Fate of soil-applied black carbon: downward migration, leaching and soil respiration

Julie Major*, Johannes Lehmann*, Marco Rondon†, and Christine Goodale‡

**Department of Crop and Soil Sciences, Cornell University, Ithaca NY 14853;*
†International Development Research Centre, Ottawa, ON K1G 3H9, Canada (formerly at the Centro Internacional de Agricultura Tropical (CIAT), A.A. 6713 Cali, Colombia);
‡Department of Ecology and Evolutionary Biology, Cornell University, Ithaca NY 14853

Author for correspondence: Johannes Lehmann, telephone: 607-254-1236, fax: 607-255-2644, email: CL273@cornell.edu

Keywords: biochar; black carbon; carbon leaching; carbon sequestration; dissolved organic carbon; particulate organic carbon; soil carbon cycling; soil respiration; Oxisol

Running title: Fate of black carbon

Abstract

Black carbon (BC) is an important pool of the global C cycle, because it cycles much more slowly than others and may even be managed for C sequestration. Using stable isotope techniques, we investigated the fate of BC applied to a savanna Oxisol in Colombia at rates of 0, 11.6, 23.2 and 116.1 t BC ha^{-1}, as well as its effect on non-BC soil organic C. During the rainy seasons of 2005 and 2006, soil respiration was measured using soda lime traps, particulate (POC) and dissolved organic C (DOC) moving by saturated flow was sampled continuously at 0.15 and 0.3 m, and soil was sampled to 2.0 m. Black C was found below the application depth of 0-0.1 m in the 0.15-0.3 m depth interval, with migration rates of 52.4 ± 14.5, 51.8 ± 18.5 and 378.7 ±196.9 kg C ha^{-1} yr^{-1} (±SE) where 11.6, 23.2 and 116.1 t BC ha^{-1}, respectively, had been applied. Over two years after application, 2.2% of BC applied at 23.2 t BC ha^{-1} was lost by respiration, and an even smaller fraction of 1% was mobilized by percolating water. Carbon from BC moved to a greater extent as DOC than POC. The largest flux of BC from the field (20-53% of applied BC) was not accounted for by our measurements and is assumed to have occurred by surface runoff during intense rain events. Black C caused a 189% increase in above-ground biomass production measured 5 months after application (2.4-4.5 t additional dry biomass ha^{-1} where BC was applied), and this resulted in greater amounts of non-BC being respired, leached and found in soil for the duration of the experiment. These increases can be quantitatively explained by estimates of greater below-ground net primary productivity with BC addition.

Introduction

Black carbon (BC) is an important C pool globally. Despite relatively low global production rates of 0.05-0.27 Pg yr^{-1} (Forbes *et al.* 2006) compared to terrestrial net primary productivity of about 60 Pg yr^{-1} (Denman *et al.* 2007), biomass-derived BC has been found to comprise about 30% of organic C in 76% of 57 soils from 6 orders worldwide (Skjemstad & Taylor 1999; Skjemstad *et al.* 1996, 1999a, 1999b). Therefore, BC cycles at a much slower rate than the non-BC fraction of soil organic C mainly due to its high content of aromatic, graphitic, or elemental refractory C (Glaser *et al.* 1998). Indeed, BC in deep-sea sediments has been found to be 2,400-13,900 years older than associated non-BC (Masiello & Druffel 1998). Black C has also been observed to represent the oldest C fraction in soils (Pessenda *et al.* 2001). Understanding the stability of BC in soil is crucial in order to balance the global C budget (Czimczik & Masiello 2007). Managing BC for actively increasing the long-term C sink of atmospheric CO_2 in the form of BC from biochar in soil has been proposed by Lehmann *et al.* (2006) and also relies on accurate information about its stability.

However, very little is known about the stability of BC under field conditions. Most studies are conducted by using laboratory incubations (Baldock & Smernik 2002; Cheng *et al.* 2008b; Liang *et al.* 2008; Kuzyakov *et al.* 2009). The few published field observations using repeated soil sampling show generally rapid disappearance rates (Bird *et al.* 1999, Hammes *et al.* 2008; Nguyen *et al.* 2008) which may be explained by the fact that a full mass balance was not possible and some BC loss may have occurred by mass transport rather than mineralization.

Despite the observed long-term stability of BC, significant initial mineralization is likely to occur. Rapid oxidation over several months of incubation was reported for experimentally-produced BC (Cheng *et al.* 2006, 2008a). BC generated in the laboratory is likely to contain a range of compounds exhibiting varying stability (Lehmann 2007). During the first 10 years after BC deposition following forest fires in Kenya, the O/C ratio significantly increased on BC surfaces (Nguyen *et al.* 2008), and 100-year-old BC particles in Kenya (Nguyen *et al.* 2008) and dissolved BC-derived molecules in the US (Hockaday *et al.* 2007) were found to be significantly altered, bearing abundant carboxyl groups. The dynamics of BC mineralization during the first years after deposition to field soil have not been studied up to now.

Several studies have argued that BC might stimulate the rates of loss of non-BC soil C (Pietikäinen *et al.* 2000; Wardle *et al.* 2008), and proposed mechanisms for this include priming of decomposition by labile C (glucose) (Hamer *et al.* 2004), and the sorption by BC of compounds which inhibit microbial growth, such as phenols (Gundale & DeLuca 2007). No data have been reported that test these processes under field conditions in mineral soil.

In addition to mineralization to CO_2, BC may be transported in the landscape (Rumpel *et al.* 2006a, 2006b; Hockaday *et al.* 2007; Guggenberger *et al.* 2008) both laterally and vertically through soil. While BC has been identified in dissolved (Guggenberger *et al*. 2008) and particulate (Mitra et al. 2002) organic C of natural river water, the magnitude of leaching with percolating soil water has not been quantified. Similarly unclear is whether BC moves primarily in dissolved or particulate form. Several authors have identified surface-deposited BC in subsoils (e.g. Brodowski *et al.*

2007; Leifeld *et al.* 2007). Yet while the BC was found to be associated with coarse silt and sand (Brodowski *et al.* 2007), actual mechanisms for its movement have not been established. Black C, alone or associated with mineral fractions, could move through soil by facilitated transport in macropores. Particles with a median size of 2-5 µm moved from topsoil through a sandy loam in the field (Laubel *et al.* 1999), and natural colloids of up to 200 µm were mobilized through a coarse disturbed soil (Totsche *et al.* 2007), also in the field. Black C found in soil has very similar size distributions, with most of the particles typically being smaller than 50 µm (Skjemstad *et al.* 1996).

This work was undertaken to assess the fate of biomass-derived BC after addition to surface soil in the field over two years. Our hypotheses were: (i) CO_2 evolution will initially increase due to rapid mineralization of the labile fraction of BC; (ii) longer-term mineralization of BC over two years will be low due to its high stability; (iii) BC will move rapidly into the subsoil; and (iv) BC will mainly travel in dissolved form through a poorly aggregated clay Oxisol.

Materials and Methods

Site characteristics

Experimental plots were established at Matazul farm in the Llanos Orientales non-flooded savanna region of Colombia (N 04º10'15.2", W 07 º36'12.9"). The soil in the experimental plots is an isohyperthermic kaolinitic Typic Haplustox sandy clay loam (Soil Survey Staff 1994), which developed from alluvial sediments originating in the Andes (Rippstein *et al.* 2001). Long-term average annual rainfall measured approximately 200 km northeast of the plot is 2200 mm, and 95% of precipitation falls between April and December. A marked dry season occurs between January and March, and average annual temperature is 26°C. Based on stable C isotope signatures, present-day native C_4 vegetation dominated the area for a very extensive period, perhaps since the Late Glaciation Period (Behling & Hooghiemstra 1998). Trees are found almost exclusively along waterways, except in areas where fire control has been practiced in modern times. This C_4 vegetation results in $\delta^{13}C$ values for soil of -10.9‰ to -13.5‰ up to a depth of 2 m as measured in this experiment.

Field experiment

The BC applied to the plots was produced in a controlled manner, in order to provide sufficient amounts for a replicated experiment that included high application rates, and yielded the most uniform BC material that could be obtained under local conditions. Black C material 1 was produced in December 2004 from prunings of old mango (*Mangifera indica* L.) trees, with a resulting $\delta^{13}C$ value of -29‰ (Table 1). A mound of tightly packed logs (approx. 2 m high, 5 m diameter) was formed, covered with dry grass and soil, and ignited through a tunnel at the base. The temperature in simple kilns may range between 400°C to 600°C and carbonization times typically last 48 hours (Emrich 1985). Black C was then ground by hand using a metallic disk pestle, to pass through a 0.9 mm sieve, and mixed well. Black C generated under natural fires (Masiello 2004) or produced using modern technology for deliberate application as biochar (Lehmann 2007) can have varying properties. Depending on the charred biomass type and charring conditions, chemical properties including decomposability may differ significantly (Baldock & Smernik 2002; Antal & Grønli 2003). The results shown here have to be viewed in the context of the BC material used (Table 1).

At the onset of the dry season in December 2004, BC was incorporated to soil under native savanna vegetation which to our knowledge had never been tilled or cropped. The fields were selected on a flat plain with no visible inclination and the slope was estimated to have been at most 2%. The site was mowed, and disked to break up the soil. A randomized complete block design was applied, with plots measuring 4 by 5 m, using 3 replications. Black C application rates were 0, 11.6, 23.2 and 116.1 t BC ha^{-1} (n=3). These rates correspond approximately to a 50% increase, doubling and five-fold increase in soil C in the top 0.1 m, respectively. Skjemstad *et al.* (1999a) reported a maximum, conservative estimate of BC content in Australian soils of 28 t BC ha^{-1} (at our soil's density and application depth), and the rate of 116.1 t BC ha^{-1} brought the soil in the range of soil organic C (SOC) stocks found in BC-rich Amazonian *Terra preta* soils (147-506 t C ha^{-1} m^{-1}) (Glaser *et al.* 2003). Black C was applied to each plot uniformly using rakes, and incorporated to 0.1 m with 2 disk harrow passes. The control plots were also disked.

Soil sampling

On 13-16 December 2006, i.e. 2 rainy seasons after application, soil was sampled in all plots, in depth increments of 0-0.15, 0.15-0.3, 0.3-0.6, 0.6-1.2, and 1.2-2 m. Sampling was carried out manually to 0.6 m with a ~50 mm diam. corer, and using a tractor-driven hydraulic version of the same corer to 2 m. Composite samples were taken in each plot, from 5 random sampling locations to 0.6 m and 3 of these locations to 2 m, and hand mixed in buckets before a ~500 g subsample was taken for analysis. These subsamples were air-dried, crushed and passed through an aluminum sieve with 2 mm circular holes.

Soil was analyzed for particle size distribution by the hydrometer technique (Bouyoucos 1927), after dispersion with sodium hexametaphosphate. In July 2006, additional samples were taken for physical property analysis, using aluminum cores. In each plot, a small pit was dug to 0.3 m, and subsamples were taken from 2 opposite sides of pits from the surface, 0.15 and 0.3 m depths. Two cores (50 mm diameter, 50 mm high) were taken at each depth and from each side of the pits. For depths of 0.6, 1.2 and 2 m, we assumed no effect of BC application on physical properties and two soil pits located 5-10 m away from the experiment were used. In each of these pits, cores were taken from 2 profiles on the side closest to the experiment. This yielded a total of 4 samples for each sampling depth.

Bulk density was determined by oven drying at 105°C for 24 h for samples from all plots and depths (84 samples). Samples to 0.3 m depth from the control and 23.2 t BC ha^{-1} treatments were used to determine saturated hydraulic conductivity with a constant head permeameter. Abnormally high saturated hydraulic conductivity data for one of the control plots, at 0.3 m, was removed before statistical analysis due to the observation of high termite activity in that plot and depth.

Carbon leaching measurement

In May 2005, free-draining lysimeters for measuring particulate and dissolved organic C (POC and DOC) in water moving by saturated flow were installed in the unamended control and the plots receiving 23.2 t BC ha^{-1}. These were inserted from soil pits at depths of 0.15 and 0.3 m, in "galleries" dug from the face of the pits and into the experimental plots. The lysimeters consisted of 0.15 m diam. Pyrex® glass funnels filled with quartz sand after washing with dilute HCl and NaOH, held by a glass wool plug. The C content of the sand after washing was 0.04% by weight. Two funnels were placed at each depth in each plot. Funnel pairs were fitted with plasticizer-free Tygon® (Formula 2075) hose and the hoses joined with a copper "T" fitting, itself draining into one amber glass bottle (washed as described above). This yielded a total of 24 funnels and 12 collection bottles (1 per depth per plot). Field collection bottles were checked at least weekly, and emptied when water had been collected. After each emptying of bottles, a solution of $HgCl_2$ was added as a biocide to achieve a final concentration of 30 μM in a 1-L sample. Water sampling was carried out between May 2005 and January 2006 (yr 1), and March and December 2006 (yr 2). At each collection time, the height of water in the bottles was recorded and converted into volume using a curve created with the bottles. Upon collection, water samples were stored in borosilicate glass vials with Teflon®-lined caps and refrigerated at 4ºC until analyzed. Samples were not refrigerated for 3-5 days while being shipped to the USA.

Depending on sample availability, 40-60 mL of each water sample was vacuum filtered using glazed ceramic Buchner funnels through binder-free glass filters (Whatman® GF/F; 13 mm diam., 0.7 µm pore size), which were then dried at 60°C for 24-36 h, cut up, and placed in tin cups for C and N analysis by dry combustion. Filtered water samples were freeze-dried in progressively smaller glass vials, and the solids weighed and prepared for C and N analysis by dry combustion. In the present study, we used 0.7 µm to separate dissolved organic C (DOC) from particulate organic C (POC). However, we recognize that this separation is operational and that some colloids may have sizes of less than 0.7 µm.

For the calculation of total C leaching, the flux in each lysimeter was multiplied by the C concentration in the sample. On two dates no samples were available for analysis for the control due to sample loss. For one of these dates the value was calculated as the average of the two adjacent dates, since these were very similar. In the other case, the date was dropped completely since no assumption of similarity could be made.

Soil respiration measurement

Static-chamber soda lime traps (after Edwards 1982) were used in order to determine the amount and isotopic composition of soil-respired CO_2. Plastic rings obtained from cutting 20-L buckets (0.273 m diameter) were buried into the soil to a depth of 0.1 m and measurements started in the second year after BC application on April

6 2006. To discern the short term response of respiration during the first year after BC addition, a second set of rings was installed on 11 May 2006 on an adjacent area, and identically treated using BC material 2 which was produced similarly to material 1, except a soil trench was used instead of a mound (Table 1). In both sets of plots, two rings were inserted at random locations away from the edges inside each replicate plot of the 23.2 t BC ha^{-1} and control treatments, for a total of 24. The soil inside the rings was kept free of vegetation. Initially, 30 g of 4-8 mesh soda lime with indicator were exposed in each chamber, based on recommendations from Edwards (1982). After 5 weekly trials, the amount of soda lime was reduced to 10 g, since CO_2 absorption did not exceed 10% of the saturation value for this amount of soda lime (as specified by the manufacturer). The highest mass of CO_2 absorbed in a single trial represented 11% of the mass of soda lime used. Prior to field exposure, soda lime was dried in wide-mouth (44 mm) glass jars for 24 h at 105°C, capped tightly, cooled and then weighed on a scale with 1 mg resolution. Uncapped jars (“traps”) were placed on elevated pieces of wire mesh, on the soil inside each ring. Chamber tops were formed from the rest of the 20 L plastic buckets, sealed using a thick, tight rubber band covering the junction. Control traps were placed in the plots, in chambers of the same internal volume as the treatment chambers. However, they consisted of two bucket bottoms sealed together, i.e. no soil was exposed. Initially, one control trap was used in each plot (total 12 control traps), but after observing low variability among control traps, their number was reduced to 6 for the entire experiment. Sampling frequency was initially weekly, but was reduced to biweekly halfway into the rainy season.

The soda lime was left in the field for 24 h, removed and dried again for 24 h as above. Final mass was obtained and the difference between initial and final mass multiplied by 1.69 to correct for chemical water generation during the process of CO_2 absorption (Grogan 1998). Control traps account for CO_2 absorbed during drying, manipulation, from the initial atmosphere inside the chamber and any additional CO_2 contributed by leaks in chamber seals. Absorbed CO_2 mass for the controls were averaged, and this average value subtracted from absorbed CO_2 mass in each treatment jar. The maximum standard error observed among control traps was 17% for the duration of the experiment. Trapped CO_2 mass was further corrected for time of exposure, which differed from 24 h by a maximum of 74 min over the duration of measurements.

The amount of CO_2 trapped depended in part on the diameter of the jars used to expose the soda lime. On four occasions it was necessary to use jars of different mouth size. To correct for this difference, a relationship was established between mouth size and CO_2 trapped by exposing jars of various sizes (30-44 mm mouth diam.) to ambient air for approximately 60 h. This trial was repeated four times and conversion factors averaged.

Exposed soda lime jars were sealed with Teflon® tape and stored in sealed drums with silica gel to absorb humidity. Samples were later composited into periods defined by peaks in amount of CO_2 absorbed over time (Fig. 4). All samples for dates within individual peak absorption periods were combined, mixed, and a subsample ground for 90 s by hand using a mortar and pestle. For the first and last sampling dates, subsamples were combined but replicates and controls were analyzed separately, to provide more detailed data as well as information on variability. Approximately 0.2 g of ground composited soda lime was placed into acid-washed glass tubes, which were then sealed and evacuated. Three milliliters of phosphoric acid (40%) were added, and the tubes were set on an orbital shaker for 1 h. The headspace gas was then sampled using a syringe and transferred to evacuated single-use tubes.

Plant biomass sampling

Plant biomass was sampled on 19 October 2006, where all above-ground vegetation inside two 1-m^2 quadrats was sampled in three replicated plots of the control and 23.2 t BC ha^{-1}, on the new set of plots used for first year soda lime measurements. Vegetation was separated into grasses, forbs and legumes, and fresh and dry mass was determined after drying at 65ºC for 72 h.

Analytical procedures

Air-dried BC and soil were ground using a ball grinder (Oscillating Mill MM400 by Retsch, Newtown PA, USA). Carbon and N contents and isotope ratios were determined by combustion on an isotope ratio mass spectrometer (IRMS; Europa Hydra 20/20 by Europa Scientific, Crewe UK) for solid samples, and gas on a GC-C-IRMS (Europa Geo 20/20-Orchid by Europa Scientific, Crewe UK). The H content of BC was measured by combustion on an oxygen analyzer (PDZ Europa 20-20, Heckatech HT by Europa Scientific, Crewe UK). Ash content was measured according to ASTM (2007). To measure pH, BC was mixed with either water or 1 N KCl in a 1:10 mass:volume ratio, stirred 3 times over 1 h and the pH was read with a gel epoxy electrode (Symphony by VWR, West Chester PA USA). Cation exchange capacity (CEC) of BC was determined by double extraction with 1 N ammonium acetate at pH 7, flushing with isopropyl alcohol followed by double 2 N KCl extraction. The ammonium content of the KCl extract was determined colorimetrically using a Technicon® flow analyzer (Technicon Corporation) and Nessler's reagent (Naude 1927). Available nutrients were extracted using 2.5 g BC and 25 mL Mehlich III solution (Mehlich 1984), followed by shaking for 5 min and filtering. Nutrients (K, Ca, Mg, P) were analyzed by ICP atomic emission spectrometry (IRIS Intrepid by Thermo Elemental, Franklin MA USA).

Calculations and statistical analyses

The amount of C contributed by BC and soil in samples was calculated from Equation 1.

$$\delta^{13}C_{A+B}(A+B) = \delta^{13}C_A A + \delta^{13}C_B B \quad \text{[Eq. 1]}$$

Where $\delta^{13}C_A$ = $\delta^{13}C$ of corresponding control soil within field replicate

$\delta^{13}C_B$ = $\delta^{13}C$ of BC

$\delta^{13}C_{A+B}$ = $\delta^{13}C$ of field sample

A = amount of C derived from C_4 sources (soil-C)

B = amount of C from BC

A+B = total amount of C in field sample

When no corresponding control was available for a specific sample, the average $\delta^{13}C$ of controls for that date was used. When no controls were available for a specific date, the average $\delta^{13}C$ of controls at the closest date was used. It is recognized that end members may not necessarily contribute C with isotopic values that are identical to the bulk $\delta^{13}C$. For example, the easily mineralizable or leachable fraction of BC may have a different

δ^{13}C than the total BC. This could be due to the fact that lignin is typically depleted in ^{13}C compared to other plant compounds (Benner *et al.* 1987) and greater BC yields from lignin than from cellulose or hemicellulose (Yang *et al.* 2007). However, C isotope ratios have not been found to change with increasing charring temperature of C_3 plants (Krull *et al.* 2003) suggesting that labile and stable BC fractions have similar δ^{13}C values compared to the large differences between the C3-derived BC and the C_4 vegetation.

Statistical analyses were conducted using the general linear model procedure of SAS (SAS Institute Inc. 2003), and means separated using the *t*-test at α=0.05.

Results

Soil

The highest rate of BC application (116.1 t BC ha^{-1}) reduced soil bulk density at the surface and 0.15 m depths ($P < 0.05$) (Fig. 1). Also, BC application significantly increased saturated hydraulic conductivity at the surface from 2.7 to 13.4 cm h^{-1} in the control and 23.2 t BC ha^{-1} application rate, respectively (data not shown).

Soil C contents were greater in plots receiving greater amounts of BC (Fig. 2), although only the highest BC application rate resulted in a significant difference from the control ($P<0.05$) at 0-0.15 m depth. For the 0.15-0.3 m depth increment, both the 23.2 and 116.1 t BC ha^{-1} rates had significantly more total C than the control ($P<0.05$, $n=3$). Black C was found mostly in the surface layer which comprised the application depth with the highest application rate producing a significantly greater BC stock than others ($P<0.05$, $n=3$). Small amounts of applied BC were also found in the 0.15-0.3 m depth increment, with the high application rate (116.1 t ha^{-1}) always resulting in significantly greater ($P<0.05$) BC stocks than the control (Fig 2). When controlling for bulk density, the non-BC stock was greater in the high BC application rate than the control at both the 0-0.15 and 0.15-0.3 m depths, after 2 years (Fig. 2). The concentration of non-BC was greater with all BC application rates ($P<0.05$) at 0-0.15 m (6.48, 8.01, 7.81 and 10.25 mg soil C g $soil^{-1}$ for 0, 11.6, 23.2 and 116.1 t BC ha^{-1} applied, respectively). Only the highest rate significantly increased non-BC content at 0.15-0.3 m, from 4.60 to 6.46 mg soil C g $soil^{-1}$ ($P<0.05$).

Plant biomass

Total above-ground plant biomass measured 5 months after biochar application increased by 189% when 23.2 t BC ha^{-1} was applied. Grasses, forbs and legumes on BC amended plots had 93, 292 and 1916% greater biomass, respectively, than on plots without BC application ($P<0.05$). The proportions of forbs and legumes were also greater when BC was applied (Table 2).

Biomass was not measured in year two, however it was observed in the second-year plot that vegetation composition had greater similarity to that which predominates in the region and which covered the plots before establishment. Grasses dominated and forbs were not as prominent, with or without BC application, than when sampling was carried out at 5 months.

Carbon leaching

The concentration of POC in water was greater at both 0.15 and 0.3 m ($P<0.0001$ and $P<0.005$, respectively) in fields with 23.2 t BC ha^{-1} than those without BC additions. Results were similar for the total flux of POC leached ($P<0.005$ at both depths) (Fig. 3). Cumulatively, 317 and 267% more POC was leached at 0.15 and 0.3 m, respectively, when BC was applied (only 23.2 t BC ha^{-1} was investigated for leaching in comparison to the control). At 0.15 m, both the volume-weighted average POC concentration and total flux of POC leached were greater with BC addition, while at 0.3 m the volume-weighted concentration of POC was not significantly affected by BC additions. Greater water flux therefore explains the increase in total POC leached only at 0.3 m (Table 3). Volume-weighted $\delta^{13}C$ values for POC were not significantly different between treatments at 0.15 m. However, at 0.3 m, BC additions resulted in significantly higher volume-weighted $\delta^{13}C$ values. Calculated amounts of BC-derived POC leached were low (Table 3), corresponding to <1% of applied BC after two years. Black C application led to greater amounts of non-BC-derived POC leached ($P<0.05$), corresponding to increases by 308 and 254% at 0.15 and 0.3 m, respectively over control plots.

Similar trends were observed for DOC, with cumulatively 158 and 199% more DOC and 131% and 122% more non-BC-derived DOC leached 0.15 and 0.3 m, respectively, when BC was applied as compared to non-amended plots. However, both volume-weighted concentrations and total amounts of DOC (in both treatments) were generally substantially greater than for POC. Also, this trend was much stronger when BC had not been applied and the relative increase of POC leaching was greater than of DOC after BC addition. Thus, the ratio of total DOC-to-total POC was 1.43 and 2.31 at 0.15 m with and without BC addition, respectively. At 0.3 m, these ratios were 1.93 and 2.37, respectively. The amount of C in DOC originating from BC was 680% greater at 0.15 m and 1342% greater at 0.3 m than that in POC, while absolute amounts of BC-induced increases in leached non-BC were similar in DOC and POC at both depths (Table 3).

Soil respiration

The amount of C respired followed similar trends as leached C but was consistently greater ($P<0.05$) when BC was added, during both the first and second year after BC application (Fig 4; only 23.2 t BC ha^{-1} was investigated in comparison to the control). Yet overall respiration rates were greater in the first than the second year. Cumulatively, 41 and 18% more C was respired when BC was applied, as compared to the non-amended control, in the first and second year, respectively (Table 4).

A small percentage of respired C originated from applied BC ($P<0.05$) (Table 4). For the two dates where replicate samples of CO_2 were analyzed, no significant difference ($P>0.05$) in $\delta^{13}C$ was found between the control and BC-amended soil. However, BC addition resulted in a 25% increase in non-BC respired ($P<0.05$). This increase was greater in the first year after application (40%) than the second (6%).

Overall, the most important fate of C originating from BC directly measured here was respiration (Table 5). The proportion which was mobilized by water was two (DOC) to three (POC) orders of magnitude lower. Increases in non-BC losses were also greatest for respiration, and again between two and three orders of magnitude greater than for DOC and POC, respectively.

Discussion

Vertical movement of total C and BC in soil

After two years, only a small proportion of soil-applied BC had moved below the 0.1 m application depth into the 0.15-0.3 m sampling depth. Leifeld *et al.* (2007) observed BC migration rates of 630 to 1160 mm yr^{-1}, where 21-69% of BC migrated below the incorporation depth of 0.3 m, down to a maximum of 1.40 m over a maximum of 95 years in peat soils with very low bulk density. In our sandy Oxisol, BC traveled from 0.1 to 0.3 m with water as both POC and DOC at the first collection date after BC application and the onset of the rainy season. For the 23.2 t BC ha^{-1} application rate, 4.25 kg BC ha^{-1} (0.02% of applied amount) over two years moved below 0.3 m depth as POC and DOC (from Table 3), while 103.4 kg BC ha^{-1} (0.45% of applied amount) was found in soil between 0.15 and 0.3 m (Fig. 2). Black C was thus retained by the mineral subsoil.

We suspect that bioturbation could have been additionally involved in BC movement below 0.15 m. Surface-applied lime was incorporated into an acidic Australian soil by earthworms to a depth of 0.15 m (Chan *et al.* 2004), and earthworm burrows were observed in our plots in cores from the 0.6-1.2 m depth increment, where the inside of the burrow was much darker in color than the surrounding soil. Termites were also observed to be active on experimental plots.

At 0-0.15 m, BC stocks after two years were 53, 41 and 20% less than applied amounts of 11.6, 23.2 and 116.1 t BC ha^{-1}, respectively, and leaching below 0.15 m accounts for a loss of 0.02% of applied BC at the 23.2 t ha^{-1} rate. Given that respiration accounted for a 2.2% loss, and that much smaller proportions were found in the soil between 0.15 and 0.3 m or leached below 0.3 m, a large proportion of applied BC may have been lost by surface runoff. This represents the largest BC flux in this study. Shortly after application, biochar might have been hydrophobic (Knicker *et al.* 2007), which could have facilitated runoff during intense rain events. On steep slopes in Laos, surface-deposited BC was preferentially eroded in comparison to other types of soil organic matter due to its lack of association with minerals shortly after deposition, its light nature and the fact that it did not significantly degrade during transport (Rumpel *et al.* 2006a). Guggenberger *et al.* (2008) also found a larger proportion of BC from vegetation fire exported by stream water than non-BC in a tundra catchment. A total export of 1 kg BC ha^{-1} yr^{-1} was observed, where 0.22 to 34.4 t BC ha^{-1} was stored in catchment soil and uniformly distributed to a depth of 1 m. In contrast to runoff, transfer of BC between plots likely did not occur in our study, since the δ^{13}C of surface soil in control plots did not change according to the direction of runoff on the field.

Lower bulk density, improved saturated hydraulic conductivity and water infiltration at the surface (Fig. 1) resulted in greater water flux at both depths when BC was added. Consequently, at 0.15 m the increase in total amounts of leached C after BC additions was proportionally greater than the increase in the volume-weighted concentration of POC as well as DOC (Table 3). At 0.3 m, volume-weighted C concentrations were lower when BC was applied, but total C leached was still greater with BC addition for both POC and DOC. This implies that greater water flux was solely responsible for the increase in total C leached at 0.3 m. Dissolved organic C concentrations found at 0.15 m (mean = 2.6 mg L^{-1}) in our study are in the range of those found at 0.1 m below an Oxisol in a Brazilian Amazon forest (mean = approx. 7 mg L^{-1}) (Johnson *et al.* 2006).

The proportion of BC in DOC was much greater than BC in POC (Table 3). This suggests that BC preferentially travels as colloidal particles < 0.7 µm, or as by-products of BC decomposition and/or weathering. On the other hand, leaching of non-BC as a result of BC additions increased 3.5-4.1 times for POC, but only 2.3-3.1 times for DOC (Table 3). The reason may lie in the adsorptivity of BC. Black C sorbs a wide range of organic compounds found in soils such as phenols (Gundale & DeLuca 2007), pesticides (Yu *et al.* 2006), glucose and DOC from birch leaf extract (Pietikäinen *et al.* 2000).

Black C effect on C cycling

The increase in respired C was mostly not related to BC, but rather to non-BC respiration (Table 4). Still, soil respiration accounted for an overwhelmingly greater proportion of BC flux than movement with water (Table 5). Black C may preferentially harbor microorganisms in its highly porous structure (Pietikäinen *et al.* 2000), and these are active as demonstrated by greater basal respiration with BC than with pumice (Pietikäinen *et al.* 2000) and by greater substrate-induced respiration by BC addition to forest humus (Wardle *et al.* 2008), in laboratory incubation studies. However, Steiner *et al.* (2004) found no difference in basal or substrate-induced respiration in the laboratory, when BC was added to either a control or synthetically fertilized Oxisol. Our results show that despite greater soil C:N ratios with BC additions (26.1 vs. 14.8 with and without BC, respectively), soil respiration increased. Possible reasons are (i) a greater amount of biomass production and hence mineralization of plant litter; (ii) possibly a larger microbial population in soils that received BC; or (iii) greater root respiration. The latter was partly captured here despite rings being kept free of vegetation, since they were only inserted to 0.1 m. The increase in non-BC respired with BC application was greater in the first year than the second year, and this may indicate that BC's stimulatory effect on soil and/or plant respiration tapers off during the first years after application.

Black C application resulted in greater amounts of non-BC in soil. This follows from greater plant biomass production. A literature review by Lehmann and Rondon (2006) found that plant biomass increased by up to 230% with BC application in 24 experiments using 10 different crops, when compared to optimally managed controls not receiving BC. Greater plant productivity and consequently greater root and leaf turnover and microbial activity likely led to greater POC and DOC leaching with BC application, and explain the observed increase in non-BC cycling and non-BC soil stocks. Induced increases were greatest, in absolute terms, for respired C and this is in agreement with the explanation that greater non-BC fluxes with BC occurred through increased plant biomass, with associated increases in both heterotrophic and autotrophic respiration. Trujillo *et al.* (2006) estimated below ground net primary productivity to 0.5 m of mature savanna vegetation at 12.5 t ha^{-1} yr^{-1} (5.0 t C ha^{-1} yr^{-1} with 41% C in roots) on the same farm where this work was carried out, using the compartment-flow model. The calculated additional C input through root turnover after BC application would represent 29 t C ha^{-1} over two years, assuming that our measured increases in above-ground biomass (Table 2) translate into similar increases below ground. This amount is greater than the sum of the additional non-BC respired (1.82 t C ha^{-1}), leached below 0.3 m (0.007 t C ha^{-1}), and of additional non-BC in soil accrued to 0.3 m (3.98 t ha^{-1}) over two years. Thus, greater biomass production alone can explain the observed increases in non-BC fluxes, and BC-induced increased fluxes of native soil organic C is unlikely the cause.

Wardle *et al.* (2008) observed that the addition of BC to litterbags containing boreal forest humus resulted in greater humus-C loss than expected from the loss of the components taken separately. Hamer *et al.* (2004) also found that BC enhanced the degradation of glucose in laboratory incubation studies. Our findings show the opposite, where applying BC to soil leads to greater non-BC content. The effect of BC on plant biomass and available substrates is not accounted for in data by Wardle *et al.* (2008), since the litter bags necessarily exclude BC-stimulated plant C input. Physical export of C might also have been attributed to C mineralization, since mineral surfaces are expected to retain leached C and this would not be the case in the organic layer of the forest floor (Lehmann & Sohi 2008). In addition, the experiment by Hamer *et al.* (2004) did not include plants. Our results represent the net effect of BC on the soil and plants.

Black C stability and implications for biochar soil management and C sequestration

The considerable proportion of BC which may have been lost by surface erosion with water could accumulate in depressions, travel mostly as sediment in waterways, potentially to the deep ocean where BC remains stable for thousands of years (Masiello & Druffel 1998).

Dissolved organic C and POC leached are likely to be adsorbed in subsoils, and thus become even more stable than topsoil C. Dissolved organic C concentrations in temperate and arctic forests decreased sharply as soil solution moved through mineral soil (Qualls & Haines 1992; Guggenberger & Zech 1994). Experimental evidence suggests that organic molecules such as plant-derived carbohydrates and lignins (Guggenberger & Zech 1994) are mainly retained abiotically by the soil matrix (Qualls & Haines 1992), and subsequently degraded *in situ* by microorganisms. Sorption of organic C to soil minerals is enhanced by the presence of Al and Fe oxides (Kaiser et al. 1996) which are abundant in Oxisols. Indeed, organic C below 0.6 m in a temperate grassland soil was > 2,000 yr old, and had a residence time 8 times greater than C found at the surface (Fontaine *et al.* 2007). The small amounts of leached BC observed here are also expected to sorb to the mineral matrix in the subsoil.

Over two years, we calculated that < 3% of applied BC had been respired, of which 75% occurred during the first year. As time passes and labile BC fractions are mineralized, respiration of BC is likely to decrease even further. Using a first-order decay model with two pools, the mean residence time (MRT) of the BC obtained from isotope recovery calculated to 600 years (r^2=0.928; *n*=12). When normalized to the same mean annual temperature of 10°C (from 26°C using a Q_{10} of 3.4 from Cheng *et al.* 2008b) the resulting MRT of 3,264 years is in the same order of magnitude as that from laboratory incubation studies of similar duration using soils from charcoal storage sites (1,335 years, Cheng *et al.* 2008b), Amazonian Dark Earths (4,035 years, calculated from Liang *et al.* 2008), or fresh ryegrass BC (2,000 years, Kuzyakov *et al.* 2009). Modeling to long-term equilibrium yielded slightly longer MRT of 1,300 and 2,600 years for BC from savanna fires in Australia at a similar mean annual temperature of 27°C (Lehmann *et al.* 2008). In addition to the degree of aging and the environmental conditions, however, also the production conditions have to be considered when comparing BC decomposition rates, for which little information is available to date.

Conclusions

Less than 3% of the applied BC was lost by CO_2 evolution, with a calculated MRT of 600 years at 26°C mean annual temperature (3,264 years at 10°C). Since 75% of the BC losses by mineralization occurred over the course of the first year, we expect that such losses will decrease even further with time as the labile BC fraction is mineralized. These low respiration losses suggest high stability of BC in soils, which confirms the role that BC plays as a C sink in the global C cycle. When linked to sustainable and renewable feedstock production, it also affirms the validity of exploring purposeful application of BC to soils as a sink enhancement of stable SOC. We did not find evidence for an enhanced loss of existing SOC due to addition of BC, but rather greater amounts of non-BC in soil, leachate and respired C explainable by greater plant productivity. In addition to higher plant productivity, greater POC and DOC leaching could also be explained by a greater water flux through soil due to influences of BC on soil structure. Black C additions therefore had effects on both soil and plant C dynamics.

The suspected physical export of BC was significantly greater than mineralization, with most of the BC likely being moved by surface erosion. This potentially large translocation of BC in the landscape must be recognized and losses of BC from soil can not be interpreted solely as mineralization. We cannot provide information about whether the leached or eroded BC is mineralized to a greater or lesser extent during transport or after accumulation. Since erosion fluxes were estimated to be a significant portion of the total loss, the fate and the effects of BC moving laterally in the landscape warrant further investigation.

Acknowledgements

Our heartfelt appreciation goes out to Pedro Herrera, Diego Molina, Gonzalo Rojas and Maria del Pilar Hurtado for their dedicated help in the field. Support for J. Major was provided by a Canada Graduate Scholarship from the Natural Sciences and Engineering Research Council of Canada, and by the Saltonstall Fellowship from the Department of Crop and Soil Sciences at Cornell University. Field and laboratory work was supported by grants from Cornell's Center for the Environment, a USAID linkage grant to CIAT, the Bradfield award from Cornell's Department of Crop and Soil Sciences, Cornell's National Science Foundation (NSF) - Integrative Graduate Education and Research Traineeship (IGERT) program, as well as research travel grants from Cornell's Graduate School.

References

American Society for Testing and Materials (ASTM) (2007) [online] Standard test method for chemical analysis of wood charcoal ASTM D1762-84 section 7.4. ASTM International, West Conshohocken PA. Available at: http://www.astm.org/Standards/D1762.htm [Accessed 7 January 2009].

Baldock JA, Smernik RJ (2002) Chemical composition and bioavailability of thermally, altered *Pinus resinosa* (Red Pine) wood. *Organic Geochemistry*, **33**, 1093-1109.

Antal MJ, Grønli M (2003) The art, science, and technology of charcoal production. *Industrial & Engineering Chemistry Research*, **42**, 1619-1640.

Behling H, Hooghiemstra H (1998) Late Quaternary palaeoecology and palaeoclimatology from pollen records of the savannas of the Llanos Orientales in Colombia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **139**, 251-267.

Bird MI, Moyo C, Veendaal EM, Lloyd J, Frost P (1999) Stability of elemental carbon in a savanna soil. *Global Biogeochemical Cycles*, **13**, 923-932.

Benner R, Fogel ML, Sprague EK, and Hodson RE (1987) Depletion of ^{13}C in lignin and its implications for carbon stable isotope studies. *Nature*, **329**, 708–710.

Bouyoucos GJ (1927) The hydrometer as a new and rapid method for determining the colloidal content of soil. *Soil Science*, **23**, 319-331.

Brodowski S, Amelung W, Haumaier L, Zech W (2007) Black carbon contribution to stable humus in German arable soils. *Geoderma*, **139**, 220-228.

Chan KY, Baker GH, Conyers MK, Scott B, Munro K (2004) Complementary ability of three European earthworms (Lumbricidae) to bury lime and increase pasture production in acidic soils of south-eastern Australia. *Applied Soil Ecology*, **26**, 257-271.

Cheng CH, Lehmann J, Thies JE, Burton SD, Engelhard MH (2006) Oxidation of black carbon by biotic and abiotic processes. *Organic Geochemistry*, **37**, 1477-1488.

Cheng CH, Lehmann J, Engelhard M (2008a) Natural oxidation of black carbon in soils: changes in molecular form and surface charge along a climosequence. *Geochimica et Cosmochimica Acta*, **72**, 1598-1610.

Cheng CH, Lehmann J, Thies JE, Burton S (2008b) Stability of black carbon in soils across a climatic gradient. *Journal of Geophysical Research*, **113**, G02027.

Czimczik CI, Masiello CA (2007) Controls on black carbon storage in soils. *Global Biogeochemical Cycles*, **21**, GB3005.

Denman KL, Brasseur G, Chidthaisong A, Ciais P, Cox PM, Dickinson RE, Hauglustaine D, Heinze C, Holland E, Jacob D, Lohmann U, Ramachandran S, da Silva Dias PL, Wofsy SC and Zhang X (2007) Couplings between changes in the climate system and biogeochemistry. In: *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (eds. Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M and Miller HL), pp. 499-587. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Edwards NT (1982) The use of soda-lime for measuring respiration rates in terrestrial systems. *Pedobiologia*, **23**, 321-330.

Emrich W (1985) *Handbook of Charcoal Making: the Traditional and Industrial Methods*. Springer, Berlin.

Fontaine S, Barot S, Barre P, Bdioui N, Mary B, Rumpel C (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature*, **450**, 277-280.

Forbes MS, Raison RJ, Skjemstad JO (2006) Formation, transformation and transport of black carbon (charcoal) in terrestrial and aquatic ecosystems. *Science of the Total Environment*, **370**, 190-206.

Glaser B, Haumaier L, Guggenberger G, Zech W (1998) Black carbon in soils: the use of benzenecarboxylic acids as specific markers. *Organic Geochemistry*, **29**, 811-819.

Glaser B, Guggenberger G, Zech, W (2003) Identifying the pre-Columbian anthropogenic input on present soil properties of Amazonian dark earths (Terra Preta). In: *Amazonian Dark Earths: Explorations in Space and Time* (eds Glaser B, Woods WI), pp. 145-158 Springer-Verlag, Berlin.

Grogan P (1998) CO_2 flux measurement using soda lime: Correction for water formed during CO_2 adsorption. *Ecology*, **79**, 1467-1468.

Guggenberger G, Zech W (1994) Composition and Dynamics of Dissolved Carbohydrates and Lignin-Degradation Products in 2 Coniferous Forests, Ne Bavaria, Germany. *Soil Biology & Biochemistry*, **26**, 19-27.

Guggenberger G, Rodionov A, Shibistova O, et al. (2008) Storage and mobility of black carbon in permafrost soils of the forest tundra ecotone in Northern Siberia. *Global Change Biology*, **14**, 1367-1381.

Gundale MJ, DeLuca TH (2007) Charcoal effects on soil solution chemistry and growth of Koeleria macrantha in the ponderosa pine/Douglas-fir ecosystem. *Biology and Fertility of Soils*, **43**, 303-311.

Hamer U, Marschner B, Brodowski S, Amelung W (2004) Interactive priming of black carbon and glucose mineralisation. *Organic Geochemistry*, **35**, 823-830.

Hammes K, Torn MS, Lapenas AG, Schmidt MWI (2008) Centennial black carbon turnover observed in a Russian steppe soil. *Biogeosciences*, **5**, 1339-1350.

Hockaday WC, Grannas AM, Kim S, Hatcher PG (2007) The transformation and mobility of charcoal in a fire-impacted watershed. *Geochimica et Cosmochimica Acta*, **71**, 3432-3445.

Johnson M, Lehmann J, Guimaraes Couto E, Novaes Filho JP, Riha SJ (2006) DOC and DIC in flowpaths of Amazonian headwater catchments with hydrologically contrasting soils. *Biogeochemistry*, **81**, 45-57.

Kaiser K, Guggenberger G, Zech W (1996) Sorption of DOM and DOM fractions to forest soils. *Geoderma*, **74**, 281-303.

Knicker H, Muffler P, Hilscher A (2007) How useful is chemical oxidation with dichromate for the determination of "black carbon" in fire-affected soils? *Geoderma*, **142**, 178-196.

Kuzyakov Y, Subbotina I, Chen H, Bogomolova I, Xu X (2009) Black carbon decomposition and incorporation into microbial biomass estimated by 14C labeling. *Soil Biology and Biochemistry*, **41**, 210-219.

Krull ES, Skjemstad JO, Graetz D, Grice K, Dunning W, Cook G, Parr JF (2003) C-13-depleted charcoal from C4 grasses and the role of occluded carbon in phytoliths. *Organic Geochemistry*, **34**, 1337-1352.

Laubel A, Jacobsen OH, Kronvang B, Grant R, Andersen HE (1999) Subsurface drainage loss of particles and phosphorus from field plot experiments and a tile-drained catchment. *Journal of Environmental Quality*, **28**, 576-584.

Lehmann J (2007) Bio-energy in the black. *Frontiers in Ecology and the Environment*, **5**, 381-387.

Lehmann J, Rondon M (2006) Bio-Char soil management on highly weathered soils in the humid tropics. In: *Biological Approaches to Sustainable Soil Systems* (eds Uphoff NT, Ball AS, Fernandes E et al.), pp. 517-530. CRC/Taylor & Francis, Boca Raton.

Lehmann J, Sohi S (2008) Comment on "Fire-derived charcoal causes loss of forest humus". *Science*, **321**, 1295.

Lehmann J, Gaunt J, Rondon M (2006) Bio-char sequestration in terrestrial ecosystems - a review. *Mitigation and Adaptation Strategies for Global Change*, **11**, 403-427.

Lehmann J, Skjemstad JO, Sohi S, Carter J, Barson M, Falloon P, Coleman K, Woodbury P, Krull E (2008) Australian climate-carbon cycle feedback reduced by soil black carbon. *Nature Geoscience*, **1**, 832–835.

Liang B, Lehmann J, Solomon D, Sohi S, Thies JE, Skjemstad JO, Luizão FJ, Engelhard MH, Neves EG, Wirick S (2008) Stability of biomass-derived black carbon in soils. *Geochimica et Cosmochimica Acta*, **72**, 6096-6078.

Leifeld J, Fenner S, Muller M (2007) Mobility of black carbon in drained peatland soils. *Biogeosciences*, **4**, 425-432.

Masiello CA (2004) New directions in black carbon organic geochemistry. *Marine Chemistry*, **92**, 201-213.

Masiello CA, Druffel ERM (1998) Black carbon in deep-sea sediments. *Science*, **280**, 1911-1913.

McClain ME, Richey JE, Brandes JA, Pimentel TP (1997) Dissolved organic matter and terrestrial-lotic linkages in the central Amazon basin of Brazil. *Global Biogeochemical Cycles*, **11**, 295-311.

Mehlich A (1984) Mehlich-3 soil test extractant - a modification of Mehlich-2 extractant. *Communications in Soil Science and Plant Analysis*, **15**, 1409-1416.

Mitra S, Bianchi TS, McKee BA, Sutula M (2002). Black carbon from the Mississippi river: quantities, sources, and potential implications for the global carbon cycle. *Environmental Science & Technology*, **36**, 2296-2303.

Naude SM (1927) Information on Nessler's reagent (in German). *Zeitschrift fur Physikalische Chemie-Stochiometrie und Verwandtschaftslehre*, **125**, 98-110.

Nguyen BT, Lehmann J, Kinyangi J, Smernik R, Riha SJ, Engelhard MH (2008) Long-term dynamics of black carbon in cultivated soil. *Biogeochemistry*, **89**, 295-308.

Pessenda LCR, Gouveia SEM, Aravena R (2001) Radiocarbon dating of total soil organic matter and humin fraction and its comparison with 14C ages of fossil charcoal. *Radiocarbon*, **43**, 595-601.

Pietikäinen J, Kiikkila O, Fritze H (2000) Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos*, **89**, 231-242.

Qualls RG, Haines BL (1992) Biodegradability of dissolved organic-matter in forest throughfall, soil solution, and stream water. *Soil Science Society of America Journal*, **56**, 578-586.

Rippstein G, Amezquita E, Escobar G, Grollier C (2001) Condiciones naturales de la sabana. In: *Agroecologia y Biodiversidad de las Sabanas en los Llanos Orientales de Colombia* (eds Rippstein G, Escobar G, Motta F), pp. 1-21.Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.

Rumpel C, Chaplot V, Planchon O, Bernadou J, Valentin C, Mariotti A (2006a) Preferential erosion of black carbon on steep slopes with slash and burn agriculture. *Catena*, **65**, 30-40.

Rumpel C, Alexis M, Chabbi A, Chaplot V, Rasse D.P, Valentin C, Mariotti A (2006b) Black carbon contribution to soil organic matter composition in tropical sloping land under slash and bum agriculture. *Geoderma*, **130**, 35-46.

SAS Institute Inc. (2003) *SAS version 9.1 for Windows*. Cary NC.

Skjemstad JO, Taylor JA (1999) Does the Walkley-Black method determine soil charcoal? *Communications in Soil Science and Plant Analysis*, **30**, 2299-2310.

Skjemstad JO, Clarke P, Taylor JA, Oades JM, McClure SG (1996) The chemistry and nature of protected carbon in soil. *Australian Journal of Soil Research*, **34**, 251-271.

Skjemstad JO, Taylor JA, Smernik RJ (1999a) Estimation of charcoal (char) in soils. *Communications in Soil Science and Plant Analysis*, **30**, 2283-2298.

Skjemstad JO, Taylor JA, Janik LJ, Marvanek SP (1999b) Soil organic carbon dynamics under long-term sugarcane monoculture. *Australian Journal of Soil Research*, **37**, 151-164.

Soil Survey Staff (1994) *Key to Soil Taxonomy*. Pocahontas Press, Blacksburg VA.

Steiner C, Teixeira WG, Lehmann J, Zech W (2004) Microbial response to charcoal amendments of highly weathered soils and Amazonian Dark Earths in central Amazonia - Preliminary results. In: *Amazonian Dark Earths: Explorations in Space and Time* (eds Glaser B, Woods WI), pp. 195-213. Springer-Verlag, Berlin.

Totsche KU, Jann S, Kögel-Knabner I (2007) Single event-driven export of polycyclic aromatic hydrocarbons and suspended matter from coal tar-contaminated soil. *Vadose Zone Journal*, **6**, 233-243.

Trujillo W, Fisher MJ, Lal R (2006) Root dynamics of native savanna and introduced pastures in the Eastern Plains of Colombia. *Soil and Tillage Research*, **87**, 28-38.

Wardle DA, Nilsson MC, Zackrisson O (2008) Fire-derived charcoal causes loss of forest humus. *Science*, **320**, 629-629.

Yang H, Yan R, Chen H, Lee DH, Zheng C (2007) Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel*, **86**, 1781-1788.

Yu XY, Ying GG, Kookana RS (2006) Sorption and desorption behaviors of diuron in soils amended with charcoal. *Journal of Agricultural and Food Chemistry*, **54**, 8545-8550.

TABLES

Table 1 Properties of BC applied to a Colombian savanna Oxisol. Values are averages of two analytical replicates

		BC material	
		1	2
pH	(H_2O)	10.14	10.07
pH	(KCl)	8.92	8.74
Total C	%	71.7	63.5
$\delta^{13}C$	‰	-28.86	-28.20
Total N	%	0.26	0.32
C/N		280	197
H/C		0.022	0.027
O/C		0.22	0.23
Ash	%	8.8	12.0
Ca*	mg g^{-1}	2.93	6.44
Mg*	µg g^{-1}	291	185
P*	µg g^{-1}	259	116
K*	mg g^{-1}	3.30	2.61
CEC	$mmol_c$ kg^{-1}	235	248

*Available nutrient contents

Table 2 Amount and proportion of above-ground biomass of spontaneous vegetation sampled on a control and BC-amended (23.2 t C ha^{-1}) Colombian savanna Oxisol.

	Dry matter (t ha^{-1})		Proportion of total (%)	
	- BC	+ BC	- BC	+ BC
Grasses	1.13	2.19	69	46
Forbs	0.47	1.86	29	39
Legumes	0.04	0.71	2	15
Total	1.64	4.75	100	100

Table 3 Total amounts and volume-weighted average concentrations of C leached over two years as POC and DOC, at 0.15 and 0.3 m depths on a Colombian savanna Oxisol. Different letters represent significant differences (*P*<0.05) between control (-BC) and BC-amended (+BC) soil

			Volume-weighted averages		Total amounts					
Depth			Total C conc.	$\delta^{13}C$	C leached	Proportion from soil	Proportion from BC	Prop. of applied BC	BC leaching rate*	BC-induced increase in non-BC leaching*
m			$\mu g\ C\ mL^{-1}$	‰	$kg\ C\ ha^{-1}$	%	%	%	$kg\ C\ ha^{-1}\ yr^{-1}$	$kg\ C\ ha^{-1}\ yr^{-1}$
0.15	POC	- BC	0.91b	-15.87a	7.3b	100a	0b	0	0b	0b
		+ BC	2.50a	-13.91a	30.2a	98.1b	1.9a	0.003	0.32a	12.2a
	DOC	- BC	2.21b	-17.57a	16.8b	100a	0b	0	0b	0b
		+ BC	5.73a	-16.69a	43.2a	89.4b	10.6a	0.020	2.49a	11.9a
0.3	POC	- BC	2.04a	-16.86a	2.2b	100a	0b	0	0b	0b
		+ BC	1.39a	-14.95b	8.0a	96.6b	3.4a	0.001	0.15a	3.0a
	DOC	- BC	5.02a	-18.39a	5.2b	100a	0b	0	0b	0b
		+ BC	2.68b	-17.91a	15.5a	74.3b	25.7a	0.017	2.16a	3.4a

*Sampling was carried out over 2 rainy seasons and 1 dry season. To generate this estimate, a second dry season was assumed to last the same number of days as the season sampled.

Table 4 Total C respired over two years from a Colombian savanna Oxisol, measured using soda lime traps during the rainy season only. Years 1 and 2 were sampled from different plots. Different letters represent significant differences (*P*<0.05) between control (-BC) and BC-amended (+BC). Data was extrapolated from 24 h soda lime assays, with assay dates taken as center points of time intervals between assays

	Total amounts						Volume-weighted average
	Total respired C	Respired BC	Prop. of C as BC	Prop. of applied BC respired	BC respiration rate*	BC-induced increase in non-BC respiration*	$\delta^{13}C$
	t ha^{-1}	kg ha^{-1}	%	%	kg ha^{-1} yr^{-1}	t ha^{-1} yr^{-1}	‰
- BC	9.97b	0b	0b	0b	0b	0b	-13.76a
+ BC	13.03a	513.8a	3.94a	2.21a	377.0a	1.82a	-14.08a

*to generate these estimates, the respired amounts for the day with lowest measured respiration in each rainy season were used to estimate respiration for the duration of the dry season. Respired BC was estimated using the average proportion of BC respired for each year. The dry season was taken to last the same number of days in 2006 as in 2005 and was defined as ending on the first day when free-draining water was collected in March.

Table 5 Fate of soil-applied BC, two years after application to soil (including dry seasons). Losses of BC by surface runoff were not measured and are excluded

Flux type		Total flux over 2 years	Proportion of total flux	Rate	Proportion of applied BC	BC-induced increase in non-BC flux	Net effect on C stocks*
		kg C ha^{-1}	%	kg ha^{-1} yr^{-1}	%	kg ha^{-1} yr^{-1}	t C ha^{-1}
Respired as CO_2		751.9	98.8	377.0	3.241	1818.0	+ 22.80
Leached below 0.15 m	as POC	0.586	0.08	0.32	0.003	12.18	
	as DOC	4.580	0.60	2.49	0.020	11.90	
	Total	5.166	0.68	2.81	0.023	24.08	+24.55
Leached below 0.3 m	as POC	0.276	0.04	0.15	0.001	3.03	
	as DOC	3.978	0.32	2.16	0.017	3.44	
	Total	4.254	0.52	2.31	0.018	6.47	+2.56
Total		761.32	100.00		3.282		
Net for respiration and leaching to 0.3 m							+22.79

*For each line, the BC-induced "loss" of non-BC was added to the amount of BC lost, and this was subtracted from the C added as BC (23.2 t ha^{-1}) and additional non-BC as found in soil samples (Fig. 2). For respiration the depth increment used was 0-0.3 m.

FIGURE CAPTIONS

Fig. 1 Bulk density of a Colombian savanna Oxisol, 18 months after BC incorporation to 0.1 m (± SE, *n*=6 to 0.3 m depth, and *n*=4 below 0.3 m). Significant differences ($P<0.05$) within a single depth are represented by different letters.

Fig. 2 Total soil C concentration (A) and C stocks from soil and BC (B and C), two rainy seasons after BC application to a Colombian savanna Oxisol under natural vegetation (± SE, *n*=3). Significant differences within a single depth are represented by different lower-case letters (significant differences in total C at 0.3 m not shown), and significant main effects are indicated by ** ($P<0.01$) and * ($P<0.05$). Letters showing differences in BC content in panels B and C are placed above bars. Points in panel A are placed at the center of the depth increment they represent.

Fig. 3 Concentration (left), total amount (center) and δ^{13}C values (right) of POC (top) and DOC (bottom) leached over two years in free-draining lysimeters placed at 0.15 and 0.3 m depth, either with (+BC, 23.2 t C ha $^{-1}$) or without BC addition (-BC) to a Colombian savanna Oxisol under natural vegetation (± SE, *n*=1, 2 or 3 depending on whether samples were collected for all replicates in the field, and whether samples were lost during transportation or storage). In the δ^{13}C panels, the upper reference line corresponds to the δ^{13}C value of the soil and the lower line to the δ^{13}C value of BC.

Fig. 4 Amount of C respired and $\delta^{13}C$ from a Colombian savanna Oxisol, measured using soda lime traps (± SE, *n*=6 for amounts of C). Years 1 and 2 were sampled from different plots. Average rainfall collected over irregular time periods is shown with bars and given per day. For $\delta^{13}C$, all samples within chosen intervals (shaded and white areas) were composited, and replicates were analyzed separately only on the first date of year 1 and the last date of year 2 (± SE, *n*=3). The upper reference line corresponds to the $\delta^{13}C$ value of the soil and the lower line to the value for BC. * indicates missing rainfall data.

FIGURES

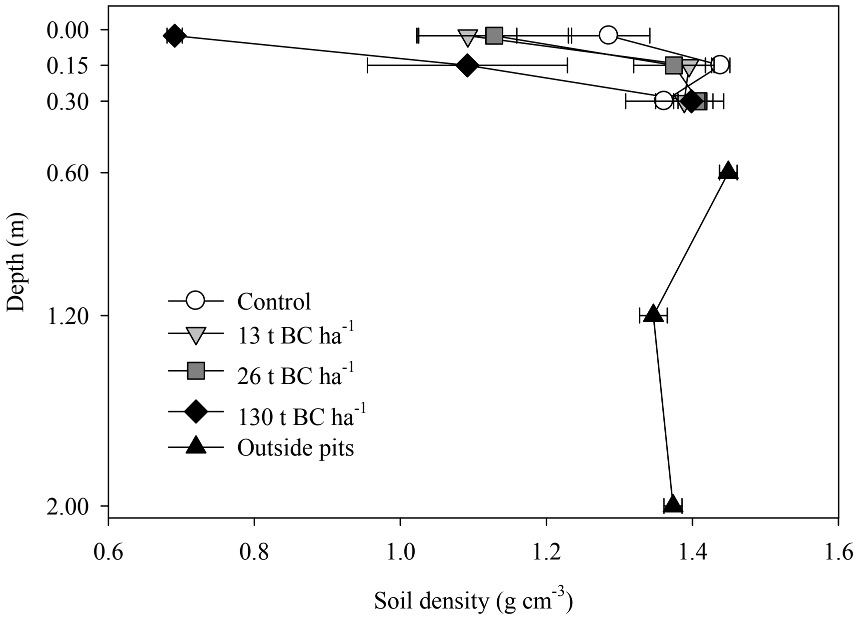

Figure 1.

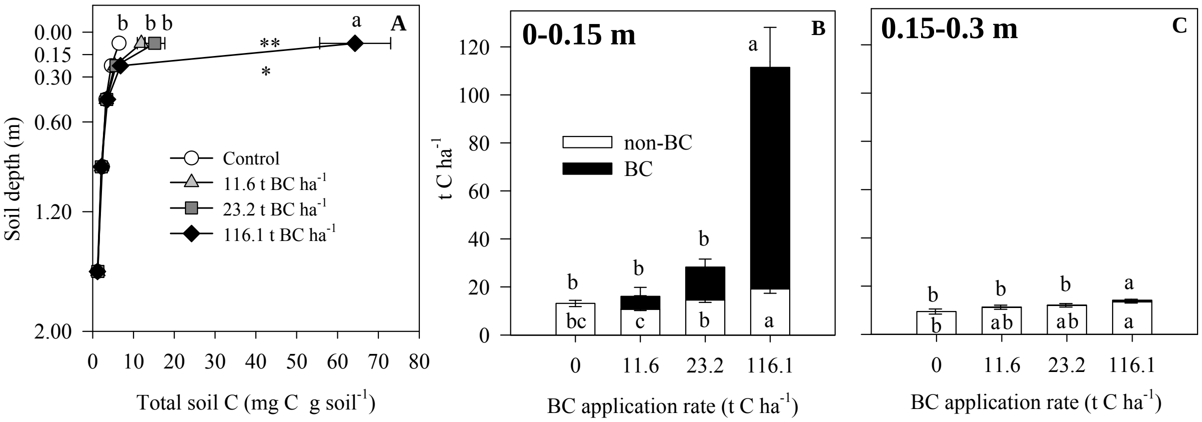
A
b
b b
a
**
*
0.00
0.15
0.30
0.60
1.20
2.00
Soil depth (m)
Control
11.6 t BC ha-1
23.2 t BC ha-1
116.1 t BC ha-1
0 10 20 30 40 50 60 70 80
Total soil C (mg C g soil-1)
0-0.15 m
B
t C ha-1
0
20
40
60
80
100
120
non-BC
BC
b b b a
bc c b a
0 11.6 23.2 116.1
BC application rate (t C ha-1)
0.15-0.3 m
C
b b b a
b ab ab a
0 11.6 23.2 116.1
BC application rate (t C ha-1)

Figure 2.

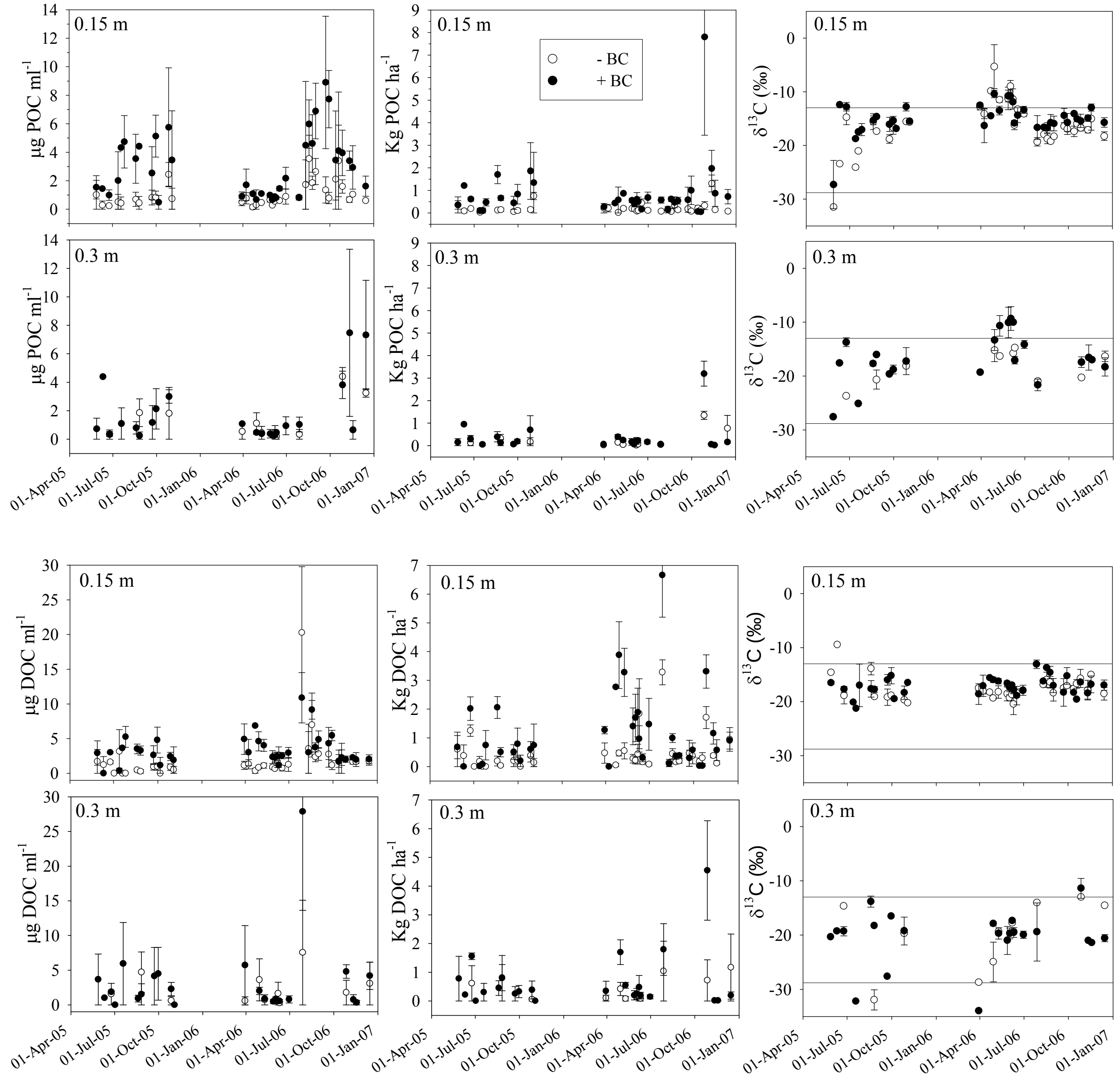
0.15 m
0.3 m
µg POC ml^{-1}
Kg POC ha^{-1}
δ^{13}C (‰)
○ - BC
● + BC
µg DOC ml^{-1}
Kg DOC ha^{-1}
01-Apr-05
01-Jul-05
01-Oct-05
01-Jan-06
01-Apr-06
01-Jul-06
01-Oct-06
01-Jan-07

Figure 3.

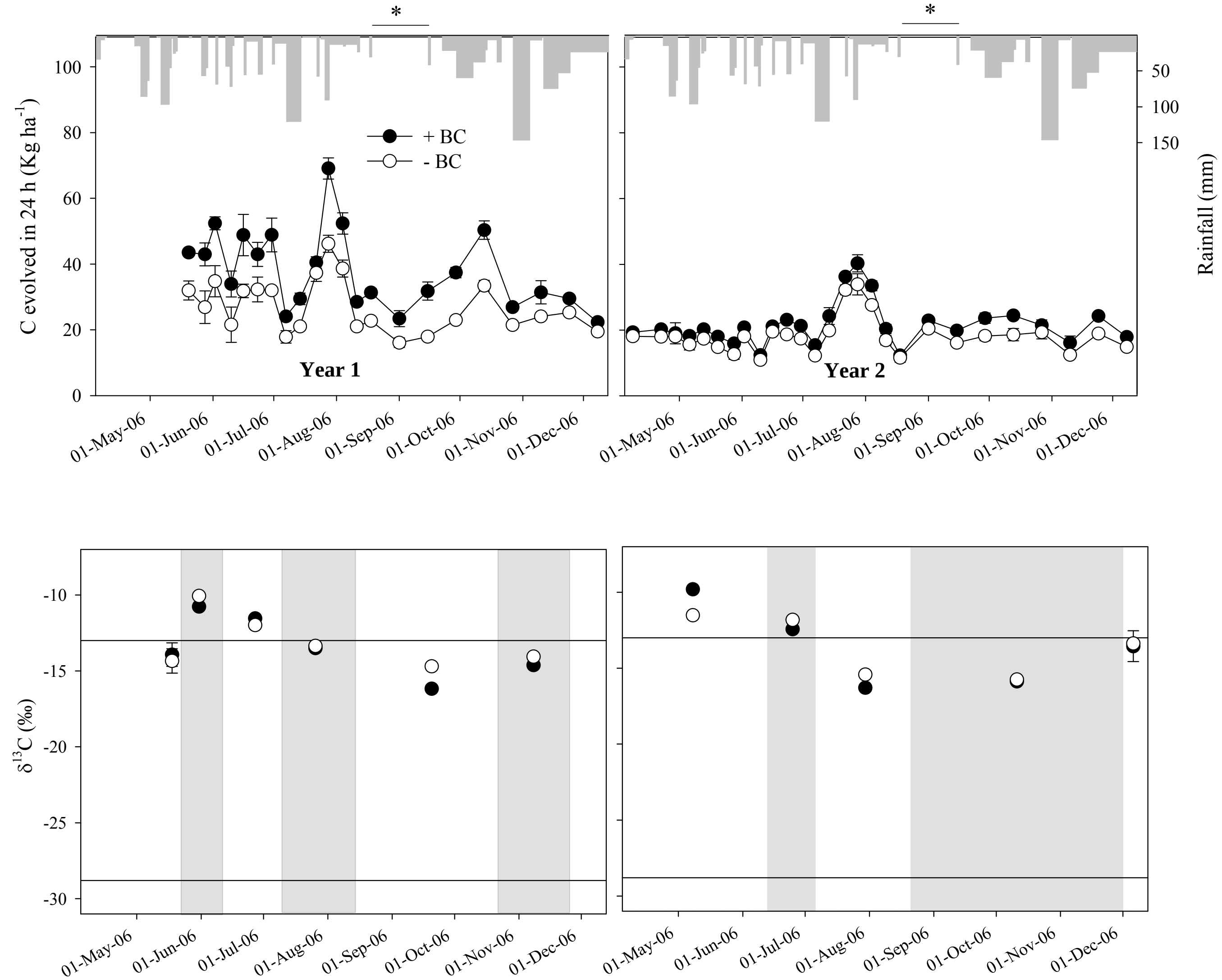
*
*
C evolved in 24 h (Kg ha^{-1})
Rainfall (mm)
0
20
40
60
80
100
50
100
150
+ BC
- BC
Year 1
Year 2
01-May-06
01-Jun-06
01-Jul-06
01-Aug-06
01-Sep-06
01-Oct-06
01-Nov-06
01-Dec-06
δ^{13}C (‰)
-10
-15
-20
-25
-30

Figure 4.