Geoderma 306 (2017) 50–57

Contents lists available at ScienceDirect

Geoderma

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

Effects of maize roots on aggregate stability and enzyme activities in soil

Amit Kumar[a,*], Maxim Dorodnikov[b], Thomas Splettstößer[b], Yakov Kuzyakov[a,b], Johanna Pausch[b]

[a] Department of Agricultural Soil Science, Georg-August University of Göttingen, Büsgenweg 2, Göttingen, Germany
[b] Department of Soil Science of Temperate Ecosystems, Georg-August University of Göttingen, Büsgenweg 2, Göttingen, Germany

ARTICLE INFO

Keywords:
Rooted soil
Root exudation
Free microaggregates
Plant density
Specific enzyme activity
Mean weight diameter

ABSTRACT

Soil aggregation and microbial activities within the aggregates are important factors regulating soil carbon (C) turnover. A reliable and sensitive proxy for microbial activity is activity of extracellular enzymes (EEA). In the present study, effects of soil aggregates on EEA were investigated under three maize plant densities (Low, Normal, and High). Bulk soil was fractionated into three aggregate size classes (> 2000 μm large macroaggregates; 2000–250 μm small macroaggregates; < 250 μm microaggregates) by optimal-moisture sieving. Microbial biomass and EEA (β-1,4-glucosidase (BG), β-1,4-*N*-acetylglucosaminidase (NAG), L-leucine aminopeptidase (LAP) and acid phosphatase (acP)) catalyzing soil organic matter (SOM) decomposition were measured in rooted soil of maize and soil from bare fallow. Microbial biomass C (C_{mic}) decreased with decreasing aggregate size classes. Potential and specific EEA (per unit of C_{mic}) increased from macro- to microaggregates. In comparison with bare fallow soil, specific EEA of microaggregates in rooted soil was higher by up to 73%, 31%, 26%, and 92% for BG, NAG, acP and LAP, respectively. Moreover, high plant density decreased macroaggregates by 9% compared to bare fallow. Enhanced EEA in three aggregate size classes demonstrated activation of microorganisms by roots. Strong EEA in microaggregates can be explained by microaggregates' localization within the soil. Originally adhering to surfaces of macroaggregates, microaggregates were preferentially exposed to C substrates and nutrients, thereby promoting microbial activity.

1. Introduction

Intensive agriculture often leads to decreases in soil carbon (C) stocks and reduces the quality of soil organic matter (SOM) (Paz-Ferreiro and Fu, 2016). The alterations to soil C stocks could have further impacts on the global C cycle (Nie et al., 2014). Soil microorganisms are one of the important biotic drivers regulating the soil C cycle. In terrestrial ecosystems, microbially mediated SOM decomposition constitutes a major part of soil C losses along with abiotic factors (Kaiser et al., 2010). Therefore, even minor changes in microbial decomposition of SOM due to intense agricultural practices may substantially impact the global climate via carbon dioxide (CO_2) efflux to the atmosphere.

Extracellular enzyme activities (EEA) are good indicators of microbially mediated SOM decomposition and are highly sensitive to environmental changes (Burns et al., 2013; Mganga et al., 2015; Sinsabaugh et al., 2005). Depending on their functions, enzymes are divided into several groups, of which oxidoreductases and hydrolases are especially relevant for SOM decomposition (Tischer et al., 2015). Among these enzymes, β-1,4-glucosidase (BG) cellulose de-polymerization, releasing two moles of glucose per mole of cellobiose (disaccharide of cellulose) (Turner et al., 2002). Degradation of various organic N compounds in soil, including proteins and chitin, are catalyzed by the hydrolyzing activities of L-leucine aminopeptidase (LAP) and β-1,4-*N*-acetylglucosaminidase (NAG), respectively (Sanaullah et al., 2011), releasing N for microbial and plant uptake. Extracellular activity of acid phosphatase (acP) in soil is associated with P mineralization through hydrolysis of organic phosphate compounds (Goldstein et al., 1988; Nuruzzaman et al., 2006).

Activities of extracellular enzymes are triggered by the presence of plants and are usually higher than in bulk soil. Release of labile substrates (i.e. root exudation) by living roots into soil enhances EEA (microbial activation hypothesis; Cheng and Kuzyakov, 2005, Kumar et al., 2016, Zhu et al., 2014). Availability of labile C from root exudation increases the microbial demand for other nutrients such as nitrogen (N) and phosphorus (P). The microbial activation enhances SOM decomposition via mining for N and P (Kuzyakov and Xu, 2013).

Soil aggregation is another factor affecting SOM decomposition as well as nutrient cycling because microbial communities and their activities differ between aggregate size classes (Caravaca et al., 2005; Duchicela et al., 2012; Gupta and Germida, 2015). Soil aggregation physically protects SOM by making it inaccessible for microbial

* Corresponding author at: Department of Agricultural Soil Science, Georg-August University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany.
E-mail addresses: akumar4@gwdg.de, aksoni089@gmail.com (A. Kumar).

http://dx.doi.org/10.1016/j.geoderma.2017.07.007
Received 24 October 2016; Received in revised form 26 June 2017; Accepted 10 July 2017

mineralization. Aggregation strongly regulates aeration, nutrient retention, and erosion (Blankinship et al., 2016) and controls the sequestration of plant-derived organic matter by occlusion into macro- and microaggregates (Lagomarsino et al., 2012; Tian et al., 2015). Based on observations, it has been identified that C content increase with increasing aggregate size classes from micro- to macroaggregates. Moreover, microaggregates constitute relatively old and recalcitrant C than macroaggregates (Six et al., 2004). Therefore, the quality of C contained within microaggregates or macroaggregates regulates the microbial community structure and associated activity (Bach and Hofmockel, 2014; Hattori, 1988).

Soil macro- (> 250 μm) and microaggregates (< 250 μm) are responsible for the heterogeneous distribution of microorganisms (Blaud et al., 2012) and therefore may affect the associated EEA. The impact of aggregate size class on EEA is inconsistent: increase, decrease or no change have been obtained. One of the possible reasons may be the methods of aggregate size fractionation (Allison and Jastrow, 2006; Dorodnikov et al., 2009a; Fang et al., 2016; Shahbaz et al., 2016). For instance, application of conventional wet- and dry sieving may substantially modify easily soluble and desiccation-sensitive enzyme molecules, and cause their redistribution from one aggregate size class to another (Dorodnikov et al., 2009a). In contrast, the proposed 'optimal moisture sieving' method was developed to minimize biases from the above-mentioned factors on EEA. The method is based on a moisture content that limits mechanical stress, to induce maximum brittle failure along natural planes of weakness in the bulk soil (Dorodnikov et al., 2009a; Kristiansen et al., 2006). This technique involves neither complete drying nor water saturation, which are respectively necessary for dry and moist sieving. Due to the optimal moisture level, macroaggregates do not disrupt completely and the microaggregates located on surfaces of macroaggregates or along natural planes of weakness are preferentially separated. This fraction comprises the free microaggregate size class, distinct from the microaggregates located inside macroaggregates (Bossuyt et al., 2005; Six et al., 2004).

In the present study, the response of EEA catalyzing the decomposition of C (BG and NAG), N (NAG and LAP), and P (acP) compounds was determined in three aggregate size classes. For this, a modified 'optimal moisture sieving' technique was used to separate bulk soil into large macroaggregates (> 2000 μm), small macroaggregates (2000–250 μm), and free microaggregates (< 250 μm). Our previous findings have shown increased enzymes activities in the rhizosphere soil as compared to bare fallow, driven by labile C inputs from roots (Kumar et al., 2016). Increase in root density will also change the distribution of the three aggregate size classes. Therefore, the following research question was addressed: could the optimally fractionated aggregates explain the effects of rhizosphere on microbial biomass distribution and measured EEA? We hypothesized that (i) EEA is higher in aggregates of planted soil than that of bare fallow, as microorganisms are fueled with C and energy-rich labile substrates by rhizodeposition; (ii) EEA is higher in free microaggregates than macroaggregates as the former should be preferentially exposed to root exudates, water and oxygen flows.

2. Materials and methods

2.1. Experimental setup

The experiment was established on a haplic Luvisol in an agricultural field (51°29′37.2″N and 9°55′36.9″E), which belongs to the research station "Reinshof" of the Georg-August-University Göttingen, Germany. Soil properties are as follow: total C (1.41 ± 0.04%), total N (0.16 ± 0.002%), pH (7.2 ± 0.01), soil bulk density (1.2 ± 0.2 g cm^{-3}).The experimental field was divided into 16 plots, each with an area of 5 × 5 m. To avoid any neighboring effects, the plots were separated by 2 m-wide buffer strips, which were kept vegetation-free throughout the experiment. A gradient of three plant densities (low, normal and high) was established in the field with completely randomized design. For this, maize was sown in plots with a plant density of 16 plants m^{-2}. When the plants were approximately 10 cm high, the plots were thinned according to the plant density gradient. Plots were thinned to 6 plants m^{-2} for low plant density; 10 plants m^{-2} for normal plant density; and 16 plants m^{-2} were left as high plant density. Four plots were kept vegetation-free throughout the experiment as control.

2.2. Soil and plant sampling

Soils were collected when the plants entered into the reproductive state (72 days after planting (DAP)) from a depth of 5–15 cm assuming maximum root growth and root exudation during plant vegetative stage (Kumar et al., 2016). This soil depth corresponded to the highest root biomass (data not presented). For soil sampling, the upper 0–5 cm soil layer was carefully removed and soil from 5 to 15 cm was collected between maize rows with a border spade. After delivery to the laboratory, soils were immediately sieved through an 8-mm sieve. A 5 g sub-sample was dried at 60 °C for 3 days to determine soil moisture content. The remaining soil was used for aggregate size fractionation. To determine shoot biomass, two plants from each plot were cut at the base, dried at 60 °C for 3 days, and weighed. Based on plot size and plant density of the respective treatment, shoot biomass was scaled up to g dry weight m^{-2}. For the total root biomass, which could not be directly quantified, the root-to-shoot ratio was used to scale measured shoot biomass to root biomass in units per area (i.e. g dry weight m^{-2}). The root-to-shoot ratio under normal plant density was 0.11 (97 DAP) and did not differ significantly between low, normal, and high plant densities at the end of the field experiment (130 DAP). The ratio was within the range of the data reported by Amos and Walters (2006), showing that the main changes of root-to-shoot ratio in maize occur within the first 60 days after planting.

2.3. Aggregate size fractionation

Aggregates of three size classes were isolated by the method described by Dorodnikov et al. (2009a) with modifications. In order to minimize disturbance to microbial activities, soils were cold dried at 4 °C to approximately 10% gravimetric water content (Bach and Hofmockel, 2015). For this, soil samples were placed in a container and spread into a thin layer. All stones and visible roots were hand-picked. Once the desired condition was achieved, approximately 700 g soil was transferred to a nest of sieves (2 mm and 0.25 mm). The nest was bolted onto a vibratory sieve shaker AS200 (Retsch, Germany) and shaken for 3 min, 2 times. Aggregates remaining on the 2 mm sieve were classified as large macroaggregates (> 2000 μm), aggregates passing through the 2 mm sieve but remaining on the 0.25 mm sieve were classified as small macroaggregates (2000–250 μm), and the remaining soil materials which passed through the 0.25 mm sieve were classified as microaggregates (< 250 μm) (Fig. 1). From each aggregate size class, soil was weighed to determine the mass distribution and mean weight diameters (MWD) of aggregates. Mean weight diameter was calculated after John et al. (2005):

$$MWD = \sum (Weight\%of\,sample\,remaining\,on\,sieve \times Mean\,inter - sieve\,size) \div 100$$

where mean inter-sieve size is the average of the two sieve sizes through which the aggregates have passed and on which the aggregates have remained after sieving.

Thereafter, post-sieving moisture content, total C and N, microbial biomass C and N, and maximal potential extracellular enzyme activities of C-, N-, and P-degrading enzymes were measured. For moisture content, a soil subsample was dried at 60 °C for 3 days. Total C and N contents were estimated with an Elementar Vario EL analyzer

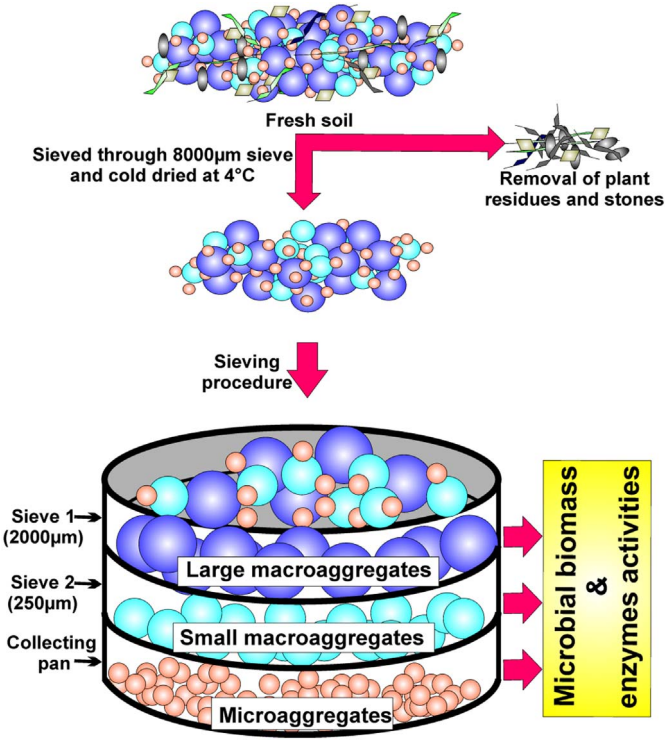

Fig. 1. Schematic diagram showing soil preparation and aggregate size fractionation.

(Elementar Analysensysteme GmbH, Germany).

2.4. Soil microbial biomass

The chloroform fumigation-extraction method was used to determine soil microbial biomass C (C_{mic}) and N (N_{mic}) (Vance et al., 1987) with slight modifications. Before microbial biomass determination, aggregates were moisten to field moisture level of 12–15% and incubated for 24 h to assure field conditions. Briefly, an 8 g soil sample (non-fumigated) was extracted with 32 ml of 0.05 M K_2SO_4 for 1 h by continuously shaking (150 rpm) on a reciprocating shaker (Laboratory shaker, GFL 3016). Afterwards, the soil suspension was filtered (grade: 3 hw, diameter 110 mm, Sartorius) and stored at 4 °C until further analyses. The same extraction procedure was used for fumigated soil. Fumigation was done with 80 ml of ethanol-free chloroform in a desiccator at room temperature for 24 h. The organic C and total N content of the filtered solution was measured with a multi N/C analyzer (multi N/C analyzer 2100S, Analytik, Jena).

Differences between extracted C and N from fumigated and non-fumigated soil were used to calculate microbial biomass C and microbial biomass N. We used K_{EC} and K_{EN} factors of 0.45 and 0.54 for microbial C and N, respectively (Joergensen and Mueller, 1996; Wu et al., 1990).

2.5. Enzyme assays

Extracellular enzyme activities were measured with fluorogenically labeled artificial substrates according to Marx et al. (2001). Fluorogenic 4-methylumbelliferone (MUB)-based substrates were used to determine the activities of β-1,4-glucosidase, β-1,4-*N*-acetylglucosaminidase and acid phosphatase. Fluorogenic 7-amino-4-methylcoumarin (AMC)-based substrate was used to determine the activity of L-leucine aminopeptidase. EEA was determined separately in distinct aggregate size class. For this, distinct aggregates (1 g) were used to make soil suspension by dissolving it in 50 ml distilled and autoclaved water. To release the enzymes trapped on soil clay particles, low-energy sonication (50 Js^{-1}) was applied for 2 min (Loeppmann et al., 2016; Razavi et al., 2015). 50 µl of soil suspension was dispensed into a black 96-well microplate (PureGrade™, GMBH + Co KG, Wertheim, Germany) while stirring the suspension on a magnetic stirrer to maintain uniformity. Thereafter, for MUB-based substrates, 50 µl of MES ($C_6H_{13}NO_4SNa_{0.5}$) buffer (pH 6.5) and for AMC-based substrate, 50 µl of TRIZMA ($C_4H_{11}NO_3{\cdot}HCl$, $C_4H_{11}NO_3$) buffer (pH 7.2) was added to each well (Hoang et al., 2016). Finally, 100 µl of substrate solutions of 4-methylumbelliferyl-β-D-glucoside, 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide, L-leucine-7-amido-4-methylcoumarine hydrochloride and 4-methylumbelliferyl phosphate were added to the wells. A substrate concentration of 400 µmol g^{-1} soil was used for the substrate-unlimited maximal potential reaction, as determined in a preliminary experiment using Michaelis-Menten kinetics (by using increasing substrate concentrations to reach V_{max}). Just after substrate addition, the microplate was gently shaken to mix the well contents and measurements were taken fluorometrically (excitation 360 nm; emission 450 nm) at 0, 30, 60, and 120 min after substrate addition with an automated fluorometric plate reader (Victor3 1420–050 Multi-label Counter, PerkinElmer, USA). Fluorescence was converted to amount of AMC or MUB, according to standards. Enzyme activities were expressed as MUB or AMC released in nanomol per gram aggregate dry weight and hour (nmol g^{-1} aggregate h^{-1}).

2.6. Statistics

The experiment was conducted with 4 field replicates. The values presented in figures are means of 4 field replicates ± standard errors (mean ± SEM). The data set was checked for normality (Shapiro-Wilk test, $P > 0.05$) and homogeneity of variance (Levene test, $P > 0.05$) prior to analysis of variance (ANOVA). For β-1,4-glucosidase and β-1,4-*N*-acetylglucosaminidase, the data did not meet the requirement for normality. Therefore, data were square-root transformed and retested for normal distribution with the Shapiro-Wilk test. Afterwards, two-factor ANOVA was performed to test the effects of aggregate size class and plant density on C_{mic} and N_{mic}, and potential and specific EEA. One-factor ANOVA was used to test the effect of plant density on MWD, relative distribution of aggregates within each aggregate size class, and root biomass. Post-hoc tests for multiple comparisons using least significant differences (Tukey-test, $P < 0.05$) were performed on each measured parameter after ANOVA. STATISTICA for Windows (version 7.0, StatSoft Inc., OK, USA) was used to perform ANOVA analyses. Figures were drawn with OriginPro 8.5G (OriginLab Corporation., Northampton, MA 01060, USA). The level of significance was defined at $P < 0.05$ for all statistical analyses, if not mentioned specifically.

3. Results

3.1. Aggregate size class distribution and mean weight diameter

Large and small macroaggregates dominated in the bare and planted soil, whereas microaggregates accounted for only a small part (Fig. 2). The relative distribution (in %) of aggregate size classes were: large macroaggregates (48–54%) > small macroaggregates (40–45%) > microaggregates (6–8%). The C and N content was 1.17 to 1.22% C and 0.13% N, respectively and did not differ significantly across the aggregate size classes.

Plant density had a minor effect on the relative distribution of aggregate size classes. The percentage of large macroaggregates in high plant density was significantly lower ($P < 0.05$) than bare fallow and low- and normal plant density. The percentage of microaggregates showed an increasing trend with increasing plant density. The MWD did not vary between bare fallow and various plant densities, except that high plant density had a minor decrease when compared to normal maize density (Fig. 3).

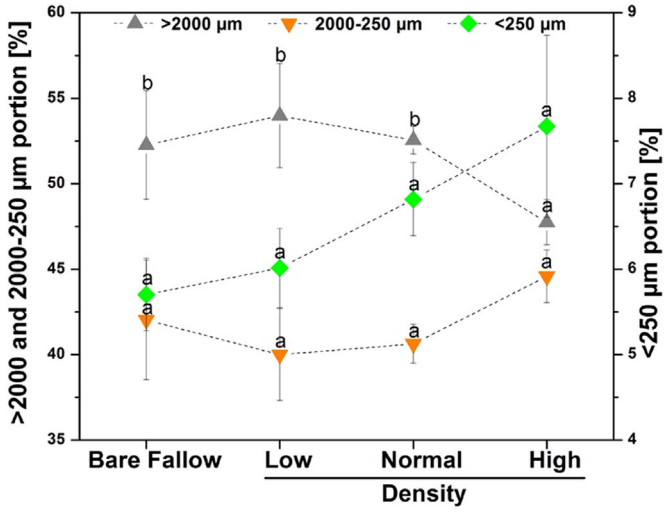

Fig. 2. The relative distribution of large and small macroaggregates (left y-axis; mean ± SEM) ($n = 4$) and microaggregates (right y-axis; mean ± SEM) ($n = 4$) in bare fallow soil and soils with Low, Normal and High maize plant densities. Letters indicate significant differences (Post-hoc LSD test, $P < 0.05$) between bare fallow and three plant densities within the same aggregate size class.

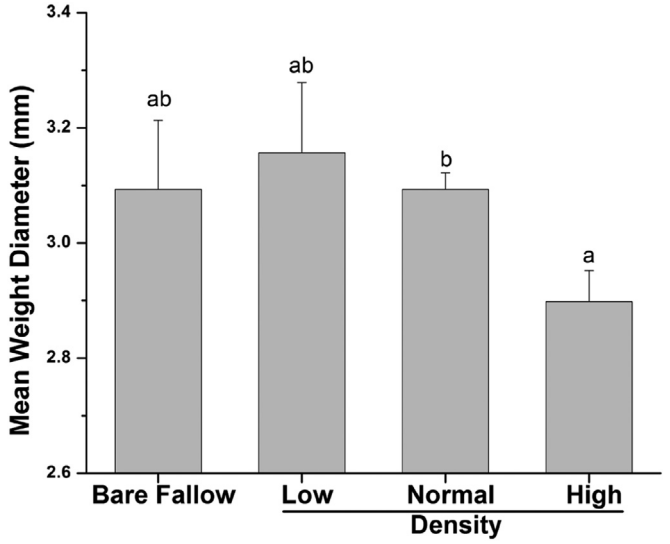

Fig. 3. Mean weight diameter (± SEM) ($n = 4$) in bare fallow soil and soils with Low, Normal and High maize plant densities. Letters indicate significant differences (Post-hoc LSD test, $P < 0.05$) in MWD between bare fallow soil and soils with low, normal and high maize plant densities.

3.2. Plant and microbial biomass

Aboveground biomass was 362 g m^{-2} under low plant density and increased by 88% and 149% under normal and high plant density, respectively. However, the increase was not significantly different ($P > 0.05$) between normal and high plant density (Supplementary Table 1). As total root biomass could not be accurately determined in the field, root biomass per area was calculated based on the measured root-to-shoot ratio. Root biomass increased from 41.2 ± 6.0 g m^{-2} for low plant density to 80.2 ± 6.1 g m^{-2} for high plant density.

Microbial biomass C decreased with decreasing aggregate size and ranged from 106.4 ± 18.5 to 138.7 ± 12.8 mg C kg^{-1} aggregate (large macroaggregates), 79.5 ± 5.4 to 121.1 ± 3.9 mg C kg^{-1} aggregate (small macroaggregates), and 77.8 ± 14.8 to 95.4 ± 8.7 mg C kg^{-1} aggregate (microaggregates) (Fig. 4). Planting had minor effects on C_{mic} relative to bare fallow. Comparing between the aggregate size classes, large macroaggregates comprised higher C_{mic}. Microbial

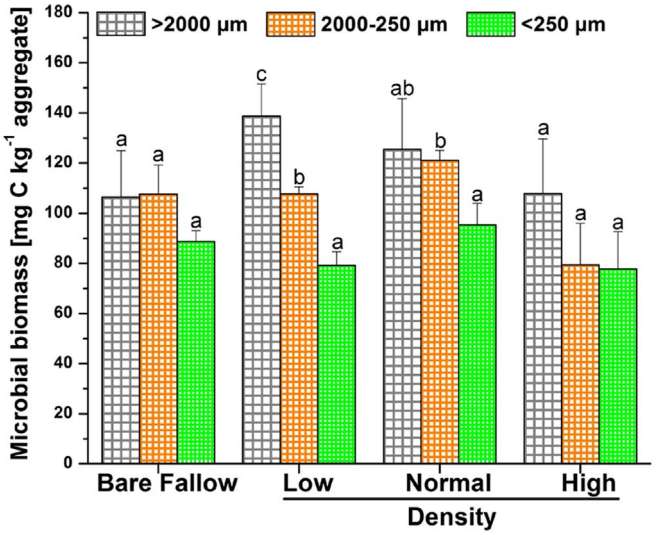

Fig. 4. Microbial biomass (mg C kg^{-1} aggregate; mean ± SEM) ($n = 4$) in bare fallow soil and soils with Low, Normal and High maize plant densities. Letters indicate significant differences (Post-hoc LSD test, $P < 0.05$) in microbial biomass C between aggregate size classes in bare fallow soil and soils with Low, Normal and High maize plant densities.

biomass N had a tendency to decrease with decreasing aggregate size classes. The content of N_{mic} was on average 27.3 ± 2.6 mg N kg^{-1} aggregate in large macroaggregates, 22.8 ± 0.8 mg N kg^{-1} aggregate in small macroaggregates, and 20.9 ± 1.1 mg N kg^{-1} aggregate in microaggregates under low plant density (Supplementary Table 1).

3.3. Extracellular enzyme activities

In contrast to microbial biomass, the potential activities of C, N, and P degrading enzymes (BG, LAP, NAG, and acP) tended to increase with decreasing aggregate size in planted soil (Fig. 5). Under bare fallow, the potential activities of BG and LAP were lower in microaggregates than macroaggregates, whereas the potential activity of NAG remained constant and that of acP was higher in microaggregates than in macroaggregates. Additionally, under bare fallow and low, normal and high plant densities, specific activities of BG, LAP, NAG, and acP remained similar, with a slight increase under high plant density (Fig. 6). Effects of planting on specific enzyme activities were strongest in microaggregates (Fig. 6). In microaggregates, the specific activity of BG was 0.47 nmol h^{-1} mg^{-1} C_{mic} in bare fallow and increased by 21–73% in the presence of roots. The specific activity of NAG was 0.57 nmol h^{-1} mg^{-1} C_{mic} in bare fallow and increased by 5–31%; specific activity of acP was 6.1 ± 0.3 nmol h^{-1} mg^{-1} C_{mic} in bare fallow and varied by −2% and 26%; and the specific activity of LAP was 0.35 nmol h^{-1} mg^{-1} C_{mic} in bare fallow and increased by about 35–92% in presence of roots under various plant densities. The specific enzyme activities were similar in each of the three aggregate size classes of the bare fallow.

4. Discussion

4.1. Aggregate fractionation

According to the aggregate hierarchy concept (Elliott and Coleman, 1988), microaggregates are located inside macroaggregates and comprise older C pools (Six et al., 2004). As shown by Dexter (1988), the maximum soil friability (tendency toward segregation of unconfined soil into smaller fragments under certain mechanical stress) occurs at about 38% of water content (field capacity). Soil colloids shrink and cracks appear, defining the boundaries of aggregates. These cracks remain as points of weakness for physical breakdown. Therefore, at this

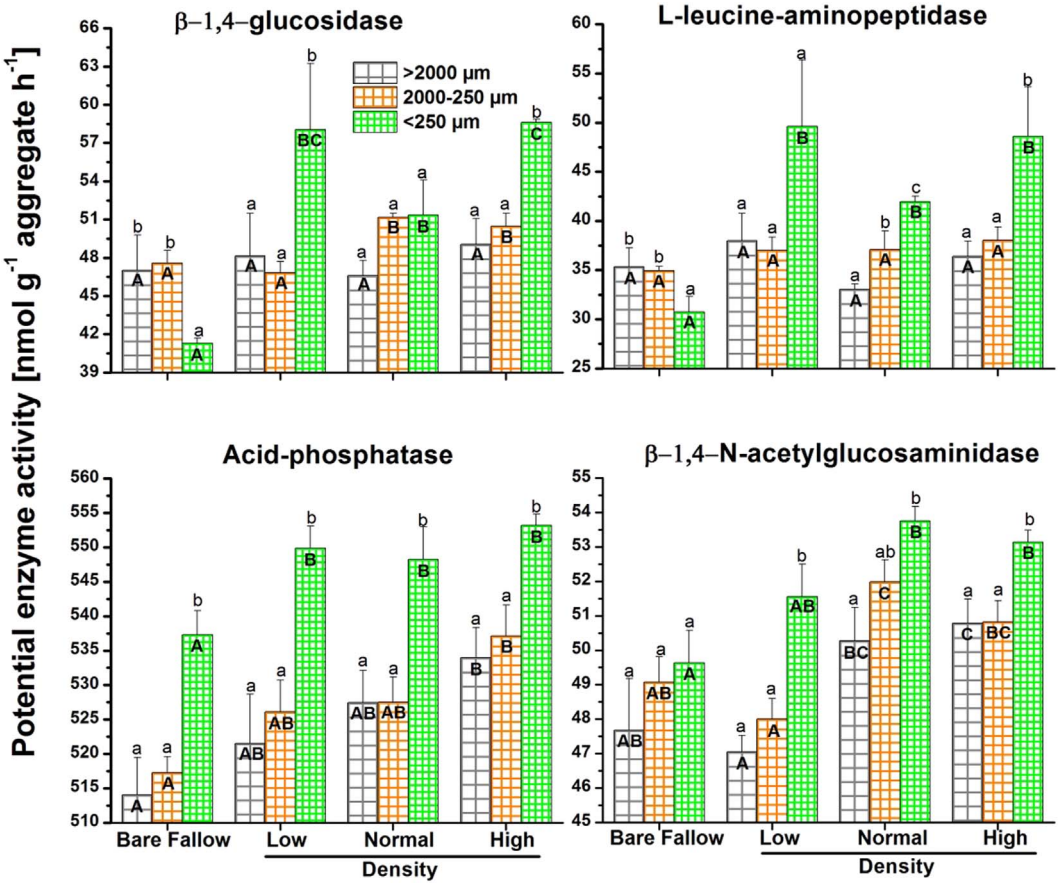

Fig. 5. Potential activity of: β-1,4-glucosidase; L-leucine aminopeptidase; acid phosphatase; and β-1,4-*N*-acetylglucosaminidase (nmol h^{-1} g^{-1} soil) (± SEM) ($n = 4$) in distinct aggregate size classes in bare fallow soil and soils with Low, Normal and High maize plant densities. Upper-case letters indicate significant differences (Post-hoc LSD test, $P < 0.05$) in potential activity within the same aggregate size class. Lower-case letters indicate significant differences (Post-hoc LSD test, $P < 0.05$) in potential activity between distinct aggregate size classes.

soil water content, aggregate fractionation results in breakdown of macroaggregates along the planes of weakness, releasing the microaggregates located on surfaces of macroaggregates and along their planes of weakness. Thus, the procedure adopted by Dorodnikov et al. (2009a) for aggregate size fractionation, termed as '*optimal moisture sieving*', accounted for free microaggregates as described in the aggregate hierarchy concept (Bossuyt et al., 2004; Oades, 1984; Simpson et al., 2004; Six et al., 2004). According to the method used in this study, the proportion of micro- to macroaggregates size classes strongly depends on the soil moisture level. Generally, the lower the moisture, the lower would be slicking and therefore, the proportion of macroaggregates is higher (Chenu et al., 2000). However, we aimed to keep the moisture under which EEA would be close to field conditions and the proportion of micro- to macroaggregates in the tested soil corresponded to field moisture conditions. In the present study, the soil moisture content was around 7 to 10% of total weight after sieving. We assume the breakdown of macroaggregates along the planes of weakness was minimal as shown by Dexter (1988). Therefore, we assume that with the aggregate fractionation technique we applied, mainly the free microaggregates and the microaggregates adhering on the surface of macroaggregates were isolated (Fig. 1). The small portion of isolated microaggregates in the present study (6–8% of total soil) further supports this concept.

4.2. Root effects on aggregate size distribution

The influence of roots on aggregate stabilization is well known (Erktan et al., 2016; Six et al., 2004), but very few studies have focused on aggregate disintegration by living roots (Materechera et al., 1994). In our field study, a gradual increase in the proportion of microaggregates and a decrease in large macroaggregates with increasing plant density may be due to disintegration of large macroaggregates by growing roots (Fig. 2). Also, the mean weight diameter, which is an indicator of aggregate stability (Tripathi et al., 2014) tended to decrease with increasing plant density. This also confirmed the redistribution of aggregate size classes in the presence of growing roots. Mechanistically, the aggregate redistribution may occur through the penetration of living roots into macroaggregates along planes of weakness and through the pores within macroaggregates, thereby decreasing their stability (Materechera et al., 1994). Hence, root morphology (root thickness, root length density, root branching, etc.) is one of the main drivers affecting aggregate redistribution (Carter et al., 1994).

4.3. Microbial biomass C in micro- and macroaggregates

Microbial biomass C decreased with decreasing aggregate size. The hierarchical aggregate concept (Elliott and Coleman, 1988) integrates the aggregate categories with the pore structure, which defines microsites of habitability for microorganisms (Gupta and Germida, 2015). Literature is replete with studies showing increased fungal abundance with increasing aggregate size (Poll et al., 2003; Zhang et al., 2015). The preferential colonization by fungal communities may occur in macroaggregates (Harris et al., 2003) by expanding their biomass through extensive hyphal growth in large pores (De Gryze et al., 2005; Dorodnikov et al., 2009b). In turn, microaggregates are inhabited predominately by bacterial communities (Ranjard and Richaume, 2001; Six et al., 2006). Higher C_{mic} / N_{mic} ratio in macroaggregates than

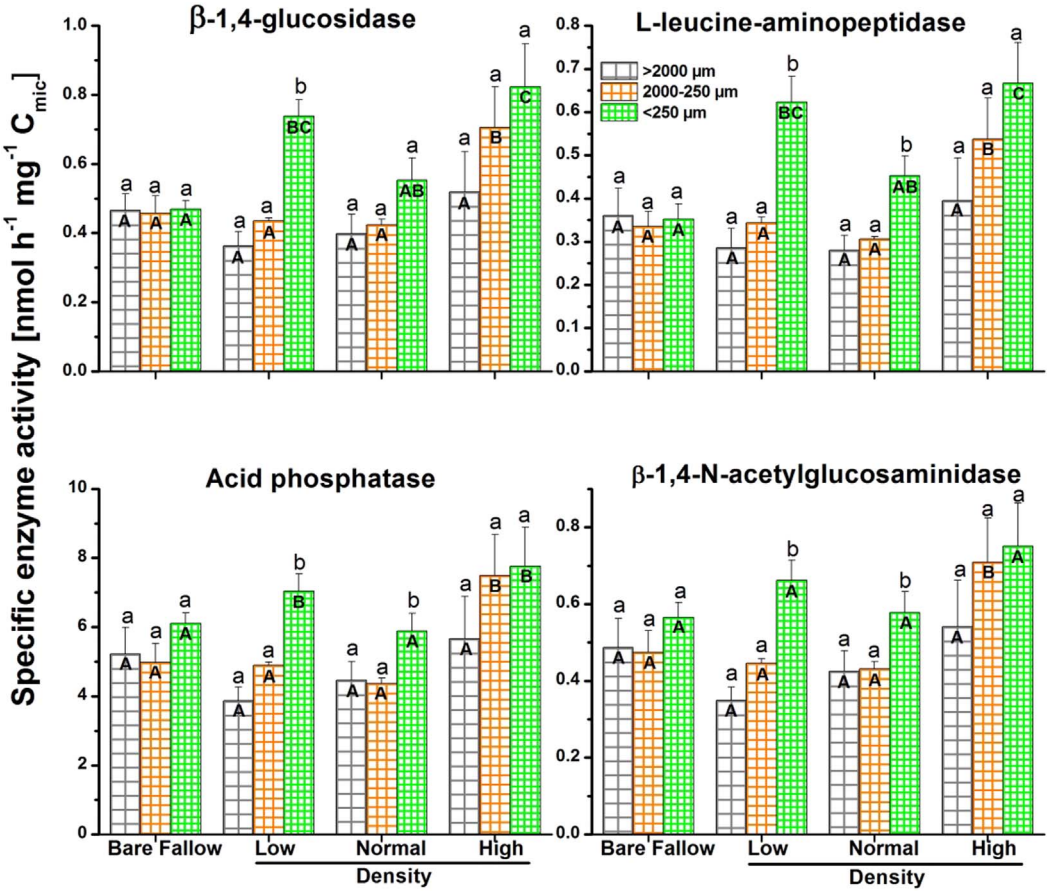

Fig. 6. Specific activity (ratio of potential activity and microbial biomass C) of: β-1,4-glucosidase; L-leucine aminopeptidase; acid phosphatase; and β-1,4-*N*-acetylglucosaminidase (nmol h^{-1} g^{-1} soil) (± SEM) ($n = 4$) in bare fallow soil and soils with Low, Normal and High maize plant densities in distinct aggregate size classes. Upper-case letters indicate significant differences (Post-hoc LSD test, $P < 0.05$) in specific activity within the same aggregate size class. Lower-case letters indicate significant differences (Post-hoc LSD test, $P < 0.05$) in specific activity between distinct aggregate size classes.

microaggregates (although significant only in low plant density) in the present study indicates fungal dominance in macroaggregates as compared to microaggregates (Supplementary Table 2) (Dorodnikov et al., 2009b). The lower microbial biomass in the microaggregates in comparison with large and small macroaggregates could reflect the distribution of fungal and bacterial communities (Gupta and Germida, 2015) as a result of different habitats.

In the short term, labile C inputs from roots did not change overall microbial growth. Such inputs predominantly activate fast-growing microbial communities (Blagodatskaya and Kuzyakov, 2008). The same amounts of microbial biomass in bare fallow and in planted soils (Fig. 4) are in line with other studies (Duineveld et al., 1998; Fontaine et al., 2007), highlighting the regulatory effect of living plants on activities rather than on the abundance of microorganisms in agricultural soil.

4.4. Effects of roots and aggregate sizes on extracellular enzyme activities

Extracellular enzyme production by microorganisms, which regulates microbially mediated SOM decomposition, may occur under nutrient limitations. In addition, root exudation may trigger extracellular enzyme production (Kumar et al., 2016; Kuzyakov and Blagodatskaya, 2015) via microbial activation. In the presence of root-released organics, which are characterized by higher C/N ratios, the microbial demand for other nutrients (especially N and P) increases (Fontaine et al., 2011). Further, plants exacerbate the nutrient limitations due to competition with microorganisms (Kuzyakov and Xu, 2013). In order to fulfill these extra nutritional demands, microorganisms produce N- and P-degrading enzymes to mine for them from SOM. Along with the P demand, acP activity reflects the overall microbial activity (as it participates in phosphorylation processes within cells and by lysis appears extracellular), which differ between macro- and microaggregates and was the highest among all enzymes tested. The results from the present study corroborate the reported increase in extracellular activities of C-, N- and P-degrading enzymes with decreasing aggregate size class (Nie et al., 2014). Similarly to the potential EEA, the specific EEA for C-, N-, and P-degrading enzymes also increased in the order: large macroaggregates < small macroaggregates < microaggregates (Fig. 6). Overall higher total and specific EEA in free microaggregates can result from the location of the latter within soil where plant root exudations as well as water, nutrient and oxygen flows are higher than in the interior of macroaggregates (Burns et al., 2013; Phillips et al., 2011). Similarly, an absence of labile substrate inputs in bare fallow soil resulted in lower enzyme activities. In summary, considering microbial activation (Cheng and Kuzyakov, 2005) by growing roots, the present study provides evidence that the influence of roots on microorganism's activities persists in different soil aggregates and such influences are more pronounced in free microaggregates (Fig. 7).

5. Conclusions

Pronounced effects of aggregate size on C_{mic}, N_{mic} as well as on EEA were demonstrated. Higher EEA in rooted soil than in bare fallow soil for three aggregate size classes highlights plant-mediated microbial activation. The presence of roots stimulated microbial activity (potential and specific EEA), which governs the catalytic reactions of SOM decomposition. Markedly higher specific EEA in free microaggregates than in large- and small macroaggregates may result from the better

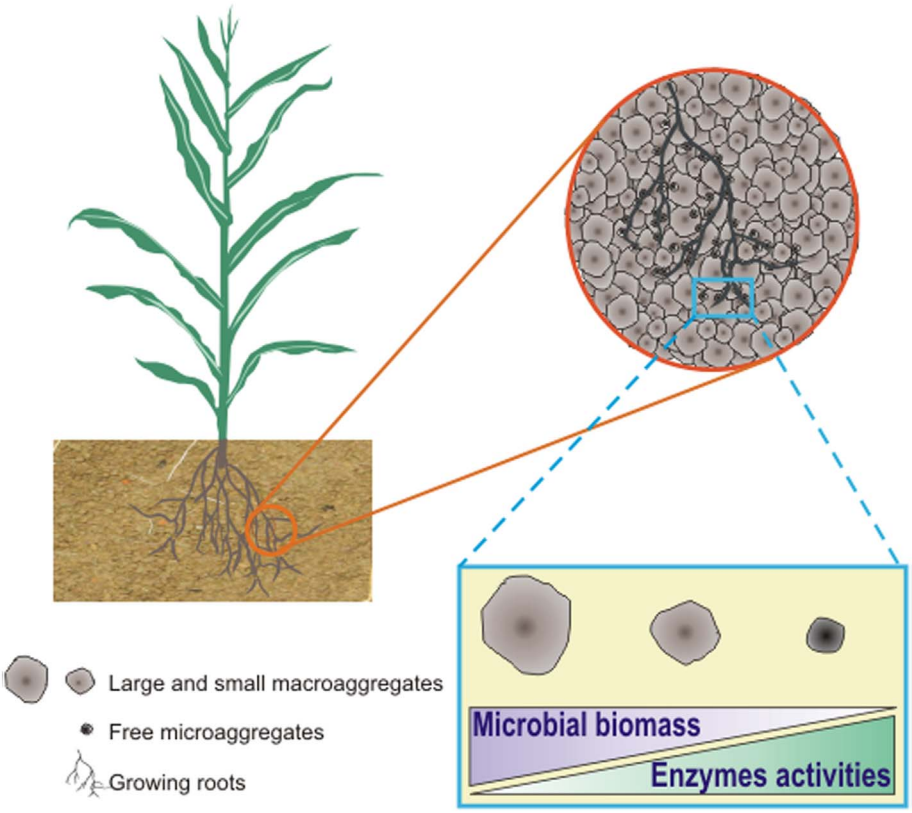

Fig. 7. Conceptual figure showing the potential effects of growing roots on extracellular enzyme activities and microbial biomass in distinct aggregate size classes in rooted soil separated by optimal moisture sieving method. Root induced microbial activities in distinct aggregate size classes are shown by higher EEA and the relations between aggregate size and microbial biomass are illustrated.

supply of root exudates, water, nutrients and oxygen to microorganisms. Minimal or no effect of aggregate size on specific EEA under bare fallow indicated microbial inefficiency in enzyme synthesis in the absence of root-released organics.

Acknowledgements

The authors thank Yue Sun for field assistance. We sincerely thank Karin Schmidt, Anita Kriegel and Ingrid Ostermeyer for laboratory assistance. We gratefully acknowledge the German Academic Exchange Service (DAAD) for a scholarship award to Amit Kumar. Funds for this research were provided by the German Research Foundation (DFG) within the project PA 2377/1-1. We would also like to thank anonymous reviewers for their critical comments/suggestions which improved the quality of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.geoderma.2017.07.007.

References

Allison, S.D., Jastrow, J.D., 2006. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biol. Biochem. 38, 3245–3256.
Amos, B., Walters, D.T., 2006. Maize root biomass and net rhizodeposited carbon. Soil Sci. Soc. Am. J. 70, 1489–1503.
Bach, E.M., Hofmockel, K.S., 2014. Soil aggregate isolation method affects measures of intra-aggregate extracellular enzyme activity. Soil Biol. Biochem. 69, 54–62.
Bach, E.M., Hofmockel, K.S., 2015. A time for every season: soil aggregate turnover stimulates decomposition and reduces carbon loss in grasslands managed for bioenergy. GCB Bioenergy doi. http://dx.doi.org/10.1111/gcbb.12267.
Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol. Fertil. Soils 45, 115–131.
Blankinship, J.C., Fonte, S.J., Six, J., Schimel, J.P., 2016. Biotic versus abiotic controls on soil aggregate stability in a seasonally dry ecosystem. Geoderma 272, 39–50.
Blaud, A., Lerch, T.Z., Chevallier, T., Nunan, N., Chenu, C., Brauman, A., 2012. Dynamics of bacterial communities in relation to soil aggregate formation during the decomposition of ^{13}C-labelled rice straw. Appl. Soil Ecol. 53, 1–9.
Bossuyt, H., Six, J., Hendrix, P.F., 2004. Rapid incorporation of carbon from fresh residues into newly formed stable microaggregates within earthworm casts. Eur. J. Soil Sci. 55, 393–399.
Bossuyt, H., Six, J., Hendrix, P.F., 2005. Protection of soil carbon by microaggregates within earthworm casts. Soil Biol. Biochem. 37, 251–258.
Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol. Biochem. 58, 216–234.
Caravaca, F., Alguacil, M.M., Torres, P., Roldán, A., 2005. Plant type mediates rhizospheric microbial activities and soil aggregation in a semiarid Mediterranean salt marsh. Geoderma 124, 375–382.
Carter, M.R., Angers, D.A., Kuneliues, H.T., 1994. Soil structure form and stability, and organic matter under cool-season perennial grasses. Soil Sci. Soc. Am. J. 58, 1194–1199.
Cheng, W., Kuzyakov, Y., 2005. Root effects on soil organic matter decomposition. Agronomy 119–144.
Chenu, C., Bissonnais, Y.L., Arrouays, A., 2000. Organic matter influence on clay wettability and soil aggregate stability. Soil Sci. Soc. Am. J. 64, 1479–1486.
De Gryze, S., Six, J., Brits, C., Merckx, R., 2005. A quantification of short-term macroaggregate dynamics: influences of wheat residue input and texture. Soil Biol. Biochem. 37, 55–66.
Dexter, A.R., 1988. Advances in characterization of soil structure. Soil Tillage Res. 11, 199–238.
Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Fangmeier, A., Kuzyakov, Y., 2009a. Stimulation of r- vs. K-selected microorganisms by elevated atmospheric CO2 depends on soil aggregate size. FEMS Microbiol. Ecol. 69, 43–52.
Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Marhan, S., Fangmeier, A., Kuzyakov, Y., 2009b. Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on soil aggregate size. Glob. Chang. Biol. 15, 1603–1614.
Duchicela, J., Vogelsang, K.M., Schultz, P.A., Kaonongbua, W., Middleton, E.L., Bever, J.D., 2012. Non-native plants and soil microbes: potential contributors to the consistent reduction in soil aggregate stability caused by the disturbance of North American grasslands. New Phytol. 196, 212–222.
Duineveld, B.M., Rosado, A.S., Elsas, J.D., Veen, J.A., 1998. Analysis of the dynamics of bacterial communities in the rhizosphere of the *Chrysanthemum* via denaturing gradient gel electrophoresis and substrate utilization patterns. Appl. Environ. Microbiol. 64, 4950–4957.
Elliott, E.T., Coleman, D.C., 1988. Let the soil work for us. Ecol. Bull. 39, 23–32.
Erktan, A., Cecillon, L., Graf, F., Roumet, C., Legout, C., Rey, F., 2016. Increase in soil aggregate stability along a Mediterranean successional gradient in severly eroded gully bed ecosystems: combined effects of soil, root traits and plant community characteristics. Plant Soil 398, 121–137.
Fang, X., Zhou, G., Li, Y., Liu, S., Chu, G., Xu, Z., Liu, J., 2016. Warming effects on biomass and composition of microbial communities and enzyme activities within soil

aggregates in subtropical forest. Biol. Fertil. Soils 52, 353–365.
Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–280.
Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revaillot, S., Maron, P.A., 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biol. Biochem. 43, 86–96.
Goldstein, A.H., Baertlein, D.A., Mcdaniel, R.G., 1988. Phosphate starvation inducible metabolism in Lycopersicon esculentum I. Excretion of acid phosphatase by tomato plants and suspension-cultured cells. Plant Physiol. 87, 711–715.
Gupta, V.V.S.R., Germida, J.J., 2015. Soil aggregation: influence on microbial biomass and implications for biological processes. Soil Biol. Biochem. 80, A3–A9.
Harris, K., Young, I.M., Gilligan, C.A., Otten, W., Ritz, K., 2003. Effect of bulk density on the spatial organisation of the fungus Rhizoctonia solani in soil. FEMS Microbiol. Ecol. 44, 45–56.
Hattori, T., 1988. Soil aggregates in microhabitats of microorganisms. Rep. Inst. Agric. Res. Tohoku Univ. 37, 23–36.
Hoang, D.T.T., Pausch, J., Razavi, B.S., Kuzyakova, I., Banfield, C.C., Kuzyakov, Y., 2016. Hotspots of microbial activity induced by earthworm burrows, old root channels, and their combination in subsoil. Biol. Fertil. Soils. http://dx.doi.org/10.1007/s00374-016-1148-y.
Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the kEN value. Soil Biol. Biochem. 28, 33–37.
John, B., Yamashita, T., Ludwig, B., Flessa, H., 2005. Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. Geoderma 128, 63–79.
Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., Rasche, F., Zechmeister-Boltenstern, S., Sessitsch, A., Richter, A., 2010. Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. New Phytol. 187, 843–858.
Kristiansen, S.M., Schjønning, P., Thomsen, I.K., Olesen, J.E., Kristensen, K., Christensen, B.T., 2006. Similarity of differently sized macro-aggregates in arable soils of different texture. Geoderma 137, 147–154.
Kumar, A., Kuzyakov, Y., Pausch, J., 2016. Maize rhizosphere priming: field estimates using 13C natural abundance. Plant Soil 409, 87–97.
Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: concept & review. Soil Biol. Biochem. 83, 184–199.
Kuzyakov, Y., Xu, X., 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol. 198, 656–669.
Lagomarsino, A., Grego, S., Kandeler, E., 2012. Soil organic carbon distribution drives microbial activity and functional diversity in particle and aggregate-size fractions. Pedobiologia 55, 101–110.
Loeppmann, S., Blagodatskaya, E., Pausch, J., Kuzyakov, Y., 2016. Substrate quality affects kinetics and catalytic efficiency of exo-enzymes in rhizosphere and detritusphere. Soil Biol. Biochem. 92, 111–118.
Marx, M.C., Wood, M., Jarvis, S.C., 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol. Biochem. 33, 1633–1640.
Materechera, S.A., Kirby, J.M., Alston, A.M., Dexter, A.R., 1994. Modification of soil aggregation by watering regime and roots growing through beds of large aggregates. Plant Soil 160, 57–66.
Mganga, K.Z., Razavi, B.S., Kuzyakov, Y., 2015. Microbial and enzymes response to nutrient additions in soils of Mt. Kilimanjaro region depending on land use. Eur. J. Soil Sci. 69, 33–40.
Nie, M., Pendall, E., Bell, C., Wallenstein, M.D., 2014. Soil aggregate size distribution mediates microbial climate change feedbacks. Soil Biol. Biochem. 68, 357–365.
Nuruzzaman, M., Lambers, H., Bolland, M.D.A., Veneklaas, E.J., 2006. Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. Plant Soil 281, 109–120.
Oades, J.M., 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76, 319–337.
Paz-Ferreiro, J., Fu, S., 2016. Biological indices for soil quality evaluation: perspectives and limitations. L. Degrad. Dev. 27, 14–25.
Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol. Lett. 14, 187–194.
Poll, C., Thiede, A., Wermbter, N., Sessitsch, A., Kandeler, E., 2003. Micro-scale distributions of microrganisms and microbial enxyme activities in a soil with long-term organic amendment. Eur. J. Soil Sci. 54, 715–724.
Ranjard, L., Richaume, A., 2001. Quantitative and qualitative microscale distribution of bacteria in soil. Res. Microbiol. 152, 707–716.
Razavi, B.S., Blagodatskaya, E., Kuzyakov, Y., 2015. Nonlinear temperature sensitivity of enzyme kinetics explains canceling effect—a case study on loamy haplic Luvisol. Front. Microbiol. 6. http://dx.doi.org/10.3389/fmicb.2015.01126.
Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2011. Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. Appl. Soil Ecol. 48, 38–44.
Shahbaz, M., Kuzyakov, Y., Heitkamp, F., 2016. Decrease of soil organic matter stabilization with increasing inputs: mechanisms and controls. Geoderma. http://dx.doi.org/10.1016/j.geoderma.2016.05.019.
Simpson, R.T., Frey, S.D., Six, J., Thiet, R.K., 2004. Preferential accumulation of microbial carbon in aggregate structures of no-tillage soils. Soil Sci. Soc. Am. J. 68, 1249.
Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M.P., Zak, D.R., 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75, 201–215.
Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. 79, 7–31.
Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in Agroecosystems. Soil Sci. Soc. Am. J. 70, 555–569.
Tian, J., Pausch, J., Yu, G., Blagodatskaya, E., Gao, Y., Kuzyakov, Y., 2015. Aggregate size and their disruption affect 14C-labeled glucose mineralization and priming effect. Appl. Soil Ecol. 90, 1–10.
Tischer, A., Blagodatskaya, E., Hamer, U., 2015. Microbial community structure and resource availability drive the catalytic efficiency of soil enzymes under land-use change conditions. Soil Biol. Biochem. 89, 226–237.
Tripathi, R., Nayak, A.K., Bhattacharyya, P., Shukla, A.K., Shahid, M., Raja, R., Panda, B.B., Mohanty, S., Kumar, A., Thilagam, V.K., 2014. Soil aggregation and distribution of carbon and nitrogen in different fractions after 41 years long-term fertilizer experiment in tropical rice–rice system. Geoderma 213, 280–286.
Turner, B.L., Hopkins, D.W., Haygarth, P.M., Ostle, N., 2002. Glucosidase activity in pasture soils. Appl. Soil Ecol. 20, 157–162.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.
Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction – an automated procedure. Soil Biol. Biochem. 22, 1167–1169.
Zhang, Q., Zhou, W., Liang, G., Sun, J., Wang, X., He, P., 2015. Distribution of soil nutrients, extracellular enzyme activities and microbial communities across particle-size fractions in a long-term fertilizer experiment. Appl. Soil Ecol. 94, 59–71.
Zhu, B., Gutknecht, J.L.M., Herman, D.J., Keck, D.C., Firestone, M.K., Cheng, W., 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biol. Biochem. 76, 183–192.