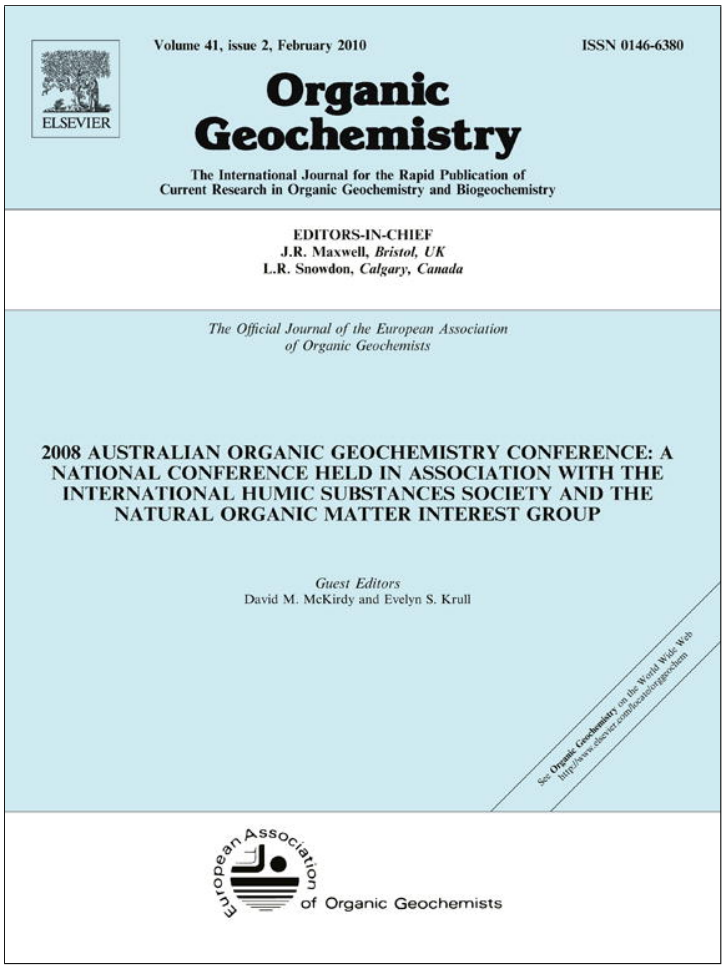

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Black carbon affects the cycling of non-black carbon in soil

Biqing Liang [a,1], Johannes Lehmann [a,*], Saran P. Sohi [b,2], Janice E. Thies [a], Brendan O'Neill [a], Lucerina Trujillo [c], John Gaunt [a], Dawit Solomon [a], Julie Grossman [a,3], Eduardo G. Neves [d], Flavio J. Luizão [c]

[a] Department of Crop and Soil Sciences, Cornell University, 909 Bradfield Hall, Ithaca, NY 14853, USA
[b] Department of Soil Science, Rothamsted Research, Harpenden AL5 2JQ, UK
[c] Instituto Nacional de Pesquisa da Amazônia (INPA), 69011-970 Manaus, Brazil
[d] Museu de Arqueologia e Etnologia, Universidade de São Paulo, Sao Paulo, SP 05508-900, Brazil

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ABSTRACT

Black carbon (BC) is an important fraction of many soils worldwide and plays an important role in global C biogeochemistry. However, few studies have examined how it influences the mineralization of added organic matter (AOM) and its incorporation into soil physical fractions and whether BC decomposition is increased by AOM. BC-rich Anthrosols and BC-poor adjacent soils from the Central Amazon (Brazil) were incubated for 532 days either with or without addition of ^{13}C-isotopically different plant residue. Total C mineralization from the BC-rich Anthrosols with AOM was 25.5% ($P < 0.05$) lower than with mineralization from the BC-poor adjacent soils. The AOM contributed to a significantly ($P < 0.05$) higher proportion to the total C mineralized in the BC-rich Anthrosols (91–92%) than the BC-poor adjacent soils (69–80%). The AOM was incorporated more rapidly in BC-rich than BC-poor soils from the separated free light fraction through the intra-aggregate light fraction into the stable organo-mineral fraction and up to 340% more AOM was found in the organo-mineral fraction. This more rapid stabilization was observed despite a significantly ($P < 0.05$) lower metabolic quotient for BC-rich Anthrosols. The microbial biomass (MB) was up to 125% greater ($P < 0.05$) in BC-rich Anthrosols than BC-poor adjacent soils. To account for increased MB adsorption onto BC during fumigation extraction, a correction factor was developed via addition of a ^{13}C-enriched microbial culture. The recovery was found to be 21–41% lower ($P < 0.05$) for BC-rich than BC-poor soils due to re-adsorption of MB onto BC. Mineralization of native soil C was enhanced to a significantly greater degree in BC-poor adjacent soils compared to BC-rich Anthrosols as a result of AOM. No positive priming by way of cometabolism due to AOM could be found for aged BC in the soils.

1. Introduction

Black carbon (BC) refers to a wide array of thermally altered products, ranging from partly charred carbonaceous material to highly condensed soot and graphite (Goldberg, 1985). It is generally considered inert or resistant to decomposition in terrestrial environments and may have a significant residence time in soils and sediments, with ages up to 5040 yrs BP in European black chernozemic soils, 3990 radiocarbon years older than bulk soil organic C, and is typically found to be the oldest C pool in soil using the ^{14}C dating method (Schmidt and Noack, 2000; Schmidt et al., 2002; Krull et al., 2006). The half-life of wood charcoal BC, estimated from ozone oxidation, was projected to be ca. 5.1×10^4 yrs in air (Kawamoto et al., 2005), while estimated mean residence time in soil ranges from about 900–9000 yrs (Cheng et al., 2008; Lehmann et al., 2008; Liang et al., 2008). Yet, a dynamic role for BC in the global C cycle was proposed only recently (Decesari et al., 2002; Kim et al., 2004; Masiello, 2004) and BC was found to react rapidly with oxygen (Cheng et al., 2006) and to possess highly oxidized surfaces (Liang et al., 2006). These properties were shown to affect the biogeochemistry of nutrients in soil (Lehmann et al., 2003; Cheng et al., 2006; Liang et al., 2006).

Despite BC being ubiquitous in natural environments, a limited number of studies have been conducted to investigate how it influences the cycling of organic C in soil. It was found by Pietikäinen et al. (2000) and Steiner et al. (2004) to promote the growth and activity of microorganisms in soil. In an incubation study using a BC-sand mixture, decomposition of added highly labile organic matter (^{14}C-glucose) was found to be accelerated by the presence

* Corresponding author. Tel.: +1 607 254 1236; fax: +1 607 255 3207.
E-mail address: CL273@cornell.edu (J. Lehmann).
[1] Present address: Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, 77 Mass Avenue, E25-653, Cambridge, MA 02139, USA.
[2] Present address: UK Biochar Research Centre, School of Geosciences, University of Edinburgh, Kings Buildings, Edinburgh, EH9 3JN, UK.
[3] Present address: Department of Crop and Soil Sciences, North Carolina State University, Raleigh, North Carolina 27695-7619, USA.

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of BC (Hamer et al., 2004). Based on the enhanced loss of humus after BC addition to a boreal forest (Wardle et al., 2008), BC was suggested to be responsible for promoting the growth of microorganisms and for enhancing the decomposition of labile C compounds rather than stabilizing them against degradation in soil. However, Wardle et al. (2008) did not directly verify whether BC promotes humus loss or humus promotes BC loss. And it remains unclear whether the presence of BC may lead to accelerated mineralization of plant litter in the presence of soil minerals (Lehmann and Sohi, 2008), which were excluded in the litterbag study by Wardle et al. (2008). Understanding the effects of BC on the cycling of other organic C [non-BC from soil and newly added organic matter (AOM)] should enhance our knowledge about the role of BC in the global C cycle and may provide insights into the consequences of deliberate BC application to soils (Lehmann et al., 2006).

Concerning the priming mechanism of other organic C on BC, it was suggested that the addition of easily mineralizable organic C may enhance the decomposition of BC by way of cometabolism as a result of the enhanced growth of microbial biomass (MB) and the concurrent increase in enzyme production (Hamer et al., 2004). Such a priming effect has been discussed for uncharred OM (Jenkinson et al., 1985) and for brown coal (Willmann and Fakoussa, 1997). Clay minerals and oxides modify the retention of OM in soil (Oades, 1988) and may therefore also alter the priming of BC decomposition. Furthermore, fresh BC contains an aliphatic fraction that may decompose much more rapidly than the aromatic fraction (Cheng et al., 2006). Decomposition of aged BC may therefore show different priming from the fresh BC studied by Hamer et al. (2004). The existence and magnitude of such priming of decomposition of aged BC has not been quantified using plant residue and in the presence of mineral matter.

Therefore, this study investigates: (i) the extent of priming of aged BC by addition of plant residues to soil and (ii) the effects of BC on the turnover and cycling of AOM, using BC-rich Anthrosols from the Brazilian Amazon vs. BC-poor adjacent soils.

2. Materials and methods

2.1. Sites and soil sampling

Pairs of BC-rich Anthrosols and BC-poor adjacent soils with identical mineralogy (Liang et al., 2006) were sampled from three different sites, Hatahara (HAT), Acutuba (ACU) and Dona Stella (DS), near Manaus, Brazil (3°8′S, 59°52′W, 40–50 m above sea level) with different soil properties between sites. The Anthrosols (locally named 'Terra Preta de Indio') were the result of pre-Columbian settlements, developed on Oxisols, Ultisols, or Spodosols. The period of occupation and therefore the age of BC and the sites have been estimated to range from 600–1000 yrs B.P. at HAT, 2000–2300 yrs B.P. at ACU and 6700–8700 yrs B.P. at DS (Neves et al., 2003; Liang et al., 2006). Sites of adjacent soils were chosen on the basis of maximum color difference vs. the BC-rich and dark Anthrosols, selecting those of typically pale yellow or white color with no visible signs of human activity. Samples were taken from a soil profile according to genetic horizons. Analyses of adjacent soils were carried out for the A horizon. All samples were air-dried and large plant debris and roots were removed. Large (ca. 5 kg) samples from the profiles were homogenized and sieved to pass 2 mm. The total C content was analyzed with a Europa Hydra 20/20 coupled to a Europa ANCA GSL sample combustion unit (PDZEuropa, Crewe, England) after grinding to fine powder using a ball mill (MM301, Retsch, Germany). The BC contents were estimated using ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy with cross polarization/magic angle spinning (CP/MAS) using a molecular mixing model (Liang et al., 2008). Properties other than mineralogy (Liang et al., 2006) were significantly different between Anthrosols and adjacent soils (Table 1). Detailed site descriptions and soil properties were shown by Liang et al. (2006, 2008).

Table 1
Properties of BC-rich Anthrosols and BC-poor adjacent soils in central Amazon.

Site	Type	Depth (m)	Organic C (mg g^{-1})	BC (mg g^{-1})	BC (% of organic C)
HAT	Anthrosol	0.43–0.69	22.0	17.5	79.4
	Adjacent soil	0–0.1	21.8	4.4	20.2
ACU	Anthrosol	0.48–0.83	15.7	11.5	73.1
	Adjacent soil	0–0.3	15.4	6.8	44.3
DS	Anthrosol	Buried (1.9–2.1)	16.5	12.4	75.1
	Adjacent soil	0–0.12	10.2	1.1	10.6

2.2. Long term incubation experiment

A long term incubation experiment over 532 days was conducted to measure the C mineralization from BC-rich Anthrosols and BC-poor adjacent soils at HAT, ACU and DS sites. OM with a distinct ^{13}C isotopic composition using the C_4 plant, sugar cane (leaves, shredded and passed through a 2 mm sieve), was added to the originally C_3 plant-dominated soil. The fate of this added organic matter (AOM) from sugar cane was tracked according to the changes of stable isotopic composition over time. Treatments with AOM and without AOM were set up. For the treatment with AOM, 2 g sugar cane (equivalent to 842.8 mg C) were mixed with 98 g soil and the samples were kept in 0.95 l wide mouth and airtight Mason jars, and incubated at a constant temperature of 30 °C. The jars were arranged in a randomized complete block design. Four replicates were used for the C mineralization experiment, three replicates for the fractionation except for the last date with four replicates (from which the C mineralization was obtained). Soil moisture content was adjusted to 55% of water holding capacity at the beginning of the incubation and maintained over the incubation period.

About 0.2–1 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. Evolved CO_2 from the total C mineralization was trapped by the soda lime and the amount of mineralized C was quantified gravimetrically (Edwards, 1982; Grogan, 1998) using a conversion factor of $[1.69 \times (\text{weight gain}) \times 12/44]$. The amount of CO_2 absorbed by the soda lime is proportional to the weight increase in soda lime during exposure (Grogan, 1998). The incubation experiment was run for 532 days, with sampling 17 times. Sample periods were kept short at the beginning and made progressively longer as the incubation progressed, with measurements after 1, 2, 4, 8, 18, 28, 38, 48, 65, 92, 128, 177, 236, 305, 366, 456 and 532 days.

A double-exponential model was run to fit the cumulative total C mineralization to define the size and turnover rate of two source pools mathematically: (i) a large stable pool with slow turnover comprising BC and/or stable organic C and (ii) a smaller and potentially more labile C pool of higher turnover rate, using the following equation for curve fitting:

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

where X_t = total mineralizable C; X_1 = size of the labile C pool; X_2 = size of the stable C pool; k_1, k_2 = mineralization rates of labile and stable pools, respectively; and t = time of incubation (days). We compared the cumulative CO_2 evolution from the BC-rich Anthrosols and BC-poor adjacent soils by normalizing on a dry soil mass basis. The units for X_t, X_1 and X_2 are mg CO_2–C g^{-1} soil.

The C content and ^{13}C isotopic composition was obtained by dissolving soda lime powder in H_3PO_3 (40% v./v.) (modified after Harris et al., 1997). The CO_2 was released into a vacutainer (Becton, Dickinson and Company, NJ) and 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).

Apparent C mineralization in response to AOM was obtained by subtracting the C mineralization without AOM from mineralization with AOM. However, the calculation of apparent mineralization of AOM did not take the interaction between AOM and soil C mineralization into account. The actual C mineralization of AOM ($C_{min(AOM)}$) was directly quantified using isotopic differences of the sources and expressed as a percentage of the total C mineralization from soil with the following equation:

$$C_{min(AOM)}[\%] = (\delta^{13}CO_2 - \delta^{13}C_{soil})/(\delta^{13}C_{AOM} - \delta^{13}C_{soil}) \times 100 \quad (2)$$

where $\delta^{13}CO_2$ is the isotopic composition of CO_2, $\delta^{13}C_{AOM}$ the isotopic composition of the AOM and $\delta^{13}C_{soil}$ the isotopic composition of the original soil C before addition of OM. Using the proportion of AOM from Eq. (2) and the total amounts of C evolved as CO_2 from soil, the amount of C mineralization from AOM and soil C was quantified.

2.3. Soil fractionation

Physical fractionation was used to quantify the amount of C from the AOM that remained in different soil fractions after 16, 35, 66, 130, 272 and 532 days incubation. Different association between OM and soil minerals renders soil C pools with different turnover time. Based on their chemical and physical attributes, free, intra-aggregate and organo-mineral fractions are considered to represent a labile soil organic matter (SOM) pool, a pool associated with stable aggregates and material strongly associated with mineral surfaces, respectively. These three fractions were measured for both BC-rich Anthrosols and BC-poor adjacent soils, using 1.83 Mg m^{-3} aqueous NaI solution (as a density separation medium), with an input of physical disruption energy (750 J g^{-1} soil) to distinguish free and intra-aggregate light fractions (Sohi et al., 2001; Liang et al., 2008). Each of the three replicate samples was fractionated in triplicate for BC-rich Anthrosols and BC-pool adjacent soils with and without AOM. Triplicates were analyzed separately for day 532 and the coefficient of variation for C content was <10% for >80% of the C-rich fractions.

The C content and $\delta^{13}C$ isotopic composition of each fraction (comprising the triplicate fractionation except for day 532) and the original soil samples was determined using a Europa ANCA-GSL CN analyzer (PDZ Europa Ltd., Sandbach, UK). The amount of C in each fraction was calculated by multiplying the C concentration (mg g^{-1}) of the fraction with the mass of each fraction. The proportion of C originating from AOM in each fraction was further estimated using $\delta^{13}C$ values:

$$C_{Fraction(AOM)}[\%] = (\delta^{13}C_{Fraction} - \delta^{13}C_{Soil})/(\delta^{13}C_{AOM} - \delta^{13}C_{Soil}) \times 100 \quad (3)$$

where $\delta^{13}C_{soil}$, $\delta^{13}C_{Fraction}$ and $\delta^{13}C_{AOM}$ represent the isotopic composition of the original soil, fraction and AOM, respectively. The amount of AOM in each fraction was calculated by multiplying the proportion obtained through Eq. (3) with the amount of C in each individual fraction, and defined as recovery of AOM.

2.4. Microbial biomass extraction

Soil microbial biomass (MB) was extracted after incubation, using a simultaneous fumigation extraction method (Witt et al., 2000). K_2SO_4 (20 ml, 0.05 M) was added to 15 g wet soil for extraction and shaken on a low speed reciprocal shaker for 4.5 h. The extractant was centrifuged at 4000 rpm for 10 min and the supernatant filtered through a No. 41 Watman filter paper. Ethanol-free $CHCl_3$ (1 ml) was added to lyse the microbes for the fumigated treatment and residual $CHCl_3$was removed by air-bubbling for 15 min (modified after Fierer and Schimel, 2003). The supernatant was evaporated in a tin cup and dried at 60 °C (modified after Brulsema and Duxbury, 1996) and analyzed for C content and isotopic composition using a dry combustion CN analyzer with an isotopic ratio mass spectrometer (Europa Hydra 20/20 and Europa TGII gas analyzer; PDZ Europa, Crewe, England). The C content of the extracted MB sample was calculated from the difference of C content between fumigated and unfumigated treatments using the following equation:

$$C_{MBC} = (C_F - C_{UF})/(K_{ec} * E) \quad (4)$$

where C_F and C_{UF} are the amounts of microbial C from fumigated and unfumigated samples, respectively; K_{ec} is the extraction efficiency factor for converting extractable C to microbial biomass C, which is 0.26, and was determined for BC-poor Amazonian soils (Feigel et al., 1995); E is an additional factor for which justification is given below.

As a result of re-adsorption of lysed MB and dissolved organic C onto BC surfaces, lower recovery and underestimation of MB occurred for BC-rich Anthrosols. Thus, an additional factor E, correcting for the extraction efficiency, was experimentally determined for each BC-rich Anthrosol and BC-poor adjacent soil by adding a ^{13}C-enriched microbial culture and quantifying its recovery. This correction factor E was designed to correct the bias in MB extraction because of the presence of BC between BC-rich Anthrosols and BC-poor adjacent soils, which is not accounted for by the K_{ec} value, which corrects for underestimation of MB because of $CHCl_3$ extraction efficiency. E is therefore not meant to improve the estimation of the absolute amount of microbial biomass in soil, but to correct for relative retention of microbial matter during fumigation extraction in the BC-rich soils investigated.

The correction factor E was expressed as a percentage of the recovered C in the extracted C to the total addition of C from a ^{13}C-enriched microbial culture, and was calculated as follows:

$$E = C_R/C_{MC} \quad (5)$$

where C_{MC} is the amount of added ^{13}C-enriched microbial culture and C_R is the recovered C in supernatant from the ^{13}C-enriched microbial culture, calculated according to the following equation:

$$C_R = C_E \times (\delta^{13}C_E - \delta^{13}C_{SMB})/(\delta^{13}C_{MC} - \delta^{13}C_{SMB}) \quad (6)$$

where C_E and $\delta^{13}C_E$ are the C content and isotopic composition of the extracted MB, respectively; $\delta^{13}C_{SMB}$ and $\delta^{13}C_{MC}$ are the isotopic compositions of soil MB and ^{13}C-enriched microbial culture, respectively. An amount of microbial culture equivalent to 402 μg C g^{-1} soil was added with an isotopic composition of +93.0‰, shaken on a reciprocal low speed shaker for 1 h and extracted using $CHCl_3$ as described above.

This ^{13}C-enriched microbial culture was obtained using ^{13}C-enriched corn biomass medium as the sole C source. The corn biomass medium was prepared by boiling 0.250 g ^{13}C-enriched corn (leaf/stem) powder in 100 ml tap water for 1 h. The extract was transferred to a graduated cylinder after removing large particles. The volume of extract was brought up to 100 ml in an Erlenmeyer flask and nutrients were added: NH_4Cl (0.008 g), $MgSO_4{\cdot}7H_2O$ (0.0002 g), $CaCl_2{\cdot}2H_2O$ (0.0015 g), K_2HPO_4 (0.004 g). The enriched corn extract medium was then sterilized for 15 min at 121 °C. Indigenous microorganisms were collected by adding 10 g air-dried soil from the HAT site to 45 ml of a 0.2 M phosphate buffer (K_2HPO_4). The buffer was shaken for 1 h. Particulate matter in the slurry was allowed to settle for 10 min and 0.5 ml of the

supernatant were used to inoculate the sterilized corn extract medium. This first culture was placed on a horizontal shaker at 200 rpm and allowed to grow for 48 h at 30 °C. An aliquot (1 ml) of this first culture was transferred to a 100 ml solution of sterilized corn extract for a second culture. The corn extract was prepared in the same manner as above without the addition of nutrients. After 36 h growth, the entire 100 ml of the second culture were transferred to a 100 ml corn extract medium for a third culture which was incubated for 7 days, by using 2.5 g ^{13}C-enriched corn biomass powder. This three-step culture procedure was chosen to eliminate interference from salt nutrients for later extraction and quantification of MB. The incubated third culture was a mixture of ^{13}C-enriched microbes and dissolved organic C, and was sterilized using UV light for 8 h on Petri dishes. Only a homogeneous portion of the ^{13}C-enriched microbial culture was used for addition to the fumigated soil samples to determine the correction factor *E*. K_2SO_4 (5 ml, 0.05 M) were added to 8 g dry soil, followed by 3 ml ^{13}C-enriched microbial culture, 10 ml 0.05 M K_2SO_4 and 1 ml distilled $CHCl_3$to the fumigated treatment.

2.5. Statistics

Analysis of variance (ANOVA) was calculated to determine significant effects of BC-rich Anthrosols and BC-poor adjacent soils on total C mineralization (CO_2–C), on the C content of microbial biomass and different soil fractions and on the proportion and amounts stemming from AOM and native soil C at different sampling times. Multiple comparisons of means for main effects were conducted using least significant difference (LSD) at $P < 0.05$, or as indicated otherwise.

3. Results

3.1. Soil properties

The BC-rich Anthrosols and BC-poor adjacent soils have comparable total organic C content (except at the DS site), but the proportions of BC are markedly different. The estimated BC content was higher in the BC-rich Anthrosols than in the adjacent soils by 293% (HAT), 65% (ACU) and 608% (DS), respectively (Table 1 and Liang et al., 2008).

3.2. Carbon mineralization

At the end of the incubation, the amount of C mineralization from total soil C of the BC-rich Anthrosols without AOM was 0.64, 0.44 and 0.54 mg C g^{-1} soil at HAT, ACU and DS, respectively. A significantly greater amount of C was mineralized in BC-poor adjacent soils, being 1.79, 1.11, 1.66 mg C g^{-1} soil, respectively (Table 2). With AOM, the total C mineralization from BC-rich Anthrosols was 7.2, 6.7, and 6.0 mg C g^{-1} soil for HAT, ACU and DS sites, respectively, 6–25% lower ($P < 0.05$) than that in BC-poor adjacent soils (Table 2 and Supplementary online material Fig. S1).

The apparent C mineralization from total soil in response to AOM was found to be 4% (ACU) and 15% (DS) lower ($P < 0.05$) in BC-rich Anthrosols than that in BC-poor adjacent soils. However, BC-rich soil at the HAT site showed a 12% greater apparent C mineralization in response to AOM than adjacent soil (Table 2 and Supplementary online material Fig. S1).

The actual C mineralization originating from AOM was calculated on the basis of the changes of ^{13}C isotopic composition over the 532 days incubation (Supplementary online material Fig. S2). At the end of incubation, C mineralization from AOM contributed 73, 80, and 68% to the total C mineralization in BC-poor adjacent soils at HAT, ACU and DS sites, respectively (Table 2). In contrast, in BC-rich Anthrosols, a significantly ($P < 0.05$) higher percentage of the total C mineralization originated from AOM, with 91%, 92%, and 92% at HAT, ACU and DS sites, respectively.

Based on the calculation of isotopic changes, similar amounts of C from AOM were mineralized in BC-poor adjacent soils and BC-rich Anthrosols at both ACU (6.13 vs. 6.19 mg C g^{-1} soil) and DS sites (5.52 vs. 5.55 mg C g^{-1} soil), except for HAT where the Anthrosol mineralized 0.98 mg C g^{-1} soil more AOM. Mineralization of native soil C was 0.63, 0.53 and 0.46 mg C g^{-1} soil for Anthrosols with AOM at HAT, ACU and DS sites, respectively, equivalent to 9%, 8% and 8% of the total C mineralization (Table 2). Mineralization of native soil C in adjacent soils with AOM was 2.07, 1.49 and 2.54 mg C g^{-1} soil at HAT, ACU and DS sites, respectively, which was 3.3, 2.8 and 5.6 times the values at corresponding Anthrosol sites, and contributed 27%, 20%, and 32% to the total C mineralization with AOM (Table 2). OM addition consistently promoted soil-derived C mineralization to a greater extent in BC-poor soils than in BC-rich Anthrosols (Table 2). In fact, mineralization of native soil C in BC-rich Anthrosols even decreased by 2% and 15% at HAT and DS sites, in response to AOM.

3.3. Carbon in different soil fractions

The recovery of AOM in the free light fraction decreased significantly faster in the BC-rich Anthrosols than in the BC-poor adjacent soils (Fig. 1B), except for the sandy DS site that had a clay content of <1% (Table 1). At the same time, a more rapid and greater proportion of AOM was found in the intra-aggregate light fraction (Fig. 1C) and in the organo-mineral fraction (Fig. 1D) of the BC-rich Anthrosols than of the BC-poor adjacent soils at all sites over the first 9 months. At the end of the incubation after 532 days,

Table 2
Total C mineralization with (+AOM) and without (−AOM) OM addition, and C mineralization from AOM and from native soil C based on calculation of isotopic composition changes ($N = 4$).

Soil type	Total C mineralization (mg CO_2–C g^{-1} soil)		Apparent AOM mineralization[a] (mg CO_2–C g^{-1} soil)	Source of evolved CO_2 with AOM[b]				
	+AOM	−AOM		Actual AOM mineralization		From soil		
				mg CO_2–C g^{-1} soil	% of total CO_2–C	mg CO_2–C g^{-1} soil	% of total CO_2–C	% increase over −AOM
HAT Anthrosol	7.23	0.64	6.59	6.60	91.3	0.63	8.7	−1.6
HAT Adjacent	7.69	1.79	5.90	5.62	73.1	2.07	26.9	+15.6
ACU Anthrosol	6.72	0.44	6.28	6.19	92.1	0.53	7.9	+20.5
ACU Adjacent	7.62	1.11	6.51	6.13	80.4	1.49	19.6	+34.2
DS Anthrosol	6.01	0.54	5.47	5.55	92.4	0.46	7.6	−14.8
DS Adjacent	8.06	1.66	6.40	5.52	68.5	2.54	31.5	+53.0

[a] Obtained by subtracting −AOM values from +AOM values.
[b] Using ^{13}C isotope tracing.

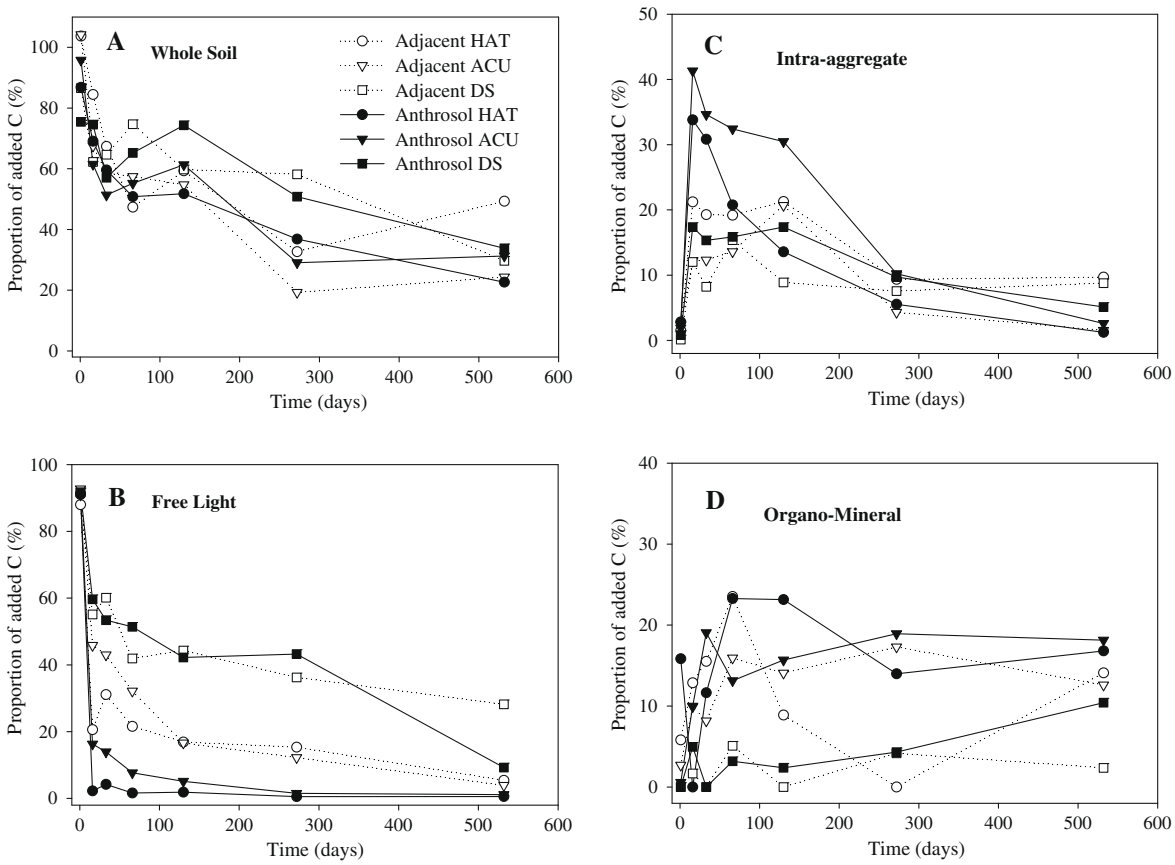

Fig. 1. Proportion of added C remaining in either: (A) whole soil, (B) free light, (C) intra-aggregate light, or (D) organo-mineral fractions for BC-rich Anthrosols and BC-poor adjacent soils at HAT, ACU and DS sites over 532 days incubation (solid lines for Anthrosols and dotted lines for adjacent soils, $N = 3$).

the organo-mineral fraction of the BC-rich Anthrosols (Fig. 1D) contained 2.7–8.1% of the AOM, which was 19–340% more than that in the BC-poor soils.

3.4. Microbial biomass

In order to account for the preferential retention of microbial matter by BC surface adsorption during $CHCl_3$ fumigation extraction, a correction factor E was calculated using the recovery of an added ^{13}C-enriched microbial culture (Table 3). Only 51%, 34% and 45% of the added ^{13}C were recovered by simultaneous fumigation extraction for BC-rich Anthrosols at HAT, ACU and DS sites, respectively. As a result of re-adsorption of MB to BC, these values were 21%, 41% and 37% lower than those for adjacent soil HAT (65%), ACU (57%) and DS (71%), respectively (Table 3).

With AOM, microbial biomass amounted to between 181–945 μg C g^{-1} soil for BC-rich Anthrosols, 43–125% higher ($P < 0.05$) than that in adjacent soils for all sampling periods pooled, except for one data pair from HAT after 16 days (Fig. 2A).

The microbial metabolic quotient (qCO_2) was expressed (Thirukkumaran and Parkinson, 2000) as the amount of CO_2–C produced per unit microbial biomass C (μg CO_2–C g^{-1} MB-C day^{-1}). qCO_2 decreased over time ($P < 0.05$) from about 0.6 to <0.001 (Fig. 2B). Overall, there was a significantly ($P < 0.05$) lower qCO_2 in Anthrosols than adjacent soils by 26–69% (Fig. 2B), with the exception of four data pairs.

4. Discussion

4.1. Effects of BC on cycling of C from soil and AOM

Mineralization of AOM was similar in both BC-rich Anthrosols and BC-poor adjacent soils except at the HAT site (Table 2). However, the mineralization of native soil C was 64–82% lower in the

Table 3
Determination of correction factor E for microbial biomass C using 402 μg C g^{-1} soil of a ^{13}C-enriched microbial culture with a $\delta^{13}C$ value of +93‰.

Soil type	C_E[a] (μg C g^{-1} soil)	$\delta^{13}C_E$[c] (‰)	$\delta^{13}C_{SMB}$[c] (‰)	C_R[b] (μg C g^{-1} soil)	E
HAT Anthrosol	284.1	3.43	−25.3	206.8	0.514
HAT adjacent	378.0	2.55	−24.7	262.3	0.652
ACU Anthrosol	178.2	4.38	−25.8	135.9	0.338
ACU adjacent	324.0	3.03	−24.6	228.4	0.568
DS Anthrosol	235.0	3.89	−26.5	179.1	0.445
DS adjacent	383.4	2.46	−27.2	283.5	0.705

[a] C_E, extracted C.
[b] C_R, recovered C from added ^{13}C-enriched microbial culture.
[c] $\delta^{13}C_E$ and $\delta^{13}C_{SMB}$, isotopic composition of extracted C and original soil microbial biomass, respectively.

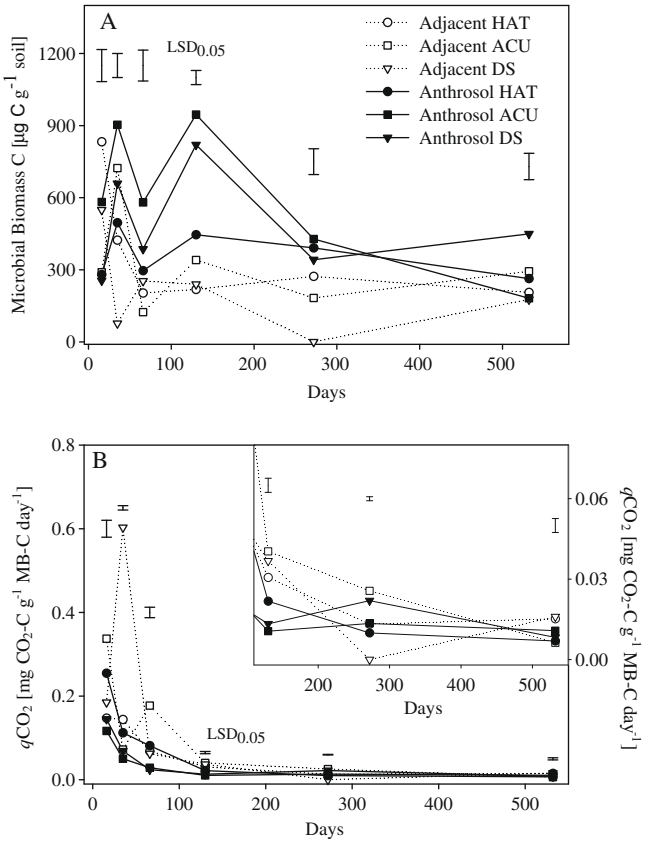

Fig. 2. Microbial biomass C (mg C g^{-1} soil) and metabolic quotient (qCO_2) when OM was added to either BC-rich Anthrosols or BC-poor adjacent soils at HAT, ACU and DS sites over 532 days incubation ($N = 3$). Inset is used to magnify the last three data points for improved clarity.

BC-rich Anthrosols compared to the BC-poor adjacent soils, which was most likely due to the higher BC content of the Anthrosols. Properties other than BC content were also different between the Anthrosols and adjacent soils, the latter having varying cation exchange capacity, lower pH, and lower base cation and P contents (Liang et al., 2006). Yet, none of these differences in properties explains a slower cycling of the soil C in the BC-rich Anthrosol. On the contrary, a higher pH should increase organic C decomposition in the Anthrosols (Waschkies and Hüttl, 1999). Therefore, the higher proportion of BC is suggested to be responsible either directly or indirectly for the lower C mineralization of native soil C in the Anthrosols.

Our results disagree with results from Hamer et al. (2004) and Wardle et al. (2008), who demonstrated that BC increased mineralization of labile C (glucose or forest humus). The discrepancy vs. Hamer et al. (2004) may be explained by the fact that simple sugars such as glucose behave differently from the more complex organic composition of the added plant material used in our study. Based on the assumption that BC did not decompose during the experiment, Wardle et al. (2008) concluded that BC caused priming of humus without providing isotope data to verify their explanation. It is therefore possible that C losses encountered by Wardle et al. (2008) in the mixture of BC and humus may in fact be explained by decomposition of BC rather than of humus.

In addition, interaction between AOM and soil minerals was not included in the studies by Hamer et al. (2004) and Wardle et al. (2008). Our study of BC effects pertained to the more complex soil environment and expands the evaluation of the role of BC in SOM cycling and turnover. The AOM was more readily transferred to the intra-aggregate light fraction of BC-rich Anthrosols than of BC-poor adjacent soils, especially during the first 9 months of incubation. This could have occurred as a result of the high surface area or the specific charge characteristics of BC (Liang et al., 2006) and requires further study.

A surplus of about 3–8% of the AOM was found in the organo-mineral fraction of BC-rich Anthrosols at the end of incubation, and can be considered an increased longer-term stabilization resulting from the presence of BC. Ladd et al. (1993) suggested that every compound is eventually degraded in soil and only physical protection, such as from interaction with mineral surfaces, can make C compounds increasingly resistant to microbial degradation. If 3–8% of the AOM that would otherwise be mineralized are stabilized in an organo-mineral fraction, this could have a profound effect on C cycling in BC-rich soils. Whether this difference found here in an incubation experiment translates into a significant long term effect in agricultural or forest ecosystems, warrants further investigation.

Interestingly, BC-rich soils maintained a greater microbial biomass and lower microbial quotient, yet incorporation of added C into physically protected soil fractions was more rapid. This observation may hint at processes of OM stabilization that are not necessarily mediated by microbial action, but processes such as improved aggregation, adsorption or compartmentalization. Other explanations may include CO_2 consumption by either abiotic or biotic mechanisms that would need to be investigated further.

The lower microbial metabolic quotient in the BC-rich Anthrosols may conform to hypotheses that microorganisms occupy pore spaces of BC (Warnock et al., 2007), similar to the process proposed by Breland and Eltun (1999), who pointed out that a smaller proportion of the microbial biomass was active when microorganisms were enclosed within aggregates. The specific habitat and lack of easily degradable C sources may also have an effect on the microbial population and structure in BC-rich soils. The microbial population in BC-rich soils was to a greater extent composed of k-strategists such as actinomycetes (O'Neill, 2006; O'Neill et al., 2009) and the bacterial community had 25% greater species richness in similar BC-rich soils (Kim et al., 2007). These microorganisms may not have the flexibility to quickly react to additions of easily available energy rich substrates (Fontaine et al., 2003) and AOM may be mineralized to a lesser extent.

4.2. Effects of OM addition on mineralization of BC

Positive priming of soil C after the incorporation of plant residues has been frequently reported (Liang et al., 1999; Kuzyakov et al., 2000; Bell et al., 2003; Malosso et al., 2004). The lack of priming of C mineralization from soil in the BC-rich soils observed in our experiment is in contrast to those studies and to the results of Hamer et al. (2004), who found increased mineralization of BC by the addition of glucose. In two out of three BC-rich Anthrosols, we even found slightly lower mineralization of soil C when OM was added. This discrepancy vs. results of Hamer et al. (2004) may be explained by: (i) the difference in added substrate quality (Fierer et al., 2001; Kuzyakov et al., 2000) and (ii) the presence of minerals as mentioned above, or, (iii) more likely, the difference in BC quality. The BC in the studied Anthrosols has been there for several hundred to a few thousand years, and any labile fraction of pyrolyzed OM may be of very small size and can be readily decomposed over a short period of time. Cheng et al. (2006) showed that aliphatic C compounds in fresh BC disappeared within several months of incubation. Therefore, priming observed by Hamer et al. (2004) may have mineralized the more labile and mainly non-aromatic fraction of their studied fresh BC. This may indeed occur shortly after forest fires or deliberate additions and accumulation of BC in soil, but is less likely to occur for the stable BC fraction.

4.3. Microbial extraction efficiency in BC-rich soils

Our study has established for the first time that an adjustment is necessary for BC-rich soils to account for the low microbial biomass recovery during fumigation extraction. Our selected K_{ec} of 0.26 is suggested for the studied highly weathered soils (Feigel et al., 1995) compared to a value of 0.33 recommended by Sparling and West (1988), within a range of conversion factors (0.2–0.48) for estimating microbial biomass C from extractable C using fumigation extraction. Such values were determined by calibration using cultured organisms in combination with biomass C estimated from CO_2–C flushes (Tate et al., 1988). BC may, however, strongly adsorb microbial matter during fumigation, leading to a low extraction efficiency (Table 3). This corresponds well with the results of low extraction efficiency for microbial DNA from these soils (O'Neill, 2006), the retention of microorganisms by charcoal (Pietikäinen et al., 2000) and the use of activated carbon as supporting materials for microorganisms (Ehrhardt and Rehm, 1985). Only with the correction factor *E*, was the MB greater in the BC-rich Anthrosols than the adjacent soils, which agrees with greater populations of culturable microorganisms in the same soils (O'Neill et al., 2009), or greater microbial growth rate in similar Anthrosols (Steiner et al., 2004).

Our results also indicate that MB cannot be accurately estimated from respiration rates when BC is present in large quantities. The values for the microbial metabolic quotient are equally affected by the proposed correction factor, but did in our case not change the fact that the quotients are lower in BC-rich than BC-poor soils regardless whether the correction factor *E* was used or not (data not shown).

A higher MB in BC-rich soils is hardly a result of greater amounts of labile OM, since the studied BC is relatively recalcitrant to microbial decay as shown for the same soils (Liang et al., 2008). One possible explanation may be that BC provides refuges for microorganisms or other services that promote a larger population (Warnock et al., 2007). For example, 1 g activated carbon was found capable of housing about 4×10^9 *Pseudomonas* cells and 3×10^8 *Candida* cells because of its surface properties (Ehrhardt and Rehm, 1985). The reason for greater microbial populations in soils that are rich in BC clearly requires further study.

5. Conclusions

The presence of BC led to more rapid incorporation of AOM into aggregate and organo-mineral fractions, whereas no positive priming effect of the AOM on BC mineralization was detected. The mechanism for the enhanced retention of AOM by BC is not clear and could involve differences in microbial population and metabolism pathway, and/or the unique surface properties of BC. More information is needed to know how unique soil microbial population and communities in BC-rich soils affect the dynamics and stabilization of AOM. Likewise, knowledge about the nature of interactions between BC surfaces and non-BC or BC and mineral surfaces is limited and warrants further research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.orggeochem.2009.09.007.

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