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Organic carbon dynamics in soils with pyrogenic organic matter that received plant residue additions over seven years

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ABSTRACT

The effect of repeated application of plant residues on mineralization of different organic carbon (OC) pools in a pyrogenic organic matter (PyOM) amended soil was determined using an incubation study conducted over 7.1 years. At five occasions during this period, sugarcane residues (C_4) were mixed with the soil (C_4) with or without PyOM (C_3) amendments. Organic C mineralized during the incubation period or remaining in different physical soil fractions after 7.1 years was partitioned into PyOM carbon (PyOM-C) and native soil organic matter C (nSOM-C) or sugarcane C plus nSOM-C (SC-C + nSOM-C). When compared to the control, total cumulative OC (comprising both nSOM-C and PyOM-C) mineralized in the presence of PyOM was 40% higher after the first 2.5 years, but equal by 6.2 years and 3% lower by the end of the incubation period. The cumulative nSOM mineralization after 7.1 years was 2.57 mg CO_2 −C g^{-1} soil with PyOM compared to 3.16 mg CO_2−C g^{-1} soil without PyOM addition ($p = 0.13$; $n = 3$). More than 60% of the added PyOM-C was present in the free-light fraction by the end of the 7.1 years. In total, 93% of the added PyOM-C remained in soil compared to 25–28% of SC-C + nSOM-C. Sugarcane residues increased the remaining PyOM-C in the occluded-light fraction by 3% ($p < 0.05$) and in the organo-mineral fraction by 4% ($p < 0.1$), suggesting a possible preferential use of SC-C or accumulation of metabolites of decomposed PyOM. However, the addition of sugarcane had no significant effect on overall mineralization of PyOM. The presence of PyOM accelerated the mineralization of SC-C + nSOM-C by 9% ($p < 0.001$). This is probably due to enhanced mineralization of sugarcane residues rather than native SOM. Although PyOM was likely to accelerate mineralization of added plant residues throughout a 7-year period, PyOM did not increase cumulative nSOM mineralization when plant residues were absent ($p > 0.05$), so PyOM may reduce nSOM mineralization in the long term.

1. Introduction

Pyrogenic carbon (PyC) is produced from vegetation fires (Czimczik and Masiello, 2007) and is thought to constitute a significant portion of soil organic C (SOC) (Preston and Schmidt, 2006; Knicker, 2011). The substantial accumulation of PyC in soil, despite estimates of relatively small amounts of PyC produced during fires (Forbes et al., 2006), may be explained by the fact that mineralization of PyC is very slow and it remains in soil for longer periods of time than uncharred OC (Baldock and Smernik, 2002; Kuzyakov et al., 2014). In some cases, pyrogenic organic matter (PyOM) improves soil fertility and other soil properties (see Biederman and Harpole, 2013 for a recent review). Therefore, it has been suggested that biomass could intentionally be converted into PyOM (typically called biochar in this context) and applied to agricultural soils as a mechanism to sequester atmospheric CO_2 and reduce global warming (Lehmann, 2007; Whitman et al., 2010; Woolf et al., 2010).

Sometimes PyOM can accelerate native soil organic matter (nSOM) mineralization, an effect referred to as positive priming (Wardle et al., 2008; Luo et al., 2011). This could partially offset the net C accrual by PyC (Woolf and Lehmann, 2012). Other studies, however, report decreased net mineralization of nSOM, often after

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an initial increase (Zimmerman et al., 2011; Whitman et al., 2014a, 2014b). The mechanisms for these divergent results are not yet clear (Whitman et al., 2015). Moreover, the addition of relatively easily-mineralizable plant residues to PyOM-containing soil may also alter the mineralization rates of PyC, the added residue and existing nSOM (Keith et al., 2011; Whitman et al., 2014a). In agricultural and forest soils, such residues repeatedly enter the soil in the form of root exudates and crop or foliage residues. To date, however, no information exists on how OC mineralization would change with repeated application of plant residues to PyOM-amended soils (Whitman et al., 2015).

Repeated addition of plant residues may affect the mineralization of either or all of the three organic matter forms: the PyOM, the existing nSOM, and the residue itself. First, PyOM mineralization itself differs whether or not relatively easily mineralizable plant residues are added (Hamer et al., 2004). Repeated residue additions may either reduce PyOM mineralization due the microorganisms' preference for organic matter that is easily mineralizable, or increase PyOM mineralization due to co-metabolism (Willmann and Fakoussa, 1997). Since mineralization of PyOM that was in the soil for centuries did not increase after plant residue additions (Liang et al., 2010), but fresh PyOM mineralized to a greater extent (Hamer et al., 2004), we expect that any increase in PyOM mineralization during the first plant residue addition will give way to a decrease in PyOM mineralization during subsequent additions.

Second, PyOM-induced changes in nSOM mineralization appear to follow a distinct temporal pattern (Woolf and Lehmann, 2012), likely affecting both nSOM and any added residues. While more often than not, increased mineralization of nSOM has been observed in an initial phase over a period of weeks or a few months after PyOM additions, decreased mineralization of nSOM has been documented thereafter (Keith et al., 2011; Zimmerman et al., 2011). Increased mineralization of nSOM in the initial period after PyOM addition is hypothesized to be caused by the stimulation of microbial activity and mineralization of easily metabolizable C present on PyOM surfaces or by changes in pH and nutrients (Whitman et al., 2014a). This stimulation of mineralization would be expected to be short-lived and likely not operate on residues that are applied several years after the PyOM entered the soil. A reduction in mineralization of repeatedly added residues may be expected as a result interactions of nSOM or added residues with PyOM surfaces that have been reported recently for one-time additions (Whitman et al., 2014b).

Therefore, the objective of this study was to determine the effects of repeated soil application of plant residues on carbon dioxide emissions and on cumulative OC amounts in physically separated OC fractions after one-time PyOM addition in an incubation study conducted over a 7-year period. We hypothesized that (i) repeated application of abundant plant residues will reduce PyOM mineralization, and (ii) one-time PyOM addition will decrease mineralization of nSOM and repeatedly added plant residues.

2. Materials and methods

2.1. Soil type and organic additions

The soil was an aniso-hyperthermic kaolinitic Typic Haplustox, sandy clay loam, which developed from alluvial sediments originating in the Andes under a mean annual temperature of 26 °C and precipitation of 2200 mm. It was taken from an experiment at the Matazul farm in the Llanos Orientales of Colombia (N 04°10′15.2″, W 72°36′12.9″) (Major et al., 2010a). The native C_4 vegetation dominated the region for a long period of time, giving rise to soil $\delta^{13}C$ value of −12.89‰, and OC content of 20 mg g^{-1} (Table 1).

Table 1
Properties of the soil, PyOM and sugarcane materials used in the experiment.

		PyOM	Soil	Sugarcane
pH	(H_2O)	10.1	nd	nd
pH	(KCl)	8.9	3.9	nd
Total C	(mg g^{-1})	717	20	415
$\delta^{13}C$	(‰)	−28.86	−12.89	−11.90
Total N	(mg g^{-1})	2.6	1.3	10.8
C/N	(w/w)	280	15	38
H/C	(mol/mol)	0.26	nd	nd
Ash	(% w/w)	8	nd	nd
Ca[a]	(mg g^{-1})	2.93	0.19	nd
K[a]	(mg g^{-1})	3.30	0.03	nd
Mg[a]	(μg g^{-1})	291	57	nd
P[a]	(μg g^{-1})	259	13	nd
CEC[b]	($mmol_c$ kg^{-1})	235	110	nd

nd: not determined.
[a] Available nutrient contents extracted with Mehlich 3.
[b] Determined at pH 7.

The PyOM was made from prunings of old mango (*Mangifera indica* L.) trees using a mound kiln with pyrolysis temperatures between 400 °C and 600 °C and carbonization times of approximately 48 h (Major et al., 2010a). The PyOM was crushed by hand using a metallic disk pestle, to pass through a 5-mm sieve, and mixed well. The PyOM had a $\delta^{13}C$ value of −28.86‰, and OC of 717 mg g^{-1} (Table 1).

The plant residue was derived from sugarcane (*Saccharum officinarum* L.) plants grown in a greenhouse and fertilized with a nutrient solution. After harvesting, the plants were separated into leaves and stems, cut, and oven-dried at 45 °C for 48 h. Only the leaves were ground to <2 mm and used for the incubation (and referred to here as 'plant residue'). The sugarcane residues had a $\delta^{13}C$ value of −11.90‰, and OC of 415 mg g^{-1} (Table 1).

2.2. Long term incubation experiment

Four treatments with and without added plant residues were arranged in a randomized complete block design with three replicates: (1) 2 g PyOM mixed with 98 g soil (PyOM); (2) 2 g sugarcane mixed with 98 g soil (SC); (3) 2 g sugarcane and 2 g PyOM mixed with 96 g soil (PyOM + SC); and (4) a control treatment with only 100 g soil (NON). Blank jars were included, as well, to account for any leakages. The PyOM and sugarcane were added to the air-dried soil before the experiment and mixed well. The long term incubation experiment spanned 7.1 years (2596 days). At four times during the incubation (at day 921, 1523, 2010, 2257), an additional 2 g sugarcane was mixed with the soil in SC and PyOM + SC treatments, and will be referred to as five different phases of the incubation. Similar mixing of the soil was done to the two treatments where no sugarcane was added. The samples were kept in 0.95-L wide-mouth, airtight Mason jars, and incubated at a constant temperature of 30 °C. Soil moisture content was adjusted to 55% of water holding capacity at the beginning of the incubation and maintained over the incubation period by watering to weight at regular intervals.

For measurements of C mineralization, evolved CO_2 was trapped by the soda lime and the amount of mineralized C was quantified gravimetrically (Edwards, 1982; Grogan, 1998). The amount of CO_2 absorbed by the soda lime is proportional to its dry-weight increase and evolved CO_2 can be calculated using a conversion factor of [1.69 (weight gain) 12/44] (Grogan, 1998). About 0.2–1.0 g soda lime (Mallinckrodt Baker, Paris, Kentucky, highest absorption capacity 26%) were added to 30-mL Qorpak vials, dried for 24 h at 105 °C before and after each CO_2 trapping. During the incubation period, CO_2 trapped in soda lime was sampled 57 times. Sampling intervals were shorter immediately after each sugarcane addition (day 1 and

6–8) and longer as the incubation progressed (weekly to monthly for the first 6 months, then approximately bimonthly). After the determination of total CO_2 trapped in soda lime during each period for all three experimental replicates, the soda lime samples were analyzed for ^{13}C isotopic composition. For two sampling points, the difference in the calculated average CO_2 derived from PyOM was found to be not significantly different (t-test p = 0.55–0.88) whether pooled or individual soda lime samples were measured for ^{13}C. Therefore, samples were combined per treatment as a weighted composite. The soda lime was crushed and ground into a fine powder inside a glove-box under N_2 environment. The ^{13}C isotopic composition was obtained by dissolving about 1 mg of soda lime powder in a vacutainer (Becton, Dickinson and Company, NJ) with 0.3–0.6 mL H_3PO_3 (40% v/v) (modified after Harris et al., 1997). The CO_2 released was measured in an exetainer (Labco, UK), using an isotope ratio mass spectrometer (Europa Hydra 20/20 and Europa TGII gas analyzer; PDZ Europa, Crewe, England). Total CO_2 fluxes from each of the three replicates were multiplied with the isotopic composition of the composite replicates. The average ^{13}C values of the CO_2 released during 0–1, 1–6, 6–12, 12–24, and 24–36 months were used to partition C mineralized between either PyOM or nSOM + SC sources. Further partitioning of C between nSOM-C and SC-C was not possible due to their similar $\delta^{13}C$ values, and the use of only two isotopes with three C pools.

2.3. Soil fractionation

Physical fractionation was used to quantify the amount of added PyOM or sugarcane that remained in different soil fractions at the end of the incubation period. Pools representing the free-light (F-L) fraction, occluded-light (O-L) fraction associated with stable aggregates, and the organo-mineral (O-M) fraction strongly associated with mineral surfaces, were measured by density fractionation in combination with sonication (Sohi et al., 2001; Liang et al., 2008). Aqueous NaI solution (1.83 kg L^{-1}), and an input of 750 J g^{-1} soil of physical disruption energy to distinguish F-L and intra-aggregate O-L fractions, were used in this method. Each of the three replicate samples was fractionated in duplicate. The C content and $\delta^{13}C$ isotopic composition of each fraction were determined using a Europa ANCAGSLCN analyzer (PDZ Europa Ltd., Sandbach, UK).

2.4. Source partitioning

The $\delta^{13}C$ signatures of the nSOM and SC were similar and therefore, C mineralized from or present in these two sources were not partitioned. It was also recognized that with the combined additions of PyOM and SC, partitioning among the three organic matter sources, PyOM-C, SC-C and nSOM-C is not possible by using two isotopes in the present experiment. Hence, the proportion of C originating from the different sources was estimated using following equations:

PyOM treatment

$$\%PyOM-C=\left(\delta^{13}C_{(PyOM)}-\delta^{13}C_{(NON)}\right)\times 100\Big/\left(\delta^{13}C-PyOM-\delta^{13}C_{(NON)}\right)$$

$$\%nSOM-C=100-\%PyOM-C$$

PyOM + SC treatment

$$\%PyOM-C=\left(\delta^{13}C_{(PyOM+SC)}-\delta^{13}C_{(SC)}\right)\times 100\Big/\left(\delta^{13}C-PyOM-\delta^{13}C_{(SC)}\right)$$

$$\%SC-C+nSOM-C=100-\%PyOM-C$$

SC treatment

$$\%SC-C+nSOM-C=100$$

NON treatment

$$\%nSOM-C=100$$

where, %PyOM-C, %SC-C + nSOM-C, and %nSOM-C are the percentages of C originating from PyOM, SC + nSOM and nSOM, respectively. $\delta^{13}C_{(PyOM)}$, $\delta^{13}C_{(PyOM+SC)}$, $\delta^{13}C_{(SC)}$, and $\delta^{13}C_{(NON)}$ are the $\delta^{13}C$ signatures of the C mineralized or obtained from PyOM, PyOM + SC, SC and NON treatments, respectively, during the same time period. $\delta^{13}C$-PyOM and $\delta^{13}C$-SC are the bulk $\delta^{13}C$ signatures of the PyOM and SC at time zero.

2.5. Statistical analyses

A one-way analysis of variance (ANOVA) was calculated to determine the effects of PyOM and/or sugarcane addition on total C mineralization (CO_2–C), on OC in different soil fractions and on the proportion and amounts arising from PyOM-C and nSOM-C in both total C mineralization and soil fractions. Repeated measures analysis was used for cumulative C mineralization data, since CO_2 evolution was repeatedly measured in the same jars. Multiple comparisons of means from the four treatments were conducted using least significant difference (LSD) at $p < 0.05$, except where indicated otherwise. Proportions were arcsin transformed prior to ANOVA and mean separation, but figures and tables show untransformed data.

A double-exponential model was used to fit the cumulative total C mineralization to define the size and turnover rate of two source pools mathematically: (i) a large pool with slow turnover likely comprising the bulk of the PyOM and/or persistent nSOC, and (ii) a smaller C pool of higher turnover rate likely comprising the most of the added sugarcane, the easily mineralizable fraction of both PyOM and nSOC (Glaser et al., 2001; Lehmann et al., 2015), using the following equation for curve fitting:

$$X_t = X_1\left(1-e^{k_1 t}\right)+X_2\left(1-e^{k_2 t}\right)$$

where X_t = total mineralized at time t; X_1 = size of the easily mineralizable C pool; X_2 = size of the persistent C pool; k_1 *and* k_2 = mineralization rates (year^{-1}) of easily mineralizable and persistent pools, respectively; and t = time (years).

3. Results

3.1. Mineralization

As expected, the total C mineralized after each addition of sugarcane leaves (called 'sugarcane' in the following) was significantly higher than that without additions (Table 2 and Supplementary Online Fig. S1a,b). This was evident even after the 5th sugarcane addition at the end of 6 years of incubation. Where no sugarcane was added, total mineralization was initially higher after the PyOM application compared to no PyOM additions. However, after the 2nd disturbance, mineralization was higher without PyOM additions compared to soils that received PyOM at the beginning of the experiment (Table 2). As a result, the cumulative CO_2–C released from soils with PyOM additions was equal to emissions from those without PyOM additions after 6.2 years and 3.1% lower after 7.1 years (Fig. 1). However, cumulative CO_2–C

Table 2
Total CO_2–C released during each incubation phase following sugarcane addition and/or disturbance and as a proportion of OC available after each sugarcane addition.

Sugarcane addition/ disturbance	Incubation period (days)	NON[a]	PyOM[a]	SC[a]	PyOM + SC[a]
		(mg CO_2–C g^{-1} soil)			
1	920	1.22c	1.71b	8.55a	8.80a
2	602	0.66c	0.59c	8.98b	10.06a
3	487	0.68c	0.40c	6.37b	7.35a
4	247	0.39c	0.26c	4.31b	4.72a
5	340	0.31c	0.19c	5.73b	6.50a
Total	2596	3.26c	3.15c	33.94b	37.40a
		(%)			
1	920	6.1c	4.8d	30.6a	20.2b
2	602	3.5c	1.7c	32.2a	23.3b
3	487	3.8c	1.2d	23.4a	17.7b
4	247	2.2c	0.8d	14.7a	11.1b
5	340	1.8c	0.6d	17.2a	14.0b
Total	2596	16.3c	8.8d	55.1a	48.5b

[a] In each row, values followed by the same letter are not significantly different at $p < 0.05$.

released when sugarcane was applied to PyOM-amended soil was significantly higher at the end of each phase than when only sugarcane was applied. Total CO_2–C released over each phase, when expressed as a proportion of OC available at the start of that phase, was significantly less after PyOM additions. This result was true for both cases when comparing (i) PyOM plus SC to SC only, or (ii) PyOM to the control, and held for all 5 phases during the 7.1 years of incubation (Table 2).

With sugarcane additions and no PyOM, the estimated size of the easily-mineralizable pool was significantly larger (4.76 mg g^{-1}) than the persistent pool (4.01 mg g^{-1}), using curve fitting (Supplementary Online Table S1). However, this relationship was reversed when sugarcane was added in the presence of PyOM (3.96 and 4.78 mg g^{-1}, for the easily-mineralizable and persistent pools, respectively). The mineralization rate constants of the two pools were significantly higher with than without PyOM during this period.

The addition of sugarcane initially decreased the mineralization of PyOM by 46% following the 1st sugarcane addition, but increased it fourfold during the 5th sugarcane additions, with variable results in-between (Table 3). However, the total PyOM mineralized by the end of the incubation was not significantly different with (0.42 mg g^{-1}) or without (0.51 mg g^{-1}) sugarcane. Mineralization of nSOM was initially higher ($p < 0.1$) with PyOM additions (1.43 mg C g^{-1} soil) than without additions (1.23 mg C g^{-1} soil) but lower thereafter ($p < 0.05$ only for the second disturbance). The cumulative nSOM mineralization after 7.1 years was 23% lower with PyOM (2.57 mg C g^{-1} soil) than without PyOM (3.16 mg C g^{-1} soil) addition ($p = 0.13$; Fig. 1a; Table 3). The C mineralization from combined nSOM plus sugarcane residues (nSOM + SC) increased as a result of PyOM applications. For 3 out of 5 sugarcane additions this effect was highly significant (11–13%, $p < 0.001$).

3.2. Organic C remaining in soil

Organic C contents in the bulk soil measured at the end of the 7.1-year incubation were highest with both PyOM and sugarcane additions (31.2 mg C g^{-1} soil) followed by PyOM (24.9 mg C g^{-1}

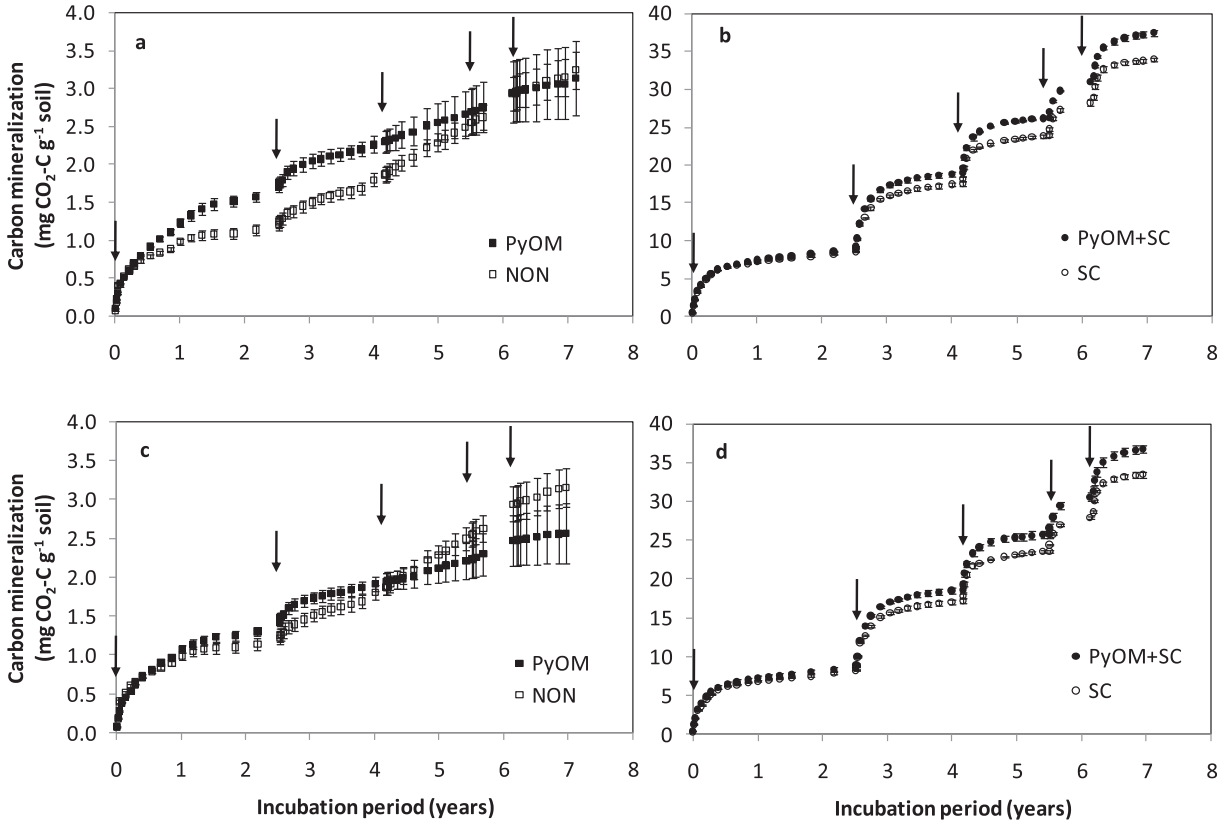

Fig. 1. Cumulative CO_2 released from all OM sources with PyOM (PyOM) and without any additions (NON) (a), with sugarcane (SC) and the combination of PyOM and sugarcane (PyOM + SC) (b), from native soil organic matter (nSOM) in treatments PyOM and NON (c), and from sugarcane plus nSOM in treatments (SC and PyOM + SC) (d). Note the differences in scales of the y-axes. Arrows indicate sugarcane additions and/or mechanical disturbance of the soil (means and standard errors; n = 3).

Table 3
Total CO_2–C released (mg CO_2–C g^{-1} soil) from mineralization of different OM sources at the end of each phase following sugarcane addition and/or disturbance. Since the ^{13}C signatures of sugarcane and native soil organic matter (nSOM) were similar, SC-C and nSOM-C were estimated as a single source (n = 3).

SC addition/disturbance	Duration (days)	PyOM-C[a] (mg CO_2–C g^{-1} soil)		SC-C + nSOM-C[a] (mg CO_2–C g^{-1} soil)		nSOM-C[a] (mg CO_2–C g^{-1} soil)	
		PyOM	PyOM + SC	PyOM + SC	SC	PyOM	NON
1	920	0.28a	0.15b	8.66a	8.55a	1.43a	1.22a
2	602	0.07a	0.08a	9.99a	8.98b	0.52a	0.66a
3	487	0.11a	0.14a	7.21a	6.37a	0.29b	0.68a
4	247	0.02a	0.00a	4.78a	4.31b	0.24a	0.39a
5	280[b]	0.02b	0.11a	6.14a	5.54a	0.09a	0.22a
Total	2536	0.51a	0.42a	36.76a	33.75b	2.57a	3.16a

[a] Values followed by the same letter in a given OC source at a given date are not significantly different at $p < 0.05$.
[b] ^{13}C contents of the CO_2 collected during the last 60 days were not analyzed.

soil), sugarcane (18.6 mg C g^{-1} soil), and no additions (10.1 mg C g^{-1} soil) (Table 4). The OC remaining in the soil as a proportion of initial contents (including all periodic additions for sugarcane) was highest with the single initial PyOM (70%) and lowest with periodic sugarcane additions (28%) (Fig. 2). Calculations done using ^{13}C values of the bulk soil revealed that, while about 93% of the added PyOM-C remained in the bulk soil at the end of the experiment, only 25% (PyOM + SC) and 28% (SC) of the sugarcane residue C plus native SOM C remained (Fig. 2). These proportions of SC-C + nSOM-C remaining in the PyOM + SC and SC treatments corroborated the observations made from CO_2 data where mineralization of sugarcane residue plus native SOM is higher with PyOM than without (Table 3).

3.3. Organic C in soil fractions

Free-light fraction in soils without PyOM addition was very low and no significant differences were observed at the end of the incubation period whether sugarcane was added (0.04 mg C g^{-1} soil) or not (0.03 mg C g^{-1} soil) (Table 4), despite the fact that undecomposed sugarcane residue would have been retrieved in this fraction. Similarly with PyOM additions, OC in the F-L fraction was not significantly different with (9.97 mg C g^{-1} soil) or without (11.18 mg C g^{-1} soil) sugarcane additions. However, sugarcane addition increased the OC allocated to O-L and O-M fractions and the highest allocation was observed when sugarcane was added to PyOM-containing soil (1.35 and 18.8 mg C g^{-1} soil for O-L and O-M fractions, respectively).

The ^{13}C signatures of the F-L fractions were −28.56‰ and −28.48‰ when just PyOM was added or together with sugarcane, respectively. This suggests that OC in that fraction was dominated by the PyOM. In comparison, F-L and O-L fractions with or without sugarcane additions but without PyOM were dominated by C_4 plant signatures.

Most (61–67%) of the added PyOM-C remained in the F-L fraction and only 23–28% in the O-M fraction (Fig. 2). In the O-L fraction, a significantly greater proportion of added PyOM-C was observed in the presence of sugarcane residues (4.8%) than without the residues (1.5%). In the absence of sugarcane, PyOM additions had no detectable effect on nSOM-C contents in the O-M fraction.

4. Discussion

4.1. Mineralization of native SOM as affected by PyOM additions

The initial increase in mineralization of nSOM after additions of PyOM (Supplementary Fig. S1a) may be a result of co-metabolism, nutrient additions or changes in pH as proposed by others (Luo et al., 2011; Zimmerman et al., 2011), and the precise cause cannot be distinguished here. By the third phase (Supplementary Fig. S1a), this net increase in nSOM mineralization changed into a net decrease in nSOM mineralization. The decrease in nSOM mineralization is not just a consequence of a reduced amount of easily mineralizable C remaining as a result of earlier increased mineralization, but showed a trajectory of a net decrease after 7.1 years. Stabilization of nSOM on PyOM surfaces and/or inactivation of enzymes by PyOM are the two most likely reasons for the observed decrease among the ones discussed by Whitman et al. (2014a). The decreased nSOM mineralization was probably not affected by: (i) Substrate switching and dilution, because these are expected to occur in the short term (weeks), whereas the decrease in nSOM mineralization was observed after several months; (ii) N inhibition, because PyOM addition did not introduce substantial amounts of N (Table 1); (iii) changes in pH, because the alkaline pH of PyOM (pH 8.9 in KCl; Table 1) would raise the low soil pH of 3.9 to a higher pH which would favor microbial activity rather than decrease it.

4.2. Mineralization of added plant residues as affected by PyOM additions

Greater mineralization of OC observed in PyOM-amended soil cannot be unequivocally attributed to more nSOM or more sugarcane mineralization, because of the inability to distinguish between

Table 4
Organic C contents and $\delta^{13}C$ signatures in the C obtained from different organic matter fractions and in the bulk soil after the 7.1 year incubation period. The $\delta^{13}C$ values of sugarcane was −11.90‰, that of PyOM −28.86‰, and that of the initial soil −12.89‰.

Treatment	Organic C[a]				$\delta^{13}C$ signature[a]			
	F-L	O-L	O-M	Bulk soil	F-L	O-L	O-M	Bulk soil
	(mg C g^{-1} soil)				(‰)			
NON	0.03b	0.04d	10.44d	10.07d	−13.33b	−12.30c	−15.40c	−15.38c
SC	0.04b	0.64b	16.45b	18.60c	−13.32b	−12.14c	−14.44d	−14.35d
PyOM	11.18a	0.29c	13.35c	24.90b	−28.56a	−25.95a	−19.13a	−23.47a
PyOM + SC	9.97a	1.35a	18.78a	31.15a	−28.48a	−21.89b	−17.84b	−21.49b

[a] In each column, values followed by the same letter are not significantly different at $p < 0.05$.

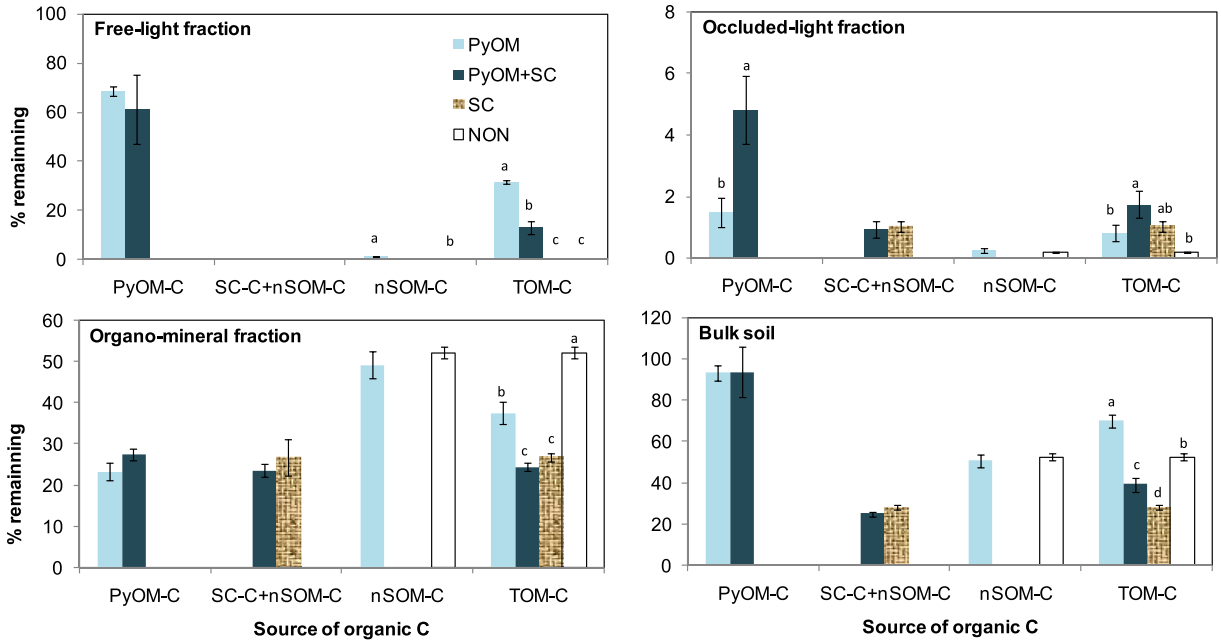

Fig. 2. Organic C remaining in free-light, occluded-light and organo-mineral fractions expressed as a proportion of OC derived from different organic matter sources; pyrogenic organic matter (PyOM-C), sugarcane residues (SC-C), native soil organic matter (nSOM-C), and total organic matter (TOM-C (including all added OC). With sugarcane additions, OC derived from SC and nSOM were not partitioned as their ^{13}C data were not significantly different. Not all pools were present for all treatments (e.g., no PyOM-C was present in NON treatment). Error bars indicate the standard error of the estimate. Columns with the same letter(s) in a given OC source of a given OM fraction are not significantly different at $p < 0.05$ ($n = 3$).

three C sources using two C isotopes in the current experiment. The significantly higher quantity and rate of CO_2 released from a persistent pool (Supplementary online Table S1) when PyOM was added to the sugarcane may point to increased mineralization of nSOM by PyOM. Increased mineralization of nSOM and decreased mineralization of added plant residues reported by Keith et al. (2011) may support this interpretation. However, it is more likely that a greater proportion of the added plant residue, not nSOM, was mineralized in the presence of PyOM. The mineralization of nSOM was reduced when PyOM was added without sugarcane, making the same response also likely with sugarcane additions. In addition, the increase in total C mineralization is much greater with sugarcane additions than with PyOM additions. If the entire increase amounting to 3 mg CO_2–C g^{-1} soil was derived from nSOM with the combined addition of PyOM and sugarcane (Table 3), the mineralization of nSOM would need to have nearly doubled relative to nSOM mineralization without any additions. Therefore, it is more likely that PyOM enhanced mineralization of sugarcane rather than that of nSOM. However, only a true three-source partitioning approach will resolve this question, and may point to intriguing interactions that could not be investigated here.

Greater mineralization of sugarcane residue in the presence of PyOM may have several causes (Blagodatskaya and Kuzyakov, 2008; Jones et al., 2011; Zimmerman et al., 2011; Whitman et al., 2014a) and effects (Liang et al., 2010), and the experimental setup allows some inferences to be made regarding their relevance to this experiment: (i) easily mineralizable OC from the added PyOM may enhance mineralization of the added residue by co-metabolism; this is unlikely to be the case here, because the effect was still seen several years after PyOM addition when minimal PyOM mineralization is expected (Kuzyakov et al., 2014); (ii) addition of nutrients with the PyOM may alleviate metabolic constraints for the microorganisms; this is also unlikely given the low amounts of nutrients added with woody PyOM (Whitman et al., 2014b) and the fact that bioavailable nutrient concentrations decrease with time; (iii) a change in water retention may promote microbial activity; this can be excluded since the water content was controlled in this experiment; (iv) increases in soil pH resulting from the addition of an alkaline PyOM may change sugarcane mineralization, but the liming effect of PyOM can be expected immediately after its addition to soil (Major et al., 2010b), which is when PyOM had the lowest impact on nSOM + SC mineralization. Therefore, none of these mechanisms provide a satisfactory explanation for our results. Possibly, a greater microbial population or a shift in microbial community composition often observed as a result of PyOM (Thies et al., 2015) may have increased sugarcane mineralization. However, this was not assessed in the current experiment and warrants further research.

We expected that the sugarcane would be more rapidly incorporated into aggregates in the presence of PyOM as shown previously (Liang et al., 2010). However, the presence of PyOM had no effect on the distribution of the SC + nSOM-derived C among the soil fractions (Fig. 2). It is possible that the difference to findings by Liang et al. (2010) can be explained by the different distribution of PyOM in soil fractions. The PyOM in the *Terra Preta* soils investigated by Liang et al. (2010) were several centuries in the soil and were all retrieved in the organo-mineral fraction (density greater than 1.83 kg L^{-3} using NaI). Consequently, a greater proportion of the added residue was incorporated into that fraction, whereas here, more than 60% of the PyOM still resided in the light fraction after 7.1 years. This is also in line with the observation that the presence of PyOM resulted in significantly more nSOM-C in the free-light fraction (Fig. 2, nSOM-C without additions of sugarcane).

4.3. Mineralization of PyOM as affected by sugarcane additions

The unchanged mineralization of PyOM-C in the presence of sugarcane residues suggests that co-metabolism did not lead to a net increase in fresh PyOM mineralization in our study, which is different than findings from shorter-term incubations (weeks to

months) of similarly fresh PyOM with glucose (Hamer et al., 2004) or with sugarcane (Keith et al., 2011). The significantly greater proportion of remaining PyOM in occluded ($p < 0.05$) and organo-mineral ($p < 0.1$) fractions as a result of sugarcane additions indicates, however, that protection of PyOM was probably enhanced by residue additions. This could also contribute to offsetting any increased mineralization of PyOM which was expected given prior studies (Hamer et al., 2004; Keith et al., 2011). The shift of PyOM to what is typically interpreted as more protected soil fractions may be a result of greater microbial activity, which enhances aggregate formation through the production of microbial metabolites (Six et al., 2002). Therefore, addition of more easily mineralizable plant residues affects mineralization of the more persistent PyOM in the soil environment in several ways that go beyond a substrate for co-metabolism or substrate switching.

4.4. Conclusions

The initial increase of nSOM mineralization that is often observed after the application of PyOM was reversed in the long run. Repeated application of sugarcane leaves increased interactions between PyOM and minerals without a net increase in PyOM mineralization by co-metabolism. Presence of PyOM appeared to enhance mineralization of the studied plant residue, but the reason for this interaction could not be unequivocally identified due to the inability to distinguish mineralization of three C sources. Future studies should specifically target such three-way interactions between nSOM, added plant residues and PyOM, which requires distinguishing three C sources with innovative use of isotope experiments. This may also reveal whether plant residues in the presence of PyOM are stabilized to a greater extent per unit C mineralized. In addition, protective effects in the form of interactions with aggregation and mineral surfaces by changed microbial activity and community composition may be an informative target for future investigations of the effects of added plant residues on PyOM and nSOM mineralization.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.06.003.

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