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BIOCHAR FOR A SUSTAINABLE ENVIRONMENT

Interactive effects of biochar and polyacrylamide on decomposition of maize rhizodeposits: implications from ^{14}C labeling and microbial metabolic quotient

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Abstract
Purpose The applications of biochar (BC) and polyacrylamide (PAM) may have interactive effects on carbon (C) dynamics and sequestration for improving the soil quality and achieving sustainable agriculture. Relative to BC and PAM, rhizodeposits act as C and energy source for microorganisms and may change the mineralization dynamics of soil organic matter (SOM). No attempt has been made to assess the effects of BC, anionic PAM, or their combination on the decomposition of different aged ^{14}C-labeled rhizodeposits. The objective of this study was to investigate the effects of the treatments mentioned above on the decomposition of different aged ^{14}C-labeled maize rhizodeposits.
Materials and methods biochar (BC) at 10 Mg ha^{-1} or anionic PAM at 80 kg ha^{-1} or their combination (BC + PAM) was applied to soils with/without 2-, 4-, 8-, and 16-day-aged ^{14}C-labeled maize rhizodeposits. After that, the soil was incubated at 22 °C for 46 days.
Results and discussion After 2 days of incubation, the total CO_2 efflux rates from the soil with rhizodeposits were 1.4–1.8 times higher than those from the soil without rhizodeposits. The cumulative $^{14}CO_2$ efflux (32 % of the ^{14}C input) was maximal for the soil containing 2-day-aged ^{14}C-labeled rhizodeposits. Consequently, 2-day-aged rhizodeposits were more easily and rapidly decomposed than the older rhizodeposits. However, no differences in the total respired $^{14}CO_2$ from rhizodeposits were observed at the end of the incubation. Incorporation of ^{14}C into microbial biomass and 66–85 % of the ^{14}C input remained in the soil after 46 days indicated that neither the age of ^{14}C-labeled rhizodeposits nor BC, PAM, or BC + PAM changed microbial utilization of rhizodeposits.
Conclusions Applying BC or BC + PAM to soil exerted only minor effects on the decomposition of rhizodeposits. The contribution of rhizodeposits to CO_2 efflux from soil and MBC depends on their age as young rhizodeposits contain more labile C, which is easily available for microbial uptake and utilization.

Keywords Biochar · Decomposition of rhizodeposits · Soil organic matter · Polyacrylamide · Responsible editor: Yu Luo

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1 Introduction

Soil organic matter (SOM) plays a significant role in maintaining soil quality and sustainable agriculture. The loss of topsoil along with SOM because of erosion decreases the soil quality and consequently reduces crop yields (Nagle 2001; Jien and Wang 2013; Lal 2004; Lee et al. 2015). The annual

loss of farmland because of water and wind erosion has been estimated at 75 billion metric tons globally and 50 million tons in Korea (Awad et al. 2012). For instance, soil erosion in England and Wales results in annual costs of £205 million because of on-farm and cleanup operations (Sojka et al. 2007). Therefore, the development of technology to reduce soil erosion and maintain soil quality is an urgent necessity.

Carbon input is an effective method to maintain SOM and enhance soil quality and productivity (Kuzyakov et al. 2007). Specifically, the following two primary sources of plant-derived C contribute to the accumulation of SOM: (1) plant residues and (2) rhizodeposits, i.e., C released into the soil during the plant growth period (Kuzyakov and Cheng 2004; Kumar et al. 2006). For instance, plant residues protect soils by increasing infiltration and decreasing surface runoff and sheet and rill erosion, resulting in a 12 % reduction of soil loss (Santhi et al. 2006; Panagos et al. 2015). Rhizodeposits increase crop yield in intercropping systems by mobilizing nutrients (Zang et al. 2015). In particular, belowground N of about 10 kg N ha^{-1} is transferred from rhizodeposition of cowpea to millet in intercropping systems. A wide range of more than 200 organic compounds is known to be released as rhizodeposits (Kuzyakov and Domanski 2000). Approximately one third of the total assimilated C released into the soil from the roots of cereal plants is respired by microorganisms (Kuzyakov and Domanski 2000). The remaining C is incorporated into SOM, microorganisms, or adsorbed on clay minerals (Kuzyakov et al. 2000).

Rhizodeposits play complex roles in soil C turnover and are more than just a source of energy for microorganisms (Marx et al. 2007; Kuzyakov et al. 2007). The decomposition of plant residues as related to soil C turnover has been widely investigated, whereas mineralization of rhizodeposits has not yet been sufficiently studied (Kumar et al. 2006). One of the main limiting factors for understanding C dynamics in the rhizosphere is the difficulty in distinguishing between C derived from the decomposition of SOM and that derived from rhizodeposits (Kuzyakov and Cheng 2004). Consequently, continuous or pulse ^{13}C- or ^{14}C-labeling techniques have been applied to separate sources of C and to estimate C turnover and root-derived C in soil CO_2 efflux and microbial biomass (Werth and Kuzyakov 2008). These tracer methods allow the separation of plant-derived C from native SOM and the quantification of C derived from plant residues or rhizodeposits (Merckx et al. 1987; Gorissen and Cotrufo 2000; Chen et al. 2009).

From a practical point of view, anionic polyacrylamide (PAM) has been commonly used as a soil conditioner to reduce erosion by means of clay flocculation and by binding particles and stabilizing the outer aggregate surfaces (Orts et al. 2007; Sojka et al. 2007). Moreover, PAM is highly resistant to microbial degradation, with a decomposition rate of ~10 % yr^{-1}, and therefore can be efficiently used as a soil conditioner for long periods (Sojka et al. 2007; Entry et al. 2008; Lee et al. 2009). Wu et al. (2012) revealed that PAM showed a minor effect on the ^{14}C allocation in plant parts and soil in a short-term ^{14}C-labeling study. Except for the observations of PAM biotransformation, little information is available on the PAM effect on microbial activity in soils (Kay-Shoemake et al. 1998; Wu et al. 2012). In contrast, knowledge of the agronomic benefits of biochar (BC) application to improve soil quality and increase SOM is growing (Cross and Sohi 2011; Zhang and Ok 2014; Ok et al. 2015; Liu et al. 2016). Applying BC improves the physicochemical properties of soil and maintains the C and N sources, leading to a possible increase in the plant growth and yield (Glaser et al. 2002; Glaser 2007; Novak et al. 2016). Moreover, wood biochar has been shown to decrease soil bulk density and enlarged the diameter of aggregates, thereby reducing soil loss (Jien and Wang 2013; Lee et al. 2015). However, little is known about the effects of BC on the soil microbial populations and activities (Lehmann et al. 2011). BC or PAM showed a minor effect on the decomposition of SOM based on CO_2 evolution because of the stability of these conditioners (Awad et al. 2012, 2013). A high portion of ^{14}C-maize residues was stabilized in the soil after adding BC and PAM as binders through the occlusion of labile residue-C into aggregates (Awad et al. 2013). Understanding the short-term changes in dynamics of C caused by PAM or BC is necessary for applying better practices and cost-effectiveness policy to reduce SOM erosion, increase C sequestration, and maintain soil quality (Lu and Zhang 2015; Borrelli et al. 2016; Chappell and Baldock 2016). For instance, the loss of SOM enriched material and plant nutrients from the topsoil because of wind and water erosion decreases soil quality and carbon content in agricultural landscapes at sites of erosion (Lee et al. 2015; Chappell and Baldock 2016; Jague et al. 2016). It is evident that fertile soil with high SOM content shows long-term stability and better C preservation compared to soil with low SOM level (Zhu et al. 2016).

A combination of oak wood BC and PAM (BC + PAM) significantly improved soil physicochemical properties and growth of maize and soybean in pot and field-plot experiments (Lee et al. 2015). In addition, BC + PAM reduced runoff and soil loss under simulated and natural rainfalls (Lee et al. 2015). Therefore, investigating the interactive effects of BC and PAM on the decomposition of rhizodeposits and soil CO_2 efflux is essential. BC, PAM, or their combination may alter the decomposition of SOM and rhizodeposits owing to the improved physicochemical properties of the soil. We hypothesized that rhizodeposits might enhance the microbial decomposition rate of SOM owing to the improved physicochemical and biological properties of rhizosphere soil, because of higher CO_2 effluxes than those from the bulk soil. To date, no attempt has been made

to assess the effects of BC, anionic PAM, or BC + PAM on the decomposition of different aged ^{14}C-labeled rhizodeposits. In this study, we therefore investigate whether or not BC, PM, or BC + PAM will enhance the decomposition of rhizodeposits in soil. Here, for the first time, we report the interactive effects of BC and PAM on the decomposition of rhizodeposits of maize in soil by measuring microbial biomass and specific respiration activity. Furthermore, we investigate the effects of the quantity and age of ^{14}C-labeled rhizodeposits on soil CO_2 efflux.

2 Materials and methods

2.1 Soil sampling and analyses

Soil, a loamy haplic Luvisol originated from loess, was collected from the upper 10 cm of an agricultural field near Göttingen, Germany (51° 33′ 36.8″ N, 9° 53′ 46.9″ E). The soil was air-dried, homogenized, and passed through a 2-mm sieve. The water-holding capacity (WHC) of the soil (17.9 %) was measured gravimetrically using the sieved samples (Veihmeyer and Hendrickson 1931). The soil parameters have been reported earlier by Kramer et al. (2012) and Pausch et al. (2013). The soil total carbon (TC) and nitrogen (TN) were analyzed using a solid sample module of multi-N/C 2100 S analyzer (Analytik, Jena, Germany) and were 13.1 mg g^{-1} TC and 1.37 mg g^{-1} TN, giving a C/N ratio of 9.58. Phosphorus and sulfur were measured in double lactate extracts, while the effective CEC was measured in 0.05-M NH_4Cl extracts. Nitrate was extracted with 0.0125 M $CaCl_2$ and measured by flow injection analysis (FIA). The concentrations of extractable/available NO_3^-, P, and S were 83, 160, and 9 mg kg^{-1}, respectively, whereas the CEC with 99.7 % base saturation was 107.8 $mmol_c$ kg^{-1}.

2.2 Production of ^{14}C-labeled rhizodeposits

Three prevernalized seedlings of maize (*Zea mays* L.) were planted per pot (pot height 30 cm, inner diameter 14 cm) containing 3-kg air-dried soil each. The plants were labeled simultaneously, 28 days after germination, for 4 h as described by Pausch et al. (2013). A solution of $Na_2{}^{14}CO_3$ (ARC Inc., St. Louis, MO, USA) containing 138.5 kBq of ^{14}C per pot was used. Further details of the labeling and partitioning of ^{14}C within the plants are reported by Pausch et al. (2013).

After labeling, the plants and rhizosphere soils were sampled destructively at 2, 4, 8, and 16 days after the ^{14}C pulse labeling. The roots were separated by handpicking, and the soil adhering to the roots, separated by slightly shaking the roots, was considered as rhizosphere soil. The wet rhizosphere soils, containing ^{14}C-labeled rhizodeposits of different amounts and ages (2, 4, 8, and 16 days), were used in the incubation experiment after removing root debris. Specifically, the ^{14}C activities of the sampled soils containing ^{14}C-labeled maize rhizodeposits were determined by combustion within an oxidizer.

2.3 Incubation

Oak wood BC produced at 400 °C (Sootgage, Gyeonggi, Korea) and synthetic PAM (Magnafloc 336, Ciba Canada Ltd., ON, Canada) were used in the experiment. The pH of the PAM and BC (1:10 conditioner and water mixtures) were 7.4 and 10.2, respectively. Characteristics of BC were described by Ahmad et al. (2012). Specifically, the C, H, O, and N contents in BC were 88.7, 1.21, 9.72, and 0.36 %, respectively. The molar H/C and O/C ratios were 0.16 and 9.72, respectively. Exchangeable cations in BC were 13.52, 0.08, 8.74, and 2.04 $cmol_{(+)}$ kg^{-1} for Ca, Na, K, and Mg, respectively. The organic matter (OM) in BC was 62.88 g kg^{-1}. The ash, mobile matter, and fixed matter contents in BC were 5, 31.4, and 56 %, respectively. BC had a surface area of 270.8 m^2 g^{-1}, and its pore size and volume were 1.1 nm and 0.12 cm^3 g^{-1}, respectively.

The incubation experiment consisted of 20 treatments with 4 replicates each in a completely randomized factorial design, including 2 factors. The first factor was the application of conditioners to soil without rhizodeposits (bulk soil): (i) BC at 10 Mg ha^{-1}, (ii) a solution of PAM (500 mg L^{-1}) at a rate of 80 kg ha^{-1}, (iii) a combination of BC + PAM, and (iv) no addition of conditioner (in the following termed "control"). The application rates of BC and PAM were selected according to the previous study by Lee et al. (2015). The second factor was rhizosphere soils with different-aged ^{14}C-labeled rhizodeposits with/without the addition of conditioners: (i) soil with 2-day-aged rhizodeposits after labeling of the shoots in $^{14}CO_2$ atmosphere, (ii) soil with 4-day-aged rhizodeposits, (iii) soil with 8-day-aged rhizodeposits, and (iv) soil with 16-day-aged rhizodeposits.

Wet bulk and rhizosphere (35-g dried soil) soils were mixed thoroughly with BC, PAM, and their combination. Rhizosphere soils with ^{14}C-labeled maize rhizodeposits had the ^{14}C activities of 0.022, 0.014, 0.013, and 0.01 kilobec querels per gram soil (kBq g^{-1}) for pots sampled at 2, 4, 8, and 16 days after labeling, respectively. The soils were mixed thoroughly with one of the conditioners (BC, PAM, or PAM + BC) and placed in sealed vessels for incubation at 22 °C for 46 days. Soil moisture was maintained at 70 % of WHC with deionized water throughout the experiment.

To trap CO_2, small vials containing 2 mL of 1 M NaOH were placed in the vessels. These vials were changed periodically at 2, 4, 8, 13, 18, 25, 32, and 46 days during the incubation period to measure CO_2 and ^{14}C activities. Four empty vessels containing only NaOH vials were used as blanks.

2.4 CO_2 efflux and ^{14}C analyses

To estimate the amount of CO_2 trapped in the 1 M NaOH, the carbonates in the solution were precipitated with 0.5 M $BaCl_2$. NaOH was then titrated with 0.1 M HCl against phenolphthalein indicator (Zibilske 1994). A 1-mL aliquot of the NaOH solution was mixed with 2 mL of Rothiscint-22× scintillation cocktail to measure the ^{14}C activity of the trapped CO_2 (Carl Roth Co., Germany). The ^{14}C activity of rhizodeposits in the soils was measured at the beginning and end of the incubation period. Specifically, 0.5 g of soil was combusted in an oxidizer unit (Feststoffmodul 1300, Analytik Jena, Germany), and the evolved CO_2 was absorbed in 10 mL of 1 M NaOH. Thereafter, 2-mL aliquots of the NaOH solution were mixed with 5 mL of the scintillation cocktail, and the ^{14}C activity was measured after the decay of chemiluminescence using a liquid scintillation counter (LSC; MicroBeta TriLux, 205 Perkin Elmer Inc., Waltham, MA, USA). Measurement error did not exceed 3 %, and ^{14}C counting efficiency was ~93 %.

2.5 Microbial biomass

The soil microbial biomass carbon (MB-C) and nitrogen (MB-N) were determined at 2 and 46 days of incubation by the chloroform fumigation-extraction method (modified after Vance et al. 1987). In particular, a 5-g portion of soil was extracted with 20 mL of 0.05 M K_2SO_4. Another portion of soil (5 g) was first fumigated with ethanol-free chloroform in a desiccator for 48 h and then extracted as described for the unfumigated soil. The extracts were frozen until analysis of TC and TN contents took place using a multi-N/C 2100 S analyzer (AnalytikJena, Germany). To calculate the ^{14}C incorporated in the microbial biomass ($^{14}C_{MB}$), the ^{14}C activity of K_2SO_4-extractable C was measured in the fumigated and unfumigated soils by mixing 2 mL of soil extract with 5 mL of scintillation cocktail (Blagodatskaya et al. 2011). The ^{14}C activity was measured by LSC as described above.

2.6 Calculations and statistical analysis

The CO_2-C efflux rates (μg C day^{-1} g^{-1} soil) and cumulative CO_2-C effluxes (mg C g^{-1} soil) were calculated according to the method described by Kuzyakov and Cheng (2004). The CO_2 efflux from the control without conditioner was subtracted from that of treatments with conditioner application to estimate the CO_2 efflux caused by the decomposition of each conditioner according to Awad et al. (2013). In addition, the CO_2 efflux from the soil with rhizodeposits was subtracted from that of treatments with each conditioner with rhizodeposits. This was done separately for each variant with and without rhizodeposits. The cumulative $^{14}CO_2$-C efflux was calculated as the increase in $^{14}CO_2$-C within each sampling interval and was represented as the percentage (%) of ^{14}C input. The initial ^{14}C activities were 0.78, 0.49, 0.47, and 0.37 kBq per vessel containing soils with 2-, 4-, 8-, and 16-day aged ^{14}C-labeled maize rhizodeposits, respectively. The ^{14}C remaining in the soil was calculated as the proportion of ^{14}C input. Microbial biomass C (MB-C) and microbial biomass N (MB-N) were calculated as described by Wu et al. (1990) and Brookes et al. (1985), respectively. Microbial biomass ^{14}C (percent of the ^{14}C input) was calculated as follows:

$$\mathrm{MB}{-}^{14}\mathrm{C} = \left[\left[\left({}^{14}\mathrm{C}_{\mathrm{MB-F}} - {}^{14}\mathrm{C}_{\mathrm{MB-Unf}}\right) : {}^{14}\mathrm{C}_{\mathrm{input}}\right] \times 100\right]/\mathrm{kEC},$$

where $^{14}C_{MB\text{-}F}$ = the activity of ^{14}C in fumigated soil extract disintegrations per minute per gram soil (DPM g^{-1}), $^{14}C_{MB\text{-}Unf}$ = the activity of ^{14}C in unfumigated soil extract (DPM g^{-1}), $^{14}C_{input}$ = activity of ^{14}C rhizodeposits input (DPM g^{-1}), and kEC = 0.45.

The metabolic quotient (qCO_2) or specific respiration activity was calculated as the production of CO_2 per unit MB-C and time (milligram of carbon mineralized per gram of microbial biomass per unit time (mg CO_2-C h^{-1} g^{-1} MBC)) in accordance with Anderson and Domsch (1993) and Leita et al. (1995).

The data were analyzed using SAS/STAT 9.1. The standard error of the means was calculated from four replicates of each treatment. Variable means were compared using a two-way factorial analysis of variance (ANOVA) and Tukey's honestly significant differences test at $P < 0.05$ (SAS 2004). In addition, multifactorial ANOVA was performed to incorporate the effects of rhizodeposit age (days after ^{14}C labeling), soil conditioners, and incubation time on the measured variables (CO_2 efflux, cumulative CO_2, $^{14}CO_2$ efflux, cumulative $^{14}CO_2$, MB-C, MB-N, MB-^{14}C, and qCO_2). Furthermore, the data were modeled as a generalized linear model (GLM) to integrate the effects of amendments (rhizodeposits and soil conditioners), incubation time (within repeated measurements of CO_2 and $^{14}CO_2$, eight repeated measurements within four replicates during 0–46 days of the incubation period and two repeated measurements of MB-C, MB-N, MB-^{14}C, and qCO_2), and their interaction (amendments × time) on the tested variables.

3 Results

3.1 Soil CO_2 efflux

The highest CO_2 efflux rates in all the treatments in soil without rhizodeposits were observed at 2 days of the incubation period (Fig. S1, Electronic Supplementary Material). CO_2 efflux rates decreased sharply during days 2–8 and then stabilized at a low level until the end of the incubation period (Fig. S1, Electronic Supplementary Material). In the treatment without rhizodeposits, no significant differences in CO_2 efflux

rates or cumulative CO_2 were observed among the three conditioners (BC, PAM, and PAM + BC) and the control soil without conditioner application during the incubation (Fig. S1, Electronic Supplementary Material and Fig. 1).

Total CO_2 efflux from the control soil with 8- and 16-day-aged rhizodeposits increased by 1.4–1.8 times as high as those of the control soil without rhizodeposits at 2–8 days of incubation (Fig. S1, Electronic Supplementary Material). Similarly, BC, PAM, and BC + PAM significantly increased CO_2 efflux rates from soils with 8- and 16-day-aged rhizodeposits at 2–4 days of the incubation period compared with those from the control soil without rhizodeposits (Fig. S1, Electronic Supplementary Material). In contrast, the CO_2 efflux rate decreased by 22.7 % at 2 days of the incubation period in BC + PAM-treated soil containing 16-day-aged rhizodeposits compared to the control soil with rhizodeposits (Fig. S1, Electronic Supplementary Material). The cumulative CO_2 after 2–8 days of the incubation period decreased by 11.1–25.4 % in BC + PAM-treated soil containing 16-day-aged rhizodeposits (Fig. 1). No significant differences in cumulative CO_2 were observed among the three conditioners (BC, PAM, and PAM + BC) and the control soils with rhizodeposits at the end of incubation.

The relatively small CO_2 efflux rates derived from the difference in CO_2 between the soil treated with conditioner and the control soil without or without rhizodeposits (Table S1, Electronic Supplementary Material) suggested a slow decomposition rate of BC, PAM, and their combination.

3.2 Decomposition of rhizodeposits and ^{14}C retained in soil

The maximum decomposition rates of the rhizodeposits occurred at 2 days of the incubation period in the range of 3.5–5.5 % of the ^{14}C input day^{-1} and then decreased during incubation. The highest cumulative $^{14}CO_2$ efflux was observed in the soil containing 2-day-aged rhizodeposits (32.1 % of the ^{14}C input at 46 days).

The PAM and BC + PAM treatments decreased decomposition of 2-day-aged rhizodeposits when compared to the control soil without PAM and BC. In particular, the PAM and BC + PAM treatments reduced the cumulative $^{14}CO_2$ effluxes in soil with 2-day-aged rhizodeposits at the end of the incubation by 17 and 17.8 %, respectively. The PAM significantly reduced the decomposition of 4-day-aged rhizodeposits by 16.0 and 14.7 % at 2 and 4 days of the incubation period, respectively, compared to those in the control soil with rhizodeposits (Fig. 2 and Table S2, Electronic Supplementary Material). Thus, the soil treatment with BC and PAM led to a better stabilization of derived C from 2-day-aged rhizodeposits (Fig. 2).

Approximately 66–85 % of the ^{14}C input were retained in the soil at 46 days of incubation, with no significant difference between treatments with ^{14}C rhizodeposits of different amounts and ages (Fig. S2, Electronic Supplementary Material). This finding indicates that a high proportion of rhizodeposits was retained in the soil and might have been stabilized into soil aggregates.

3.3 Metabolic quotient and microbial biomass

No significant differences in the metabolic quotient (qCO_2), MB-C, or MB-N were observed at 2 and 46 days of the incubation period among all treated soils without rhizodeposits (Fig. S3, Electronic Supplementary Material). In contrast, the BC-treated soils showed higher qCO_2 at 2 days of incubation than that of the control soils (no conditioner) containing 2-day-aged rhizodeposits (Fig. 3). No significant differences in MB-C or MB-N were observed at 2 and 46 days of the incubation period among all treated soils with rhizodeposits (Fig. 3).

The MB-N increased significantly at 2 days of the incubation period in soil with rhizodeposits and conditioners when compared with soil without rhizodeposits (Fig. S3, Electronic Supplementary Material and Fig. 3).

At 2 days of the incubation period (Fig. 4), all the treated soils containing 2-day-aged rhizodeposits showed higher MB-^{14}C than that of the soils containing longer-aged rhizodeposits (from 4 to 16 days). No significant effect on the MB-^{14}C of soil treated with conditioners was observed at the end of the incubation period compared to that of the control soil (Fig. 4).

The multifactorial ANOVA of the tested variables showed significant responses to the age of rhizodeposits, soil conditioners, and time of incubation (Table S3, Electronic Supplementary Material); furthermore, the GLM repeated measures analysis (Table S4, Electronic Supplementary Material) revealed a significant linear relationship between amendments and time or their interaction (amendments × time) and the tested variables (CO_2 efflux, cumulative CO_2, $^{14}CO_2$ efflux, cumulative $^{14}CO_2$, and qCO_2). Time (two repeated measurements with three replicates at 2 and 46 days of the incubation) appeared not to have a significant effect on MB-C, MB-N, and MB-^{14}C, whereas amendments and their interaction with time (amendments × time) exerted a profound effect on the tested variables.

4 Discussion

4.1 Effects of conditioners on soil CO_2 efflux

Soil CO_2 efflux is an important component of the C cycle, and a major proportion of CO_2 is released by microbial decomposition of SOM. During the first days of incubation (Fig. S1, Electronic Supplementary Material and Fig. 1), high soil CO_2 efflux rates from all the treatments were connected to the accessibility of the biodegradable SOM

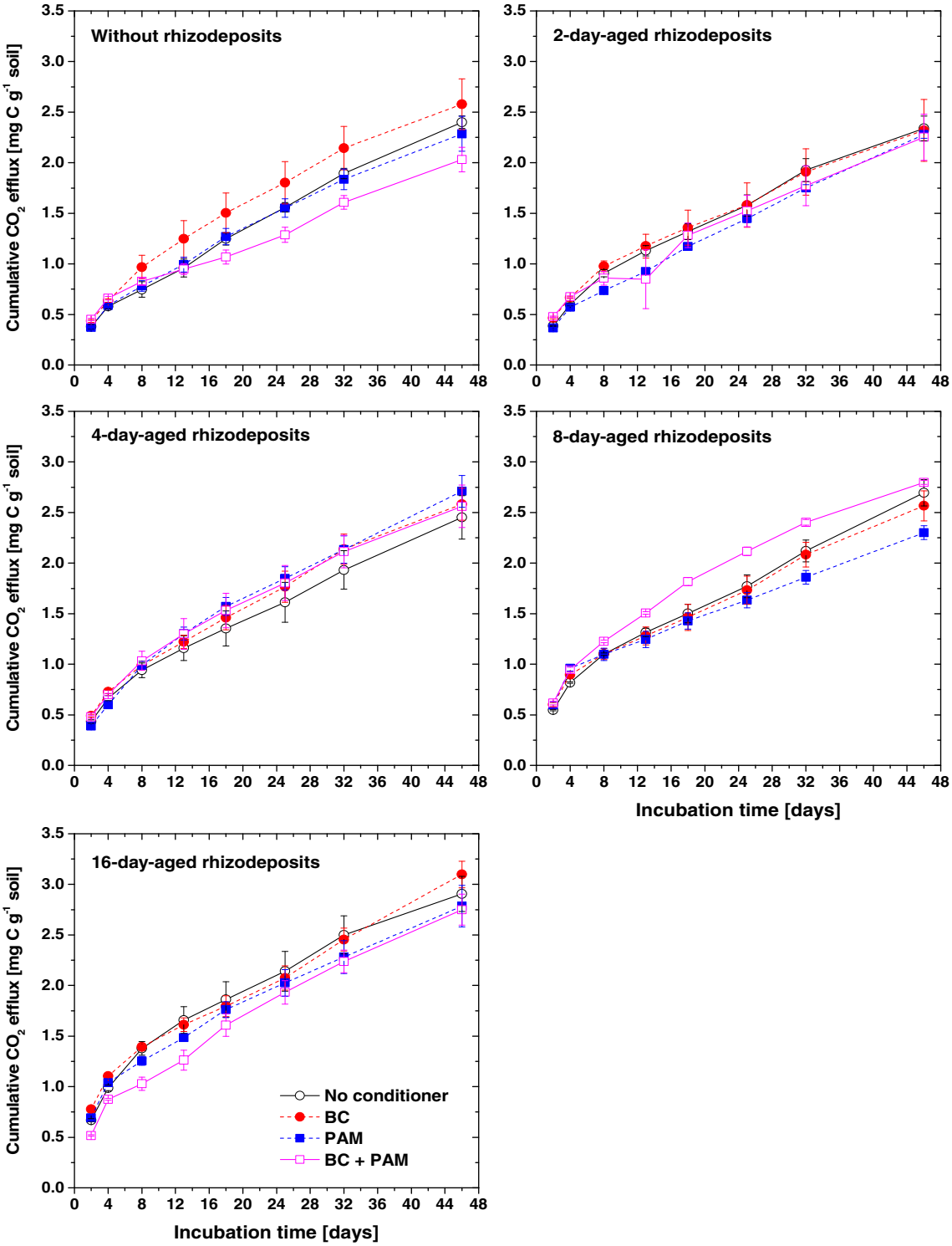

Fig. 1 Cumulative CO_2 (mg C g^{-1} soil) from the soil without and with 2-, 4-, 8-, and 16-day-aged rhizodeposits in response to the addition of 10 t ha^{-1} of biochar (BC), 80 kg ha^{-1} of polyacrylamide (PAM), and their combination (BC + PAM), compared to the control soils (no conditioner) without/with rhizodeposits. *Error bars* represent the standard error of the mean ($n = 4$)

pool to microorganisms, followed by subsequent exhaustion of labile OC (Awad et al. 2012, 2013). Moreover, BC, PAM, and BC + PAM did not show pronounced effects on CO_2 evolution or cumulative CO_2 efflux, because of their slow decomposition in the soil (Kuzyakov et al. 2009, 2014; Awad et al. 2012). PAM had no significant effect on soil CO_2 efflux during the 46 days of the incubation period, because of its slow decomposition rate of

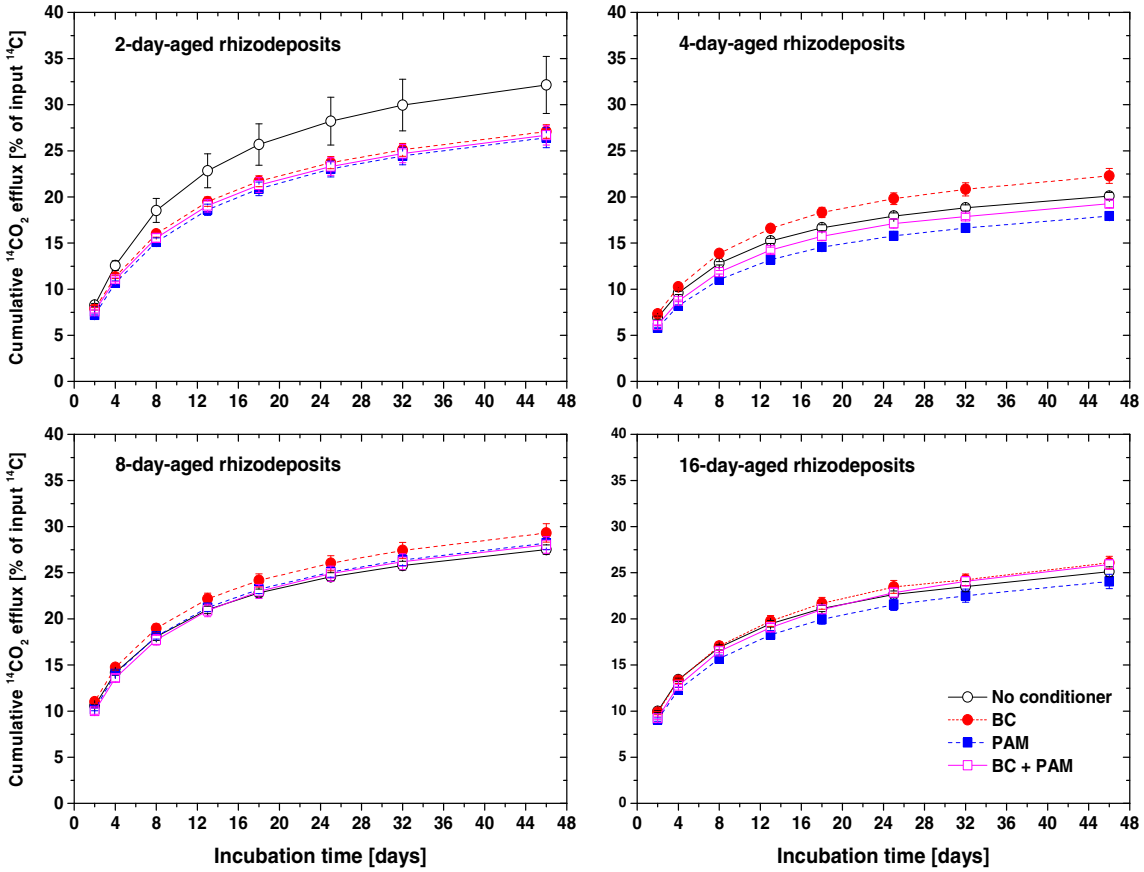

Fig. 2 Cumulative $^{14}CO_2$ efflux (percent of the ^{14}C input) from the decomposition of 2-, 4-, 8-, and 16-day-aged rhizodeposits in the soils in response to the addition of 10 t ha^{-1} of biochar (BC), 80 kg ha^{-1} of polyacrylamide (PAM), and their combination (BC + PAM), compared to the control soil (no conditioner) with rhizodeposits. *Error bars* represent the standard error of the mean ($n = 4$)

9.8 % yr^{-1} (Entry et al. 2008). Our findings are in agreement with the results reported in previous studies, where the applications of BC and PAM to soils showed a minor effect on soil CO_2 efflux because of the stability of these conditioners (Zimmerman et al. 2011; Awad et al. 2012, 2013).

4.2 Effect of rhizodeposits on soil CO_2 efflux and microbial biomass

Soil CO_2 efflux is derived from the microbial decomposition of SOM, rhizodeposits, and conditioners. In our study, the presence of rhizodeposits significantly increased the total CO_2 and $^{14}CO_2$ effluxes from soil during days 2–8 of the incubation period (Fig. S1, Electronic Supplementary Material and Figs. 1 and 2). Rhizodeposits exhibited a positive effect on soil CO_2 efflux when compared to soil without rhizodeposits. In addition, the decomposition of rhizodeposits in soil was shown to be dependent on the age of rhizodeposits and time of incubation, as indicated by the statistical analysis (Tables S3 and S4, Electronic Supplementary Material). This result reveals that the quantity and composition of rhizodeposits may alter microbial biomass and C turnover (Amos and Walter 2006; Fischer et al. 2010). Specifically, the age of rhizodeposits after labeling altered the quantity and quality of ^{14}C in the soil. Thus, it is evident that the 2-day-aged rhizodeposits contain readily available substances for microbial uptake and utilization than the old rhizodeposits, contributing to the relatively higher cumulative $^{14}CO_2$ effluxes in the soil at 46 days of incubation (Fig. 2). This result clearly indicates that 2-day-aged rhizodeposits were more accessible to microorganisms than longer-aged rhizodeposits (from 4 to 16 days). This can be explained as follows: rhizodeposits, which are complex substrates containing low- and high-molecular-weight compounds, function as essential substrates for microorganisms and play a significant role in microbial community composition in the rhizosphere (Rovira 1956; Kumar et al. 2006; Kuzyakov and Larionova 2006). In this instance, rhizodeposits provided available C to the microorganisms and increased their biomass, enabling them to take up more available N, as indicated by the higher MB-N (Fig. 3). In addition, rhizodeposits modify physicochemical characteristics of rhizosphere in soil systems and increase soluble nutrients,

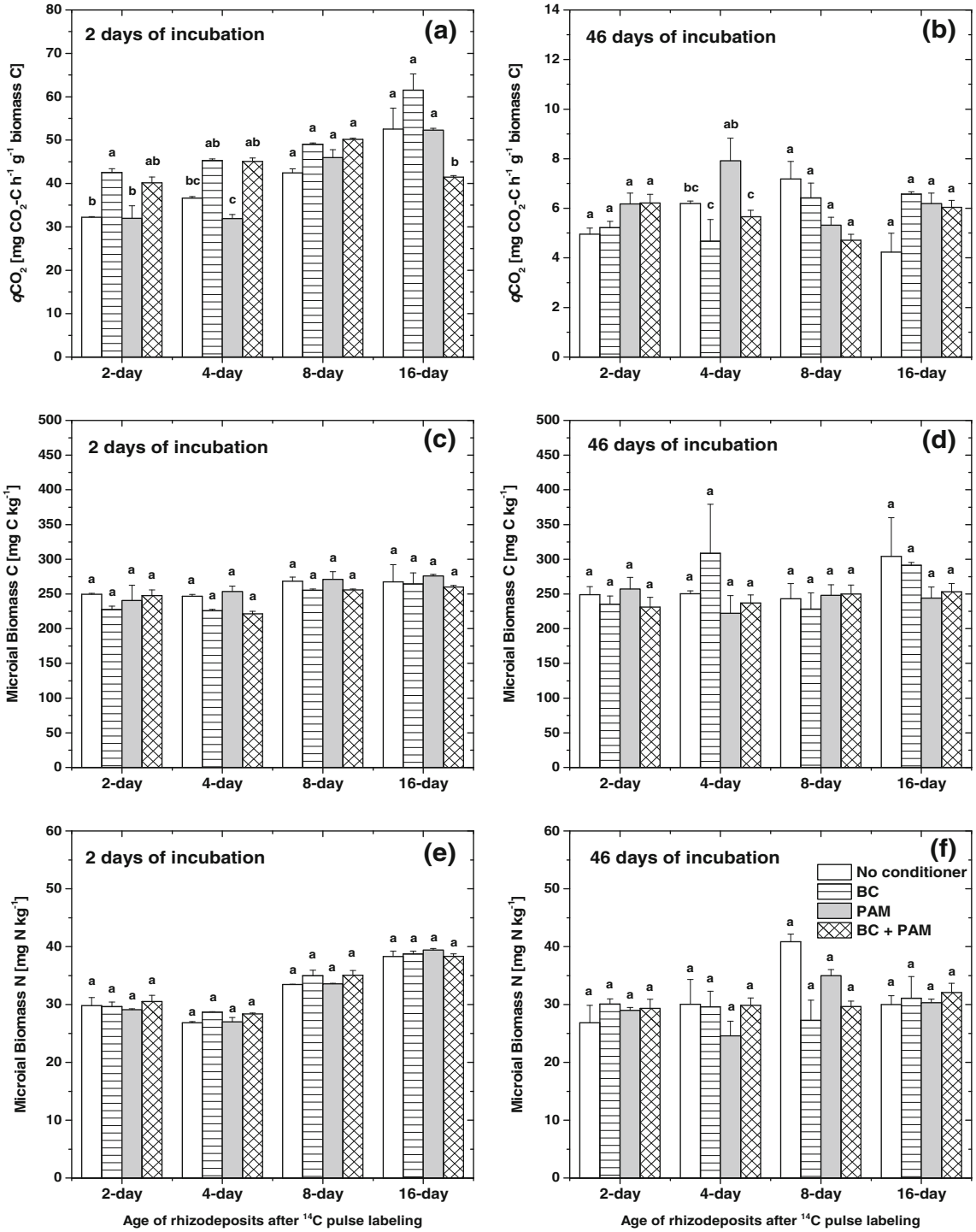

Fig. 3 Microbial metabolic quotient (qCO_2) and microbial biomass carbon (MBC) and nitrogen (MBN) in the soil at 2 and 46 days of the incubation containing 2-, 4-, 8-, and 16-day-aged rhizodeposits in response to the addition of 10 t ha^{-1} of biochar (BC), 80 kg ha^{-1} of polyacrylamide (PAM), and their combination (BC + PAM), compared to the control soil (no conditioner) with rhizodeposits. *Bars with the same letters* are not significantly different at $p \leq 0.05$ ($n = 4$)

owing to the increased microbial biomass and subsequent OM mineralization in the soil (Hinsinger 1998; Farrar and Jones 2003; Marschner and Rengel 2007).

Other studies have similarly reported that the decomposition of rhizodeposits increases soil-respired CO_2 by microorganisms (Kuzyakov and Cheng 2004; Marx et al. 2007). Specifically, soluble maize rhizodeposit compounds provide energy, C, and nutrients for microorganisms during the first days of incubation and may increase soil respiration and hence soil-derived CO_2 (Marx et al. 2007). The ^{14}C pulse

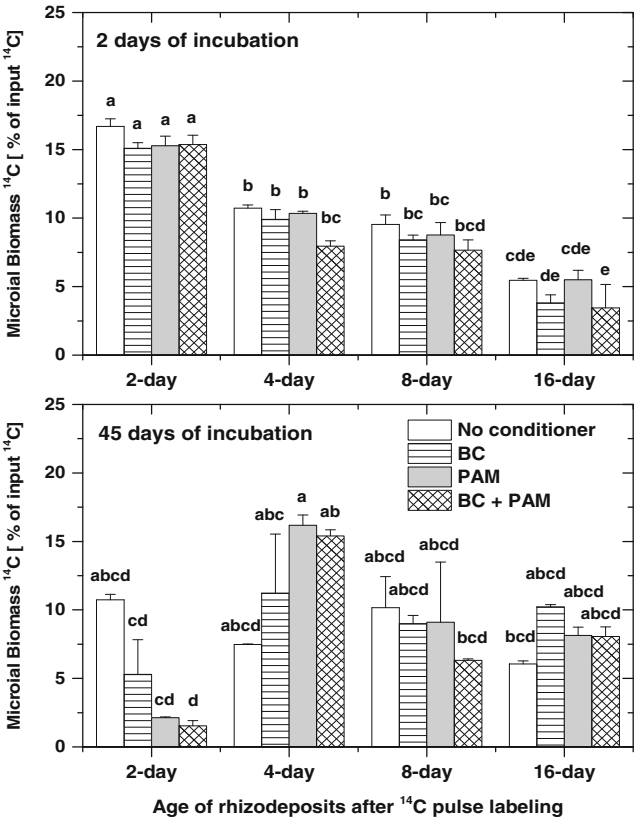

Fig. 4 Microbial biomass ^{14}C in the soil containing 2-, 4-, 8-, and 16-day-aged rhizodeposits in response to the treatments with 10 t ha^{-1} of biochar (BC), 80 kg ha^{-1} of polyacrylamide (PAM), and their combination (BC + PAM), compared to the control soil (no conditioner) with rhizodeposits. *Bars with the same letters* are not significant different at $p \leq 0.05$ ($n = 4$)

labeling technique of rhizodeposits proved that they play a major role in microbial respiration, contributing to the evolved CO_2 from SOM decomposition when compared to soil without rhizodeposits. Yevdokimov et al. (2007) reported that $^{13}CO_2$ was evolved from ^{13}C-labeled maize rhizodeposits by microbial decomposition, while 33–42 % of ^{13}C from rhizodeposits remained in the soil. Results indicate that BC and BC + PAM stabilized a high portion of C derived from rhizodeposits. The BC, PAM, and BC + PAM in the soils with rhizodeposits led to low estimated CO_2 relative to the soil without rhizodeposits. BC alone or in combination with PAM suppressed the microbial decomposition of 2-day-aged rhizodeposits based on $^{14}CO_2$ efflux (Figs. 2 and 4). BC and BC + PAM had negative priming effects on decomposition of young rhizodeposits due to its high sorption affinity for OM or labile C to its surface or within its pores as reported previously by Awad et al. (2016), Lehmann et al. (2011), Liang et al. (2010), and Zimmerman et al. (2011). Also, Bandara et al. (2015) reported lowered respired CO_2 efflux because of a reduction in activities of extracellular enzymes in BC-treated soil. However, the amount and age of rhizodeposits (2–16 days after ^{14}C labeling) have a stronger effect on their decomposition than those of the conditioners.

The higher percentage of ^{14}C retained in the soil (66–85 % of the ^{14}C input) at 46 days indicates that rhizodeposits in the soil incorporated into SOM. Specifically, the large amount of remnant rhizodeposits provides a valuable insight into the fate of rhizodeposit-derived C and its contribution to C storage in soil even during the first day after labeling. At the end of incubation, no significant differences were observed in the cumulative CO_2 and $^{14}CO_2$ effluxes, MB-C, MB-N, qCO_2, or MB-^{14}C among the treated and untreated soils (Figs. 1, 2, 3, and 4). These findings are explained by the reduction of available native or added C in the soil during incubation, accompanied by lower mineralization rates of more recalcitrant rhizodeposits or SOM (Fontaine et al. 2003; Hamer and Marschner 2005; Marx et al. 2007). The smaller metabolic quotient (qCO_2) values at the end of incubation indicated lower microbial respiration compared to that in the soil at 2 days of the incubation period (Fig. 3). As mentioned above, this was primarily because of the reduction of available rhizodeposit-C and SOM over the incubation time, decreasing microbial activity (Griffiths et al. 1999; Benizri et al. 2002; Marx et al. 2007).

5 Conclusions

We conclude that BC and PAM, both individually and in combination, had no significant effect on total CO_2 efflux because of their very slow decomposition. The contribution of rhizodeposits to CO_2 release from soil and MBC depends on their age as young rhizodeposits, containing higher amounts of labile C compared to aged rhizodeposits, are more easily available for microbial uptake and utilization. However, incorporation of ^{14}C into microbial biomass indicated that 66–85 % of the ^{14}C input remained in the soil after 46 days, and neither the age of ^{14}C-labeled rhizodeposits nor BC, PAM, or BC + PAM changed microbial utilization of ^{14}C rhizodeposits. Rhizodeposits increased the SOM mineralization and CO_2 release than do soil conditioners.

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