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Effects of polyacrylamide, biopolymer and biochar on the decomposition of ^{14}C-labelled maize residues and on their stabilization in soil aggregates

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Summary

The efficacy of applying plant residues to agricultural soils as a carbon (C) source for microorganisms and C sequestration is dependent on soil physiochemical properties, which can be improved by aggregation using soil conditioners. However, no attempt has been made to assess the effects of soil conditioners such as biochar (BC), biopolymer (BP) or polyacrylamide (PAM) on plant residue decomposition. We assessed the effects of BC, synthesized BP and anionic PAM on the decomposition of ^{14}C-labelled maize residues and on their stabilization in aggregate fractions in sandy and sandy loam soils. Polyacrylamide and BP were applied at 400 kg ha^{-1} and BC was applied at 5000 kg ha^{-1}, and the soils were incubated for 80 days at 22°C. The conditioners improved the physical and biological properties of both soils, as shown by a 24% increase in the 1–2 mm aggregates. Biochar and BP accelerated the decomposition of plant residues as indicated by $^{14}CO_2$ efflux, and resulted in reduced stabilization of residues in both soils relative to that observed in the control and PAM treatments. The reduction in ^{14}C incorporation and C stabilization in the BC- and BP-treated soils was observed mainly in the < 0.25-mm aggregates. This was confirmed by reduction of activity of hydrolytic enzymes (β-cellobiosidase and β-glucosidase). Decomposition of plant residues in sandy soil was more sensitive to BP and PAM application than that in sandy loam soil. Improved soil structure after applying BC and BP increased aeration and decreased the contact between plant residues and mineral soil particles and consequently accelerated plant residue decomposition and reduced C sequestration.

Introduction

Soil quality decreases in response to a loss of carbon (C) from the upper fertile horizons through erosion. The annual loss of soil derived from water and wind erosion on agricultural land has been estimated at more than 70 billion metric tons globally (Pimentel *et al*., 1995). Accordingly, it is necessary to develop methods to reduce soil degradation and to maintain soil organic carbon (SOC). In particular, SOC is an important soil quality indicator, primarily through its effects on physiochemical and biological properties (Mikha & Rice, 2004). Notably, changes in agricultural practices, such as adding plant residues, can stimulate both SOC decomposition and C sequestration (Chen *et al*., 2009). Soil microaggregates may occlude added plant residues, thereby rendering them inaccessible to decomposing microorganisms (Majumder & Kuzyakov, 2010). Thus, a clear understanding of the effects of soil management on C dynamics is a prerequisite to ensure soil quality.

From a practical point of view, C input in the form of plant residues is effective for maintaining SOC and is a major energy source for microorganisms, thereby influencing C turnover, soil quality and crop production (Kuzyakov *et al*., 2007; Ok *et al*., 2011). Moreover, decomposable plant C and SOC bind soil microaggregates (< 0.25 mm) together to form stable macroaggregates (> 0.25 mm), and these enhance aggregate stability and perform a critical function in storing C (Abiven *et al*., 2008) through protecting SOC against degradation by organisms and their enzymes (Kögel-Knabner *et al*., 2008). Intensification of agriculture and frequent cropping (double or even triple cropping systems) make it necessary for plant residue to decompose rapidly to maintain soil quality and nutrient release and avoid pathogen transfer.

Since the 1990s, polyacrylamide (PAM) has been applied to reduce soil erosion by stabilizing the outer surfaces of aggregates

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and penetrating the inner parts (Sojka *et al*., 2007; Wu *et al*., 2011). Thus, PAM binds particles and forms larger aggregates and in so doing prevents crust formation on the soil surface, thereby reducing erosion (Sojka *et al*., 2007). Overall, PAM alters the physical, chemical and biological properties of soil, thereby improving aeration, water and root penetration and erosion resistance (Sojka *et al*., 2007). The efficiency of PAM tends to be greater in soils with a large clay content because of a larger number of charged bonding sites (Lee *et al*., 2010). Polyacrylamide is highly persistent in soil, with a decomposition rate of 9.8% per year (Entry *et al*., 2008) and is more resistant to microbial degradation than other commonly used polymers (Sojka *et al*., 2007). Improvement effects therefore persist over a longer period. However, little is currently known about the activities of soil enzymes and microorganisms in response to PAM (Kay-Shoemake *et al*., 1998).

Use of biopolymers (BPs) is an eco-friendly alternative to PAM to prevent soil erosion (Sojka *et al*., 2007). Biopolymers are produced from starch, cellulose, chitin, lignin, microfibril suspensions, polysaccharides and protein derivatives (Sojka *et al*., 2005). Biopolymers stabilize soil aggregates through their surface charge and retain their stability in aqueous suspensions (Orts *et al*., 2000). In particular, cellulose microfibrils in BPs (crystalline units of cellulose) are dispersed in water during acid hydrolysis through the charge on their outer surface, thereby resulting in clay flocculation and stabilization of aggregates (Orts *et al*., 2000). In contrast to PAM, BPs are highly biodegradable and represent a source of C for saprophytic fungi (Baldrian *et al*., 2011). Therefore, applying a BP may result in an increase in soil microorganism and enzyme activities, followed by an increase in plant residue decomposition. Transformation of the C present in BP is an important process during soil C turnover (Baldrian *et al*., 2011). However, it remains unclear as to whether the potential for soil microorganisms and litter-associated fungi to degrade plant residues can be altered by BPs (Baldrian *et al*., 2011).

The use of biochar (BC) as a fertilizer improves soil quality by increasing cation exchange capacity, SOC content and microbial activity (Lehmann *et al*., 2006). Biochar also improves the physical and biological properties of soil, thereby allowing it to retain nutrients and enhance plant growth (Glaser *et al*., 2002) and large applications enhance C stock in soil (Lehmann *et al*., 2006). Biochar is a by-product of the pyrolysis of agricultural residues such as crop residues, carbonaceous sources and natural or anthropogenic wastes (Bruun & Luxhoi, 2008). Consequently, BC degradation is dependent on its origin and the production procedure. Thus, both the quality and quantity of BC affect the soil microbial population (Lehmann *et al*., 2006). However, little information is currently available regarding the effects of BC on soil microorganisms and aggregation (Brodowski *et al*., 2006).

Soil enzyme activity is a sensitive indicator used to evaluate the effects of amendments or land-use changes (Katsalirou *et al*., 2010). Increases in β-cellobiosidase and β-glucosidase activity (responsible for cellulose and hemicellulose decomposition, major constituents of plants) actually reflect the availability of plant residues for microbial decomposition and perform vital functions in the soil C cycle (Dorodnikov *et al*., 2009).

We showed earlier that the sensitivity of SOC mineralization is affected by the incorporation of plant residues into BC- and BP-treated soils when compared with control or PAM-treated soils (Awad *et al*., 2012). Accordingly, the present study was conducted to determine whether adding BP and BC accelerates plant residue decomposition by improving physical and biological properties of different textured soils. Specifically, we tested whether the decomposition of plant residues might be more sensitive to conditioners in sandy soil with a small initial C content than that in sandy loam soil with a relatively large C content. We attempted to determine whether or not the readily degradable substrates in BC and BP might enhance enzyme activity, thereby increasing the plant residue decomposition rate in sandy and sandy loam soils. If these conditioners altered the aggregate distribution, we expected to observe direct effects on the stabilization of plant residues by aggregate production as evaluated by the activity of hydrolytic enzymes in each aggregate-size fraction at the end of the incubation experiment.

Materials and methods

Soil sampling and characteristics

Soil samples were collected from the upper 5 cm from two agricultural fields in Haean Catchment, Korea. Sandy soil was collected from an agricultural field, and sandy loam soil was collected from an agricultural highland adjacent to a forest. From our previous study, the sand, silt and clay contents were 90.9, 4.6 and 4.5% for the sandy soil, and 67.3, 25.9 and 6.8% for the sandy loam soil, respectively (Awad *et al*., 2012). The sandy loam soil had a larger TC content (3.1%) and C:N ratio (18.2) than the sandy soil (0.1% TC and 10.0 C:N). The very small TC content noted in the sandy soil was attributable to the local farmer's use of sand to compensate for substantial erosion losses in the hilly landscape. Water-holding capacities (WHCs) were 14.5 and 28.2% for the sandy and sandy loam soils, respectively.

Soil amendments and plant residues

Biochar BC250 (BC) was purchased from Sonnenerde GmbH (Riedlingsdorf, Austria), and PAM (Magnafloc 336) from Ciba Canada, Ltd (Mississauga, Ontario, Canada). The BP was synthesized by the method described by Liu *et al*. (2008). The BC contained 2.73% N and 67.0% C, PAM contained 16.2% N and 42.2% C, and BP contained 5.3% N and 28.4% C. Before the soil conditioner experiment, maize plants were labelled three times in a $^{14}CO_2$ atmosphere to produce uniformly ^{14}C-labelled plant residues, based on the method described by Gocke *et al*. (2011). The plant residues were dried and ground using a ball mill and had a final ^{14}C activity of 30 Bq mg^{-1}.

Incubation

This incubation experiment was a continuation from our previous study to understand the role of soil conditioners in decomposition of plant residues and their stabilization in different aggregate-size fractions, evaluated by extracellular enzyme activity at the end of the incubation experiment (Awad *et al*., 2012). The experiment consisted of eight treatments with four replicates. Two factors, (i) soil type (sandy or sandy loam) and (ii) plant residues (PR) alone or in combination with soil conditioners (soil + PR (Control), BC at 5000 kg ha^{-1} + PR, BP at 400 kg ha^{-1} + PR and PAM at 400 kg ha^{-1} + PR), were evaluated with a completely randomized factorial design. ^{14}C-labelled maize residue (100 mg) was mixed thoroughly with 30 g air-dried soil containing none or one of the three aforementioned conditioners. The amended soils were placed in closed vessels and incubated for 80 days at 22°C. Soil moisture was maintained at 70% of WHC with deionized water throughout the experiment. The CO_2 and $^{14}CO_2$ efflux rates from soils with and without PAM, BP and BC were compared in order to evaluate plant residue and soil organic matter decomposition after adding the conditioners.

Small vials containing 2 ml 1.0 M NaOH were placed in the incubation vessels to trap CO_2. These vessels were changed periodically at 2, 4, 8, 12, 16, 24, 34, 48, 62 and 80 days during the incubation to measure CO_2 and ^{14}C activity. Four vessels containing NaOH vials but without soil were used as blanks.

Aggregate-size fractionation

Aggregates were separated at the end of the 80-day incubation period using the method described by Dorodnikov *et al*. (2009). Briefly, soil samples were spread as a thin layer and dried to optimal moisture to ameliorate mechanical stress. Thereafter, soil samples were gently sieved through 1.0- and 0.25-mm sieves and then shaken for 90 s, after which the 1–2-mm aggregates were collected. Next, the soil remaining on the 0.25-mm sieve was shaken as described above, and the 0.25–1-mm aggregates and the < 0.25-mm aggregates were collected. The recovery after sieving was > 95% of soil mass.

CO_2 efflux and ^{14}C analyses

Carbon dioxide trapped in the 1.0 M NaOH solution was precipitated with 0.5 M $BaCl_2$ solution to estimate total CO_2 efflux and the NaOH then titrated with 0.01 M HCl against the indicator phenolphthalein (Zibilske, 1994). A 0.4-ml aliquot of NaOH solution was mixed with 2-ml of Rothiscint-22x scintillation cocktail (Roth Co., Karlsruhe, Germany) to measure ^{14}C activity. $^{14}CO_2$ (% plant residue input) was calculated as described by Van Groenigen *et al*. (2005). Cumulative $^{14}CO_2$ efflux was calculated as the increase in $^{14}CO_2$ within each sampling interval, and presented as a percentage (%) of ^{14}C input from days 0 to 24 and 24 to 80 of the incubation, respectively. We estimated total CO_2 and $^{14}CO_2$ effluxes and cumulative CO_2 and $^{14}CO_2$ during days 0–24 and 24–80 to understand the role of plant residue decomposition in C mineralization in soil. Similarly, the ^{14}C remaining in each aggregate-size fraction was calculated as the percentage of plant residue-^{14}C input. Carbon dioxide efflux from soil without amendments (control) was subtracted from that collected from soil amended with conditioner to estimate CO_2 efflux caused by decomposition of each conditioner. These calculated data were adopted from previous findings (Awad *et al*., 2012). At the end of the incubation period, 0.5 g of soil was combusted within an oxidizer unit (multi N/C 2100; Analytik Jena AG, Jena, Germany) and the released CO_2 was absorbed in 1.0 M NaOH to measure ^{14}C activity in each aggregate-size fraction. Then, 1-ml aliquots of NaOH solution were mixed with 2-ml scintillation cocktail to measure ^{14}C activity. ^{14}C counting efficiency was approximately 93%, and the measurement error did not exceed 3%.

Enzyme activities

Extracellular enzyme activities in each aggregate-size fraction were determined with fluorogenically-labelled substrates based on 4-methylumbelliferone (MUF). MUF-β-D-cellobioside and MUF-β-D-glucopyranoside were used to measure β-cellobiosidase EC 3.2.1.91 and β-glucosidase EC 3.2.1.21 activities, respectively, using the technique described by Dorodnikov *et al*. (2009). Briefly, soil samples (0.5 g) were suspended in 25 ml water and shaken for 15 minutes at 220 rpm and room temperature. Then, 0.5 ml soil suspension was added to 0.5 ml of the substrate solution in deep-well plates (24-wells × 10 ml, HJ-Bioanalytik GmbH, Göckelsweg, Germany). The plates were incubated at 22°C for 3 hours for MUF-β-D-cellobioside and 1 hour for MUF-β-D-glucopyranoside. After centrifugation (402 ***g*** for 10 minutes), fluorescence was measured in a 1-ml aliquot of supernatant with a Victor^3 1420–050 Multilabel Counter (PerkinElmer, Waltham, MA, USA) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm with a slit width of 25 nm. Enzyme activities were calculated as released MUF in nanomoles per gram of bulk soil per dry mass per hour (nmol g^{-1} hour^{-1}).

Statistical analysis

All variables (CO_2 efflux, cumulative CO_2, estimated CO_2 rates from the decomposition of conditioners, $^{14}CO_2$ efflux, and cumulative $^{14}CO_2$) were tested by a three-factor ANOVA to study their responses to soil texture, amendments (soil conditioners and plant residues) and incubation time. In addition, a two-factor ANOVA was performed to integrate the effects of soil type and amendment on aggregate-size fractions and extracellular enzyme activity in each fraction. Data were modelled as a generalized linear model (GLM) to integrate the effects of incubation time (within repeated measurements of CO_2: 10 repeated measurements with four replicates during 0–80 days of incubation), amendments and their interaction (time × amendments) with soil amendments on the tested variables. The standard error of the mean was calculated from four replicates of each treatment. A *P* value of

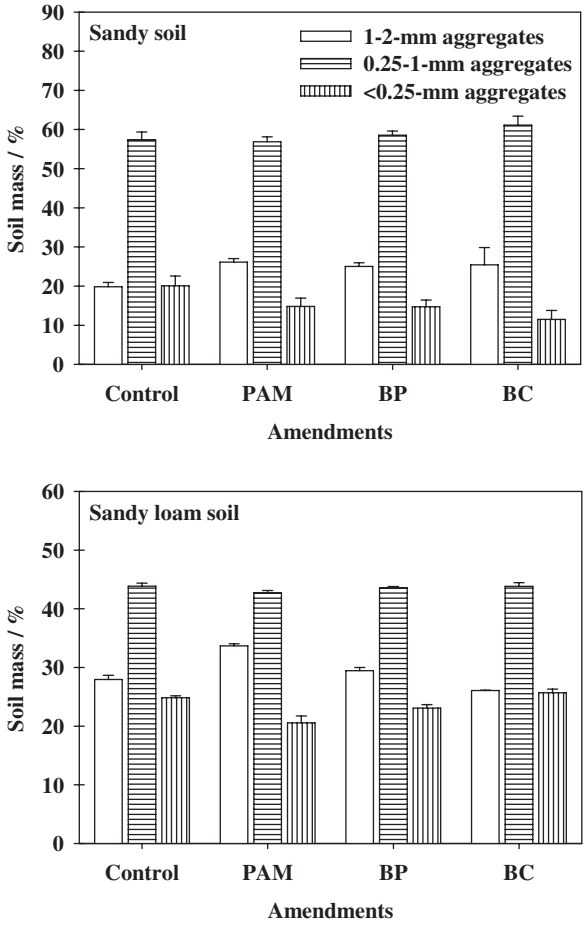

Figure 1 Aggregate size fractions of sandy and sandy loam soils mixed with plant residues in response to amendments with 400 kg ha^{-1} polyacrylamide (PAM), 400 kg ha^{-1} biopolymer (BP) and 5 Mg ha^{-1} biochar (BC) compared with no additives (control). Error bars indicate the standard error of the mean.

less than 0.05 was considered to be significant and SAS software was used for all analyses (SAS Institute, 2004).

Results

Effects of soil conditioners on aggregate fractions

PAM, BP and BC in the sandy soil with added plant residues increased the percentage of 1–2-mm aggregates by an average of 28.7% and reduced the percentage of < 0.25-mm aggregates by an average of 31.8% relative to those in the control soil with plant residues only (Figure 1). In the sandy loam soil, PAM with added plant residues increased the 1–2-mm aggregates by 20.5% and reduced the < 0.25-mm aggregates by 17.2%, relative to those in control soil with plant residues (Figure 1). Such strong effects of the soil conditioners led us to expect changes in the soil's physicochemical and biological properties and, consequently, changes in the decomposition of plant residues and their stabilization.

CO_2 efflux and plant residue decomposition

From our previous study (Awad *et al*., 2012), two phases of plant residue decomposition occurred from 0 to 24 and 24 to 80 days of incubation and appeared to have an influence on carbon mineralization that was independent of soil texture and characteristics (data from Awad *et al*., 2012 for 0–24 and 24–80 days of incubation are presented in Table S1). Carbon dioxide efflux rates from both soils decreased sharply during days 0–24 and thereafter the rates decreased slowly during days 24–80. No effects of the conditioners on cumulative CO_2 efflux were observed in the sandy soil. Polyacrylamide applied with plant residues led to 11 and 10.5% reductions in cumulative CO_2 efflux during days 0–24 and 24–80, respectively, in the sandy loam soil relative to that in the control soil with plant residues (Table S1). Biopolymer applied with plant residues reduced the cumulative CO_2 efflux by 13.2% during days 24–80 in the sandy loam soil relative to that in the control soil with plant residues. Additionally, PAM and BP with plant residues reduced the cumulative CO_2 efflux by 11.9 and 5.6%, respectively, after 80 days of incubation relative to that in the control soil with plant residues. Cumulative CO_2 efflux was 1.5 times greater in sandy loam soil mixed with plant residues than in sandy soil, as an average across all treatments.

The maximum increase in cumulative $^{14}CO_2$ of 16 and 7.4% of ^{14}C input in sandy soil occurred in response to treatment with BC during days 0–24 and 24–80, respectively, compared with that in control soil (Table S1). Polyacrylamide and BP increased the cumulative $^{14}CO_2$ efflux by 11.5 and 7.7% of ^{14}C input in sandy soil during days 0–24, respectively, compared with that in control soil. Only BC increased the cumulative $^{14}CO_2$ efflux from plant residue decomposition in the sandy loam soil by 13.5% of the input of ^{14}C during days 0–24, relative to that observed in control soil with plant residues (Table S1). From the evidence of $^{14}CO_2$ evolution, BC induced the greatest increase in the rate of plant residue decomposition in both soils followed by BP, when compared with PAM and/or the control soil with plant residues. These findings indicate that decomposing plant residues in the sandy soil were more sensitive to PAM and BP than those in the sandy loam soil. The sandy loam soil had the greatest cumulative $^{14}CO_2$ efflux (23.1%) after 80 days of incubation compared with that in the sandy soil without adding conditioners.

All variables (CO_2 efflux, cumulative CO_2, estimated CO_2 rates from the decomposition of conditioners, $^{14}CO_2$ efflux and cumulative $^{14}CO_2$) tested by the three-factor ANOVA showed significant responses to soil texture, amendments and incubation time (Table 1). The repeated measurements GLM analysis showed a significant linear relationship between amendments or time or their interaction and the tested variables ($^{14}CO_2$ and CO_2 effluxes and cumulative $^{14}CO_2$ and CO_2 in both sandy and sandy loam soils). Using the GLM analysis (Table 2), treatments, time (10 repeated measurements with four replicates during 0–80 days of incubation) and their interaction (time × amendments) exerted significant effects on plant residue decomposition. Similarly, time

Table 1 Multifactor ANOVA for the effects of soils, amendments and incubation time on the tested dependent variables

Source	Degrees of freedom	Sum of squares	Mean square	*F* ratio	*P* > *F*
CO_2 efflux / μg C day^{-1} g^{-1} soil					
Soils	S-1 = 1	1320.85	1320.85	20.23	<0.0001
Amendments	A-1 = 3	226.40	75.47	1.16	NS
Incubation time	T-1 = 9	222 211.26	24 690.14	378.24	<0.0001
Total	13	223 758.52	17 212.19	263.68	<0.0001
$^{14}CO_2$ efflux / % of the input ^{14}C day^{-1}					
Soils	S-1 = 1	0.43	0.43	0.69	NS
Amendments	A-1 = 3	19.67	6.56	10.46	<0.0001
Incubation time	T-1 = 9	942.47	104.72	167.01	<0.0001
Total	13	962.58	74.04	118.09	<0.0001
CO_2 efflux from conditioner / μg C day^{-1} g^{-1} soil					
Soils	S-1 = 1	32.78	32.78	17.99	<0.0001
Amendments	A-C-1 = 2[a]	68.20	34.10	18.71	<0.0001
Incubation time	T-1 = 9	172.69	19.19	10.53	<0.0001
Total	12	273.67	22.81	12.51	<0.0001
Cumulative CO_2 / mg C g^{-1}					
Soils	S-1 = 1	5.52	5.52	931.91	<0.0001
Amendments	A-1 = 3	0.136	0.045	7.63	<0.0001
Incubation time	T-1 = 9	21.24	2.36	397.96	<0.0001
Total	13	26.90	2.07	348.95	<0.0001
Cumulative $^{14}CO_2$ / % of input ^{14}C					
Soils	S-1 = 1	8889.80	8889.80	473.45	<0.0001
Amendments	A-1 = 3	10 094.54	3364.84	179.20	<0.0001
Incubation time	T-1 = 9	140 904.13	15 656.01	833.79	<0.0001
Total	13	159 888.47	12 299.11	655.02	<0.0001

[a]Amendments (*A*) = 3 as CO_2 efflux from conditioner was estimated by subtracting the CO_2 efflux from the amended soil from the CO_2 efflux from control soil.
Where *S* = 2, *A* = 4, *T* = 10 and *C* = 1 (control soil). NS = no significant difference.

(0–24 and 24–80 days of incubation), amendments and their interaction acted significantly on the individual variables such as estimated cumulative $^{14}CO_2$ effluxes from sandy and sandy loam soils (GLM analysis in Table S2).

Amendment with plant residues appeared to have a profound effect on CO_2 and $^{14}CO_2$ effluxes but depended on soil texture and time, as indicated by a strong linear relationship between time of repeated measurements and the tested variable (CO_2 and $^{14}CO_2$ effluxes) in both soils (Tables 1 and 2). Taken together, the statistical analysis indicated that the decomposition of plant residues was dependent on applying conditioners to sandy and sandy loam soils and the time of the measurement, attributed to substrate accessibility of microorganisms.

Decomposition of soil conditioners and CO_2 efflux from soil

The CO_2 efflux rate from PAM, BP or BC treatments was estimated by subtracting the CO_2 efflux from soil amended with each conditioner from the CO_2 efflux from soil without amendments (Awad *et al*., 2012). The maximum decomposition rate of BC (0.86 μg C day^{-1} g^{-1}) was observed in the sandy soil after 8 days incubation, whereas a rate of 5.0 μg C day^{-1} g^{-1} was observed in the sandy loam soil (Figure 2). Similarly, BP had a maximum decomposition rate in the sandy soil of 1.6 μg C day^{-1} g^{-1} at 8 days, whereas a rate of 4.2 μg C day^{-1} g^{-1} was noted in the sandy loam soil. No PAM decomposition was noted in either soil during the incubation period, except at day 12 in the sandy soil (0.9 μg C day^{-1} g^{-1}) and at days 8, 12 and 80 (< 3.2 μg C day^{-1} g^{-1}) in the sandy loam soil (Figure 2); PAM was much more stable than the other two conditioners. The effect on efflux rate from each conditioner was generally much larger in the sandy loam soil than in the sandy soil. Taken together, the conditioners appeared to exert no effects on SOM decomposition in either type of soil as assessed by the short-term incubation.

Plant residue incorporation in aggregate-size fractions

The BC-induced reductions in the ^{14}C remaining per mass unit of < 0.25-mm aggregates were 35.5 and 44% in sandy and sandy loam soils, respectively (Figure 3). Similarly, only BP reduced the ^{14}C remaining in the < 0.25-mm aggregates by 31.5% in sandy soil compared with those in the control. No differences in ^{14}C plant residue retention among the conditioners and controls for the 1–2-mm and 0.25–1-mm aggregates in either soil were noted, indicating a less profound reduction in ^{14}C in both aggregate fractions in response to conditioners than in the

Table 2 Generalized linear model (GLM) analysis for the effects of soils, amendments and incubation time on the tested dependent variables

		Sandy soil				Sandy loam soil			
Source	Degrees of freedom (DF)	Sum of squares	Mean square	*F* ratio	*P* > *F*	Sum of squares	Mean square	*F* ratio	*P* > *F*
CO_2 efflux / $\mu g\,C\,day^{-1}\,g^{-1}$ soil									
Amendments	A-1 = 3	3671.864	1223.955	781.600	<0.0001	3281.242	1093.747	45.010	<0.0001
Incubation time	T-1 = 9	103 177.130	11 464.126	7320.840	<0.0001	120 604.922	13 400.547	551.410	<0.0001
Time × amendments	27	3632.920	134.553	85.920	<0.0001	4939.788	182.955	7.530	<0.0001
Total	39	110 481.914	2832.870	1809.030	<0.0001	128 825.950	3303.230	135.920	<0.0001
$^{14}CO_2$ efflux / % of the input $^{14}C\,day^{-1}$									
Amendments	A-1 = 3	18.977	6.326	1671.160	<0.0001	4.235	1.412	490.690	<0.0001
Incubation time	T-1 = 9	465.204	51.689	13 655.700	<0.0001	479.792	53.310	18 528.400	<0.0001
Time × amendments	27	76.587	2.837	749.380	<0.0001	108.421	4.016	1395.650	<0.0001
Total	39	560.768	14.379	3798.670	<0.0001	592.449	15.191	5279.740	<0.0001
CO_2 efflux from conditioner / $\mu g\,C\,day^{-1}\,g^{-1}$ soil									
Amendments	A-C-1 = 2[a]	15.381	7.690	15.850	<0.0001	62.010	31.005	32.35	<0.0001
Incubation time	T-1 = 9	34.842	3.871	7.980	<0.0001	255.631	28.403	29.64	<0.0001
Time × amendments	18	42.239	2.347	4.840	<0.0001	114.519	6.362	6.64	<0.0001
Total	N-1 = 29	92.461	3.188	6.570	<0.0001	432.159	14.902	15.55	<0.0001
Cumulative CO_2 / $mg\,C\,g^{-1}$									
Amendments	A-1 = 3	0.022	0.007	1.990	NS	0.310	0.103	577.280	<0.0001
Incubation time	T-1 = 9	6.517	0.724	194.790	<0.0001	15.815	1.757	9812.890	<0.0001
Time × amendments	27	0.025	0.001	0.250	NS	0.030	0.001	6.130	<0.0001
Total	39	6.564	0.168	45.280	<0.0001	16.155	0.414	2313.170	<0.0001
Cumulative $^{14}CO_2$ / % of input ^{14}C									
Amendments	A-1 = 3	5663.445	1887.815	469.920	<0.0001	5868.975	1956.325	1185.630	<0.0001
Incubation time	T-1 = 9	63 289.624	7032.180	1750.450	<0.0001	80 639.410	8959.934	5430.170	<0.0001
Time × amendments	27	559.183	20.710	5.160	<0.0001	43.649	1.617	0.980	NS
Total	39	69 512.252	1782.365	443.670	<0.0001	86 552.034	2219.283	1345.000	<0.0001

[a]Amendments (A) = 3 as CO_2 efflux from conditioner was estimated by subtracting the CO_2 efflux from the amended soil from the CO_2 efflux from control soil.
Where A = 4 and T = 10. NS = no significant difference.

controls (Figure 3). Biochar clearly exerted a stronger effect on the changes in ^{14}C remaining in the < 0.25-mm aggregates of both soils compared with that of BP and PAM. The ^{14}C plant residues persisting in the < 0.25-mm aggregates in sandy soil were more sensitive to BP and BC than those in the sandy loam soil.

Enzyme activities in aggregate-size fractions

Sandy soil mixed with plant residues showed greater β-cellobiosidase activity in the < 0.25-mm aggregates than in the BC-amended soil (Figure 4). In particular, BC applied to the plant residues resulted in a 47.1% reduction in β-cellobiosidase activity in the < 0.25-mm aggregates at the end of the incubation in sandy soil relative to that in the control soil (Figure 4). PAM and BP applied to plant residues in sandy loam soil reduced β-cellobiosidase activity in the 0.25–1-mm and < 0.25-mm aggregates (Figure 5). However, BC applied with plant residues increased β-cellobiosidase activity in sandy loam soil by 2.9 times in the < 0.25-mm aggregates (Figure 5).

BP applied with plant residues increased β-glucosidase activity by 1.4 times in the < 0.25-mm aggregates of the sandy soil relative to that in the control soil with plant residues (Figure 4). Greater β-glucosidase activity was noted in all aggregate-size fractions in the sandy loam soil mixed with plant residues relative to that in the PAM-, BP- and BC-amended soils with plant residues. Soil conditioners applied with plant residues reduced β-glucosidase activity by 1.4 times in all aggregate-size fractions of the sandy loam soil. These results clearly demonstrate that β-cellobiosidase and β-glucosidase activities in the aggregate-size fractions were much greater in the sandy loam soil than in the sandy soil (Figures 4 and 5). The large amount of plant residues reflected by the persistence of ^{14}C in each aggregate fraction of the control soil resulted in an increase in β-cellobiosidase activity relative to soils amended with conditioners.

All variables tested by the two-factor ANOVA showed a significant response to soil texture (Table 3). Specifically, soil texture exerted profound effects on cumulative CO_2, cumulative $^{14}CO_2$ efflux and enzyme activities in each aggregate-size fraction, because of differences in the physicochemical and biological properties of both soils (Table 3). This explains the larger CO_2 efflux rate from the sandy loam soil than the sandy soil, as it was mainly attributable to the incorporation of added C from decomposing

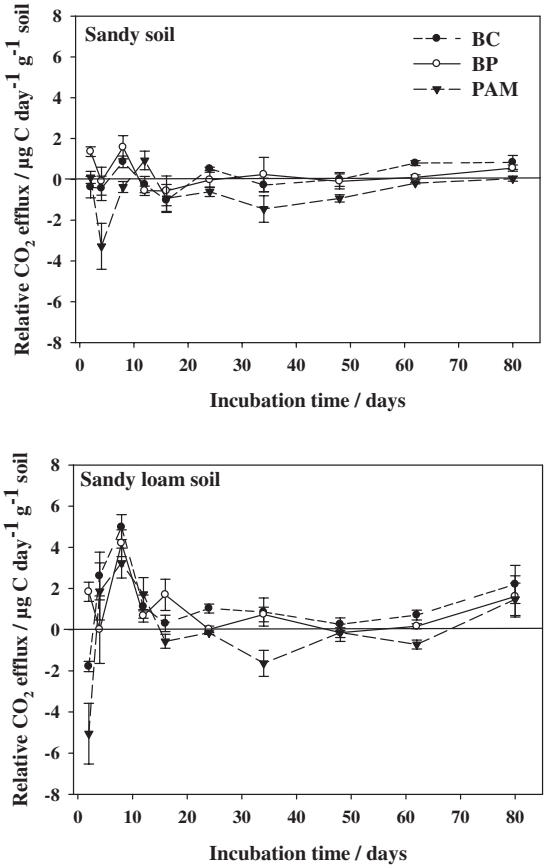

Figure 2 CO_2 efflux rates ($\mu g\ C\ day^{-1}\ g^{-1}$ soil) from decomposition of $5\ Mg\ ha^{-1}$ biochar (BC), $400\ kg\ ha^{-1}$ biopolymer (BP) and $400\ kg\ ha^{-1}$ polyacrylamide (PAM) in sandy and sandy loam soils relative to no amendments (control). Error bars indicate the standard error of the mean ($n = 4$). Data adopted and estimated from Awad *et al*. (2012).

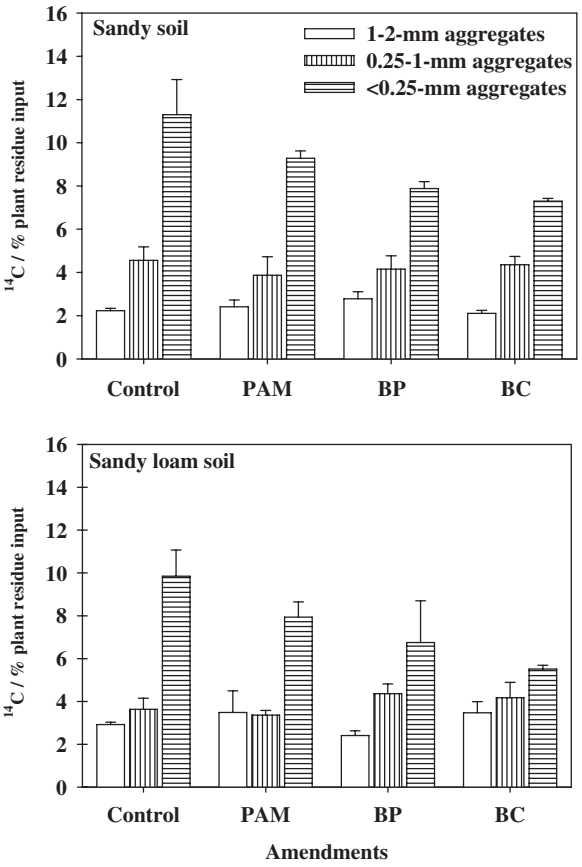

Figure 3 Percentage of ^{14}C from added plant residues retained in three aggregate-size fractions of sandy (a) and sandy loam (b) soils in response to amendments with $400\ kg\ ha^{-1}$ polyacrylamide (PAM), $400\ kg\ ha^{-1}$ biopolymer (BP) and $5\ Mg\ ha^{-1}$ biochar (BC) compared with no amendments (control). Error bars indicate the standard error of the mean ($n = 4$).

plant residues into aggregates associated with differences in initial C content. Additionally, the multifactorial ANOVA and GLM analysis revealed a direct correlation between the CO_2 from decomposition of conditioner and soil texture in addition to the incubation time and its interaction with amendments (Tables 1 and 2).

Discussion

Aggregate formation

Substances produced by decomposing plant residues bind microaggregates (< 0.25-mm) together into stable macroaggregates (> 0.25-mm) (Abiven *et al*., 2008). Addition of plant residues to both soils stabilized aggregates and improved soil structure (Figure 1). Specifically, labile organic compounds in plant residues bind particles together to form aggregates (Abiven *et al*., 2008). Our results are consistent with the findings of Martens (2000) who noted that adding maize residues to soil improved aggregation over 84 days and that a large amount of organic C from maize residues resulted in an increase in the masses of the macro-aggregates.

In our study, PAM applied with plant residues reduced the percentage of < 0.25-mm aggregates by binding the particles into large aggregates, thereby increasing the percentage of 1–2-mm aggregates in both soils as reported by Sojka *et al*. (2007). Polyacrylamide efficacy was greater in the sandy loam soil with a greater clay content, resulting in more charged sites being available for bonding relative to that observed in the sandy soil (Lee *et al*., 2010). However, in the present study, adding maize residues to sandy soil improved the efficacy of PAM and the other conditioners on aggregate formation. Similarly, BP applied with plant residues increased the amount of 1–2-mm aggregates in sandy soil because of the cellulose microfibrils, resulting in clay flocculation and stabilized aggregates (Orts *et al*., 2000).

In our study, BC may have acted as a binding agent for OM during aggregate formation, as previously reported by Brodowski *et al*. (2006), and this resulted in an increase in 1–2-mm aggregates in the sandy soil. Brodowski *et al*. (2006) showed that up to 7.2% of organic C in the < 0.053-mm aggregates was BC, whereas only a small quantity of BC was located in the > 2-mm

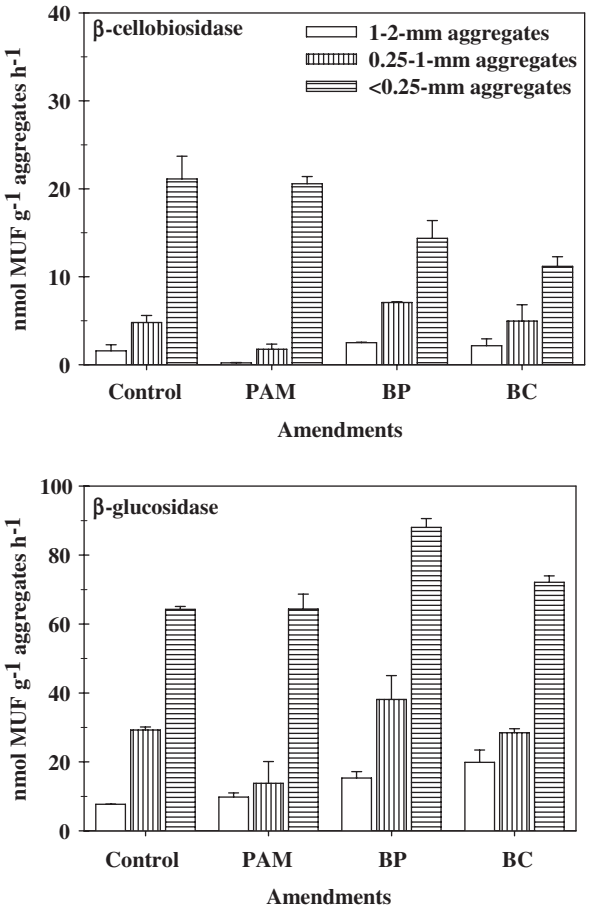

Figure 4 Enzyme activities (nmol g^{-1} aggregates h^{-1}) in three aggregate size fractions, 1–2 mm, 0.25–1 mm and < 0.25 mm, of sandy soil mixed with plant residues in response to amendments with 400 kg ha^{-1} polyacrylamide (PAM), 400 kg ha^{-1} biopolymer (BP) and 5 Mg ha^{-1} biochar (BC) compared with no amendments (control). Error bars indicate the standard error of the mean.

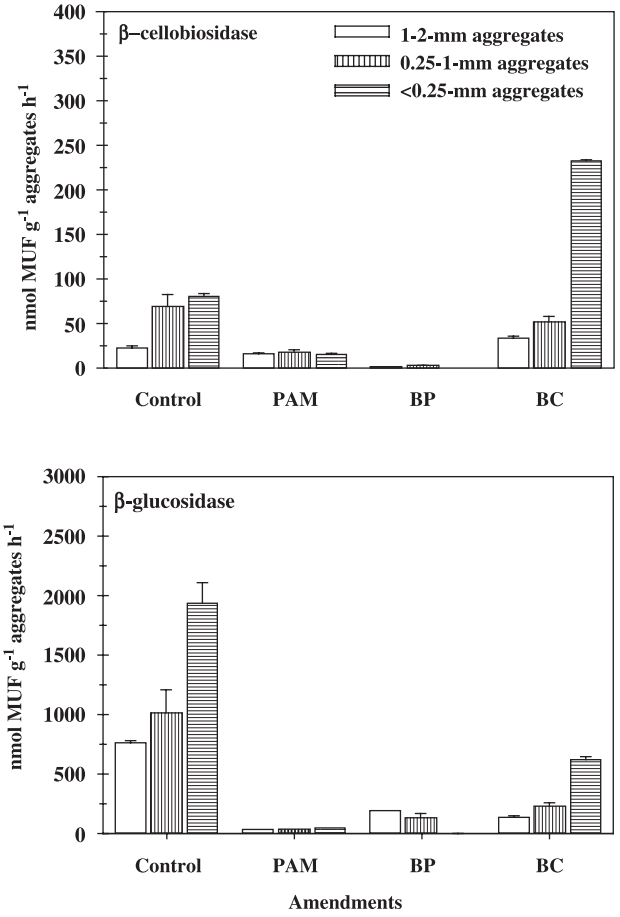

Figure 5 Enzyme activities (nmol g^{-1} aggregates h^{-1}) in three aggregate size fractions of 1–2, 0.25–1 and < 0.25 mm in sandy loam soil mixed with plant residues in response to amendments with 400 kg ha^{-1} polyacrylamide (PAM), 400 kg ha^{-1} biopolymer (BP) and 5 Mg ha^{-1} biochar (BC) compared with no amendments (control). Error bars indicate the standard error of the mean.

aggregates. Moreover, BC improves soil aggregation because of its surface charge characteristics and sorption of labile OM (Yu *et al*., 2006).

Total CO_2 efflux from soil and contribution of conditioners

Soil conditioners applied with plant residues exerted no effect on cumulative CO_2 efflux after 80 days of incubation in sandy soil (Table S1). Polyacrylamide exerted no effect on CO_2 in sandy soil, as its decomposition rate was small (Entry *et al*., 2008); this is probably because PAM showed a minor effect on coarse-textured soils with small contents of exchangeable cations (Sojka *et al*., 2007), as observed in the current study. Biopolymer decomposition also showed minor effects on cumulative CO_2 efflux. Our results indicate that PAM was more recalcitrant than BP. Microbial BC decomposition in both soils was slow, and its contribution to CO_2 efflux was also small relative to that of SOM and plant residues (Figure 2). This could be explained by the insignificant differences in cumulative CO_2 among the BC-amended soils relative to the control soil mixed with plant residues (Awad *et al*., 2012).

Polyacrylamide and BP induced a reduction in cumulative CO_2 efflux relative to control soil mixed with plant residues in sandy loam soil (Table S1). This might be attributable to the negative effects of a large OC content on PAM efficacy in the sandy loam soil relative to the sandy soil after applying plant residues. Lee *et al*. (2010) noted that large contents of OM lead to a reduction of PAM sorption in soil aggregates. Synthesized BP contains polyacrylamide; therefore, it functions similarly to PAM in soil.

In contrast, the CO_2 efflux rate from decomposition of BP and BC in the sandy loam soil was greater than that in the sandy soil because of their different C contents. Biochar contains readily degradable components, leading to an increase in microbial activity, followed by a minor increase in CO_2 efflux rates from decomposition relative to the control soils during the incubation period (Bruun & Luxhoi, 2008). Similarly, labile C in BP

Table 3 Two-factor ANOVA for the effects of soils and amendments on the tested dependent variables after 80 days of incubation

Source	Degrees of freedom	β-cellobiosidase				β-glucosidase			
		Sum of squares	Mean square	F ratio	$P > F$	Sum of squares	Mean square	F ratio	$P > F$
1–2-mm aggregates									
Soils	S-1 = 1	1142.408	1142.408	20.170	0.0009	280 925.365	280 925.365	9.590	0.0102
Amendments	A-1 = 3	589.277	196.426	3.470	NS	309 511.538	103 170.513	3.520	NS
Total	4	1731.684	432.921	7.640	0.0034	590 436.903	147 609.226	5.040	0.0148
0.25–1-mm aggregates									
Soils	S-1 = 1	3758.566	3758.566	12.560	0.0046	432 120.761	432 120.761	7.110	0.0219
Amendments	A-1 = 3	2722.704	907.568	3.030	NS	601 795.134	200 598.378	3.300	NS
Total	4	6481.270	1620.318	5.410	0.0117	1 033 915.895	258 478.974	4.250	0.0254
<0.25-mm aggregates									
Soils	S-1 =1	16 880.413	16 880.413	5.180	0.0438	1 341 308.114	1 341 308.114	5.790	0.0349
Amendments	A-1 = 3	32 162.532	10 720.844	3.290	NS	2 429 323.058	809 774.353	3.490	NS
Total	4	49 042.945	12 260.736	3.760	0.0365	3 770 631.171	942 657.793	4.070	0.0291

Source	Degrees of freedom	Aggregate size fractions			
		Sum of squares	Mean square	F ratio	$P > F$
1–2-mm aggregates					
Soils	S-1 = 1	133.114	133.114	18.090	0.0014
Amendments	A-1 = 3	78.242	26.081	3.540	NS
Total	4	211.356	52.839	7.180	0.0043
0.25–1-mm aggregates					
Soils	S-1 =1	852.786	852.786	299.420	<0.0001
Amendments	A-1 = 3	18.274	6.091	2.140	NS
Total	4	871.060	217.765	76.460	<0.0001
<0.25-mm aggregates					
Soils	S-1 =1	312.052	312.052	32.480	0.0001
Amendments	A-1 = 3				NS
Total	4	368.242	92.061	9.580	0.0014

Where $S = 2$ and $A = 4$. NS = no significant difference.

is easily biodegradable and represents a source of C for soil microorganisms such as fungi (Baldrian *et al*., 2011). However, the estimated CO_2 efflux rate from each conditioner was small and contributed in a minor way to cumulative CO_2 efflux in the amended soils.

Plant residue decomposition and incorporation in aggregates relative to enzyme activity

Readily biodegradable organic C in plant residues passes through microbes and increases microbial respiration, resulting in increased $^{14}CO_2$ emissions (Chen *et al*., 2009; Paterson *et al*., 2011). During the first phase (days 0–24) of plant residue decomposition, the readily degradable substrates provide energy, C and nutrients to microorganisms (Turner *et al*., 2002). The evolution of $^{14}CO_2$ during days 24–80 (Table S1) followed a trend similar to that noted by Chotte *et al*. (1998), probably as a result of decomposition of recalcitrant substrates including lignin, cellulose and phenolic compounds (Chen *et al*., 2009). Decomposition of cellulose fragments is related to β-cellobiosidase activity. In particular, β-cellobiosidase decomposes polymeric cellulose from the ends of molecules and releases cellobiose. β-glucosidase subsequently cuts the monomers from oligomeric compounds such as cellobiose and releases glucose as well as soluble C and carbohydrates, which are employed as energy sources by microorganisms (Turner *et al*., 2002). In our study the activities of these enzymes decreased at the end of the incubation period (Figures 4 and 5) because of mineralization of labile or dissolved organic C from soil and plant residues by microorganisms (Chen *et al*., 2009).

From our results, BC accelerated plant residue decomposition and thereby reduced the remaining ^{14}C plant residues and enzyme activity in < 0.25-mm aggregates in both soils after 80 days. Our findings are consistent with previous studies demonstrating that BC accelerates the decomposition of ryegrass and switchgrass residues (Hilscher *et al*., 2009; Novak *et al*., 2010). Zimmerman *et al*. (2011) noted that BC produced at low temperatures (250–400°C) stimulates C mineralization through decomposition of labile BC components over the short-term as observed in the current study. In particular, BC provides additional C for cellulose-decomposing microorganisms such as saprophytic fungi (Lehmann *et al*., 2006). This might explain the more rapid decomposition of plant residue and reduced content of remaining ^{14}C and enzyme activity in the < 0.25-mm aggregates in the BC treatment compared with soil mixed with plant residues

(Table S1 and Figure 3). In contrast, BC induced an increase of β-cellobiosidase activity in the < 0.25-mm aggregates in sandy loam soil, which might be attributable to the decomposition of recalcitrant substrates relative to the poorly textured sandy soil. Further research is required to understand BC decomposition in soils. PAM had no effect on the ^{14}C remaining in the < 0.25-mm aggregates of either soil type. As mentioned earlier, PAM is a synthetic polymer, whereas BC contains natural organic compounds showing different microbial availabilities. In a short-term experiment, Land *et al*. (2011) observed that polymers (xanthan gum and guar gum) at 1% (w/w) exerted no effects on basal microbial respiration, as they are independent of soil type and OM content. The greater activity of these enzymes in control versus treated soils was associated with an increase in the ^{14}C remaining in the < 0.25-mm aggregates relative to the > 0.25-mm aggregates (Figure 3).

More ^{14}C remained in the < 0.25-mm aggregates than in the > 0.25-mm aggregate-size fractions (Figure 2), which was consistent with Majumder & Kuzyakov (2010). Aoyama *et al*. (2000) noted that macroaggregates had a larger microbial biomass than that of microaggregates during 14 days of incubation after adding ^{13}C-glucose to soil. They noted that organic C and biomass C mineralization rates were larger in macroaggregates than in microaggregates. Notably, the macroaggregates induced a more rapid decomposition of plant residues during incubation in both soils than the microaggregates in the present study, resulting in the depletion of the ^{14}C remaining in the soil. These findings are consistent with the results of Cosentino *et al*. (2006), who reported that fungi and bacteria had similar activities with regard to the evolution of $^{14}CO_2$ from soil containing glucose in macroaggregates. Consequently, β-cellobiosidase and β-glucosidase activities were linked to the ^{14}C remaining in the aggregate-size fractions and the microaggregates (< 0.25 mm) contained more ^{14}C than the macroaggregates (> 0.25 mm) (Figures 3–5), as reported by Lagomarsino *et al*. (2012).

From the two-factor ANOVA (Table 3), it can be seen that soil texture was a major factor controlling extracellular enzyme activity, cumulative CO_2 and plant residue decomposition. In particular, the larger C and N contents as well as exchangeable cations in the sandy loam soil than in the sandy soil may have contributed to greater decomposition of plant residues. This result explains the greater β-cellobiosidase and β-glucosidase activity in sandy loam soil than in sandy soil (Figures 4 and 5). Turner *et al*. (2002) noted that β-glucosidase activity is correlated with total C and clay content in soil. Ladd *et al*. (1995) reported that the levels of ^{14}C-labelled plant residues and glucose decomposition in sandy loam soil are dependent on soil texture (or clay content), which influenced the accessibility of substrates to soil microorganisms. They demonstrated that microbial biomass-^{14}C in sandy loam soil was about 1.5 times that observed in clay-rich soil during 101 days of incubation. This observation confirms our hypothesis that the effect of each conditioner is primarily dependent on soil physiochemical properties. Schmidt *et al*. (2011) reported that the transformation of organic C (SOC or plant residues) in soil might be limited as a consequence of micro-environmental conditions, which could restrict decomposer-enzyme activity. No significant correlations (data not shown) were found between enzyme activity and the ^{14}C remaining at aggregate-size because of C-limitation for microorganisms after 80 days of incubation; this was probably attributable to the decomposition of the available C during the incubation period (Chen *et al*., 2009).

Conclusions

Soil conditioners applied with plant residues exerted no effect on cumulative CO_2 efflux after 80 days of incubation in sandy soil. However, PAM and BP induced a reduction in cumulative CO_2 efflux in the sandy loam soil relative to the control mixed with plant residues, and this was attributable to the negative effects of high levels of organic C on the sorption of polymers to soil. Biochar and BP increased maize residue decomposition and, consequently, reduced its stabilization in both soils, as indicated by the greater depletion of ^{14}C in the < 0.25-mm aggregates than in the controls and PAM-treated soils. The greater β-cellobiosidase and β-glucosidase activity in the control soils than in the amended soils was attributable to the mineralization of labile or dissolved organic C (from native SOC or decomposable plant C) by microorganisms during the incubation period. Additionally, the GLM analysis showed a significant effect of time and its interaction with amendments on the tested variables. Polyacrylamide exerted no effects on decomposition of native or added C in either soil type, despite improved soil aggregation. The results of this study indicate that applying BC to soils is effective for inducing rapid plant residue decomposition between cropping seasons. In contrast, applying PAM profoundly improved soil aggregation without accelerating decomposition.

Supporting Information

The following supporting information is available in the online version of this article:

Table S1. Cumulative CO_2 and $^{14}CO_2$ effluxes in sandy and sandy loams amended with conditioners mixed with plant residues (data from Awad *et al*., 2012).

Table S2. Generalized linear model (GLM) analysis for the effects of soils, amendments and incubation time on the tested dependent variables.

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