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REGULAR ARTICLE

Maize rhizosphere priming: field estimates using ^{13}C natural abundance

Amit Kumar · Yakov Kuzyakov · Johanna Pausch

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Abstract
Introduction Root-mediated changes in soil organic matter (SOM) decomposition, termed rhizosphere priming effects (RPE), play crucial roles in the global carbon (C) cycle, but their mechanisms and field relevance remain ambiguous. We hypothesize that nitrogen (N) shortages may intensify SOM decomposition in the rhizosphere because of increase of fine roots and rhizodeposition.
Methods RPE and their dependence on N-fertilization were studied using a C_3-to-C_4 vegetation change. N-fertilized and unfertilized soil cores, with and without maize, were incubated in the field for 50 days. Soil CO_2 efflux was measured, partitioned for SOM- and root-derived CO_2, and RPE was calculated. Plant biomass, microbial biomass C (MBC) and N (MBN), and enzyme activities (β-1,4-glucosidase; N-acetylglucosaminidase; L-leucine aminopeptidase) were analyzed.
Results Roots enhanced SOM mineralization by 35 % and 126 % with and without N, respectively. This was accompanied by higher specific root-derived CO_2 in unfertilized soils. MBC, MBN and enzyme activities increased in planted soils, indicating microbial activation, causing positive RPE. N-fertilization had minor effects on MBC and MBN, but it reduced β-1,4-glucosidase and L-leucine aminopeptidase activities under maize through lower root-exudation. In contrast, N-acetylglucosaminidase activity increased with N-fertilization in planted and unplanted soils.
Conclusions This study showed the field relevance of RPE and confirmed that, despite higher root biomass, N availability reduces RPE by lowering root and microbial activity.

Keywords C_3/C_4 vegetation change · Soil CO_2 · SOM decomposition · Enzyme activities · Microbial biomass · N-fertilization

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Introduction

Agricultural soils are central with regard to global climate change because they may act either as potential C sinks (Smith et al. 2013) or as net sources of greenhouse gases (Lal 2011; Smith 2012). This makes it important to evaluate and balance the C inputs via living roots (rhizodeposits) and dead plants (litter) versus outputs via SOM decomposition. Altered dynamics of SOM decomposition in the rhizosphere play a significant role in the global C cycle (Coleman et al. 1992). This calls for a better understanding of the SOM dynamics in the rhizosphere in the field. In a meta-analysis, Finzi et al. (2015) showed that microbially mediated SOM decomposition is

enhanced in the rhizosphere of various vegetation types and concluded that rhizospheric processes in SOM decomposition and subsequent nutrient release are quantitatively important at the ecosystem level. Thus, along with the importance of soil moisture and temperature for SOM decomposition, increasing recognition is being given to biotic processes in the rhizosphere regulating SOM decomposition (Zhu and Cheng 2012). Besides various processes occurring in the rhizosphere, rhizodeposition is the most important link between plant growth and microbially mediated processes in soils (Pausch et al. 2013a). The availability of easily utilizable C substrates is a key limiting factors for microbial activity in soil, and C availability is a main factor controlling SOM turnover (Fontaine et al. 2007; Paterson and Sim 2013). Thus, labile C input, e.g. root exudates, may alter the microbial decomposition of SOM, a process termed rhizosphere priming effects (Kuzyakov 2002).

Living roots may either inhibit or stimulate the decomposition of SOM (Dijkstra et al. 2006; Fu et al. 2002; Reid and Goss 1982) via synergistic or antagonistic interactions, or both. The RPE of maize on the decomposition of SOM ranged from −30 % to more than 300 % considering the effects of soil types, time period, N and CO_2 regimes in various studies (summarized by Cheng et al. 2014). Although RPE has been investigated in many studies, the underlying mechanisms are currently widely debated, but there is evidence that RPE mainly depends on decomposable C (Dormaar 1990; Meier et al. 2015) and the mineral N content in soil (Craine et al. 2007).

Rhizodeposition is an ecologically important part of rhizosphere processes because it serves as the primary energy source for microorganisms. This may enhance the metabolic activity of microorganisms and consequently affects the dynamics of SOM decomposition and, thus, rhizosphere priming (Microbial activation hypothesis, Cheng and Kuzyakov 2005; De Nobili et al. 2001; Kuzyakov et al. 2007; Pausch et al. 2013b). A trace amount of root exudates (μg g^{-1}) may enhance the microbially mediated decomposition of SOM (De Nobili et al. 2001). Furthermore, altered root exudation may change the structure and function of microbial communities in the rhizosphere. This subsequently affects the SOM decomposition. Moreover, microbial N mining (Craine et al. 2007) may enhance SOM decomposition when nutrients are limited. Microorganisms as well as plants may thus benefit from nutrients released by extra decomposition of SOM (via RPE).

Here, we investigate the mechanisms of RPE and address the ecological importance of RPE. We applied a C_3-to-C_4 vegetation change in the field to estimate RPE. This approach is based on the discrimination of heavier (^{13}C) and lighter (^{12}C) C isotopes during CO_2 assimilation by C_3 and C_4 plants, which are characterized by distinct photosynthesis types (Balesdent and Mariotti 1996; Kuzyakov and Domanski 2000). Hence, by planting maize, a C_4 plant, on a soil which developed solely under C_3 vegetation, we introduced a distinct isotopic signal. This enabled partitioning total soil CO_2 efflux for root- and SOM-derived CO_2 and thus to estimate the RPE of field-grown maize. Moreover, the extracellular activity of three enzymes (BG, NAG and LAP) was determined to link rhizosphere priming to microbial activities.

We hypothesized that (i) planting increases SOM decomposition via microbial activation through root exudates, and that (ii) mineral N application reduces RPE because plants alter their root activities and microbes are less dependent on nutrient gains from SOM decomposition.

Materials and methods

Experimental setup

The experiment was established on an agriculture field at the experimental research station Reinshof of the Georg-August University, Göttingen, and was solely under C_3 crops. Therefore, the organic C in the soil originated from C_3 vegetation. In this experiment, a vegetation change from C_3 to C_4 (maize) crops was used to introduce a distinct ^{13}C signal into the soil and to partition the total soil CO_2 efflux into root-derived and SOM-derived CO_2.

Four plots (5 × 5 m^2) were established: bare fallow (Unplanted), bare fallow with N-fertilization (Unplanted + N), maize-planted (Planted) and maize-planted with N-fertilization (Planted + N). In both planted plots, maize was grown with a plant density of 6 plants m^{-2}. For N-fertilization, urea (Weiterer, Landhandel GmbH) was applied at the soil surface at a rate of 160 kg N ha^{-1}.

Before the incubation started, maize (*Zea mays* L.) was sown in the field for 10 days. For incubation, mesh pots (height 35 cm, diameter 18 cm) were constructed from stainless metal mesh covered with nylon gauze to

avoid soil losses from the pot. The nylon gauze allowed water and other solute transport across the mesh. Four undisturbed soil cores were collected from each plot (Unplanted, Unplanted + N, Planted, and Planted + N) with a soil corer (height 35 cm, diameter 18 cm) and transferred to the pots. In both planted plots, each soil core contained one 10 cm-high maize plant. The pots were then placed back in the holes made with the corer and incubated in the field.

CO_2 trapping

The pots were incubated in the field for 50 days. We have chosen the time point of 50 days after planting in order to sample during the period of maximum growth and root exudation. Afterwards, the pots were removed from the field and brought to laboratory and placed in a growth chamber for 30 h with conditions adapted to those in the field. Total soil respiration was measured using a closed-circulation CO_2 trapping system (Fig. 1). Briefly, each pot was placed in a PVC column (KG tubes; height 40 cm, diameter 20 cm). Air inlet tubing at the upper end and outlet tubing at the lower end of the PVC column were connected to a membrane pump. An aliquot of 1 M NaOH solution was inserted between the air outlet tubing and membrane pump (Fig. 1). The planted pots were sealed with plastic foil, and at the base of plant stem with a non-toxic gel (Wasserfuhr, GmbH), to avoid any leakage. Prior to CO_2 trapping, CO_2 inside each pot was removed by circulating the isolated air through 1 M NaOH for 2 h. Afterwards, the CO_2 produced in each pot was trapped in 400 ml of 1 M NaOH solution for a period of 24 h by periodic air circulation for 1 h at 6 h intervals. Blanks were included (empty but closed PVC columns) and treated in the same way to correct inorganic C for handling errors. One subsample from each NaOH solution was analyzed for total inorganic C (SHIMADZU,TOC-5050) and another subsample was precipitated as $SrCO_3$ with 1 M $SrCl_2$ for $\delta^{13}C$ analysis using an isotopic ratio mass spectrometer (Delta V Advantage, Conflo III) coupled to an elemental analyzer 2000 (Thermo Fischer Scientific, Cambridge UK).

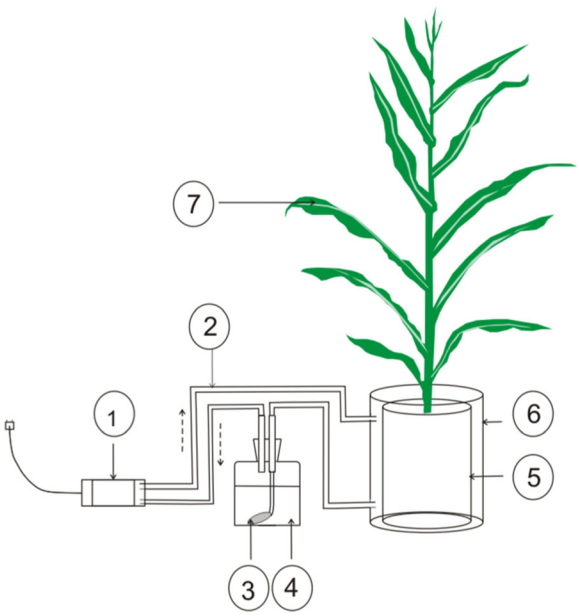

Fig. 1 Experimental setup of the CO_2 trapping system. 1 - membrance pump, 2 - PVC tube (diameter 5 mm), 3 - air stone, 4 - NaOH, 5 - pot, 6 - PVC column, 7 - maize plant. Arrows show the direction of air flow in the closed-circulation system

Total soil CO_2 efflux (C_{total}) was separated into SOM-derived CO_2 (C_{SOM}) and root-derived CO_2 (rhizosphere respiration) (C_{root}) using a two-source mixing model (Pausch et al. 2013b).

$$C_{SOM} = C_{TOTAL}\left(\delta^{13}C_{TOTAL} - \delta^{13}C_{ROOT}\right) / \left(\delta^{13}C_{SOM} - \delta^{13}C_{ROOT}\right) \quad (1)$$

$$C_{ROOT} = C_{TOTAL} - C_{SOM} \quad (2)$$

where, δ_{TOTAL}, δ_{SOM} and δ_{ROOT} are the $\delta^{13}C$ values in ‰ for total CO_2 efflux, SOM- and root-derived CO_2. C_{TOTAL}, C_{SOM} and C_{ROOT} are the CO_2 concentrations (mg C day^{-1} kg^{-1} soil).

RPE was calculated as the difference of C_{SOM} between planted and unplanted soils (Pausch et al. 2013b) as shown below in Eq. 3 and Eq. 4.

$$RPE = C_{SOM(Planted)} - C_{SOM(Unplanted)} \quad (3)$$

$$RPE = C_{SOM(Planted+N)} - C_{SOM(Unplanted+N)} \quad (4)$$

Note, the ^{13}C isotopic fractionation between root C and root-derived CO_2 was not considered in the present study, which may have affected the calculated RPE. As reviewed by Werth and Kuzyakov (2010), the fractionation for C_4 plants is on average − 1.3 ‰ with variations up to ±2 ‰. Since the fractionation is unknown for our plant-soil system, we decided not to include the literature value, as this would not add greater certainty to the results.

Harvest

Directly after CO_2 trapping, the total weight of each pot was determined and the pots were destructively harvested. Shoots were cut at the base and dried at 60 °C for 3 days. The soil cores were pulled out of the pot and the "main" root system was carefully removed. A representative homogenized soil sample (400–500 g) was taken from each pot to determine soil moisture, microbial biomass C and N, and extracellular enzyme activities. Soil moisture was about 12.5 to 14 % and did not differ significantly between the planted and unplanted soils.

For root analyses, a soil subsample (300 g fresh soil) was taken and fine roots were picked from the soil for 15 min. Afterwards, the "main" roots and the fine roots (from root picking) were scanned by an EPSON (PERFECTION™ V700 PHOTO) scanner and the root length density was determined using WinRhizo (Regents Instruments Inc., Quebec, Canada) software. All roots with diameters <2 mm were considered as fine roots. The root length density of roots picked from 300 g soil was up-scaled to the whole pot weight. After scanning, all roots ("main" and picked roots) were dried at 60 °C for 3 days. Roots were analyzed for $\delta^{13}C$ values using the isotopic ratio mass spectrometer and elemental analyzer 2000 noted above. All the isotopic analyses were performed at the Center for Stable Isotope Research Analysis (KOSI) at the University of Göttingen, Germany.

Soil microbial biomass

Soil microbial biomass C (MBC) and N (MBN) were analyzed on fresh samples using the chloroform fumigation-extraction method (Vance et al. 1987). Briefly, a non-fumigated soil sample (8 g fresh soil) was extracted with 40 ml of 0.05 M K_2SO_4 by continuously shaking (Laboratory shaker, GFL 3016) (150 rpm) for 1 h. After shaking, the soil suspension was filtered through Ahlstrom-Munktell filters (Grade: 3hw, diameter 110 mm). The organic C and N contents of filtered solution were measured with a multi N/C analyzer (multi N/C analyzer 2100S, Analytik Jena). The same extraction procedure was followed for fumigated soil. Fumigation was carried out in a desiccator with 80 ml of ethanol-free chloroform at room temperature for 24 h.

MBC and MBN were calculated by dividing the difference between extracted C and N from fumigated and non-fumigated soil samples with a K_{EC} and K_{EN} factor of 0.45 and 0.54, respectively (Joergensen and Mueller 1996). The C and N contents from non-fumigated soil samples were considered as dissolved organic C (DOC) and dissolved N (DN), respectively.

Enzyme assays

Extracellular enzymes activities were measured using the method described by Marx et al. (2001). Fluorogenic methylumbelliferone (MU)-based artificial substrates were used to estimate the activities of β-1,4-glucosidase (EC 2.2.1.21) (BG), which catalyzes the terminal reaction in hydrolyzing structural carbohydrates (i.e. cellulose) and the activities of β-1,4-N-acetylglucosaminidase (EC 3.2.1.14) (NAG), which catalyzes the terminal reaction in chitin and other N-acetylglucosamine-containing polymer hydrolysis. Fluorogenic 7-amino-4-methycoumarin (AMC)-based artificial substrate was used to estimate the activity of L-leucine aminopeptidase (EC 3.4.11.1) (LAP), which hydrolyses the terminal reaction in peptide breakdown, releasing leucine and other amino acids (Sinsabaugh and Shah 2012).

Briefly, soil suspension was made by dissolving 1 g fresh soil sample in 50 ml autoclaved water using a low-energy sonication (50 Js^{-1}) for 120 s (Koch et al. 2007; Stemmer et al. 1998). An aliquot of 50 µl was dispensed in a 96-well black microplate (Puregrade, Germany) while stirring the soil suspension to ensure uniformity. Afterwards, 50 µl of MES buffer (pH 6.5) was added to the well. Finally, 100 µl serial concentrations of substrate solutions (20, 40, 60, 80, 100, 200, 400 µmol substrate g $soil^{-1}$) were added to the wells. The microplate was rippled and measured fluorometrically (excitation 360 nm; emission 450 nm) at 0, 30, 60, 120 m after substrate addition with an automated fluorometric plate-reader (Victor3 1420–050 Multi-label Counter, PerkinElmer, USA).

To estimate enzyme activity (V), we used the Michaelis-Menten equation for enzyme kinetics (Marx et al. 2001, 2005; Razavi et al. 2015):

$$V = (V_{max} \times [S])/(K_m + [S]) \quad (5)$$

where, V_{max} is the maximal rate of enzyme activity; K_m (Michaelis constant) is the substrate concentration at which V_{max} is half; and [S] is the substrate concentration.

Statistics

The experiment was carried out with 4 field replicates for each measured parameter. The values for RPE, microbial biomass C and microbial biomass N, plant biomass, and enzymes activity were expressed as means ± standard errors (mean ± SEM). Prior to analysis of variance (ANOVA), the data were tested for normality (Shapiro-Wilk test, $P > 0.05$) and homogeneity of variance (Levene-test, $P > 0.05$). We used factorial ANOVA to test the effects of plantation and N-fertilization on MBC and MBN, SOM-derived CO_2, and V_{max} of extracellular enzymes. The ANOVAs were followed by post-hoc tests for multiple comparisons using least significant differences (Tukey-test). We used Student's t-test to test the differences in plant biomass (root- and shoot biomass), RPE, root-derived CO_2, specific RPE, and RPE as percent of control in Planted and Planted + N soils. In general, a significance level of $P < 0.05$ was used for ANOVA and t-test if not mentioned specifically. Statistical analyses were performed with STATISTICA for Windows (version 7.0; StatSoft Inc., OK, USA).

Results

Plant biomass

Total plant biomass (shoot and root biomass) per pot was higher in the N-fertilized plants (Planted + N) (36.3 ± 8.1 g pot^{-1}) than in unfertilized plants (Planted) (20.7 ± 2.2 g pot^{-1}) (Fig. 2). The shoot to root ratio was lower in N-fertilized plants (Planted + N) (7.5 ± 0.9) than unfertilized plants (Planted) (10.2 ± 1.3), although the difference was not statistically significant ($P < 0.05$).

Total soil CO_2 efflux and source-partitioning

Plants increased the total soil CO_2 efflux in both N-fertilized and unfertilized soils (Fig. 3). However, the CO_2 efflux was lower in N-fertilized treatments (both in Unplanted + N and Planted + N) compared with unfertilized treatments (Unplanted and Planted). Total CO_2 efflux ranged from 27.7 ± 5.9 to 116.0 ± 26.2 mg C day^{-1} kg^{-1} soil, being lowest in bare fallow with N-fertilization (Unplanted + N) and highest in unfertilized soils planted with maize (Planted).

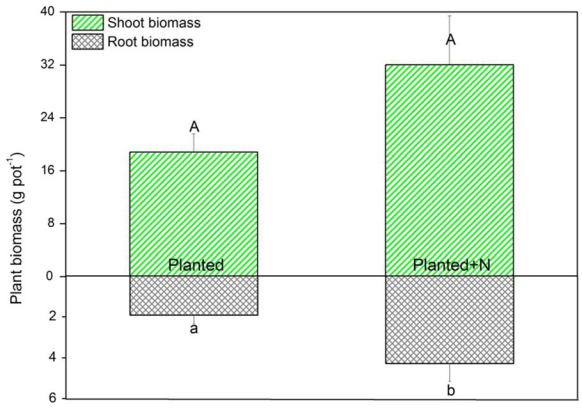

Fig. 2 Plant biomass (root and shoot biomass) (g pot^{-1}) (±SEM) for unfertilized and N-fertilized maize plants. Lower-case letters indicate significant differences for root biomass, upper-case letters indicate significant differences for shoot biomass between N-fertilized and unfertilized maize ($P < 0.05$)

A linear two-source isotopic mixing model was used to calculate the contribution of SOM-derived and root-derived CO_2 to total CO_2 efflux in unfertilized and N-fertilized soils planted with maize. SOM-derived CO_2 was higher (87.4 ± 16.1 mg C day^{-1} kg^{-1} soil) ($P < 0.05$) in unfertilized soils with maize (Planted), whereas N-fertilization resulted in less SOM-derived CO_2 emission (37.2 ± 2.6 mg C day^{-1} kg^{-1} soil) (Planted + N) (Fig. 3). Furthermore, specific root-derived CO_2 was calculated by dividing root-derived CO_2 to total root biomass.

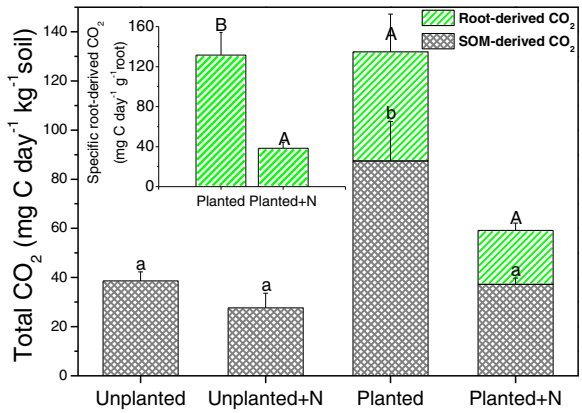

Fig. 3 Total CO_2 efflux (mg C day^{-1} kg^{-1} soil) (±SEM) from bare fallow (Unplanted), bare fallow with N-fertilization (Unplanted + N), unfertilized maize-planted (Planted) and N-fertilized maize-planted (Planted + N) soils. Total CO_2 efflux was partitioned by source (SOM-derived CO_2 and root-derived CO_2). Lower-case letters indicate significant differences between bare fallow, bare fallow with N-fertilization, unfertilized and N-fertilized maize-planted soils (ANOVA, $P < 0.05$). Upper-case letters in root-derived CO_2 and specific root-derived CO_2 (inset) indicate significant differences according to the t-test ($P < 0.05$)

Specific root-derived CO_2 was higher ($P < 0.05$) in unfertilized plants (Planted) (131.6 ± 22.5 mg C day^{-1} g^{-1} root) than in N-fertilized plants with (Planted + N) (38.4 ± 5.8 mg C day^{-1} g^{-1} root) (Fig. 3; inset).

Rhizosphere priming effect

Positive RPE was found in both planted soils (Planted and Planted + N), resulting from enhanced decomposition of SOM. Nonetheless, the RPE was lower ($P < 0.1$) in N-fertilized soils with maize (Planted + N) (9.6 ± 2.6 mg C day^{-1} kg^{-1} soil) compared to unfertilized soils with maize (Planted) (48.8 ± 16.1 mg C day^{-1} kg^{-1} soil). SOM-decomposition in unfertilized and N-fertilized soils with maize increased by 126.2 ± 41.7 % and 34.5 ± 9.2 %, respectively, compared to the unplanted soils (Fig. 4). Specific RPE was calculated by dividing RPE by total root biomass. Specific RPE was higher ($P < 0.05$) in unfertilized soils (Planted) than N-fertilized soil (Planted + N) (Fig. 4; inset).

Microbial biomass and extracellular enzyme activity

Plants had stimulating effects on MBC and MBN. The lowest MBC was in bare fallow (Unplanted), whereas N-fertilization increased MBC (20 %) especially in planted soils (Planted + N) versus bare fallow. There was a trend of increasing MBC with N-fertilization and under plants with and without N-fertilization in the sequence: Unplanted < Unplanted + N < Planted < Planted + N (Fig. 5). Planting also increased MBN ($P < 0.05$) (Planted and Planted + N) compared to bare fallows (Unplanted and Unplanted + N). N-fertilization, however, had only a minor effect on MBN. The ca. 30 % increase in MBN in planted soils reflected microbial activation.

The activities of three enzymes were stimulated by planting (Planted and Planted + N), resulting in increased reaction rates. Planting increased the potential activity of BG (84 % and 97 % for N-fertilized and unfertilized soils with maize), NAG (80 % and 65 % for N-fertilized and unfertilized soils with maize), and LAP (27 % and 53 % for N-fertilized and unfertilized soils with maize) in comparison with N-fertilized and unfertilized bare fallow (Fig. 6). N-fertilization lowered the potential activity of BG and LAP by lowering V_{max} in planted soils, but it increased the activity in bare fallow (not statistically significant). When compared to BG and LAP, NAG showed a different pattern. V_{max} of NAG followed a pattern in ascending order: bare fallow < bare fallow with N-fertilization < unfertilized maize-planted soil < N-fertilized maize-planted soil (Fig. 6).

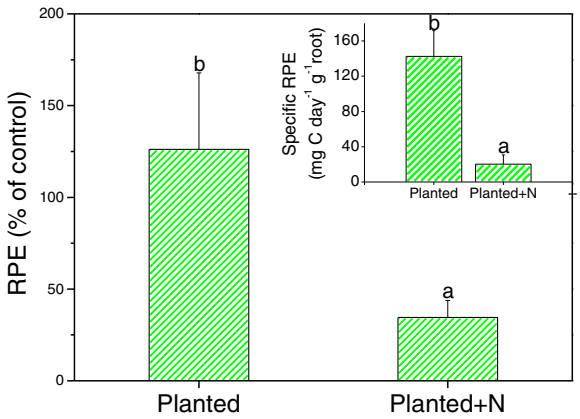

Fig. 4 Rhizosphere priming effect (RPE) (±SEM) as % of CO_2 efflux from bare fallows for unfertilized (Planted) and N-fertilized (Planted + N) maize plants. The inset shows specific RPE (mg C day^{-1} g^{-1} root) (±SEM). Letters indicate the significant differences for RPE ($P < 0.1$); and for specific RPE ($P < 0.05$) between unfertilized and N-fertilized maize-planted soils

Discussion

Effects of living roots on SOM decomposition

The rhizosphere priming effect has been widely observed in numerous studies under controlled conditions, but field studies are still rare (Cheng et al. 2014). Here, we provide measurements of RPE of SOM decomposition in a maize field based on SOM-derived CO_2. In agreement with other studies (Dijkstra et al. 2013; Finzi et al. 2015; Mwafulirwa et al. 2016; Pausch et al. 2013b), the plants accelerated the decomposition of SOM.

Positive priming has often been explained by the microbial-activation hypothesis (Chen et al. 2014; Cheng and Kuzyakov 2005). The secretion of labile C compounds by roots enhances microbial growth and activity, leading to higher extracellular enzyme activities and, hence, accelerated SOM decomposition (Fig. 7) (Fontaine et al. 2003; Kuzyakov 2010; Loeppmann et al. 2016; Neumann and Römheld 2007).

SOM-derived CO_2 was about 35 % higher for N-fertilized and 126 % higher for unfertilized planted soils

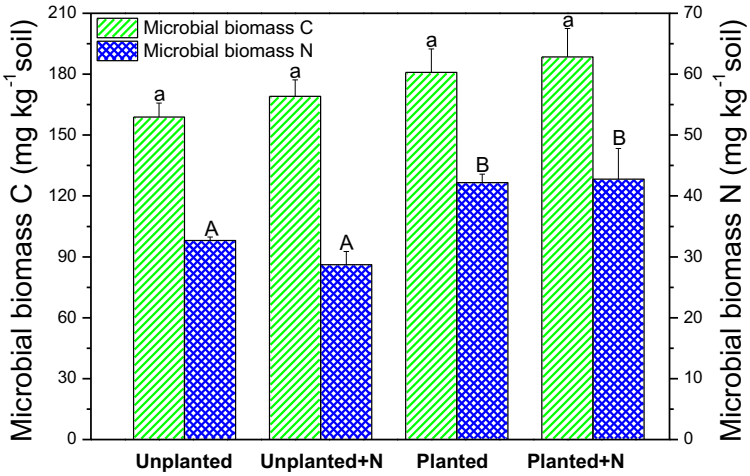

Fig. 5 Microbial biomass C (left y-axis; mg C kg^{-1} soil) and N (right y-axis; mg N kg^{-1} soil) (±SEM) in bare fallow (Unplanted), bare fallow with N-fertilization (Unplanted + N), unfertilized maize-planted (Planted) and N-fertilized maize-planted (Planted + N) soils. Lower-case letters indicate significant differences for MBC, upper-case letters indicate significant differences for MBN between bare fallow, bare fallow with N-fertilization, unfertilized and N-fertilized maize-planted soils (ANOVA, $P < 0.05$)

compared to the bare fallows. Accompanied by positive rhizosphere priming, MBC and MBN were increased through planting (Fig. 4). Furthermore, in the rhizosphere; the higher microbial activity in response to root exudation (root-released easily available substrates) was characterized by increased V_{max} for BG, NAG and LAP in comparison with bare fallows. The rhizosphere priming effect increased with activities of BG, LAP and NAG. In a similar study, BG activity and SOM decomposition were positively correlated (Zhu et al. 2014).

BG is involved in the degradation of structural carbohydrates (i.e. cellulose). It catalyzes terminal hydrolysis in cellulose degradation by producing two moles of glucose per mole of cellobiose, and glucose is an

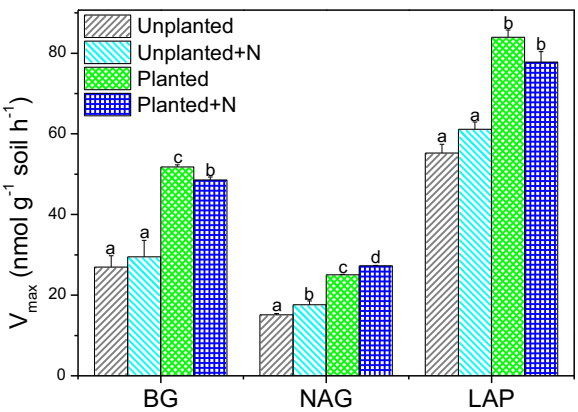

Fig. 6 Potential activity (Vmax; nmol g $soil^{-1}$ h^{-1}) (±SEM) of three extracellular enzymes (BG: β-1, 4-glucosidase; NAG: β-1, 4-N-acetylglucosaminidase; LAP: L-leucine aminopeptidase) in bare fallow (Unplanted), bare fallow with N-fertilization (Unplanted + N), unfertilized maize-planted (Planted) and N-fertilized maize-planted (Planted + N) soils. Letters indicate significant differences between bare fallow, bare fallow with N-fertilization, unfertilized and N-fertilized maize-planted soils (ANOVA, $P < 0.05$)

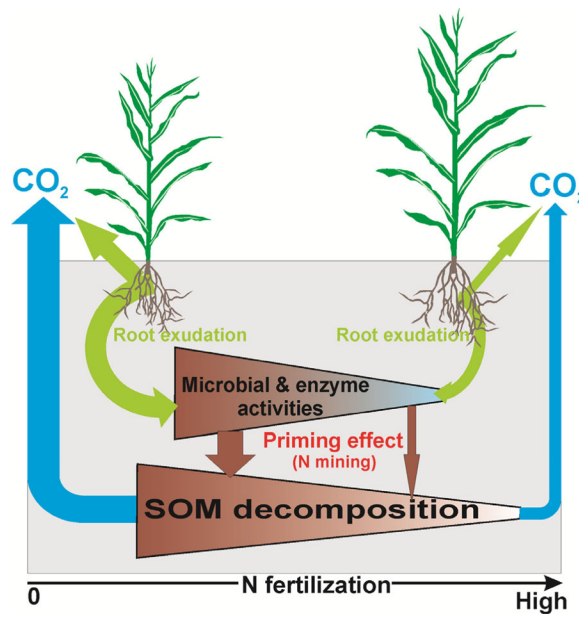

Fig. 7 Conceptual figure showing rhizosphere priming on SOM decomposition accompanied by microbial activation and N mining. Arrow thickness indicates process intensity

important energy source for microorganisms (Turner et al. 2002). Furthermore, BG synthesis is triggered by the presence of cellobiose, glucose and other metabolites of cellulose degradation (Stewart and Leatherwood 1976). In the rhizosphere, root exudation triggers enhanced synthesis of BG, which is accompanied by RPE in the decomposition of SOM.

When labile C sources with high C/N ratios are available for microorganisms, they start producing N-degrading enzymes to obtain N from SOM (Fontaine et al. 2011). Proteins and chitins are the most abundant organic N sources (Moorhead et al. 2012). For proteins, LAP is involved in the terminal hydrolysis of polypeptides, releasing amino acids. For chitins, NAG hydrolyses N-acetylglucosamine (monosaccharide derivative of glucose) from chito-oligosaccharides (i.e. chitobiose) (Sinsabaugh 1994). LAP and NAG are the most commonly detected N-degrading enzymes for SOM decomposition (Moorhead et al. 2012). Taking into account the microbial activation hypothesis (Cheng and Kuzyakov 2005), the present study provides evidence that, also under field conditions, living roots activate microorganisms for SOM decomposition.

Effect of N-fertilization on SOM decomposition

Planting induced positive priming effects in the rhizosphere (Fig. 4). When N was added, however, the extra SOM decomposition (positive RPE) was much lower in N-fertilized soils (35 % of bare fallow with N-fertilization) than in unfertilized soils (126 % of bare fallow), despite a higher shoot and root biomass of N-fertilized maize plants. Increased N supply may increase net assimilation, and plants produce higher biomass (Hodge et al. 1996; Warembourg and Estelrich 2001; Zhu et al. 2015). Although the root biomass of N-fertilized maize was about twice as high, the rhizosphere respiration (root-derived CO_2) was lower, indicating lower root activity (Fig. 7). It is well known that plants invest more C resources for root exudation under nutrient limitations (Hodge et al. 1996; Kraffczyk et al. 1984; Ratnayake et al. 1978). Root exudation also stimulates microbial activity and nutrient availability (Smith 1976; Yin et al. 2013). Both theoretical (Cheng et al. 2014; Wutzler and Reichstein 2013) and experimental studies (Drake et al. 2013; Phillips et al. 2011) have shown that enhanced root exudation may accelerate RPE for SOM decomposition, thus increasing the flux of nutrients to forms available for plants. Reduced root exudation in N-fertilized maize plants, indicated by lower root-derived CO_2 (consisting of CO_2 from root respiration and CO_2 released by decomposition of exudates), showed that these plants do not rely solely on nutrients from SOM decomposition. RPE can increase with increasing root activity (Zhu et al. 2014). Rhizosphere respiration was positively correlated with RPE ($P < 0.01$). In addition, the specific root length density (fine root length density (< 2 mm) per gram root) was higher ($P < 0.059$) for unfertilized maize plants (data not presented). This altered root architecture of unfertilized maize may help to make the limiting resources accessible by maximizing root surface area and enabling roots to have greater contact with soil surfaces (Paterson and Sim 1999).

These findings suggested that root activity is intimately connected with microbially mediated SOM decomposition. Furthermore, root exudates are characterized by high C to N ratios (Cheng and Kuzyakov 2005). Such high ratios results in higher C availability and a severe N limitation for microorganisms (Kuzyakov and Blagodatskaya 2015; Kuikman et al. 1990; Liljeroth et al. 1990; Merckx et al. 1987). Moreover, there is a strong competition for mineral N between roots and microorganisms (Kuzyakov and Xu 2013). With addition of N-fertilizer, microorganisms are less dependent on extra N released via priming because the competition between plant roots and microorganisms for mineral N becomes weaker (Van Veen et al. 1989): microorganisms start utilizing exudates and the available mineral N (preferential substrate utilization) (Kuzyakov 2002; Sparling et al. 1982). The present study detected no differences for the effect of N-fertilization on microbial biomass C and N. Nonetheless, N-fertilization altered extracellular enzyme activity differently. Inorganic fertilizers may either maintain or reduce the activities of many extracellular enzymes in planted soils, but increase their activities in unplanted soils (Ai et al. 2012). In the present research, the activities of BG and LAP were generally lowered in maize-planted soils with N-fertilization, whereas their activities increased in bare fallow with N-fertilization. Moreover, the NAG activity increased in both bare fallow and maize-planted soils with N-fertilization. This could be an indirect evidence for shifts in microbial communities in favor of fungi. Various studies showed this shift in microbial taxonomic groups in favor of fungi with N-fertilization (Bardgett et al. 1999; Paul and Clarke. 1996 in Keeler et al. 2009; Weand et al. 2010). In summary, N-fertilization lowered the root release of available C, which subsequently

lowered microbial activity by decreasing extracellular enzyme production. The net result is less SOM decomposition in the rhizosphere.

Conclusions

RPE were measurable in the field. Higher MBC and MBN in the rhizosphere support the microbial activation by root exudation. This microbial activation is accompanied by increased extracellular enzyme activities, which further confirm that extracellular enzyme production is an important mechanism of SOM decomposition in the rhizosphere. The N status of soils largely controls the magnitude of rhizosphere priming. N fertilization substantially reduced rhizosphere priming by lowering SOM decomposition. Lower root-derived CO_2 and enzyme activities in the rhizosphere with N-fertilization confirmed that the availability of mineral N weakens the competition between roots and microorganisms. However, increased root-derived CO_2 and enzyme activities without N fertilization intensify the root and microbial competition for N and the dependence of microorganisms on N mining.

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