoot-B contained up to 24–27 times more C_{mic} than the Bulk-S and 4 times more C_{mic} than the Root-B (Fig. 1). Although C_{org} did not differ between the Worm-B and WRoot-B, C_{mic} was twice as high in the WRoot-B. C_{mic} in the Root-B was five- and eightfold higher than in the bulk soil in upper and lower horizons, respectively. The largest differences between pores of either origin and the

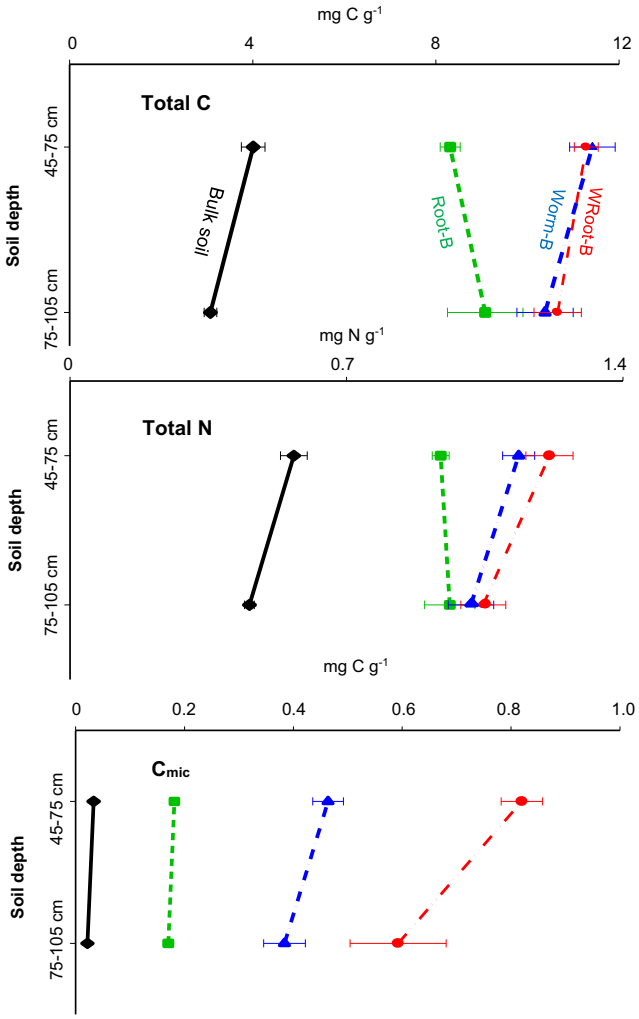

Fig. 1 Organic C content (C_{org}), microbial biomass (C_{mic}), and total N under effect of biopore origins and bulk soil in two soil depths (45–75 and 75–105 cm). WRoot-B (*red line*), Worm-B (*blue line*), Root-B (*green line*), and Bulk-S (*black line*) expressed mixed-origin pores by earthworms and old root channels, earthworm pores, old root channels, and bulk soil, respectively. *Error bars* indicated standard errors (of the means)

bulk soil were in the upper horizon with the highest values (0.82 mg C_{mic} g^{-1} soil) in the WRoot-B and the lowest values (0.03 mg C_{mic} g^{-1} soil) in the Bulk-S. C_{mic} in the WRoot-B sharply decreased with depth, while C_{mic} in the Worm-B, Root-B, and Bulk-S were stable.

The C_{mic} proportions in both subsoil depths ranged from 0.71 to 7.3 % of C_{org}, which were affected only by biopore origin ($p < 0.001$). The metabolic quotient (qCO_2) decreased in the order Bulk-S > Root-B > Worm-B > WRoot-B, regardless of soil depth (Fig. 2). Although the qCO_2 slightly increased, the effect of depth on qCO_2 was not significant (with the exception of the bulk soil).

The increase of microbial biomass was associated with an increase in organic matter content in biopores in comparison with the Bulk-S. The effect of soil depth on microbial biomass was clear in the WRoot-B.

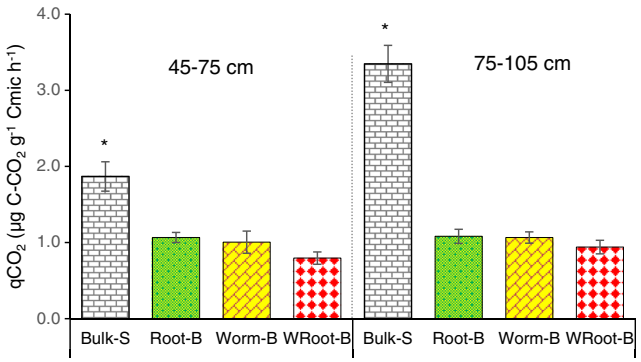

Fig. 2 The qCO_2 values decreased from bulk soil to biopores (Root-B, Worm-B, and WRoot-B). Depth only affected qCO_2 of bulk soil significantly (*)

Enzyme activities

Earthworms increased the activities of the seven enzymes (β-glucosidase, cellobiohydrolase, xylanase, chitinase, chitotriosidase, leucine aminopeptidase, and tyrosine aminopeptidase), which target the decomposition of C- and N-containing substrates compared to root pores and bulk soil at both soil depths (Fig. 3). In contrast, acid phosphomonoesterase activity was lower in biopores than in the bulk soil, but increased with depth.

Generally, C and N enzyme activities increased under earthworm presence, but this depended on enzyme type. For example, earthworms raised cellobiohydrolase activities (C-cycle) fivefold, but raised leucine-aminopeptidase (N-cycle) up to 11-fold. The combination of decomposed roots and earthworms increased activities of cellobiohydrolase, xylanase, chitotriosidase, leucine aminopeptidase, and tyrosine aminopeptidase by 1.2–1.9 times at 45–75-cm depth compared with earthworm pores. These differences, however, were very small or even absent at 75–105 cm. Along with earthworm biopores, the Root-B also positively affected enzyme activities of C and N cycles, but 1.2–3.9 times less than the Worm-B and WRoot-B. Most of enzyme activities in the WRoot-B and Worm-B decreased ($p < 0.05$) with soil depth, by a factor of 1.2–3.4. Nevertheless, enzyme activities in the Root-B and Bulk-S remained the same or even increased with soil depth, as did cellobiohydrolase.

For acid phosphomonoesterase activity, the bulk soil had the highest V_{max} values in both soil horizons, being 10–40 % times higher than in all the biopores. The three biopore types, in contrast, presented almost the same acid phosphomonoesterase activities, which significantly ($p < 0.05$) increased with depth in biopores and bulk soil.

The constrained variance accounts for 67.3 % of the total variance whereby the first and second eigenvalues accounted for 66.4 and 0.82 % of the total variance, respectively (Fig. 4a). All enzyme activities (except acid phosphomonoesterase) were positively correlated with C_{org} and C_{mic} and negatively

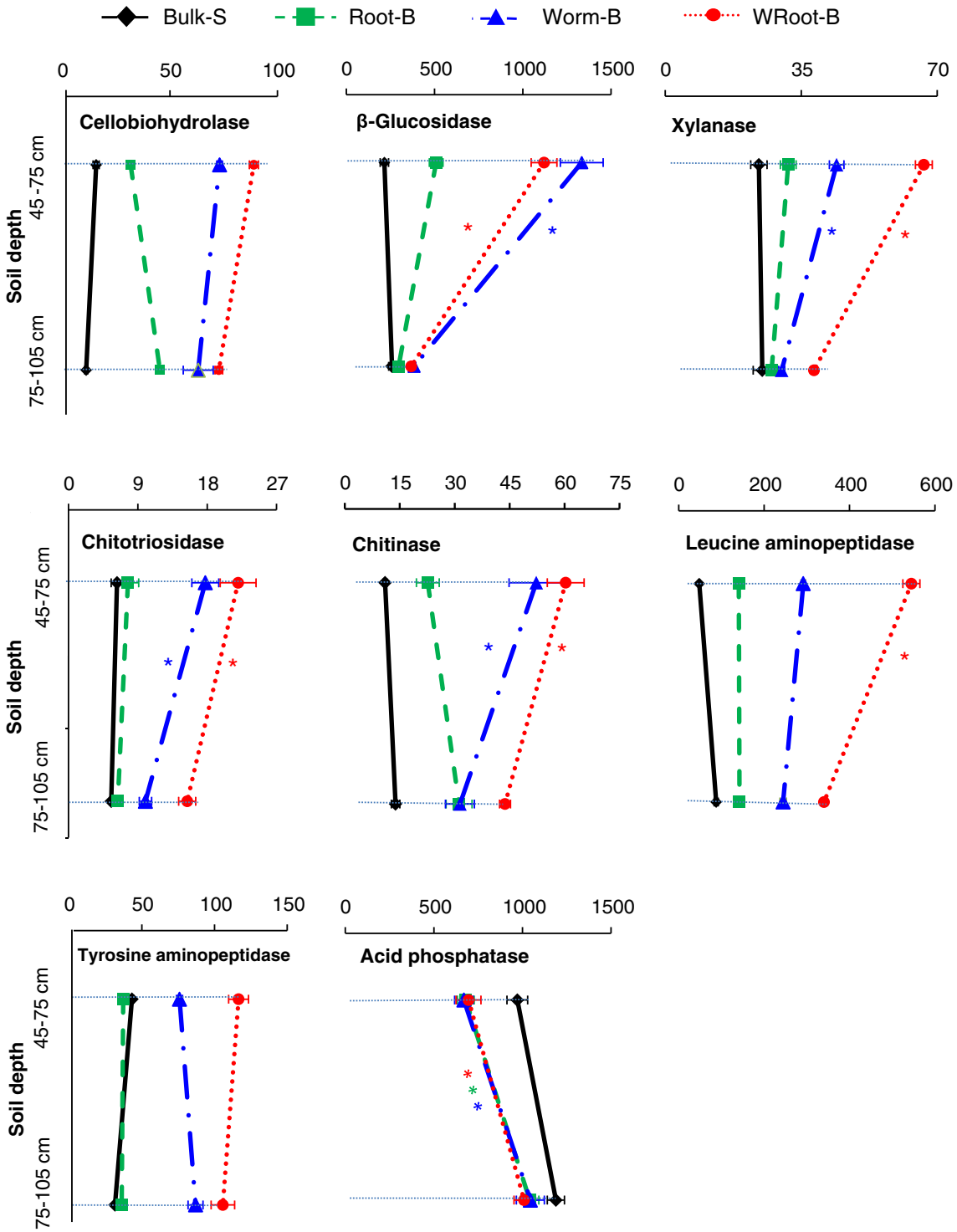

Fig. 3 Enzyme activities (nmol g^{-1} MUF h^{-1} or nmol g^{-1} AMC h^{-1}) at two soil depths (45–75 and 75–105 cm) under effects of different biopore types and bulk soil. The *asterisk* (*) illustrated a significant effect of soil depth

correlated with qCO_2. The RDA result of the object focus type I scaling plot showed that microbial activities were distinctive between biopore types and bulk soil, and also differed between soil depths (Fig. 4b).

L. terrestris presence positively affected all enzyme activities (except acid phosphomonoesterase). Enzyme activities of earthworm biopores decreased with depth, but those of root pores and bulk soil remained the same.

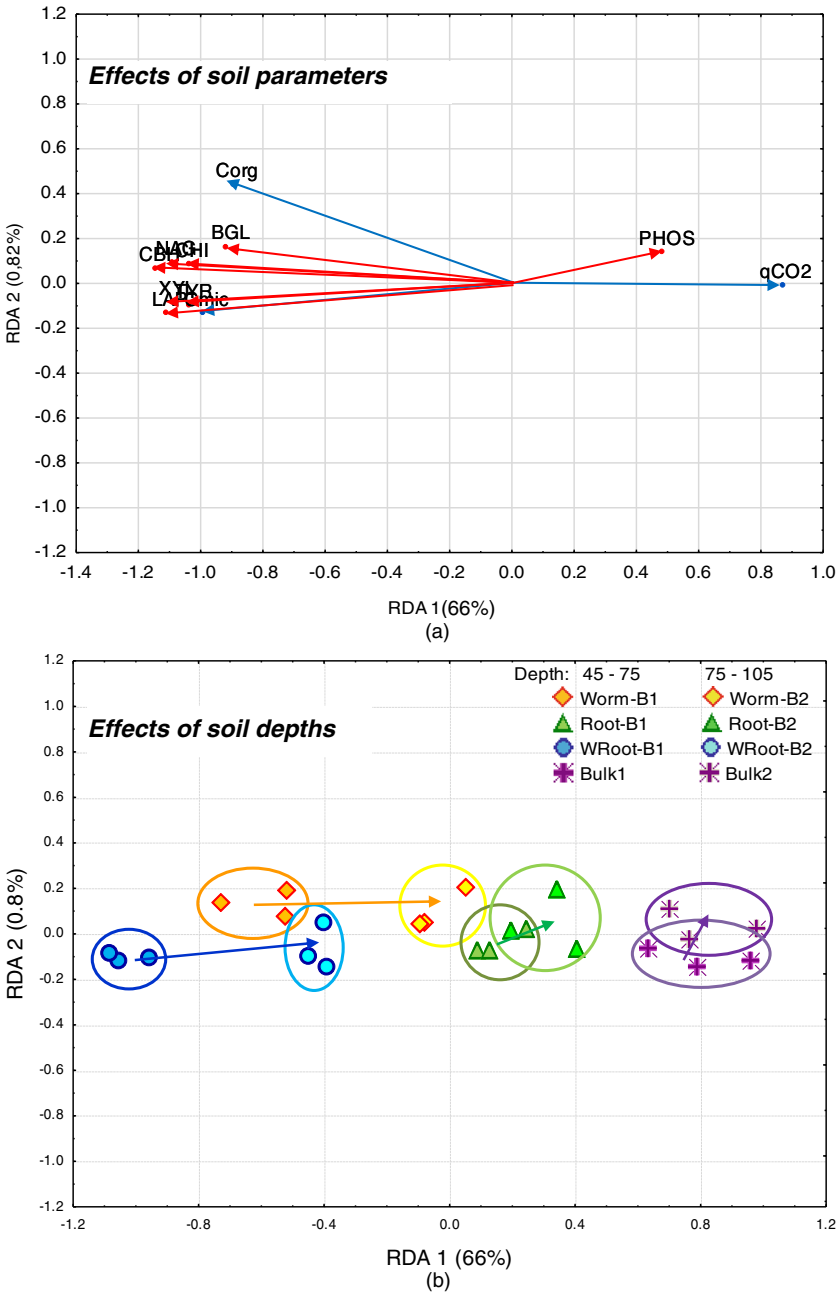

Fig. 4 **a** The redundancy analysis (type II scaling plots are shown) illustrated that the constrained variance of enzyme activities accounts for 67.3 % of the total variance. All enzyme activities are positively correlated with C_{org} and C_{mic} and negatively correlated with qCO_2 (except acid phosphatase). **b** The redundancy analysis (type I scaling) showed distinct microbial activity not only between biopore origins and bulk soil, but also between the two soil depths. *Arrows* showed the different depth effect on biopore types and bulk soil nmol MUF μmol^{-1} substrate h^{-1} (PHO2)

Enzyme activities and catalytic efficiency in dependence on substrate affinity

Substrate affinities (K_m) varied differently among biopore origins and Bulk-S in association with enzyme types and soil depth (Appendix 1). Chitotriosidase, tyrosine aminopeptidase, and acid phosphomonoesterase illustrated a similar K_m among bulk soil, Worm-B, and WRoot-B. However, the ratio between V_{max} and K_m, i.e., reflecting the catalytic efficiency of enzymes (K_a), presented a clear variation both among biopores types and between depths (Fig. 5). In all C- and N-involved enzymes, the catalytic efficiency increased in biopores versus Bulk-S. The K_a of acid phosphomonoesterase declined sharply from the bulk soil to root pores, then gradually to earthworm biopores and mixed pore origins. The decrease of acid phosphomonoesterase was stronger at the lower than the upper depth, reflecting the simultaneous increase of K_m and V_{max} with depth. We found a significantly positive relationship ($R^2 > 0.8$) between K_a and C_{mic} for cellobiohydrolase, β-glucosidase, N-acetylglucosaminidase,

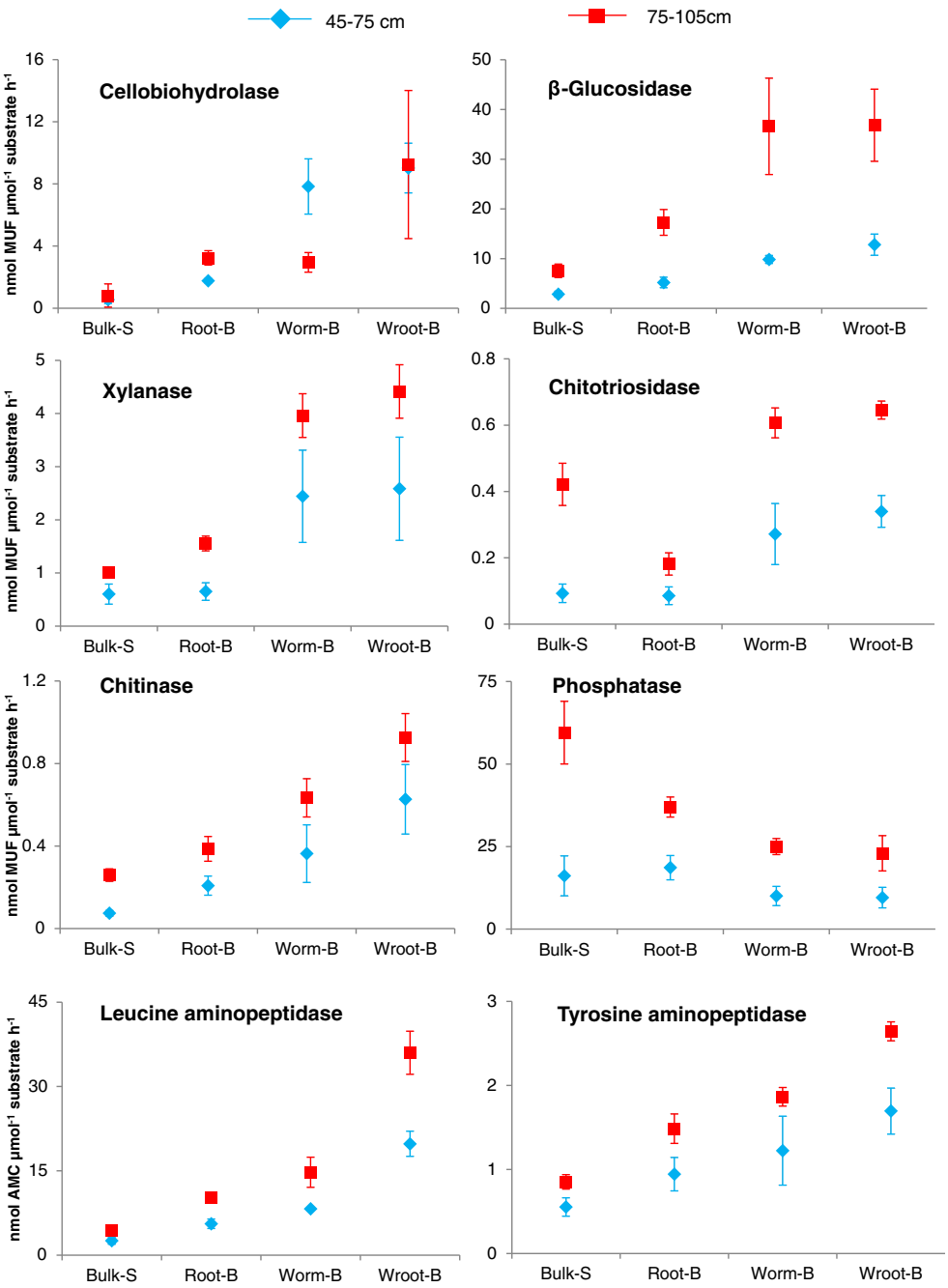

Fig. 5 The catalytic efficiency (K_a) of all C-, N-, and P-involved enzymes was illustrated in two colors associated with soil depths: *blue* for upper layer and *red* for lower layer

chitotriosidase, and leucine aminopeptidase at the upper soil depth and xylanase at the lower depth (Supplementary S1).

To conclude, microbial biomass C, enzyme activities, and C accumulation increased from the bulk soil to biopores with a maximum in worm-root pores. After the introduction of epi-earthworms into root pores, the enzymes involved in chitin, protein, and organic P decomposition shift from root effect to epi-earthworm effect. Soil depth exerts a stronger effect in the drilosphere and the detritus-drilosphere than in the detritusphere and the bulk soil (Fig. 6).

Discussion

Organic matter accumulation and microbial activities in biopores

The presence of earthworms in general and epi-anecic earthworms (*L. terrestris*) in particular significantly modifies the amount and composition of organic matter through their burrowing, ingesting, and casting activities (Marinissen and de Ruiter 1993; Tiunov and Scheu 2000; Aira et al. 2003;

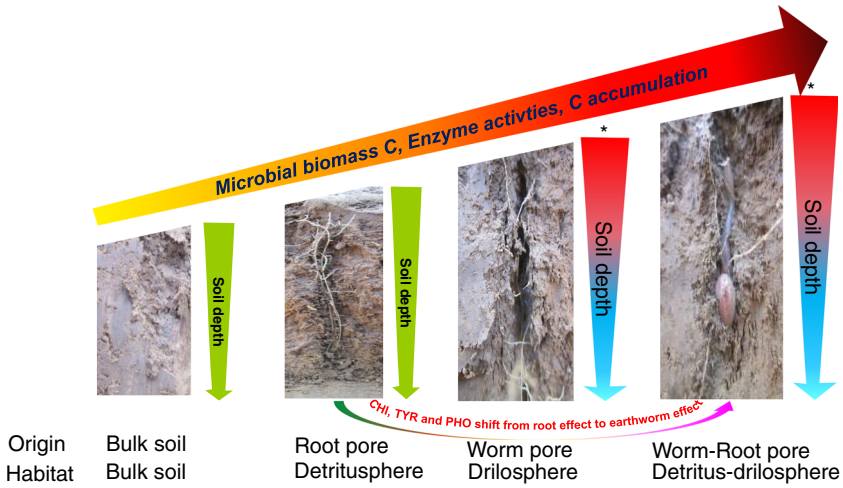

Fig. 6 The increase of microbial biomass C, enzyme activities, and C accumulation from bulk soil to biopores with maximum in worm-root pores. The *asterisk* (*) illustrated a significant difference between the two depths

Capowiez et al. 2015). The role of biopores becomes more important in the subsoil because these deeper soil horizons are strongly limited in fresh C input. We explain the higher content of C_{org} and TN in both pore types under epi-earthworm presence (Fig. 1) by the enrichment of fresh C and N in their cast crashed along the burrow walls. Moreover, the formation of macro-aggregates by epi-earthworms protected SOM from microbial decomposition (Martin 1991; Lavelle and Martin 1992; Gilot 1997). We consider the combination of decaying roots, ryegrass incorporated into earthworm bodies, and earthworm activities to be the main reasons for the increase of C_{org} and TN in earthworm burrows. After about 2 years of root decomposition, there were no visible main root residues in the pores. Hence, chicory roots were decomposed because soil moisture, O_2 concentration, and pH (Silver and Miya 2001) in biopores are optimal for high microbial activities. Root detritus increases the amount of organic matter in the Root-B versus Bulk-S. A slight decrease of C_{org} with depth in all biopores and Bulk-S showed that organic matter was uniformly distributed along subsoil horizons (from 45 down to 105 cm). The *L. terrestris* burrow wall is highly variable because casts are crashed along the burrow wall but not at the same vertical density and not at the same date. These results are in line with the findings of Don et al. (2008), who showed the absence of C_{org} differences from 60 down to 140-cm soil depth in the drilosphere.

Microorganisms play a key role in soil nutrient cycles. They proliferate in habitats rich in organic matter and featuring harmonized moisture and temperature conditions. Our results of C_{mic} were consistent with the C_{org} content, where C_{mic} in the WRoot-B and Worm-B were the highest, and the Bulk-S showed the lowest values. C_{mic} in the drilosphere exceeded that in the bulk soil by 14–18 times. In comparison, Tiunov and Scheu (2000) only documented 2.3–4.7 times higher C_{mic} in the drilosphere compared to the bulk soil. We explain the difference in these findings by the difference in organic properties of forest soils in Tiunov and Scheu's study: forest soils were more stable for decomposition by microorganisms than crop residues in an agricultural soil. The 25 to 27 times higher C_{mic} in detritus-drilosphere than bulk soil is explained by two processes: (i) complex SOM structures are weakened and broken down after passing through earthworm guts, becoming more available to microbes (Svensson and Friberg 2007); and (ii) the mucus secreted with casts is like a "kiss" to wake up the "sleeping beauty" (microorganisms) because of the labile C richness (Brown et al. 2000). Decaying roots increased microbial biomass with respect to the bulk soil by providing C_{org} and N (Poll et al. 2008; Bastian et al. 2009). Nonetheless, the lifetime of the detritusphere may be very short, and the root mass sharply decreased after a few weeks (Spohn and Kuzyakov 2014), so that C_{mic} in the Root-B was lower than those in the Worm-B and WRoot-B. Whereas C_{mic} of the WRoot-B dropped off at the lower depth, those of the Worm-B slightly decreased but those of the Root-B and Bulk-S remained unchanged. Combining C_{mic} and C_{org} results, we conclude that the organic matter quality (labile C amount) controlled microbial activity more than organic matter quantity (Anderson and Domsch 1989).

The percentage of C_{mic}-to-C_{org} can be used to indicate the state of soil C: equilibrium, accumulating, or decreasing (Beck 1984). A wide range of C_{mic}-to-C_{org} from 0.71 to 7.3 % (Appendix 2) revealed distinct effects of epi-earthworms and roots on soil C content compared to the bulk soil. At both soil depths, C_{mic} in the bulk soil contributed 0.7–0.8 % to total organic C, but this contribution was eightfold and threefold higher in the WRoot-B (5.6–7.3 %) and Root-B (2.2 %), respectively. Thus, a unit of C_{org} in the burrow wall contained a larger share of microbial biomass than its counterpart in root pores or bulk soil (Tiunov and Scheu 1999). According to Jenkinson and Ladd (1981) and McGill et al. (1986), C

accumulates or decreases if the percentage of C_{mic}-to-C_{org} is larger or smaller than the C equilibrium of 1.8–3.3 %. Consequently, C declined in bulk soil, whereas an accumulation in biopores (Appendix 2). Elsewhere, the increase of C_{mic}-to-C_{org} was caused by an increase of organic matter input to a soil (Bauhus et al. 1997). As epi-earthworms were introduced into root pores, feeding activities of this species in the organic-mineral soil mixture provided a frequent C source in the drilosphere. The equilibrium of C in root biopores was only a transient state that then fell back to a base level after dead roots were degraded. The decline of C_{mic}-to-C_{org} in the bulk soil was accompanied by a decrease in available organic matter content.

Basal respiration in biopores and the metabolic quotient (qCO_2) strongly supported the above explanations about the status of soil C. Biopore characterized by a high C_{mic}-to-C_{org} also had a low qCO_2, but the bulk soil was characterized by a low C_{mic}-to-C_{org} and a high qCO_2. The higher metabolic efficiency of the microbial community in the Bulk-S compared to that in biopores means that relatively less C from organic substrates was channeled into anabolic processes (Miller and Dick 1995). In contrast, the reverse trend in the bulk soil implied a slower C metabolism or unavailability of C sources (Dippold and Kuzyakov 2013). However, the differences of qCO_2 among biopores and bulk soil might be affected by microbial compositions (the ratio between bacterial and fungal biomass) as suggested by Nannipieri et al. (2003). Soil depth, nevertheless, had a minor effect on biopore qCO_2 but strongly affected the bulk soil.

Thus, roots and epi-earthworms induced C accumulation in the subsoil and increased microbial biomass, with stronger effects in the drilosphere and detritus-drilosphere compared to the detritusphere.

Enzyme activities

The earthworm-induced increase of the activities (V_{max}) of all the tested enzymes, with the exception of acid phosphomonoesterase, supported previous studies (e.g., Don et al. 2008; Hoang et al. 2016). Enzyme activities are related to microbial biomass (C_{mic}) (Aira et al. 2007), so higher C_{mic} in earthworm pores led to higher enzyme activities compared to root pores and bulk soil. The increases in the activities of C and N cycle enzymes in the WRoot-B and Worm-B (Fig. 3) are explained by microbial abundance inducing an increase of enzyme activity (German et al. 2011). Nevertheless, this increase was only temporary as long as C was available in the pores. Cellulose and hemicellulose are two substances provided by fine root detritus via their decomposition (Kögel-Knabner 2002). These polymer structures are ruptured during earthworm gut transit. The higher availability of these organic compounds stimulates microbial enzyme production (Allison and Vitousek 2005). Although main roots in our experiment were completely decomposed at the time of sampling, these two polymers were still absorbed on soil particles along pore walls. The existence of enzyme variety is an index for the presence of complex substrates (German et al. 2011).

The activities of enzymes (cellobiohydrolase and chitotriosidase) cleaving the polymers into sub-units were lower than those (β-glucosidase and N-acetylglucosaminidase) involved in the second step of decomposition by factors of 5–18 times and 2–3 times, respectively. This pattern supports the microbial economic theory (Koch 1985) and Allison's statement (Allison 2005), that microorganisms are stimulated to produce enzymes only because this production leads to greater resource acquisition. In comparison, enzyme activities in root pores were 1.2–4 times higher than in the bulk soil, but 1.2–2.8 times lower than those in the WRoot-B and Worm-B. Activities of C-functioning enzymes were 1.5–2 times lower in the Root-B than those in the biopores made by epi-earthworms. Labile substrates were probably exhausted in the detritusphere after a short time (within 20 days as suggested by Spohn and Kuzyakov 2014). In contrast, substrate input in the drilosphere and the detritus-drilosphere was more continuous as epi-earthworms incorporated fresh litter. Thus, there was more accumulated organic matter in burrow linings than that in the Root-B, which increased soil enzyme activities.

The activities of most enzymes decreased with depth. Both C_{org} content and C_{mic}, however, remained stable with depth. This indicates a "stressed" condition of microorganisms in enzyme production. The reduction of enzyme activity was stronger under the WRoot-B and Worm-B than that under the Root-B and Bulk-S. Based on soil data (Kautz et al. 2014), bulk density increased with depth because the clay content increased (from 27 % at 45–75 cm to 30 % at 75–87 cm). Soil organic matter was adsorbed to clay minerals, making it more difficult for microorganisms to access (Chenu and Stotzky 2002). Briefly, these changes imply that enzyme production by microorganisms was affected not only by substrate concentration but also by microbe population size and microbial demand.

Acid phosphomonoesterase activity reacted differently from C-involved enzymes. They were highest in the bulk soil and as similarly low in epi-earthworms and root biopores. This contradicts the enhancing effect of epi-earthworms on acid phosphomonoesterase in burrow linings proposed by Le Bayon and Binet (2006). Although most studies confirmed the promotion of acid phosphomonoesterase activity in worm casts (Satchell and Martin 1984; Buck et al. 1999; Flegel and Schrader 2000), but less attention was given to acid phosphomonoesterase activities on burrow surfaces. Clearly, epi-earthworms do increase water-soluble and exchangeable P content after soil ingestion (Mackay et al. 1982; Wan and Wong 2004). On the one hand, the higher acid phosphomonoesterase activities in the Bulk-S compared to those in the

biopores in our experiments were indicated by "microbial economics" (Allison and Vitousek 2005; Nannipieri et al. 2011), in which the enzyme production drops in the event of more readily available assimilable resources. On the other hand, the increase of acid phosphomonoesterase at lower depth indicated a lack of available P along the soil profile. Nannipieri et al. (2012)) also announced the enhancement of acid phosphomonoesterase activities as a consequence of P deficiency. Furthermore, soil pH increase with depth (Vetterlein et al. 2013) could be put forward as a reason for the decrease of acid phosphomonoesterase activities, indicating a higher contribution of alkaline phosphomonoesterase activities, which was not measured in this study.

Biopores with favorable organic matter supply increased enzyme activities. Lower acid phosphomonoesterase activities in biopores than in the bulk soil indicated a higher availability of inorganic P by root degradation and epi-earthworm burrowing.

Substrate affinities in dependence on epi-earthworm and root effects

Substrate affinities are an important indicator for the enzymatic degradation of SOM (German et al. 2011). Accordingly, the response of K_m to biopore presence reflects the corresponding SOM decomposition by microbes. While V_{max} increased for most enzymes in earthworm biopores and root pores (except acid phosphomonoesterase activities), K_m varied among enzymes and soil groups (Appendix 1). This variation of K_m can be explained by a variation in the composition of microbial communities depending on the presence of epi-earthworms and root detritus. At 45–75-cm depth, K_m was similar in the Bulk-S, WRoot-B, and Worm-B in chitotriosidase, tyrosine aminopeptidase, and acid phosphomonoesterase. A similar set of enzymes or isoenzymes (i.e., enzymes with the same functions, but different structure) (Farrell et al. 1994; Tabatabai et al. 2002) with similar substrate affinity were therefore responsible for the decomposition of organic matter in these two biopores and the bulk soil. These enzyme systems in earthworm pores (Worm-B) were the same as those in the bulk soil but different from root pores (Root-B) (Appendix 1). However, as epi-earthworms were introduced into root pores to make the WRoot-B, the K_m values of old root channels became closer to those of the Worm-B and bulk soil. This implies a "call-back" effect of epi-earthworms, i.e., the enzyme systems involved in chitin, protein, and organic phosphorus were expressed by microorganisms in old root channels to adapt to the presence of epi-earthworms. These findings confirmed that the role of dead roots in shaping microbial communities was minor compared with epi-earthworms.

The general trend of catalytic efficiency demonstrated an increase from the bulk soil to the Worm-B and WRoot-B, but this increase was enzyme-dependent. The catalytic efficiency of enzymes was highly correlated with the soil organic matter content ($R^2 > 0.8$) (except chitotriosidase at 75–105 cm, Supplementary S1). This close connection, however, was negative for acid phosphomonoesterase. Thus, given an increase of K_a and C_{mic} from the bulk soil to the Worm-B and WRoot-B, we conclude that organic matter decomposition within biopores is more efficient because of higher microbial activity.

Conclusions

The combination between *L. terrestris* and old root channels fosters the microbial activities than mere earthworm effect in the subsoil. Nonetheless, biochemical properties of biopores (C_{org}, C_{mic}, and enzyme activities) depend on their origins: epi-earthworms or roots. Earthworms, with their burrowing activities and input of pre-digested organics, have stronger effects on C content, microbial biomass, and enzyme activities in biopores than decomposed roots after 2 years. Epi-earthworms increased enzyme activities involved in the C and N cycles by up to 500 %, but decreased acid phosphomonoesterase activities by 28 %. We attribute the decrease of acid phosphomonoesterase activities in biopores compared to bulk soil to more available inorganic P in biopores due to epi-earthworms and root decomposition. The change of microbial biomass and activities of five enzymes (xylanase, N-acetylglucosaminidase, chitotriosidase, leucine aminopeptidase, and tyrosine aminopeptidase) with soil depth was negligible in root biopores and the bulk soil, but significantly decrease in earthworm-induced biopores. Our study raises the questions on the difference of soil organic matter decomposition in top—compared to subsoil under effects of distinctive biopore types as the C input in topsoil is supposed to be "younger" in top—than sub-layers.

Acknowledgments We thank Dr. Timo Kautz for the field experiment setup, Dr. Evgenia Blagodatskaya for the fruitful suggestions on the experimental setup, Dr. Norman Loftfield for the support in laboratory, and the Centre for Stable Isotope Research and Analysis—KOSI (Göttingen, Germany) for the TOC measurement. We gratefully acknowledge the Vietnamese government for supporting DH, and DAAD for supporting BSR. The study was supported by the German Research Foundation in the framework of the project PAK 888.1.

Appendix 1

(Fig. 7).

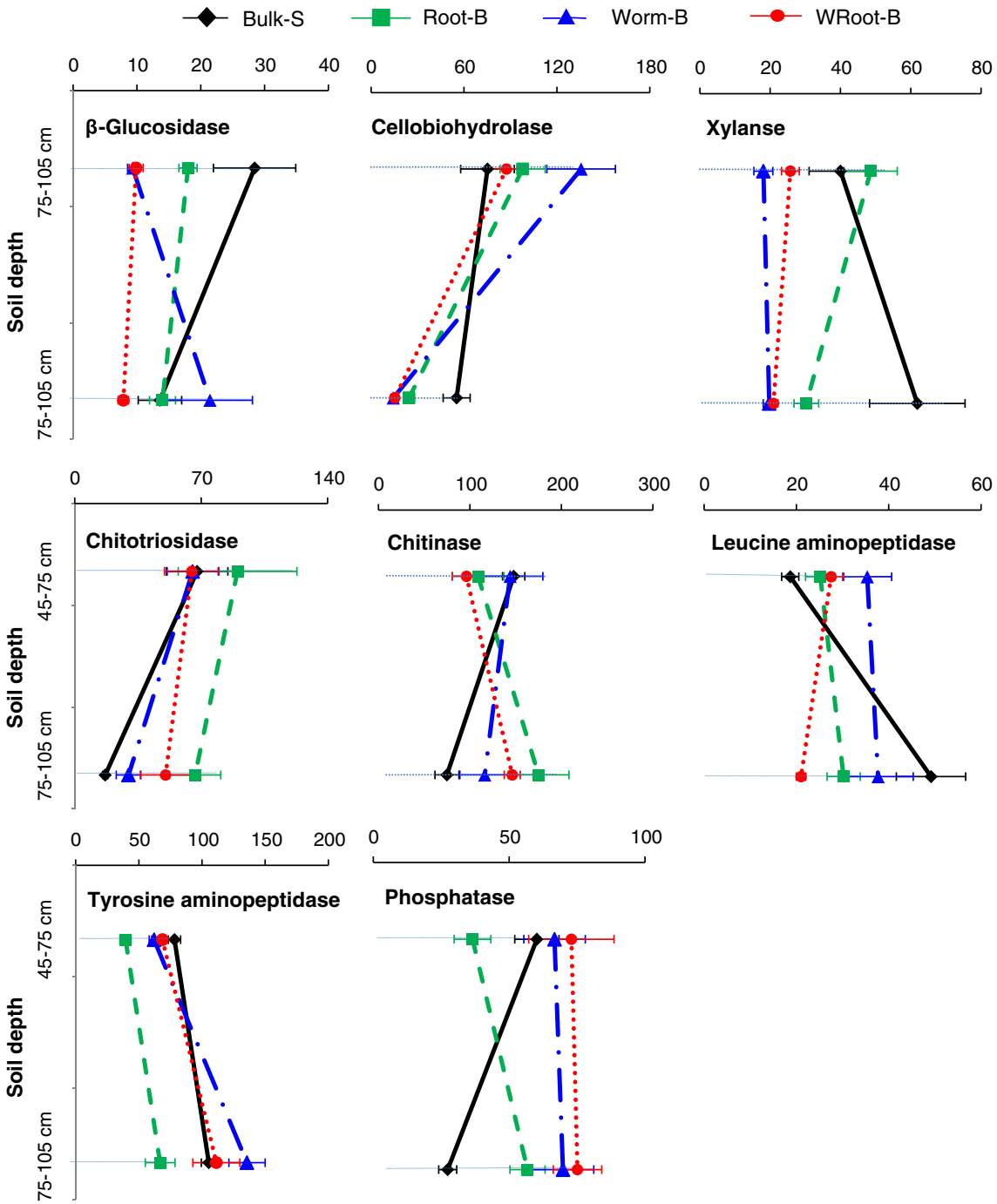

Fig. 7 The variation of substrate affinity (μmol g^{-1} soil) at the two soil depths (45–75 and 75–105 cm)

Appendix 2

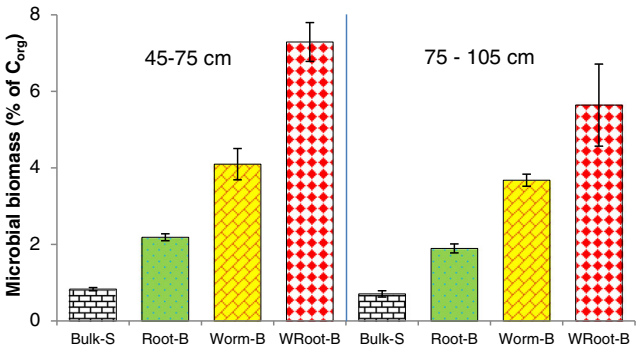

Fig. 8 The percentage of C_{org} presented as C_{mic} at the two soil depths (45–75 and 75–105 cm)

References

Aira M, Monroy F, Dominguez J (2003) Effects of two species of earthworms (*Allolobophora* sp.) on soil systems: a microfaunal and biochemical analysis. Pedobiologia 47:877–881

Aira M, Monroy F, Domínguez J (2007) Earthworms strongly modify microbial biomass and activity triggering enzymatic activities during vermicomposting independently of the application rates of pig slurry. Sci Total Environ 385:252–261

Allison SD (2005) Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecology Letter 8:626–635

Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol Biochem 37:937–944

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 3:215–221

Anderson TH, Domsch KH (1989) Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biol Biochem 21:471–479

Bamminger C, Zaiser N, Zinsser P, Lamers M, Kammann C, Marhan S (2014) Effects of biochar, earthworms, and litter addition on soil microbial activity and abundance in a temperate agricultural soil. Biol Fertil Soils 50:1189–1200

Barois I, Lavelle P (1986) Changes in respiration rate and some physicochemical properties of a tropical soil during transit through *Pontoscolex core-thrurus* (Glossoscolecidae, Oligochaeta). Soil Biol Biochem 18:539–541

Bastian F, Bouziri L, Nicolardot B, Ranjard L (2009) Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biol Biochem 41:262–275

Bauhus J, Paré D, Côté L (1997) Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. Soil Biol Biochem 30:1077–1089

Beck T (1984) Mikrobiologische und biochemische Charackterisierung landwirtschaftlich genutzter Böden II Mitteilung Beziehung zum Humusgehalt. Zeitschrift für Pflanzenernährung und Bodenkunde 147:467–475

Blagodatskaya EV, Anderson TH (1999) Adaptive responses of soil microbial communities under experimental acid stress in controlled laboratory studies. Appl Soil Ecol 11:207–216

Blagodatskaya E, Yuyukina T, Blagodatsky S, Kuzyakov Y (2011) Three-source-partitioning of microbial biomass and of CO_2 efflux from soil to evaluate mechanisms of priming effects. Soil Biol Biochem 43:778–786

Blagodatsky SA, Yevdokimov IV, Larionova AA, Richter J (1998) Microbial growth in soil and nitrogen turnover: model calibration with laboratory data. Soil Biol Biochem 30:1757–1764

Brown GG, Barois I, Lavelle P (2000) Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. Eur J Soil Biol 36:177–198

Buck C, Langmaack M, Schrader S (1999) Nutrient content of earthworm casts influenced by different mulch types. Eur J Soil Biol 35:23–30

Buttigieg PL, Ramette A (2014) A guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses. FEMS Microbiol Ecol 90:543–550.

Capowiez Y, Bottinelli N, Sammartino S, Michel E, Jouquet P (2015) Morphological and functional characterisation of the burrow systems of six earthworm species (*Lumbricidae*). Biol Fertil Soils 51: 869–877

Chenu C, Stotzky G (2002) Interactions between microorganisms and soil particles: an overview. In: Huang PM, Bollag J-M, Senesi N (eds) Interaction between soil particles and microorganisms. John Wiley and Sons, Ltd, West Sussex, pp 4–40

Creamer RE, Schulte RPO, Stone D, Gal A, Krogh PH, Papa GL, Murray PJ, Pérès G, Foerster B, Rutgers M, Sousa JP, Winding A (2014) Measuring soil respiration across Europe: do incubation temperature and incubation period matter? Ecol Indic 36:409–418

Dippold MA, Kuzyakov Y (2013) Biogeochemical transformations of amino acids in soil assessed by position-specific labelling. Plant Soil 373:385–401

Don A, Steinberg B, Schöning I, Pritsch K, Joschoko M, Gleixner G, Schulze ED (2008) Organic carbon sequestration in earthworm burrows. Soil Biol Biochem 40:1803–1812

Eivazi F, Tabatabai MA (1977) Phosphatases in soils. Soil Biol Biochem 09:167–172

Farrell RE, Gupta VVSR, Germida JJ (1994) Effects of cultivation on the activity and kinetics of Arylsulfatase in Saskatchewan soils. Soil Biol Biochem 26:1033–1040

Flegel M, Schrader S (2000) Importance of food quality on selected enzyme activities in earthworm casts (*Dendrobaena octaedra*, Lumbricidae). Soil Biol Biochem 32:1191–1196

German DP, Chacon SS, Allison SD (2011) Substrate concentration and enzyme allocation can affect rates of microbial decomposition. Ecology 92:1471–1480

Gill RA, Burke IC (2002) Influence of soil depth on the decomposition of *Bouteloua gracilis* roots in the shortgrass steppe. Plant Soil 241:233–242

Gilot C (1997) Effects of a tropical geophageous earthworm, *M. anomala* (Megascolecidae), on soil characteristics and production of a yam crop in Ivory Coast. Soil Biol Biochem 29:353–359

Goberna M, Insam H, Klammer S, Pascual JA, Sanchez J (2005) Microbial community structure at different depths in disturbed and undisturbed semiarid mediterranean forest soils. Microb Ecol 50:315–326

Hamid R, Khan MA, Ahmad M, Ahmad MM, Abdin MZ, Musarrat J, Javed S (2013) Chitinases: an update. J Pharm Bioallied Sci 5:21–29

Hoang TTD, Razavi BS, Kuzyakov Y, Blagodatskaya E (2016) Earthworm burrows: kinetics and spatial distribution of enzymes of C-, N- and P- cycles. Soil Biol Biochem 99:94–103

Hodge A, Robinson D, Filter A (2000) Are microorganisms more effective than plants at competing for nitrogen? Trends Plant Sci 5: 304–308

Jégou D, Schrader S, Diestel H, Cluzeau D (2001) Morphological, physical and biochemical characteristics of burrow walls formed by earthworms. Appl Soil Ecol 17:165–174

Jenkinson DS, Ladd JN (1981) Microbial biomass in soil: measurement and turnover. In: Paul EA, Ladd JN (eds) Soil biochemistry, vol 5. Dekker, New York, pp 415–471

Kautz T (2014) Research on subsoil biopores and their functions in organically managed soils: a review. Renew Agr Food Syst 30:318–327

Kautz T, Amelung W, Ewert F, Gaiser T, Horn R, Jahn R, Javaux M, Kemna A, Kuzyakov Y, Munch JC, Pätzold S, Peth S, Scherer HW, Schloter M, Schneider H, Vanderborght J, Vetterlein D, Walter A, Wiesenberg GLB, Köpke U (2013) Nutrient acquisition from arable subsoils in temperate climates: a review. Soil Biol Biochem 57:1003–1022

Kautz T, Lüsebrink M, Pätzold S, Vetterlein D, Pude R, Athmann M, Küpper PM, Perkons U, Köpke U (2014) Contribution of anecic earthworms to biopore formation during cultivation of perennial ley crops. Pedobiologia 57:47–52

Kibblewhite MG, Ritz K, Swift MJ (2007) Soil health in agricultural systems. Philos Trans R Soc B 363:685–701

Koch AL (1985) The macroeconomics of bacterial growth. In: Fletcher M, Floodgate GD (eds) Bacteria in their natural environments. Academic, London, pp 1–42

Koch O, Tscherko G, Kandeler E (2007) Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. Global Biogeochem Cy 21:1–11

Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34:139–162

Kuzyakov Y (2010) Priming effects: interactions between living and dead organic matter. Soil Biol Biochem 42:1363–1371

Kuzyakov Y, Blagodatskaya E (2015) Microbial hotspots and hot moments in soil: concept and review. Soil Biol Biochem 83:184–199

Lavelle P, Martin A (1992) Small-scale and large-scale effects of endogeic earthworm on soil organic matter dynamics in soils of the humid tropics. Soil Biol Biochem 24:1491–1498

Le Bayon RC, Binet F (2006) Earthworms change the distribution and availability of phosphorous in organic substrates. Soil Biol Biochem 38:235–246

Lin Q, Brookes PC (1999) An evaluation of the substrate-induced respiration method. Soil Biol Biochem 31:1969–1983

Lopez-Hernandez D, Lavelle P, Niño M (1993) Phosphorus transformations in two P-sorption contrasting tropical soils during transit through *Pontoscolex corethrurus* (Glossoscolecidae: Oligochaeta). Soil Biol Biochem 25:789–792

MacKay AD, Syers JK, Springett JA, Gregg PEH (1982) Plant availability of phosphorus in superphosphate and a phosphate rock as influenced by earthworms. Soil Biol Biochem 14:281–287

Malcolm RE (1983) Assessment of phosphatase activity in soils. Soil Biol Biochem 15:403–408

Marinissen JCY, de Ruiter PC (1993) Contribution of earthworm to carbon and nitrogen cycling in agro-ecosystems. Agric Ecosyst Environ 47:59–74

Martin A (1991) Short- and long-term effects of the endogeic earthworm *Millsonia anomala* (Omodeo) (Megascolecidæ, Oligochæta) of tropical savannas, on soil organic matter. Biol Fert Soils 11:234–238

Marx MC, Kandeler E, Wood M, Wermbter N, Jarvis SC (2005) Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particale-size fractions. Soil Biol Biochem 37:35–48

Matson PA, Parton W, Power A, Swift M (1997) Agricultural intensification and ecosystem properties. Science 277:504–509

Mcgill MB, Cannon KR, Robertson JA, Cook FD (1986) Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Can J Soil Sci 66:1–19

Menichetti L, Ekblad A, Kätterer T (2014) Contribution of roots and amendments to soil carbon accumulation within the soil profile in a long-term field experiment in Sweden. Agric Ecosyst Environ 200:79–87

Mganga KZ, Razavi BS, Kuzyakov Y (2015) Microbial and enzymes response to nutrient additions in soils of Mt. Kilimanjaro region depending on land use. Eur J Soil Biol 69:33–40

Miller M, Dick RP (1995) Dynamics of soil C and microbial biomass in whole soil and aggregates in two cropping systems. Appl Soil Ecol 2:253–261

Nakamoto T (2000) The distribution of wheat and maize roots as influenced by biopores in a subsoil of the Kanto loam type. Plant Prod Sci 3:140–144

Nannipieri P, Gianfreda L (1998). Kinetics of enzyme reactions in soil environments. In: Huang PM, Senesi N, Buffle J (eds) Environmental particles-structure and surface reactions of soil particles. J. Wiley and Sons, pp 449–479

Nannipieri P, Ceccanti B, Bianchi D (1988) Characterization of humus-phosphatase complexes extracted from soil. Soil Biol Biochem 20:683–691

Nannipieri P, Sequi P, Fusi P (1996) Humus and enzyme activity. In: Piccolo A (ed) Humic substances in terrestrial ecosystems. Elsevier, Amsterdam, pp 293–328

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54:655–670

Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Role of phosphatase enzymes in soil. In: Bunemann EK, Obreson A, Frossard E (eds) Phosphorus in action. Springer, Berlin, pp 215–243

Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, Masciandaro G, Fornasier F, Moscatelli MC, Marinari S (2012) Soil enzymology: classical and molecular approaches. Biol Fertil Soils 48:743–762

Orchard VA, Cook FJ (1983) The relationship between soil respiration and soil moisture. Soil Biol Biochem 15:447–453

Pathan SI, Ceccherini MT, Pietramellara G, Puschenreiter M, Giagnoni L, Arenella M, Varanini Z, Nannpieri P, Renella G (2015) Enzyme activitiy and microbial community structure in the rhizosphere of two maize lines differing in N use efficiency. Plant Soil 387:413–424

Perkons U, Kautz T, Uteau D, Peth S, Geier V, Thomas K, Holz KL, Athmann M, Pude R, Köpke U (2014) Root-length densities of various annual crops following crops with contrasting root systems. Soil Till Res 137:50–57

Poll C, Marhan S, Ingwersen J, Kandeler E (2008) Dynamics of litter carbon turnover and microbial abundance in a rye detritusphere. Soil Biol Biochem 40:1306–1321

Rawlings ND, Morton FR, Barrett AT (2006) MEROPS: the peptidase database. Nucleic Acids Res 32:D160–D164

Razavi BS, Blagodatskaya E, Kuzyakov Y (2015) Nonlinear temperature sensitivity of enzyme kinetics explains canceling effect: a case study on loamy haplic Luvisol. Front Microbiol 6:1126

Ritz K, Wheatley RE (1989) Effects of water amendment on basal and substrate-induced respiration rates of mineral soils. Biol Fert Soils 8:242–246

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech Adv 17:319–339

Salomé C, Nunan N, Pouteau V, Lerch TZ, Chenu C (2010) Carbon dynamics in topsoil and in subsoil may be controlled by different regulatory mechanisms. Glob Chang Biol 16:416–426

Sanaullah M, Chabbi A, Leifeld J, Bardoux G, Billou D, Rumpel C (2011) Decomposition and stabilization of root litter in top- and subsoil horizons: what is the difference? Plant Soil 338:127–141

Satchell JE, Martin K (1984) Phosphatase activity in earthworm faeces. Soil Biol Biochem 16:191–194

Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–563

Silver WL, Miya RK (2001) Global patterns in root decomposition: comparision of climate and litter quality effects. Oecologia 129:407–419

Spohn M, Kuzyakov Y (2014) Spatial and temporal dynamics of hotspots of enzyme activity in soil as affected by living and dead roots: a soil zymography analysis. Plant Soil 379:67–77

Stehouwer RC, Dick WA, Traina SJ (1993) Characteristics of earthworm burrow lining affecting atrazine sorption. J Environ Qual 22:181–185

Svensson K, Friberg H (2007) Changes in active microbial biomass by earthworms and grass amendments in agricultural soil. Biol Fert Soils 44:223–228

Tabatabai MA, García-Manzanedo AM, Acosta-Martínez V (2002) Substrate specificity of arylamidase in soils. Soil Biol Biochem 34:103–110

Thielemann U (1986) The octet-method for sampling earthworm populations. Pedobiologia 29:296–302

Tiunov AV, Scheu S (2000) Microfungal communities in soil, litter and casts of *Lumbricus terrestris* L. (Lumbricidae): a laboratory experiment. Appl Soil Ecol 14:17–26

Uksa M, Schloter M, Kautz T, Athmann M, Köpke U, Fischer D (2015) Spartial variability of hydrolytic and oxidative potential enzyme activities in different subsoil compartments. Biol Fertil Soils 51:517–521

Uteau D, Pagenkemper SK, Peth S, Horn R (2013) Root and time dependent soil structure formation and its influence on gas transport in the subsoil. Soil Till Res 132:69–76

Van Bruggen AHC, Semenov AM (2000) In search of biological indicators for soil health and disease suppression. Appl Soil Ecol 15:13–24

Van Der Waerden BL (1952) On the method of saddle points. Appl Sci Res B 2:33–45

Vetterlein D, Kühn T, Kaiser K, Jahn R (2013) Illite transformation and potasium release upon changes in composition of the rhizosphere soil solution. Plant Soil 371:267–279

Wan JHC, Wong MH (2004) Effects of earthworm activity and P-solubilizing bacteria on P availability in soil. J Soil Sci Plant Nutr 167:209–213

West AW, Sparling GP (1986) Modification to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. J Microbiol Meth 3:177–189