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Effects of polyacrylamide, biopolymer, and biochar on decomposition of soil organic matter and plant residues as determined by ^{14}C and enzyme activities

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ABSTRACT

Application of polymers for the improvement of aggregate structure and reduction of soil erosion may alter the availability and decomposition of plant residues. In this study, we assessed the effects of anionic polyacrylamide (PAM), synthesized biopolymer (BP), and biochar (BC) on the decomposition of ^{14}C-labeled maize residue in sandy and sandy loam soils. Specifically, PAM and BP with or without ^{14}C-labeled plant residue were applied at 400 kg ha^{-1}, whereas BC was applied at 5000 kg ha^{-1}, after which the soils were incubated for 80 days at 22 °C. Initially, plant residue decomposition was much higher in untreated sandy loam soil than in sandy soil. Nevertheless, the stimulating effects of BP and BC on the decomposition of plant residue were more pronounced in sandy soil, where it accounted for 13.4% and 23.4% of ^{14}C input, respectively, whereas in sandy loam soil, the acceleration of plant residue decomposition by BP and BC did not exceed 2.6% and 14.1%, respectively, compared to untreated soil with plant residue. The stimulating effects of BP and BC on the decomposition of plant residue were confirmed based on activities of β-cellobiohydrolase, β-glucosidase, and chitinase in both soils. In contrast to BC and BP, PAM did not increase the decomposition of native or added C in both soils.

1. Introduction

Wind and water erosion causes annual soil losses of 75 billion metric tons worldwide [1]. Total soil losses amounted for 6.9 billion Mg yr^{-1} in the United States [1] and 50 × 10^6 Mg yr^{-1} in Korea [2]. The actual erosion rates for tilled and arable land in Europe are 3–40 times greater than the upper limit of tolerable erosion (1.4 tons ha^{-1} yr^{-1}), and this has resulted in a cost of £205 million in England and Wales [3]. This cost is due to loss of soil organic matter (SOM), CO_2 emissions, on-farm costs (fertilizers, manure, etc.), clean-up operations, and accidents such as flooding [3]. Additionally, loss of fertile topsoil reduces water quality, plant available nutrients, crop yields, and soil quality due to soil degradation [1,2]. Accordingly, this has led to erosion control technologies receiving a great deal of attention.

Application of polyacrylamide (PAM) and biopolymers are reliable soil conservation technologies [4]. The use of PAM for reduction of soil erosion has been extensively studied since the 1990s [4,5], and PAM has become a standard method to reduce the outflow and turbidity of runoff by improving soil structure and infiltration [6,7]. Moreover, increased water viscosity occurs after application of PAM, which leads to reduced water flow and better water retention [4,8]. Anionic PAM has also been shown to prevent seal formation on soil surfaces and reduce soil loss [9]. PAM also stabilizes the soil structure and reduces surface hydraulic conductivity by preventing the formation of surface seals [4]. Particle flocculation of PAM in water with sufficient electrolytes attracts soil particles due to coulombic and van der Waals forces, thereby increasing aggregation [10]. The degree of adsorption of polymers has been shown to be dependent on soil exchangeable cations, clay content, pH, and polymer molecular size [11]. PAM efficacy has also been shown to be high in soil with increased clay content due to the adsorption of its molecules to clay minerals through bridging with polyvalent exchangeable cations [10,12]. Specifically, PAM penetrates into aggregates and stabilizes exterior surfaces, thereby stabilizing soil aggregates [12,13]. On the other hand, the use of biopolymers for the prevention of soil erosion has been recommended as a cost-effective alternative to PAM. Materials such as starch, sugar, cellulose, and other compounds obtained from chitosan, potato, corn, wheat, and xanthate are commonly used as biopolymers [14,15]. Biopolymers are known to reduce soil loss and runoff [15]; however, the effects of polymers on the activity and functions of soil microorganisms are not yet clear [16].

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Biochar (BC), or biomass-derived black carbon, is a by-product of pyrolysis and is known to improve crop yields when applied as a fertilizer, especially under low-fertility soil conditions [17–19]. BC is produced from carbonaceous sources, plant tissues, and natural or anthropogenic sources [20]. BC increases cation exchange capacity (CEC) and water-holding capacity, thereby improving soil quality and increasing SOM [21]. BC can also increase the activities of soil microorganisms such as mycorrhizal fungi, which can improve soil aggregation [20]. Moreover, high levels of BC have been shown to increase C stocks in soil [22]. However, stimulation and suppression of C mineralization in soils following addition of BC have been reported in many studies [23]. Based on CO_2 evolution, Zimmerman et al. [23] previously observed that BC produced at low temperatures (250–400 °C) stimulated C mineralization due to decomposition of labile components of BC over the short term. Conversely, BC produced at high temperatures (525–650 °C) suppressed C mineralization due to the sorption and physical protection of organic matter (OM) by BC [23]. Despite these positive or negative effects, little is known about the effects of BC on soil enzymes and the decomposing activities of soil microorganisms [20]. Therefore, the soil-BC interaction may enhance the decomposition of OM and plant residue.

Application of plant residue to soils is a method for reducing soil degradation, helping to maintain [24] or even increase the OM content of the soil [25] and replace eroded C with new organic C [26]. On one hand, plant residue improves soil quality by increasing the microbial biomass carbon (MBC) by 1.7–5.4 fold when compared to soil without added plant residue [27]. In particular, labile plant residue acts as a C or N source for microorganisms, accelerating their decomposing activity. A labile pool of plant residue has been shown to pass through the microbial biomass (MB), contributing to increased microbial respiration and the release of large quantities of CO_2 from the soil [25,28]. An understanding of the C dynamics involved in the decomposition of plant residue is necessary to predict the effects of land use on soil C storage [25,29,30]. In Korea, rapid decomposition of plant residue is desired in double cropping systems in order to maintain a proper supply of nutrients for crop growth and substrates for soil microorganisms.

Plant residue-derived C and C from native SOM can be differentiated through application of ^{14}C-labeled plant residue, which provides a precise estimate of the decomposition rate of the residue [31] independent of SOM content and the addition of polymers. This makes ^{14}C-labeled plant residue a useful tool for investigating C flux in plant/soil systems [25]. Changes in land use have also been shown to lead to significant changes in the decomposition of SOM and plant residue. Therefore, it is necessary to evaluate mineralized C as CO_2 efflux from agricultural soils in response to polymer addition.

Changes in agricultural practices such as variation in the cultivation of crops and the addition of plant residue can lead to higher SOM sequestration rates [25,32]. The effects of polymers, BP, and BC on soil microorganisms and the soil C balance are not yet well understood [16,17]. Specifically, investigation of the decomposition dynamics of plant residue as a source of organic C input is needed to improve soil fertility and productivity [33].

Decomposition of plant residue is mediated by a variety of extracellular enzymes [34] that are responsible for the initial stage of plant residue transformation (degradation of long chain polymeric molecules to oligomers) as well as the final stage (production of monomers). Increased activities of enzymes responsible for the decomposition of cellulose, which is a major compound of plant tissues, indicate the availability of plant residues to microbial decomposition in the soil [35]. The activities of β-cellobiosidase and β-glucosidase play major roles in the C cycle and cellulose decomposition in soil [36]. Specifically, cellulose in plant residue is broken down by β-cellobiosidase into smaller oligosaccharides through the removal of cellobiose or glucose from the ends of the cellulose molecules [34]. These oligosaccharides are then broken down by β-glucosidase into glucose [36]. Application of polymeric stabilizers to soil can alter various stages of plant residue decomposition.

To date, decomposition of plant residue as affected by application of PAM, BP, and BC has not been investigated. Therefore, this study was conducted to determine if application of the aforementioned amendments in combination with plant residue can conserve SOM by improving the physical and chemical characteristics of soil. It was assumed that these polymeric amendments may improve soil structure by the formation of macroaggregates, thereby increasing the decomposition of plant residue in soil. In addition, an improved soil-BC interaction may enhance the accessibility of added C to microorganisms and enzymes. Specifically, improved soil properties induced by BC as a source of C may increase microbial activity, resulting in rapid decomposition of plant residue and SOM. However, the counterbalancing of these controversial effects on plant residue and SOM decomposition has not yet been demonstrated. Moreover, the effects of these polymers in soils with different textures and chemical properties have not been reported to date. Therefore, the effects of PAM, BP, and BC on potential changes in SOM and decomposition of ^{14}C-labeled maize residue were evaluated in different textured soils in an incubation experiment. Specifically, we evaluated the extracellular enzyme activities involved in plant residue decomposition in sandy and sandy loam soils.

2. Materials and methods

2.1. Soil sampling and properties

Two agricultural soils with different textures were evaluated. Specifically, sandy soil was collected from an agricultural field (site 1) (128°8′51.6″E and 38°16′39.6″N), and sandy loam soil was collected from an agricultural highland (site 2) (128°9′22.4″E and 38°16′21.7″N) adjacent to a forest in Haean Catchment, Korea. Soil samples were collected from the upper 5 cm of the surface soil in September of 2009 using a soil corer with an inner diameter of 6 cm. The soil pH and EC of 1:5 soil and water mixtures were determined using a pH meter (Orion 3 Star, Thermo, USA). The exchangeable cations were analyzed by inductively coupled plasma (ICP) spectrometry after 1 M NH_4OAc extraction [37]. Soil texture was determined using the pipette method [38]. Soil total carbon (TC) and total nitrogen (TN) contents were analyzed using a multi N/C 2100 S analyzer (Analytik Jena, Germany). The soil-water holding capacity was determined on a gravimetric basis using the core and sieved samples [39].

The pH was much lower in sandy loam soil than in sandy soil (Table 1). The silt and clay contents were 3.6 fold higher in sandy loam soil than in sandy soil. The sandy loam soil also had a relatively higher total carbon (TC) content and C/N ratio than did sandy soil, as well as a higher level of exchangeable cations (Table 1).

2.2. Characteristics of soil stabilizers

Synthetic polyacrylamide polymer (PAM, Magnafloc 336, Ciba Canada Ltd., Canada) and biopolymer (BP) were applied to both soils in an incubation experiment. BP was synthesized from a suspension of lignin, starch, acrylamide, and acrylic acid according to the method described by Liu et al. [40]. Biochar BC250 (BC) composed of 250 kg of charcoal, which was mixed into 1 ton of compost material (50% sewage sludge + 25% freshly chopped lop,

Table 1
Physiochemical properties of soils collected from two different locations in Haean.

Location	Sand	Silt	Clay	Texture	pH	EC[a]	WHC[b]	TC[c]	TN[d]	C/N	NH_4OAc extractable bases[e]			
											Ca^{2+}	Mg^{2+}	K^+	Na^+
		%				dS m^{-1}	%		g kg^{-1}		$cmol_{(+)}$ kg^{-1}			
Site 1	90.9	4.6	4.5	Sand	6.8	0.02	14.6	1.0	0.1	10.0	4.4	0.1	0.2	0.1
Site 2	67.3	25.9	6.8	Sandy loam	6.1	0.09	28.2	31.0	1.7	18.2	6.0	0.9	1.6	0.1

[a] Electrical conductivity.
[b] Water-holding capacity.
[c] Total carbon.
[d] Total nitrogen.
[e] Ammonium acetate.

grass and leaves + 25% soil, and coarse wood branches (1:1)) was purchased from Sonnenerde GmbH (Austria). Charcoal in BC contained 2.73% N and 67% C, whereas PAM contained 16.2% N and 42.2% C and BP contained 5.3% N and 28.4% C.

2.3. Experimental design and incubation experiment

The experiment consisted of 16 treatments including three factors performed four times each. The first factor was soil texture (sandy or sandy loam), the second was soil stabilizers (PAM or BP at 400 kg ha^{-1}, BC at 5000 kg ha^{-1}, and untreated soil as the control), and the third was addition of 100 mg per 30 g of soil ^{14}C-labeled (1793 DPM mg^{-1}) plant residue (PR). To produce ^{14}C-labeled maize residue, maize plants were labeled three times in $^{14}CO_2$ atmosphere as described by Gocke et al. [41]. ^{14}C-PR was ground using a ball mill (MM2, Fa Retsch) and then thoroughly mixed with 30 g of air-dried soil containing one of the four aforementioned stabilizers. Amended soils with moisture adjusted to 70% water-holding capacity (WHC) were placed in closed vessels and incubated at 22 °C for 80 days. Soil moisture was maintained at 70% WHC with deionized water throughout the experiment. To trap CO_2, small vials containing 2 ml of 1.0 M NaOH were placed in the vessels, which were changed at 2, 4, 8, 12, 16, 24, 34, 48, 62, and 80 days during the incubation period for measurement of CO_2 and ^{14}C activity. Additionally, four vessels without soil containing only NaOH vials served as blanks.

2.4. CO_2 efflux and ^{14}C analyses

To estimate total CO_2 efflux, CO_2 trapped in 1.0 M NaOH was precipitated with 0.5 M $BaCl_2$. Next, NaOH was titrated with 0.01 M HCl using phenolphthalein as an indicator [42].

To estimate $^{14}CO_2$–C in total CO_2 trapped in NaOH, a 0.4 ml aliquot of NaOH was mixed with 2 ml of scintillation cocktail Rothiscint-22x (Roth Company, Germany) to measure ^{14}C activity after the decay of chemiluminescence. The efficiency of ^{14}C counting was always higher than 93%, and the measurement error of ^{14}C activity did not exceed 3%. $^{14}CO_2$ efflux from labeled plant residue was calculated as the percentage of initial input ^{14}C activity. Detailed information regarding calculation of the CO_2 and $^{14}CO_2$ (% plant residue input) efflux rates is available in Kuzyakov and Cheng [43] and van Groenigen et al. [44].

2.5. Analyses of enzyme activity

Enzyme activities in the soil were measured using fluorogenically labeled substrates [45]. For the analyses, the following fluorogenic enzyme substrates based on 4-methylumbelliferone (MUF) were used: MUF-ß-D-cellobioside for measurement of cellobiohydrolase activity (β-cellobiosidase EC 3.2.1), MUF-ß-D-glucopyranoside for measurement of β-glucosidase EC 3.2.1.21 activity, and MUF-N-acetyl-ß-D-glucosaminide hydrate for measurement of chitinase EC 3.2.1.14 activity. L-Leucine-7-amino-4-methyl coumarin (AMC) substrate was used for measurement of leucine aminopeptidase EC 3.4.11.1 activity. In addition, 2 ml of 2-methoxyethanol was used to dissolve the MUF-substrates [46]. All chemicals were purchased from Fluka (Germany). Soil samples (0.5 g) were suspended in water (25 ml) and shaken at 220 rpm for 15 min. Next, 1.0 ml of the soil suspension was added to 1.0 ml of the substrate solution (containing either 200 μmol of MUF- or AMC-substrate) in deep-well plates (24-wells × 10 ml, HJ-Bioanalytik GmbH, Germany), after which the samples were incubated at the temperature of the incubation experiment (22 °C) for 3 h for MUF-β-D-cellobioside and 1 h for the other enzymes. The saturation concentrations of the fluorogenic substrates were determined in preliminary experiments. Calibration curves were then prepared based on the soil suspension (1 ml) and samples containing serial concentrations of MUF or AMC (0–32 μmol, 1 ml). To accomplish this, soil-calibration-MUF samples were added to deep-well plates and then centrifuged (3000 rpm, 10 min). Then, 1 ml of supernatant was pipetted into 24-well microplates (Becton Dickinson, USA), and fluorescence was measured using a Victor^3 1420–050 Multilabel Counter (PerkinElmer, USA) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a slit width of 25 nm. Enzyme activities were calculated as released MUF or AMC in nanomoles per gram of bulk soil/dry weight per hour (nmol g^{-1} h^{-1}).

2.6. Statistics

The standard error of the means was calculated from four replicates of each treatment. We compared the means of the variables using three-way factorial ANOVA and Tukey's HSD test at a probability level (P) < 0.05 [47]. In addition, Pearson correlation coefficients between the enzyme activities and decomposition rates of the plant residue were estimated at $P < 0.05$.

3. Results

3.1. Effect of soil stabilizers on SOM decomposition

The highest CO_2 efflux rates were recorded after 2 days in both soils without added ^{14}C-labeled plant residue (Fig. 1a,b). The CO_2 evolution rates decreased sharply during 2–24 days of incubation in both sandy and sandy loam soils. Thereafter, the CO_2 efflux rates became generally constant (Fig. 1). Furthermore, sandy loam soil had a higher CO_2 efflux rate than sandy soil (Fig. 1a,b). In sandy loam soil, only PAM induced a strong short-term decrease in CO_2 efflux rate (26.4%; 16.2 μg C day^{-1} g^{-1}) after 2 days of incubation compared to untreated soil (22 μg C day^{-1} g^{-1}) (Fig. 1a). During incubation, the effects of PAM, BP, and BC without added

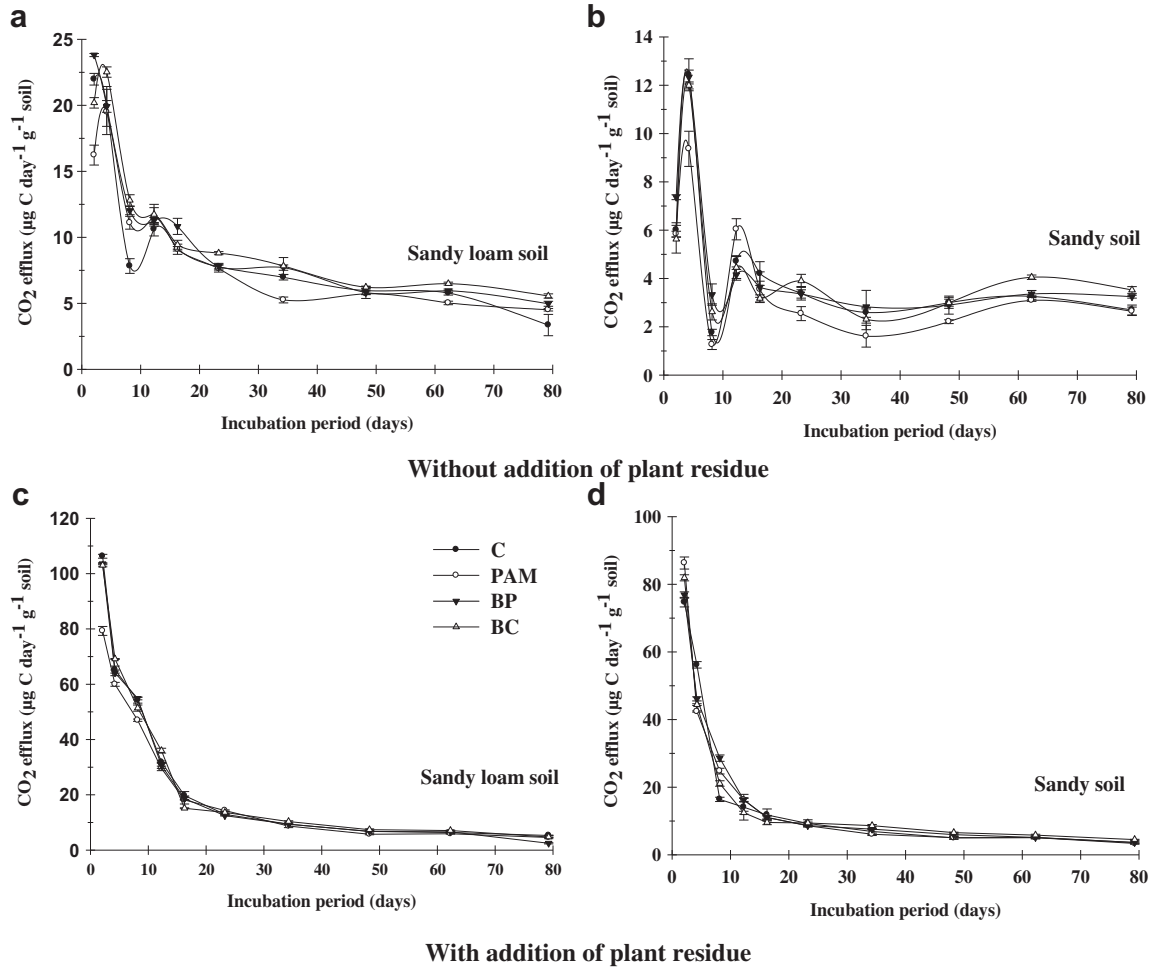

Fig. 1. CO_2 efflux rates (µg C day^{-1} g^{-1} soil) from sandy loam and sandy soils in response to soil stabilizers without (a, b)/with (c, d) addition of plant residue (C: control soil, BC: biochar 250, BP: biopolymer, and PAM: polyacrylamide). Error bars represent the standard error of the mean ($n = 4$).

^{14}C-labeled plant residue on CO_2 evolution in either soil were insignificant compared to those in untreated soil (Fig. 1a,b).

Cumulative CO_2 varied slightly in both soils without plant residue after 80 days of incubation and ranged from 0.23 to 0.29 and 0.54–0.65 mg C g^{-1} in sandy and sandy loam soils, respectively (Table 2). In sandy loam soil, cumulative CO_2 increased by 18.2% and 12.7% in BC- and BP-amended soils after 80 days of incubation, respectively, compared to that in untreated soil (Table 2). PAM slightly decreased cumulative CO_2 in both soils compared to that in untreated soil; however, the decrease was much greater in sandy soil than in sandy loam soil. No significant differences in CO_2 efflux rate or cumulative CO_2 were observed in either soil amended with PAM compared with those of untreated soil.

3.2. Effect of plant residue on CO_2 efflux

The addition of plant residue led to a significant increase in total CO_2 efflux from sandy and sandy loam soils to 74.7 and 106 µg C day^{-1} g^{-1}, respectively, compared to that from soil without plant residue (Fig. 1c,d). PAM led to a significant decrease in CO_2 efflux rate (25.4%) after 2 days of incubation from sandy loam soil, whereas no significant differences were observed for the other amendments compared to that from untreated soil with plant residue (Fig. 1c). After 2 days of incubation, PAM and BC increased the CO_2 efflux rate from sandy soil amended with plant residue by 15.5% and 9.6%, respectively, compared to that from untreated soil with plant residue (Fig. 1d). Except for the first few days of incubation, there were no significant effects of soil stabilizers on total CO_2 efflux in soil amended with plant residue.

The effluxes of cumulative CO_2 for the sandy and sandy loam soils subjected to addition of plant residue were increased by 0.79 and 1.25 mg C g^{-1}, respectively, after 80 days incubation (Table 2). No significant differences were observed upon BP and BC treatment to sandy loam soil compared to untreated soil with plant residue (Table 2). However, PAM decreased cumulative CO_2 by 11.2% in sandy loam soil after 80 days of incubation compared to untreated soils with plant residue. Compared to untreated soil with plant residue, BP and BC increased cumulative CO_2 in sandy soil after 80 days of incubation by 6.3% and 6.7%, respectively (Table 2).

3.3. Effect of soil stabilizers on ^{14}C plant residue decomposition

There were two phases of plant residue decomposition. The first phase occurred from days 2–24 and the second from days 24–80. Based on $^{14}CO_2$ evolution (Table 3), the maximum rate of ^{14}C maize residue mineralization occurred after 2 days of incubation and ranged from 3.5% to 10% of the input ^{14}C day^{-1}. Plant residue was mineralized for about 10% of the input ^{14}C day^{-1} in response to treatment with BC after 2 days of incubation in both soils. Plant residue decomposition in sandy loam soil was significantly

Table 2
Cumulative CO_2 efflux (mg C g^{-1} soil) from sandy loam and sandy soils in response to soil stabilizers.

Treatments	Incubation time (days)									
	2	4	8	12	16	24	34	48	62	80
Sandy loam soil										
C[a]	0.05 c[b]	0.09 c	0.12 d	0.16 d	0.20 cd	0.25 d	0.33 d	0.41 de	0.50 de	0.55 e
PAM	0.03 d	0.08 d	0.12 d	0.17 cd	0.20 cd	0.26 d	0.31 d	0.39 e	0.46 e	0.54 e
BP	0.05 c	0.09 c	0.14 c	0.19 cd	0.23 c	0.28 c	0.37 c	0.45 cd	0.53 cd	0.62 d
BC	0.04 cd	0.09 c	0.14 c	0.19 cd	0.23 c	0.29 c	0.37 c	0.46 c	0.55 c	0.65 d
C + PR	0.22 a	0.36 a	0.58 a	0.71 a	0.79 a	0.88 a	0.98 a	1.07 a	1.16 a	1.25 a
PAM + PR	0.17 b	0.3 b	0.48 b	0.60 b	0.67 b	0.77 b	0.87 b	0.95 b	1.03 b	1.11 c
BP + PR	0.22 a	0.36 a	0.57 a	0.70 a	0.77 a	0.86 a	0.96 a	1.06 a	1.15 a	1.19 b
BC + PR	0.22 a	0.36 a	0.57 a	0.71 a	0.77 a	0.87 a	0.98 a	1.09 a	1.19a	1.27 a
Sandy soil										
C	0.01 d	0.04 b	0.05 b	0.07 b	0.08 b	0.11 b	0.13 b	0.18 b	0.22 b	0.27 b
PAM	0.01 d	0.03 b	0.04 b	0.06 b	0.08 b	0.09 b	0.11 b	0.14 b	0.19 b	0.23 b
BP	0.02 d	0.04 b	0.06 b	0.07 b	0.09 b	0.11 b	0.14 b	0.18 b	0.23 b	0.28 b
BC	0.01 d	0.04 b	0.05 b	0.07 b	0.08 b	0.11 b	0.13 b	0.17 b	0.23 b	0.29 b
C + PR	0.16 c	0.28 a	0.34 a	0.40 a	0.45 a	0.51 a	0.59 a	0.66 a	0.73 a	0.79 a
PAM + PR	0.18 a	0.27 a	0.37 a	0.44 a	0.48 a	0.54 a	0.61 a	0.68 a	0.75 a	0.81 a
BP + PR	0.16 bc	0.26 a	0.37 a	0.44 a	0.48 a	0.54 a	0.63 a	0.71 a	0.78 a	0.84 a
BC + PR	0.17 ab	0.27 a	0.35 a	0.40 a	0.44 a	0.51 a	0.60 a	0.69 a	0.77 a	0.85 a

[a] C: control soil, BC: biochar 250, BP: biopolymer, PAM: polyacrylamide, PR: plant residue addition.
[b] The different letters in each column indicate significant differences at $P < 0.05$.

decreased by 19% after 2 days of incubation in response to treatment with PAM (3.5% of input ^{14}C day^{-1}) compared to that of untreated soil (4.4% of input ^{14}C day^{-1}). After 2 days of incubation, PAM slightly increased the decomposition rate of plant residue in sandy soil by 4.7% of the input ^{14}C day^{-1} compared to that of untreated soil (4.13% of input ^{14}C day^{-1}). BP had no significant effect on the rate of plant residue decomposition in either soil after 2 days of incubation compared to that of untreated soil.

After 80 days of incubation, the decomposition rate of plant residue in sandy soil was still 50% and 75% higher in BP (0.06% of input ^{14}C day^{-1}) and BC (0.07% of input ^{14}C day^{-1})-treated soils than in untreated soil (0.04% of input ^{14}C day^{-1}). PAM had no significant effect on the plant residue decomposition rate in sandy soil after 80 days of incubation compared to that of untreated soil. In sandy loam soil, no significant differences were observed in the plant residue decomposition rate in response to treatment with soil stabilizers compared to that of untreated soil.

The order of cumulative $^{14}CO_2$ efflux after 80 days of incubation was as follows: C < PAM ≤ BP < BC in sandy soil; PAM < C < BP < BC in sandy loam soil (Table 3). After 80 days of incubation, BP and BC increased cumulative $^{14}CO_2$ in sandy loam soil by 2.6% and 14.1% of input ^{14}C, whereas it increased cumulative $^{14}CO_2$ in sandy soil by 13.4% and 23.4% of input ^{14}C compared with corresponding untreated sandy and sandy loam soils (Table 3). PAM significantly decreased the decomposition rate of plant residue in sandy loam soil during 2–24 days of incubation, whereas there was no significant effect on cumulative $^{14}CO_2$ during days 24–80 compared with that of untreated soil. However, PAM significantly increased cumulative $^{14}CO_2$ efflux in sandy soil by 21% compared to that in untreated soil after 80 days of incubation.

Overall, BC and BP induced an increase in plant residue decomposition in soil compared to PAM, which led to a slight decrease in plant residue decomposition in sandy loam soil.

3.4. Enzyme activities

Without plant residue, no significant differences in the activities of β-cellobiosidase, β-glucosidase, chitinase, and leucine aminopeptidase were observed in response to stabilizers in either soil compared to those in untreated soils after 34 days of incubation (Figs. 2 and 3). However, remarkably higher enzyme activities were observed in sandy loam soil when compared to those in sandy soil.

Only BC with added plant residue significantly increased the activity of β-cellobiosidase by 3.6 and 2.1 fold in sandy and sandy loam soils, respectively, compared to those in untreated soil with plant residue after 34 days of incubation (Figs. 2 and 3). Similarly, BC with added plant residue induced a 1.7 fold increase in the

Table 3
Cumulative $^{14}CO_2$ efflux (% of input ^{14}C) from decomposition of ^{14}C-labelled maize residue in sandy loam and sandy soils in response to soil stabilizers.

Treatments	Incubation time (days)									
	2	4	8	12	16	24	34	48	62	80
Sandy loam soil										
C[a]	9.17 b[b]	22.19 b	44.29 a	55.23 b	61.25 b	66.58 b	70.31 bc	72.84 bc	74.59 bc	76.44 bc
PAM	7.43 c	20.36 c	40.42 c	52.47 c	58.71 c	64.06 c	68.31 c	70.98 c	72.55 c	74.29 c
BP	9.41 b	22.54 b	44.74 b	56.88 b	63.19 b	68.34 b	72.56 b	75.23 b	77.08 b	79.04 b
BC	20.21 a	35.41 a	56.60 a	69.16 a	74.41 a	80.03 a	84.43 a	87.08 a	88.78 a	90.47 a
Sandy soil										
C	8.66 c	17.48 d	27.47 d	33.50 d	38.03 c	43.84 c	50.57 c	55.69 c	58.75 c	62.11 c
PAM	9.95 b	21.11 b	34.29 b	43.05 b	48.73 a	55.29 b	61.99 b	67.88 b	71.21 b	75.16 b
BP	8.58 c	19.46 c	31.14 c	39.28 c	44.67 b	51.53 b	60.56 b	66.98 b	70.87 b	75.49 b
BC	20.91 a	30.55 a	40.64 a	47.91 a	52.33 a	59.82 a	70.04 a	75.42 d	80.00 a	85.47 a

[a] C: control soil, BC: biochar 250, BP: biopolymer, PAM: polyacrylamide.
[b] The different letters in each column indicate significant differences at $P < 0.05$.

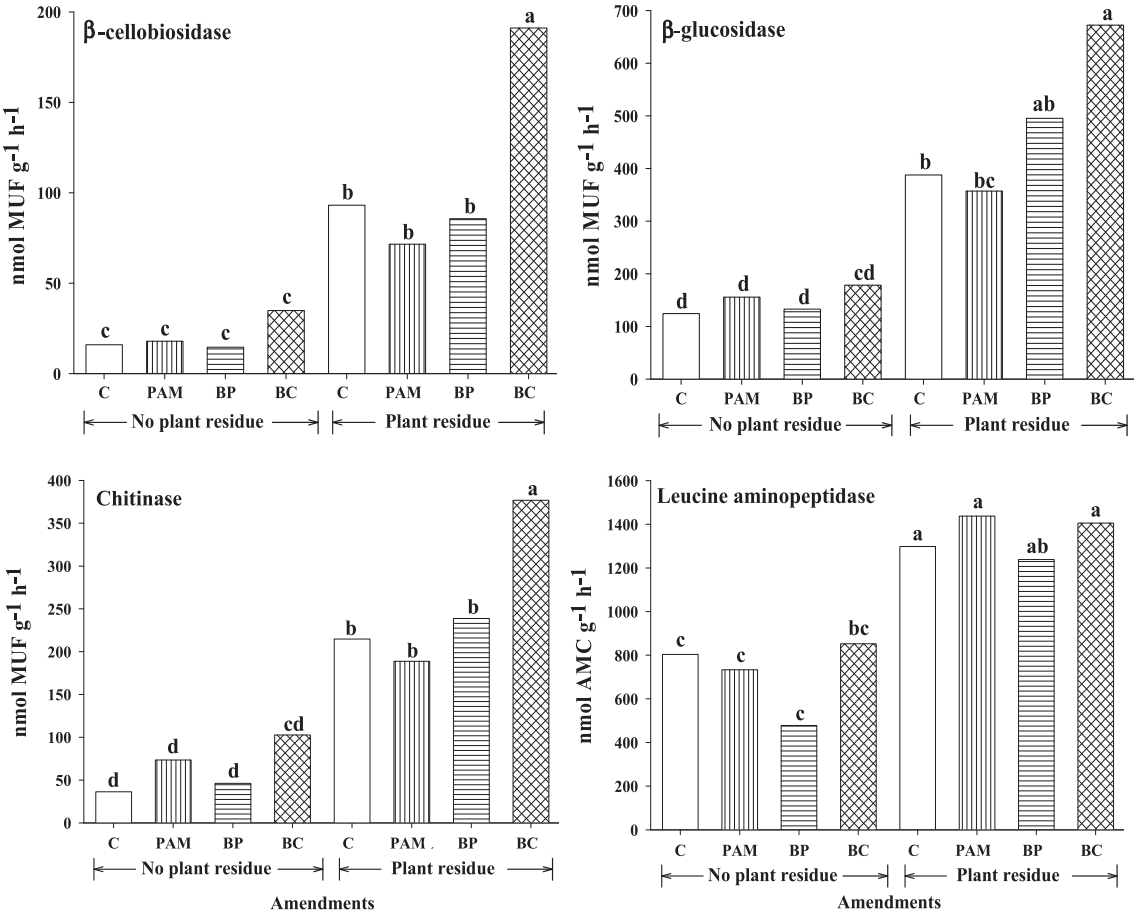

Fig. 2. Activities of cellobiohydrolase β-cellobiosidase, β-glucosidase, chitinase, and leucine aminopeptidase enzymes (nmol g^{-1} h^{-1}) in sandy loam soil in response to soil stabilizers (C: control soil, BC: biochar 250, BP: biopolymer, PAM: polyacrylamide and PR: ^{14}C plant residue). Bars with the same letters are not significantly different at the 0.05 probability level.

activity of β-glucosidase in sandy loam soil compared to that in untreated soil with plant residue. BP with added plant residue significantly increased β-glucosidase activity in sandy soil by 1 fold, whereas no significant difference in sandy loam soils was observed compared to that in untreated soil with plant residue (Figs. 2 and 3). In sandy soil, BC and PAM with added plant residue increased chitinase activity by 1.5 and 1.3 fold, respectively, compared to that of untreated soil with plant residue. PAM and BC with added plant residue also significantly increased leucine aminopeptidase activity in sandy soil by 1.7 and 1.4 fold, respectively, compared to that in untreated soil with plant residue (Fig. 3).

β-cellobiosidase activity was significantly correlated with the $^{14}CO_2$ decomposition rate of plant residue in sandy loam soil after 2, 34, and 80 days of incubation, with r values of 0.997 ($P < 0.001$), 0.980 ($P < 0.05$), and 0.950 ($P < 0.05$), respectively. Similarly, β-glucosidase activity was significantly correlated with the $^{14}CO_2$ decomposition rate of plant residue in sandy loam soil (Table 4). However, no significant correlations between β-cellobiosidase activity and the $^{14}CO_2$ decomposition rate of plant residue were observed in sandy soil, except after 2 days of incubation ($r = 0.984$, $P < 0.01$). Chitinase activity was significantly correlated only with the $^{14}CO_2$ decomposition rate of plant residue in sandy loam soil after 2, 34, and 80 days of incubation, with r values of 0.993 ($P < 0.01$), 0.999 ($P < 0.001$), and 0.988 ($P < 0.01$), respectively. There was no significant correlation between $^{14}CO_2$ decomposition rate and the other enzymes in sandy soil (Table 4).

Three-way factorial ANOVA showing the effects of soil texture, plant residue, and amendments on cumulative CO_2, $^{14}CO_2$ efflux, and the activities of β-cellobiosidase, β-glucosidase, chitinase, and leucine aminopeptidase is given in Table 5. Soil texture and plant residue had significant effects on the tested variables. Soil stabilizers had significant effects only on β-cellobiosidase activity (Table 5).

4. Discussion

4.1. Effects of soil stabilizers on SOM decomposition

In this study, stabilizers were not found to have had significant effects on native SOM mineralization in either soil (Fig. 1). The decomposition and chemical transformation of BC by soil microorganisms is very slow, and its contribution to CO_2 efflux is also very small compared to that of SOM and other sources such as plant residue [48]. Moreover, Kuzyakov et al. [48] reported that oxidation of BC is very slow, and it might be bound to soil mineral components.

PAM also had no significant effect on CO_2 in either soil. This failure to increase CO_2 efflux from either soil indicates that the decomposition rates are very low [49]. These results demonstrate that PAM is highly persistent in soil and more resistant to microbial degradation than other polymers [4].

4.2. CO_2 efflux and plant residue decomposition

Decomposition of plant residue contributed to the significant increase in total CO_2 efflux compared to that in soil without added

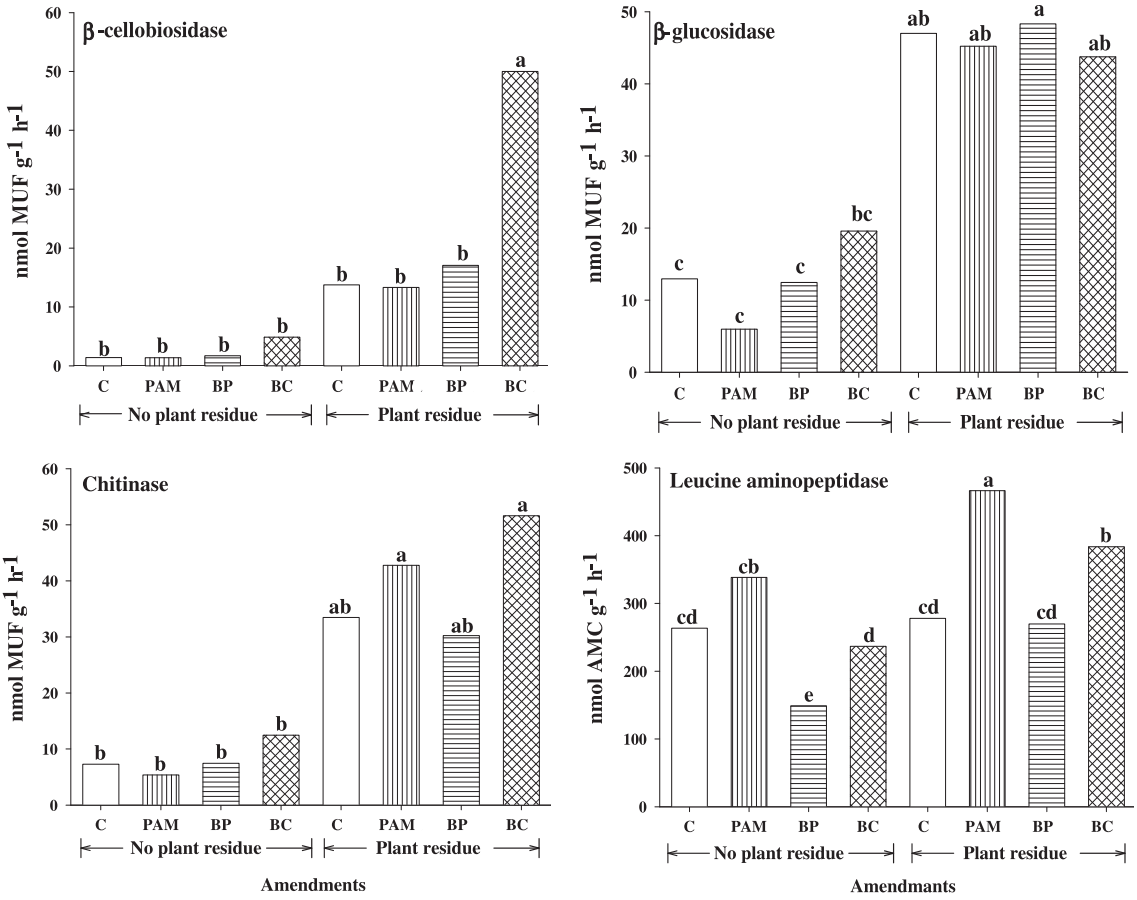

Fig. 3. Activities of cellobiohydrolase β-cellobiosidase, β-glucosidase, chitinase, and leucine aminopeptidase enzymes (nmol g^{-1} h^{-1}) in sandy soil in response to soil stabilizers (C: control soil, BC: biochar 250, BP: biopolymer, PAM: polyacrylamide) with or without plant residue. Bars with the same letters are not significantly different at the 0.05 probability level.

^{14}C residue (Fig. 1). These results were in accordance with those of many other studies [e.g. [23,50]]. Previous studies [51,52] have shown that BC increases the decomposition of ryegrass and switchgrass residues. BC application also induced higher enzyme activities due to improved soil chemical properties, resulting in faster decomposition of plant residue compared to that in untreated soil. The activities of soil microorganisms increased as indicated by the higher activity of chitinase in soil amended with BC. Similar findings on plant residue decomposition have been reported in response to changes in land use in studies conducted by Dilly and Munch [53] and Chen et al. [25]. The mineralization rate of the available part of fresh plant residue is highest during the first 24 days of incubation [54]. Our findings are similar to those of Chen et al. [25], who found that addition of plant residue to soil leads to

Table 4
Pearson correlation coefficients (r) between $^{14}CO_2$ decomposition rate of plant residue and the activity of β-cellobiosidase, β-glucosidase and chitinase in sandy loam and sandy soils.

	$^{14}CO_2$ decomposition rate (% of input ^{14}C day^{-1})			Enzymes activities (nmol g^{-1} h^{-1})		
	2 days	34 days	80 days	β-celliobiosidase	β-glucosidase	Chitinase
Sandy loam soil						
$^{14}CO_2$ decomposition rate after 2 days of incubation	1.000***	0.991**	0.970*	0.997**	0.946*	0.993**
$^{14}CO_2$ decomposition rate after 34 days of incubation		1.000***	0.990**	0.980*	0.981**	0.999***
$^{14}CO_2$ decomposition rate after 80 days of incubation			1.000***	0.950*	0.990**	0.988**
β-celliobiosidase (nmol g^{-1} h^{-1})				1.000***	0.923	0.983**
β-glucosidase (nmol g^{-1} h^{-1})					1.000***	0.978*
Chitinase (nmol g^{-1} h^{-1})						1.000***
Sandy soil						
$^{14}CO_2$ decomposition rate after 2 days of incubation	1.000***	0.802	0.786	0.984**	−0.832	0.887
$^{14}CO_2$ decomposition rate after 34 days of incubation		1.000***	0.997**	0.783	−0.674	0.774
$^{14}CO_2$ decomposition rate after 80 days of incubation			1.000***	0.780	−0.622	0.730
β-celliobiosidase (nmol g^{-1} h^{-1})				1.000***	−0.719	0.791
β-glucosidase (nmol g^{-1} h^{-1})					1.000***	−0.989***
Chitinase (nmol g^{-1} h^{-1})						1.000***

Correlation significant levels: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Table 5
Multifactor ANOVA showing the effects of soils, plant residue and amendments on the tested variables.

Source	DF	Cumulative CO_2	Cumulative $^{14}CO_2$	β-cellobiosidase	β-glucosidase	Chitinase	Leucine aminopeptidase
				$P > F$			
Soil	1	<0.0001***	0.0004***	<0.0001***	<0.0001***	<0.0001***	<0.0001***
Plant residue	1	<0.0001***	–	<0.0001***	<0.0001***	<0.0001***	<0.0001***
Amendments	3	0.0002 ***	<0.0001***	<0.0011**	0.3178	0.1798	0.0819
Error *MS*		0.003	14.551	3093.303	43,195.429	14,511.980	112,817.480
R^2		0.978	0.814	0.740	0.771	0.747	0.884

Significant levels: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

increased decomposition of litter based on $^{14}CO_2$ evolution. Furthermore, Chen et al. [25] previously showed that the decomposition rates of maize residue in soil range from 4.7% to 5.8% of the input ^{14}C day^{-1} after 2 days of incubation, whereas they are 0.04% and 0.05% of the input ^{14}C day^{-1} at the end of the incubation period.

The two phases of plant residue decomposition observed in our study indicate 1) rapid decomposition of easily available compounds in plant residue (sugars, starch, cellulose, and simple proteins) and 2) very slow decomposition of substrates that were not readily available (lignin and phenolic compounds) [25].

The effect of PAM differs according to the type of soil it is applied to as well as its interaction with residue in the soil [16]. PAM may not be useful as an amendment for sandy soil since its efficacy is highly dependent on clay content [12]. However, in our study, BP and BC consistently increased the decomposition rate of plant residue. These results can likely be attributed to the higher activities of decomposing microorganisms or higher substrate availability due to aggregation after addition of soil stabilizers. Soil aggregates and chemical properties play essential roles in the availability of SOM or plant residue to the microbial biomass (as evaluated by enzyme activities) [55].

4.3. Enzyme activities

We investigated the activities of β-cellobiosidase, β-glucosidase, chitinase, and leucine aminopeptidase since they reflect different parts of the C and N cycles [56]. Decomposition of cellulose in plant residue occurred during 34 days of incubation and was found to be responsible for the major loss of litter mass in the soil in a study by Teklay [33]. The high activity of the cellulolytic enzymes β-cellobiosidase and β-glucosidase observed in our study after 34 days suggest a shorter period of cellulose decomposition than that reported by Teklay [33]. The slower decomposition of leaf residue in the study conducted by Teklay was likely due to a higher total polyphenol content in the stem residue in their study compared to that in the present study [33].

Cellulose in plant residue is an insoluble molecule that is broken down by cellobiohydrolase into smaller oligosaccharides through the removal of cellobiose or glucose from the ends of cellulose molecules [34]. β-glucosidase leads to the breakdown of oligosaccharides into glucose, as well as soluble C and carbohydrates [36]. During the initial stage of decomposition of plant residue, β-cellobiosidase decomposes polymeric cellulose molecules and releases cellobiose, after which it cuts monomers from oligomeric compounds such as cellobiose and releases glucose, which is used as an energy source for microbial biomass [34,35].

β-glucosidase is an index of high microbial activity and contributes to the decomposition of plant residue [57]. This enzyme is produced by a variety of bacteria and fungi and appears to play a vital role in the decomposition of cellulose [35].

Based on correlation coefficients between the $^{14}CO_2$ decomposition rate of plant residue and enzyme activities, β-cellobiosidase was determined to be the most effective enzyme after 34 days of plant residue decomposition in either soil (Table 4). These results indicate that decomposition of cellulose was responsible for the major loss of plant residue [33]. The highly significant correlation between chitinase activity and $^{14}CO_2$ decomposition rate of plant residue (observed only in sandy loam soil) indicates that fungi made a greater contribution to decomposition than did plant residue in sandy loam as compared to in sandy soil [28]. Therefore, soil texture was a major factor controlling the decomposing microorganisms in the soil as indicated by significant correlations with cumulative CO_2, $^{14}CO_2$ evolution, and enzyme activities (Table 5). Specifically, the higher contents of C and N, clay, and exchangeable cations in sandy loam soil compared to sandy soil may have contributed to the higher enzyme activities. Turner et al. [36] observed that β-glucosidase activity is positively correlated with the chemical and physical properties of soil such as clay content and TC. Furthermore, enzyme activities are related to the soil organic C content, which is relatively higher in sandy loam soil when compared to that of sandy soil in our study [58]. Compared to previous studies [59], the low contents of soil C, N, and exchangeable cations contributed to the low rate of plant residue decomposition in sandy soil compared to that in sandy loam soil in the present study [25,26]. The lower C/N ratio (10–18.2) in our study indicates that the high litter quality increased the decomposition rate of plant residue by microbial biomass to balance the nutrients required to build their cells [33,53].

In this study, we observed high activity of leucine aminopeptidase after the addition of PAM and BC to both soils amended with plant residue. These findings could be due to the fact that proteins in plant residue contain about 16% N and decompose easily [28]. Stimulation of the degradation of proteins and N-rich substances by BC could be attributed to increased aminopeptidase activity.

After 34 days of incubation, BC and BP stimulated soil microorganisms, leading to a significant increase in extracellular enzyme activities and subsequently faster decomposition of plant residue, thereby promoting the growth of soil fungi [28]. This is partly associated with better soil aeration after the formation of larger aggregates stimulated by the addition of BC and BP to soil amended with plant residue. In the present study, during the first days of incubation, soil stabilizers increased the decomposition of the labile fraction of plant residue by improving the physical and chemical properties of the soil.

Taken together, BC and BP only increased microbial activity in soil amended with plant residue, which is similar to the finding that CO_2 is only increased after activation of microorganisms with glucose [56]. BC improved the soil quality by increasing the CEC, nutrient retention, and the physical and biological properties of the soil [19,23] (based on higher activities of β-cellobiosidase, β-glucosidase, and aminopeptidase). The effectiveness of PAM is greater in soil with high clay content since it increases the number of charged sites available for bonding compared to that in poorly structured soil [60]. Accordingly, PAM had a minor effect on poorly textured soils with low levels of exchangeable cations.

5. Conclusions

The effects of PAM, BP, and BC on decomposition of SOM and ^{14}C maize residue were evaluated based on cumulative CO_2 and $^{14}CO_2$ efflux during 80 days of incubation. Compared with unamended soils, the addition of BC stimulated the highest decomposition rate of ^{14}C-labeled plant residue in either soil, followed by BP. The highest cellobiohydrolase, β-glucosidase, and chitinase activities were observed in BC-amended soil, followed by BP-amended soil. β-cellobiosidase was the major enzyme responsible for plant residue decomposition in both soils after 34 days of incubation. Analysis of enzyme activities indicated that there were higher microbial activities in the sandy loam soil than in the sandy soil. The results of 3-way factorial ANOVA indicated that soil texture was a major factor controlling decomposition of plant residue. Soil texture is an important factor controlling the decomposing microorganisms in the soil. For instance, the lower enzyme activity in sandy soil led to a lower decomposition rate of plant residue compared with that in sandy loam soil. In addition, our study revealed a higher contribution of fungi to the decomposition of plant residue in sandy loam soil compared to that in sandy soil based on a highly significant correlation between chitinase activity and the $^{14}CO_2$ decomposition rate. Further research is needed to understand the effects of polymers on C sequestration, especially in long-term incubation experiments. Due to the readily available C in the tested BC in our study, we recommend the addition of BC as an effective agricultural practice in Korea in areas in which rapid decomposition of plant residue is needed between crop seasons. In addition, PAM can be recommended as a carbon conservation technology.

Acknowledgments

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References

[1] D. Pimentel, C. Harvey, P. Resosudarmo, K. Sinclair, D. Kurz, M. McNair, S. Crist, L. Shpritz, L. Fitton, R. Saffouri, R. Blair, Environmental and economic costs of soil erosion and conservation benefits, Science 267 (1995) 1117–1123.
[2] K.H. Jung, Y.K. Sonn, S.Y. Hong, S.O. Hur, S.K. Ha, Assessment of national soil loss and potential erosion area using the digital detailed soil maps, Korean J. Soil Sci. Fert 38 (2005) 59–65 (in Korean with English abstract).
[3] F.G.A. Verheijen, R.J.A. Jones, R.J. Rickson, C.J. Smith, Tolerable versus actual soil erosion rates in Europe, Earth Sci. Rev. 94 (2009) 23–38.
[4] R.E. Sojka, D.L. Bjorneberg, J.A. Entry, R.D. Lentz, W.J. Orts, Polyacrylamide in agriculture and environmental land management, Adv. Agron. 92 (2007) 75–162.
[5] R.D. Lentz, R.E. Sojka, Field results using polyacrylamide to manage furrow erosion and infiltration, Soil Sci. 158 (1994) 274–282.
[6] M. Agassi, M. Ben-Hur, Stabilizing steep slopes with soil conditioners and plants, Soil Technol. 5 (1992) 249–256.
[7] D.C. Flanagan, L.D. Norton, I. Shainberg, Effect of water chemistry and soil amendments on a silt loam soil – Part 1: infiltration and Runoff, Trans. Am. Soc. Agric. Eng. 40 (1997) 1549–1554.
[8] J. Letey, Effective viscosity of PAM solution through porous media. in: R.E. Sojka, R.D. Lentz (Eds.), Proceedings of Managing Irrigation-induced Erosion and Infiltration with Polyacrylamide (1996), pp. 94–96 University of Idaho Miscellaneous Publication No. 101–96.
[9] I. Shainberg, G.J. Levy, Organic polymers and soil sealing in cultivated soil, Soil Sci. 158 (1994) 267–273.
[10] G.J. Levy, W.P. Miller, Polyacrylamide adsorption and aggregate stability, Soil Till. Res. 51 (1999) 121–128.
[11] C.A. Seybold, Polyacrylamide review: soil conditioning and environmental fate, Commun. Soil Sci. Plant Anal. 25 (1994) 2171–2185.
[12] A.I. Mamedov, S. Beckmann, C. Huang, G.J. Levy, Aggregate stability as affected by polyacrylamide molecular weight, soil texture, and water quality, Soil Sci. Soc. Am. J. 71 (2007) 1909–1918.
[13] L. Wu, Y.S. Ok, X.L. Xu, Y. Kuzyakov, Effects of anionic polyacrylamide on maize growth: a short term ^{14}C labeling study, Plant Soil (2011). doi:10.1007/s11104-011-0911-y.
[14] R. Parker, S.G. Ring, The physical chemistry of starch. in: S. Dumitriu (Ed.), Polysaccharides: Structural Diversity and Functional Versatility, second ed. Marcel Dekker, New York, 2005, pp. 591–604.
[15] R.E. Sojka, J.A. Entry, W.J. Orts, D.W. Morishita, C.W. Ross, D.J. Horne, Synthetic- and bio-polymer use for runoff water quality management in irrigated agriculture, Water Sci. Technol. 51 (2005) 107–115.
[16] T.C. Caesar-TonThat, W.J. Busscher, J.M. Novak, J.F. Gaskin, Y. Kim, Effects of polyacrylamide and organic matter on microbes associated to soil aggregation of Norfolk loamy sand, Appl. Soil Ecol. 40 (2008) 240–249.
[17] S. Bruun, T. El-Zahery, L. Jensen, Carbon sequestration with biochar - stability and effect on decomposition of soil organic matter, IOP Conf. Series, Earth Environ. Sci. 6 (2009) 242010.
[18] B. Glaser, L. Haumaier, G. Guggenberger, W. Zech, The ‘Terra Preta’ phenomenon: a model for sustainable agriculture in the humid tropics, Naturwissenschaften 88 (2001) 37–41.
[19] J. Lehmann, J.P. da Silva Jr., C. Steiner, T. Nehls, W. Zech, B. Glaser, Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments, Plant Soil 249 (2003) 343–357.
[20] D.D. Warnock, J. Lehmann, T.W. Kuyper, M.C. Rillig, Mycorrhizal responses to biochar in soil – concepts and mechanisms, Plant Soil 300 (2007) 9–20.
[21] M.W.I. Schmidt, A.G. Noack, Black carbon in soils and sediments: analysis, distribution, implications, and current challenges, Glob. Biogeochem. Cycles 14 (2000) 777–793.
[22] J. Lehmann, J. Gaunt, M. Rondon, Bio-Char sequestration in terrestrial ecosystems – A review, Mitig. Adapt. Strateg. Glob. Change 11 (2006) 403–427.
[23] A.R. Zimmerman, B. Gao, M.Y. Ahn, Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils, Soil Biol. Biochem. 43 (2011) 1169–1179.
[24] Y.S. Ok, A.R.A. Usman, S.S. Lee, S.A.M. Abd El-Azeem, B. Choi, Y. Hashimoto, J.E. Yang, Effects of rapeseed residue on lead and cadmium availability and uptake to rice plants in heavy metal contaminated paddy soil, Chemosphere (2011). doi:10.1016/j.chemosphere.2011.06.073.
[25] H. Chen, M. Fan, N. Billen, K. Stahr, Y. Kuzyakov, Effect of land use types on decomposition of ^{14}C-labeled maize residue (*Zea mays* L.), Eur. J. Soil Biol. 45 (2009) 123–130.
[26] R. Lal, Soil carbon sequestration impacts on Global climate change and food security, Science 304 (2004) 1623–1627.
[27] B. Majumder, Y. Kuzyakov, Effect of fertilization on decomposition of ^{14}C labelled plant residues and their incorporation into soil aggregates, Soil Till. Res. 109 (2010) 94–102.
[28] S. Steinbeiss, G. Gleixner, M. Antonietti, Effect of biochar amendment on soil carbon balance and soil microbial activity, Soil Biol. Biochem. 41 (2009) 1301–1310.
[29] S. Fu, D.C. Coleman, R. Schartz, R. Potter, P.F. Hendrix, D.A. Crossley Jr., ^{14}C distribution in soil organisms and respiration after the decomposition of crop residue in conventional tillage and no-till agroecosystems at Georgia Piedimont, Soil Till. Res. 57 (2000) 31–41.
[30] Y. Kuzyakov, Sources of CO_2 efflux from soil and review of partitioning methods, Soil Biol. Biochem. 38 (2006) 425–448.
[31] A. Gorissen, M.F. Cotrufo, Decomposition of leaf and root tissue of three perennial grass species grown at two levels of atmospheric CO_2 and N supply, Plant Soil 224 (2000) 75–84.
[32] J.G. Guzman, M. Al-Kaisi, Landscape position and age of reconstructed prairies effect on soil organic carbon sequestration rate and aggregate associated carbon, J. Soil Water Conserv 65 (1) (2010) 9–21.
[33] T. Teklay, Decomposition and nutrient release from pruning residues of two indigenous agroforestry species during the west and dry seasons, Nutr. Cycl. Agroecosyst 77 (2007) 115–126.
[34] P. Nannipieri, E. Kandeler, P. Ruggiero, Enzyme activities and microbiological and biochemical processes in soil. in: R.G. Burns, R.P. Dick (Eds.), Enzymes in the Environment: Activity, Ecology, and Applications. Marcel Dekker, Inc, 270 Madison Avenue, New York, 2002, pp. 1–33.
[35] N.C. Brady, R.R. Weil, Elements of the Nature and Properties of Soils, third ed. Pearson Education, Inc., USA, 2010, Publishing as Prentice Hall.
[36] B.L. Turner, D.W. Hopkins, P.M. Haygarth, N. Ostle, β-Glucosidase activity in pasture soils, Appl. Soil Ecol. 20 (2002) 157–162.
[37] M.E. Sumner, W.P. Miller, Cation exchange capacity and exchange coefficients. in: D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, M.E. Sumner (Eds.), Methods of Soil Analysis, Part 3 – Chemical Methods. Am. Soc. Agron.-Soil Sci. Soc. Am. Inc., Madison, Wisconsin, USA, 1996, pp. 1201–1229.

[38] B.H. Sheldrick, C. Wang, Particle-size distribution. in: M.R. Carter (Ed.), Soil Sampling and Methods of Analysis. Canadian Society of Soil Science Lewis Publishers, Boca Raton, Florida USA, 1993.
[39] F.J. Veihmeyer, A.H. Hendrickson, The moisture equivalent as a measure of the field capacity of soils, Soil Sci. (1931) 181–194.
[40] C. Liu, G. Wu, H. Mu, Z. Yuan, L. Tang, X. Lin, Synthesis and application of lignin-based copolymer LSAA on controlling non-point source pollution resulted from surface runoff, J. Environ. Sci. 20 (2008) 820–826.
[41] M. Gocke, K. Pustovoytov, Y. Kuzyakov, Carbonate recrystallization in root-free soil and rhizosphere of *Triticum aestivum* and *Lolium perenne* estimated by ^{14}C labeling, Biogeochem 103 (2011) 209–222.
[42] L.M. Zibilske, Carbon mineralization. in: R.W. Weaver, S. Angle, P. Bottomley, D. Bezdicek, S. Smith, A. Tabatabai, A. Wollum (Eds.), Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties, Soil Science Society of America Book Series, vol. 5. Soil Sci. Soc. Am., Inc., Madison, 1994, pp. 835–864.
[43] Y. Kuzyakov, W. Cheng, Photosynthesis controls of CO_2 efflux from maize rhizosphere, Plant Soil 263 (2004) 85–99.
[44] K.J. van Groenigen, A. Gorissen, J. Six, D. Harris, P.J. Kuikman, J.W. van Groenigen, C. van Kessel, Decomposition of ^{14}C-labeled roots in a pasture soil exposed to 10 years of elevated CO_2, Soil Biol. Biochem. 37 (2005) 497–506.
[45] K. Pritsch, S. Raidl, E. Marksteiner, H. Blaschke, R. Agerer, M. Schloter, A. Hartmann, A rapid and highly sensitive method for measuring enzyme activities in single mycorrhizal tips using 4-methylumbelliferone-labelled fluorogenic substrates in a microplate system, J. Microbiol. Methods 58 (2004) 233–241.
[46] H.G. Hoppe, Significance of exoenzymatic activities in the ecology of Brackish Water: measurements by means of Methylumbelliferyl-substrates, Mar. Ecol. Prog. Ser. 11 (1983) 299–308.
[47] SAS Institute Inc, SAS, SAS/STAT® 9.1 User's Guide. SAS Institute Inc., Cary, NC, USA, 2004.
[48] Y. Kuzyakov, I. Subbotina, H. Chen, I. Bogomolova, X. Xu, Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling, Soil Biol. Biochem. 41 (2009) 210–219.
[49] J.A. Entry, R.E. Sojka, B.J. Hicks, Carbon and nitrogen stable isotope ratios can estimate anionic polyacrylamide degradation in soil, Geoderma 145 (2008) 8–16.
[50] W.J. Wang, J.A. Baldock, R.C. Dalal, P.W. Moody, Decomposition dynamics of plant materials in relation to nitrogen availability and biochemistry determined by NMR and wet-chemical analysis, Soil Biol. Biochem. 36 (2004) 2045–2058.
[51] A. Hilscher, K. Heister, C. Siewert, H. Knicher, mineralization and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil, Org. Geochem. 40 (2009) 332–342.
[52] J.M. Novak, W.J. Busscher, D.W. Watts, D.A. Larid, M.A. Ahmeden, M.S.A. Niandou, Short-term CO_2 mineralization after additions of biochar and switchgrass to a typic kandiudult, Geoderma 154 (2010) 281–288.
[53] O. Dilly, J.C. Munch, Litter decomposition and microbial characteristics in agricultural soils in northern, central and southern Germany, Soil Sci. Plant Nutr. 50 (2004) 843–853.
[54] J.L. Chotte, J.N. Ladd, M. Amato, Sites of microbial assimilation, and turnover of soluble and particulate ^{14}C labelled substrates decomposing in a clay soil, Soil Biol. Biochem. 30 (1998) 205–218.
[55] M. Dorodnikov, E. Blagodatskaya, S. Blagodatsky, S. Marhan, A. Fangmaier, Y. Kuzyakov, Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on soil aggregate size, Glob. Change Biol. 15 (2009) 1603–1614.
[56] R.G. Burns, R.P. Dick, Enzymes in the Environment: Activity, Ecology and Applications. Marcel Dekker, Inc., 270 Madison Avenue, New York, 2002.
[57] M. Stemmer, M.H. Gerzabek, E. Kandeler, Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication, Soil Biol. Biochem. 30 (1998) 9–17.
[58] D. Landgraft, S. Klose, Mobile and readily available C and N fractions and their relationship to microbial biomass and selected enzyme activities in a sandy soil under different management systems, J. Plant Nutr. Soil Sci. 165 (2002) 9–16.
[59] E.A. Kaiser, T. Mueller, R.G. Joergensen, H. Insam, O. Heinemeyer, Evaluation of methods to estimate the soil microbial biomass and the relationship with soil texture and organic matter, Soil Biol. Biochem. 24 (1992) 675–683.
[60] S.S. Lee, C.J. Gantzer, A.L. Thompson, S.H. Anderson, Polyacrylamide and gypsum amendments for erosion and runoff control on two soil series, J. Soil Water Conserv 65 (2010) 233–242.