1 IMPACT OF NATURAL AND ANTHROPOGENIC PYROGENIC CARBON IN
2 SOILS AND SEDIMENTS

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4 **Soil chemical properties and organic matter composition of a subtropical Cambisol**
5 **after charcoal fine residues incorporation**

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Abstract

Purpose Brazil is the world's greatest charcoal producer with an annual production of 10 million tons. However, about 15 % is lost as charcoal fines, which can be used as soil fertilizer and source of soil stable carbon (C). In this study we investigated the impact of charcoal fines application on soil chemical properties and organic matter (SOM) stability and composition.

Materials and Methods Four doses (0 - control, 10, 20 and 40 Mg ha⁻¹) of charcoal fines were incorporated to the soil at 10 cm. Soil samples were collected at four soil depths (0-5, 5-10, 10-20 and 20-30 cm) 20 months after charcoal incorporation. Soil chemical properties were determined and SOM composition was evaluated by infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), scanning electron microscopy (SEM-EDX) and carbon stable isotopic analysis ($\delta^{13}\text{C}$ IRMS).

Results and discussion Soil chemical properties were affected mainly in the upper depth (0-5 cm) and only at the highest dose (40 Mg ha⁻¹), which also increased the total C content in 37.7 % compared to the control. Probably, the observed effects solely on the surface are due the concentration of charcoal on the upper depth, despite its incorporation. The dose of 40 Mg ha⁻¹ also increased the total C content at 10-20 cm depth compared to the control. Such result was assigned to the leaching of some finest charcoal particles. The SEM-EDX and SOM $\delta^{13}\text{C}$ signature confirmed the presence of charcoal in both 0-5 cm and 10-20 cm soil depths. FTIR and TGA indicated an increase in SOM aromaticity and thermostability until 20 cm depth after charcoal incorporation. No effects were observed at the deepest soil depth (20-30 cm), suggesting that charcoal was not leached to this depth within 20 months.

Conclusions The application of charcoal fines ameliorated the soil chemical properties. In general, the charcoal increased the SOM aromaticity and thermostability and changed the $\delta^{13}\text{C}$ signature at 0-5 and 10-20 cm. Charcoal leaching from the 0-10 to the 10-20 cm depth was observed, but further downward transport was not evidenced by our data. Charcoal fine residues show a potential to be used as an effective soil fertilizer, as well as a stable C source into the soil.

Keywords Soil fertilizer • Carbon stable isotope ratio • Infrared spectroscopy • Organic matter thermostability • Scanning electron microscopy

1 Introduction

Brazil is the world's greatest charcoal producer contributing with approximately 38.5 % of the global amount and supplying mainly the steel industry and the pig iron sector. The annual Brazilian production adds up to around 10 million tons of charcoal. However, 15 % of this production is lost as charcoal fines (Pennise et al. 2001; Maia 2011) which are not suitable for the steel industry due to clogging of the gas flow, which reduces the efficiency of the blast furnaces (Angelo et al. 2014).

During the last decade there is a raising interest in the application of charcoal (biochar) aiming to improve the soil properties. In addition, the charcoal seems to have a potential to increase the soil carbon (C) sink on a long-term scale (Kuzyakov et al. 2014). This charcoal characteristic is usually related to its high C content and aromaticity, which is believed to be associated to its inherent biochemical recalcitrance (Verheijen et al. 2012). This recalcitrance, in turn, is supposed to increase the slow-cycling soil organic matter (SOM) pool. Besides the impact on the global C cycle, charcoal application can also improve soil structure and soil fertility (Novak et al. 2009; Knicker 2013).

Depending on pyrolysis conditions (temperature, time, source material and oxygen supply) the characteristics of the final pyrogenic C (PyC) can vary broadly (Steinbeiss et al. 2009; Cao and Harris, 2010; Keiluweit et al. 2010; Paz-Ferreiro et al. 2014). And these, in turn, cause variations in their behavior into the soil and their effect on soil properties and SOM composition. Charcoal is usually assumed to be a highly condensed polyaromatic network. However, an alternative concept describes PyC as a heterogeneous mixture, composed mainly of partially degraded and heat altered biomacromolecules (Knicker et al. 2008).

87 Despite the long mean residence time commonly assumed for charcoal in soils,
88 its contact with the soil components may promote chemical and biological changes in
89 the original charcoal structure, and a functionalization of the charcoal may occur. This
90 so called aging increases the amount of functional groups, mostly of carboxyl, leading
91 to the increase of polar adsorption sites. In fact, this process can start very quickly after
92 entering the soil (Hilscher and Knicker 2011).

93 Several studies with charcoal fines application to soils have been developed in
94 Brazil. However, these studies usually focus on soil fertility, crop yield, impact on soil
95 microorganisms, greenhouse gas emissions and charcoal functionalization (Zanetti et al.
96 2003, Steiner et al. 2007, Angelo et al. 2014). Therefore, there is still scarce information
97 about how SOM stability and quality are affected by charcoal application. This is in
98 particular true with respect to charcoal fines, whose potential as soil fertilizer and as a
99 relevant source of stable SOM pool still needs scientific support.

100 During aging of charcoal, larger pyrogenic condensed structures tend to be
101 released into the soil, which can be leached along the soil profile (Abiven et al. 2011).
102 The leaching of charcoal components has been verified by the increase of the SOM
103 aromaticity degree, which may suggest that at least some pyrogenic structures can be
104 transported to deeper soil depths (Dieckow et al. 2005, Knicker et al. 2006, 2012).

105 Thermal analysis and Fourier transform infrared spectroscopy (FTIR) have been
106 used to evaluate the PyC characteristics and its contribution to the SOM composition in
107 fire affected and charcoal/biochar amended soils (Leifeld 2007, De La Rosa et al. 2008,
108 Dick et al. 2008a, Potes et al. 2012, Dai et al. 2013). In this way, these techniques could
109 also be of value for assessing changes in the stability and composition of the SOM after
110 the application of charcoal fines. Studying alterations of the SOM as function of soil
111 depths allows monitoring of vertical charcoal movements. An additional tool to study

such mobilization of charcoal in soils is the use of stable carbon isotope signature ($\delta^{13}\text{C}$), which provides representative data about the average isotopic signal of the entire sample. Thus, changes in the SOM ^{13}C signature after charcoal incorporation can be traced based on the different $^{13}\text{C}/^{12}\text{C}$ isotope ratios of the natural SOM and of the charcoal (Muccio and Jackson 2009).

In order to obtain more insights on the impact of charcoal fines on soil fertility and on the SOM dynamics, we determined some soil chemical properties and the SOM composition by means of TGA, FTIR and $\delta^{13}\text{C}$ analysis of the whole soil and of C physical pools of a subtropical Cambisol after 20 months of pyrogenic material incorporation.

2 Materials and methods

2.1 Site description, experimental design and soil sampling

The experimental area is located in Irati, Center-South of Paraná State (PR), Brazil, at approximately 855 m above sea level. The climate is humid subtropical mesothermic - Cfb (Köppen, 1948), with frequent and severe frosts occurrence during winter. The annual mean temperature is 17.2 °C, with an average rainfall of 193.97 mm month⁻¹ and a relative humidity of 79.58 %. The study was carried out in the *Campus Irati*, at the State University of *Centro-Oeste* (25° 27' 56" S 50° 37' 51" W). The relief varies between undulated and strong undulated, and the soil is classified as a Haplic Cambisol (FAO 2006). The experimental area has an agricultural use history (Soybean - *Glycine max*, five years), but for the last three years, before the beginning of the experiment, the soil was under fallow.

The experiment was implemented in February 2010 when the area was manually and mechanically mowed. About 2.5 Mg ha⁻¹ of dolomitic limestone (85% of relative

power of total neutralization) was firstly applied on the soil surface and subsequently incorporated at 10 cm using a light disk harrow.

The charcoal was originated from the pyrolysis of hardwood native Brazilian species, mainly *Mimosa Scabrella* Bentham. The greatest part of the charcoal was used to supply the steel industry, whilst the fine residues of the charcoal, which are unsuitable for such purpose, were collected to be used in this experiment. About 45 % of the charcoal particles were smaller than 2 mm (Table 1). The pyrolysis was performed under not controlled and artisanal conditions, which may have contributed to its low fixed C and high volatile matter contents (Table 1). These data characterize this material as a low condensed charcoal, produced at low temperature. High values for volatile matter and low values for fixed C for biochars produced under controlled conditions were already observed for wood and grass feedstock pyrolyzed at low temperatures (300 to 400°C) (Zimmerman et al. 2011; Enders et al., 2012).

The total content of the main nutrients from the charcoal (Table 1) was determined in a concentrated acid extract (12.5 % HNO₃ and 37.5 % HCl, digested for 24 h at 200 °C) by inductive coupled plasma (ICP).

Directly after soil liming, three different doses of charcoal fines, 10, 20 and 40 Mg ha⁻¹, were applied onto the soil surface and incorporated at 10 cm using a light disk harrow. In March 2010 seedlings of *Eucalyptus benthamii* were planted.

The study was performed in a randomized block design with four treatments, which were arranged in four blocks: T1 = 0 Mg ha⁻¹ (without charcoal - control); T2 = 10 Mg ha⁻¹; T3 = 20 Mg ha⁻¹ and T4 = 40 Mg ha⁻¹. Each field replicate collected in each block was composed by three subsamples, which were sampled within a 144 m² plot.

Soil sampling was performed in September 2012, 20 months after charcoal incorporation. Soil samples were collected at four soil depths: 0-5; 5-10; 10-20 and 20-30 cm. Before analysis all samples were air dried and passed through a 2.00 mm sieve.

2.2 Soil chemical and elemental analysis

Soil chemical properties (Tedesco et al. 1995) and granulometric distribution (Embrapa, 1997) were determined. Effective cation exchange capacity (ECEC) was calculated as the sum of the exchangeable cations ($ECEC = K + Al + Ca + Mg$). The average particle size distribution of T1 (control) (determined by the pipette method) was: 441 g kg⁻¹ of sand, 167 g kg⁻¹ of silt and 392 g kg⁻¹ of clay in the 0-5 cm soil depth; 492 g kg⁻¹ of sand, 170 g kg⁻¹ of silt and 338 g kg⁻¹ of clay in the 5-10 cm soil depth; 467 g kg⁻¹ of sand, 162 g kg⁻¹ of silt and 371 g kg⁻¹ of clay in the 10-20 cm soil depth and 437 g kg⁻¹ of sand, 182 g kg⁻¹ of silt and 381 g kg⁻¹ of clay in the 20-30 cm soil depth. Total soil C (TC) and total nitrogen (N) (TN) contents were determined by dry combustion (975 °C) in an elemental analyzer (Perkin Elmer 2400). The C content was assigned exclusively to the SOM, as all soil samples were carbonate free.

2.3 SOM fractionation

The SOM granulometric fractionation was carried out according to Cambardella and Elliot (1992). Soil samples (20 g) were horizontally shaken (16 h) with 60 ml of sodium hexametaphosphate solution (5 g L⁻¹). Subsequently, the suspension was passed through a 0.053 mm sieve. The fraction retained on the sieve corresponds to the particulate organic matter, while the fraction that passed through the sieve corresponds to the silt+clay size organic matter fraction.

The C and N contents of the particulate fraction ($C_{\text{particulate f.}}$; $N_{\text{particulate f.}}$) were determined by dry combustion (975 °C) (Perkin Elmer 2400), whereas the C and N

contents of the silt+clay size fraction were calculated by difference: $C_{\text{silt+clay f.}} = TC - C_{\text{particulate f.}}$; $N_{\text{silt+clay size f.}} = TN - N_{\text{particulate f.}}$

2.4 Isotope ratio mass spectrometry (IRMS) - stable isotope signature $\delta^{13}\text{C}$

Charcoal and soil samples (T1 and T4) were analyzed in quadruplicates with a Flash 2000 HT (N, C, S, H and O) elemental analyzer (Thermo Scientific) in combustion mode and coupled to a Delta V Advantage IRMS (Thermo Scientific). Stable carbon isotopic ratios are reported as part per thousand (‰) deviation from that of Pee Dee Belemnite (PDB) which is the standard recognized by the international atomic energy agency (IAEA).

The proportion of the TC derived from the charcoal ($F_{\text{tcharcoal}}$) was calculated based on Bernoux et al. (1998) and considering that the original isotopic signature before charcoal application and *Eucalyptus* plantation was that of the 20-30 cm depth. Firstly, the proportion of the TC derived from *Eucalyptus* plants ($F_{\text{teucalyptus}}$) was estimated, accordingly to the equation 1:

$$F_{\text{teucalyptus}} = (\delta t_1 - \delta A_1) / (\delta B_1 - \delta A_1) \quad (1)$$

where,

δt_1 = soil $\delta^{13}\text{C}$ signature after *Eucalyptus* cultivation (T1);

δA_1 = original soil $\delta^{13}\text{C}$ signature before *Eucalyptus* cultivation (T1 20-30 cm);

δB_1 = *Eucalyptus* $\delta^{13}\text{C}$ signature, assumed as -27 ‰ (Schulze et al. 2006).

Thereafter, the proportion of the TC derived from charcoal+*Eucalyptus*, ($F_{\text{tcharcoal+eucalyptus}}$) was estimated as follows:

$$F_{\text{tcharcoal + eucalyptus}} = (\delta t_2 - \delta A_2) / (\delta B_2 - \delta A_2) \quad (2)$$

where,

δt_2 = soil $\delta^{13}\text{C}$ signature after *Eucalyptus* cultivation and charcoal addition (T4);

δA_2 = original soil $\delta^{13}\text{C}$ signature before *Eucalyptus* cultivation (T4 20-30 cm);

δB_2 = charcoal $\delta^{13}\text{C}$ signature (-25.99 ‰).

Finally, the solely charcoal contribution was estimated by the difference $F_{t_{\text{charcoal}}} =$

$F_{t_{\text{charcoal+eucalyptus}}} - F_{t_{\text{eucalyptus}}}$.

2.5 10 % HF solution treatment

The soil samples were treated with 10 % hydrofluoric acid (HF) solution to concentrate the SOM (Gonçalves et al. 2003). Using a plastic container, soil samples (1 g) were treated with 10 ml of a 10 % (w/w) HF solution. After 2 h of mechanical agitation with subsequent centrifugation of the closed containers at 2000 g for 10 minutes, the supernatant was removed and discarded. This procedure was repeated eight times. The remaining sediment was washed five times with deionized water and dried at 50 °C in a vacuum oven.

2.6 Infrared spectroscopy, thermogravimetric analysis and scanning electron microscopy

The HF treated soil samples were analyzed by fourier transform infrared spectroscopy (FTIR) and by thermogravimetric analysis (TGA). For the FTIR spectroscopy the samples were analyzed in KBr pellets (1.0 mg sample:100 mg KBr) (Shimadzu FTIR 8300) using 32 scans and a resolution of 4.0 cm^{-1} within the range of 4,000 to 400 cm^{-1} .

The absorption band attributions were made according to Tan (2003) and the aromaticity index was calculated ($I_{\text{C}=\text{C}}/I_{\text{C}-\text{H}}$) (Chefetz et al. 1996), in which $I_{\text{C}=\text{C}}$ is the absorption intensity around 1,630 cm^{-1} and $I_{\text{C}-\text{H}}$ is the absorption intensity around 2,920 cm^{-1} .

The TGA analyses were carried out in duplicate in a thermo-gravimetric analyzer (TGA Q5000IR - TA instruments) using approximately 5 to 10 mg of HF treated sample. Firstly, the initial weight was stabilized at 40 °C and thereafter a heating curve was obtained with a heating rate of 10 °C min⁻¹ up to 105 °C (holding time of 10 min), followed by a heating rate of 5 °C min⁻¹ up to 800 °C. Six regions were identified in the thermo-decomposition curves, according to the samples decomposition behaviour along the heating. Such regions were separated as follows: 40-105 °C; 105-240 °C; 240-340 °C; 340-440 °C; 440-520 °C and 520-800 °C. The thermogravimetric index (TGI) (adapted from Benites et al. 2005) was calculated as: $TGI = \text{mass loss}_{(5^a+6^a \text{ regions})} / \text{mass loss}_{(2^a+3^a+4^a \text{ regions})}$.

In addition to the soil samples, pure charcoal samples (before the application to the soil) were also analyzed by FTIR and TGA. The charcoal presented different thermal decomposition behaviour compared to the soil samples and consequently different temperature intervals were used for the calculation of the TGI ($TGI = \text{mass loss}_{(3^a+4^a \text{ regions})} / \text{mass loss}_{(2^a \text{ region})}$).

The particulate fraction, silt+clay size fraction and the charcoal were analyzed by scanning electron microscopy (SEM) (JSM 5800) with dispersive energy detector of X-ray (EDX). The samples were fixed on top of aluminum stubs with an aid of double-sided tape and metalized with gold (Au). The micrographs were obtained with a voltage of 10 kV.

2.7 Statistical analysis

The treatments were compared at the same soil depth by Tukey test ($p < 0.10$) and by standard deviation. All statistical analyses were performed using the software Statistical Analysis System (SAS) 9.3.

3 Results and discussion

3.1 Effect of charcoal application on soil chemical properties

The main effect of charcoal application on soil chemical properties was observed in the upper depth (0-5 cm) with the application of 40 Mg ha⁻¹ of charcoal. This dose increased significantly the soil pH, available P, exchangeable K and Ca and the ECEC in comparison to the control (T1) (Table 2). Furthermore, the content of exchangeable Al at the 0-5 cm depth decreased in T4. The intermediate doses (10 and 20 Mg ha⁻¹) did not differ from the control regarding the soil pH and the exchangeable cations content.

The pH increase in the upper depth from 4.85 (T1) to 5.20 (T4) can be explained by the charcoal pH itself, which showed an alkaline pH level, 7.59 (Table 1). Also the increase of available P from 6.38 mg dm⁻³ (T1) to 11.78 mg dm⁻³ (T4) in the same depth was probably caused both by the input of P to the soil via charcoal (Table 1) and by the raise of the soil pH. Concerning the latter factor, the previously strongly sorbed P on the mineral surfaces is desorbed from the inner sphere complexes due to the raise of the negative charge of variable charge sites, forming a more labile sorbed form (Wang et al. 2013). Biederman and Harpole (2013) evaluated ecosystem responses to biochar application with a meta-analysis of 371 independent studies culled from 114 publications and observed that despite the variability introduced by soil and climate, the addition of biochar to soil resulted, on average, in an increased of soil available P, which was related to P desorption. Furthermore, the observed decrease of 135 % in the exchangeable Al (from 1.85 to 0.65 cmol_c dm⁻³) in T4 is also explained by the soil pH raise in T4.

The increase of exchangeable K and Ca contents at 0-5 cm depth in T4 can be attributed to the charcoal itself, whose ash usually contains a large amount of these

elements (Table 1). The higher pH value observed in T4 compared to T1 (Table 2) promoted an increase of the amount of negative surface charges in this pH dependent charge soil, and consequently it increased the ECEC as well (Table 2). In Anthrosols from Brazil an increase of the ECEC due to PyC presence was also observed (Liang et al. 2006).

Increase in the soil pH, available P, exchangeable Ca and a decrease in the soil exchangeable Al were also found after the incorporation of 32 Mg ha⁻¹ of charcoal in a Plinthosol (Petter et al. 2012) and in loamy sand soil of South Carolina, US (Novak et al. 2009).

Despite the charcoal incorporation at 10 cm depth, the effects on the chemical properties were observed mainly in the top 5 cm depth. A possible explanation is that the ploughing was not efficient enough for an uniform incorporation of the charcoal and a greater amount of it still remained close to the soil surface.

3.2 Effect of charcoal application on C and N contents, SOM $\delta^{13}\text{C}$ signature and aromaticity

Compared to that of T1, TC of T4 increased 37.7% in the upper depth (0-5 cm) and 22.9 % at the 10-20 cm depth. The TC contents of T2 and T3 did not differ from that determined for T1 (Table 3). The increase of TC content at 10-20 cm depth in T4 may be caused by the leaching of small charcoal particles (over 45 % of the particles of the charcoal applied were smaller than 2 mm) after strong rains. In this region the average monthly rainfall comprises 194 mm and is well distributed within the year. The accumulation of leached particles at the 10-20 cm depth might be explained in light due the soil particle size distribution: at the 5-10 cm depth the content of sand (492 g kg⁻¹) is greater and that of clay (338 g kg⁻¹) is smaller than those in the above (441 g kg⁻¹ of

sand, 392 g kg⁻¹ of clay) and in the beneath (467 g kg⁻¹ of sand, 371 g kg⁻¹ of clay) depths. Therefore the leached particles tended to percolate through the 5-10 cm depth and to accumulate in the 10-20 cm. Our data are consistent with those from Petter et al. (2012) who also observed an increase of TC content at the 10-20 cm soil depth during the second year after charcoal fines incorporation at 10 cm. Such results were attributed due to the leaching of small charcoal particles (≤ 2 mm).

The partial oxidation of charcoal leading to the introduction of carboxylic groups on its surface during aging increases its hydrophilicity. Thus, it facilitates the translocation of pyrogenic compounds with the soil solution down to 20 cm. The transport of functionalized PyC along the soil profile by the soil solution was verified by Knicker et al. (2012) in Leptosols under grassland managed by biannual prescribed burning.

The highest C/N ratio observed at the 0-5 cm depth in T4 compared to T1 (Table 3) is in line with the presence and contribution of charcoal to the greater TC content observed in T4 at this soil depth. Regarding the 10-20 cm depth, the C/N ratio in T4 tended to be greater, but did not differ from that in T1. This behaviour is probably due to the fact that the N content did not change relevantly enough at this depth to affect the C/N ratio after charcoal incorporation.

TN content of all treatments varied between 1.78 and 3.30 g kg⁻¹ and only a slight difference (0.30 g kg⁻¹) between T4 and T3 at 10-20 cm depth was observed (Table 3).

The results obtained from the physical SOM fractionation allowed to distinguish the physical pool that was preferably affected by the charcoal application. In the upper soil depth (0-5 cm) in T4, the C_{particulate f.} increased significantly by 127, 55 and 76 % compared to T1, T2 and T3, respectively. Considering that T4 increased the C_{particulate f.}

content in 15.7 g kg^{-1} compared to T1, it follows that charcoal particles accumulated in this fraction and still remained in the topsoil after 20 months of its application. The increase of the C/N ratio of the particulate SOM from 22.5 (T1) to 39.7 (T4) (Table 3) supports this assumption. For the deeper depths, no difference concerning $C_{\text{particulate f.}}$ was observed in T4.

In contrast, at the 10-20 cm depth, the difference in C content between T1 and T4 was observed for the silt+clay fraction: $C_{\text{silt+clay f.}}$ in T4 was 26 % greater than that found for T1 (Table 3). This result corroborates the assumption that small particles of charcoal were leached to this depth accumulating in the silt+clay size fraction.

The SEM-EDX image of the charcoal shows its characteristic morphology with internal pores and cracks which contribute to its high surface area and pore volume (Fig 1a) as described by Dai et al. (2013). The SEM images and the high intensity of the C signal give a further evidence of the charcoal contribution to both $C_{\text{particulate f.}}$ (0-5 cm) and $C_{\text{silt+clay f.}}$ (10-20 cm) in T4 (Fig 1b, 1c). This technique was previously used by Glaser (2000) to identify the PyC contribution to SOM fractions in an anthropogenic soil from the Brazilian Amazon region. Using the SEM-EDX technique, Laird et al. (2008) could observe the presence of PyC compounds associated to different clay fractions of agricultural soils.

The $\delta^{13}\text{C}$ signature of the charcoal (-25.99 ‰) and that of the *Eucalyptus* plant (-27 ‰) showed the relevant contribution of the ^{12}C isotope. This is in line with the isotopic signature of plants that photosynthesise glucose via the C_3 mechanisms (O'Leary, 1981). The $\delta^{13}\text{C}$ determined for the SOM in T1 (control) increased from -19.12 ‰ to -16.62 ‰ along the soil profile (Table 4). Considering that C_4 plants show a less negative isotopic signature, it follows that in the topsoil there is an input derived from C_3 photosystem plants (*Eucalyptus*). The change of the signature with soil depth

in T1 indicates further that formerly a vegetation was present, which was predominantly composed of C₄ photosystem plants. According to the literature, the native vegetation in the experimental area was mainly composed by the local poaceae Brazilian satintail (*Imperata brasiliensis*) (Bauer et al. 2008). However, herein we have to bear in mind that liming (2.5 Mg ha⁻¹) occurred and dolomite represents an extra source of the heavy ¹³C isotope. Although, the carbonate will quickly be released and transformed at the low pH values of the soil, some of this C could be incorporated into newly growing biomass (Miltner et al. 2005). In T4, δ¹³C values increased from -23.42 ‰ to -16.41 ‰ along the soil profile and the values at the depths 0-5 cm and 10-20 cm in T4 were more negative than those in T1 (Table 4). This results corroborate the relevant contribution of the charcoal (δ¹³C of -25.99 ‰) to the SOM of these two depths.

Based on the soil isotopic signature of the T1 depths, and assuming that the value at the 20-30 cm depth was that from the original vegetation (before *Eucalyptus* plantation and charcoal application), the contribution of *Eucalyptus* plant to the SOM was estimated as ranging from around 25 to 8 % within 20 cm soil depth (Table 4). Assuming that the effect of *Eucalyptus* plant to the SOM was similar in T4 and T1, the contribution solely of charcoal was estimated. At the 0-5 cm depth the contribution of charcoal to the SOM was 49 % while in the 5-10 and 10-20 cm the contributions varied around 15 % (Table 4). The increase of TC content at the 0-5 cm depth observed for T4 was around 38 %, and thus lower than the estimated charcoal contribution. This difference in the values may suggest that some of the endogenous SOM was preferentially mineralized leading to a relative enrichment of charcoal derived SOM. In the 10-20 cm the increase in TC was around 23 % and thus greater than the charcoal contribution. Possibly in this depth the increase of SOM occurred due to the increase of microbial products as shown by the Csilt+clay f. content (Table 3), which have a less

negative $\delta^{13}\text{C}$ value than the source. It is reported in the literature that during the microbial decomposition of C_3 residues a selective enrichment of the heavy ^{13}C isotope occurs (Henn and Chapela, 2000). The contribution of charcoal for the 5-10 cm depth was around 14 % and this value is coherent with the TC increase of 12 %.

For all treatments and depths the SOM FTIR spectra were quite similar and the same pattern that is exemplified in figure 2 was observed. The main identified absorption bands and their respective attributions were: a broad band at $3,405\text{ cm}^{-1}$ due to O-H stretching; two bands at $2,920\text{ cm}^{-1}$ and $2,850\text{ cm}^{-1}$ due the aliphatic C-H stretching; $1,730\text{ cm}^{-1}$ attributed to the C=O stretching of carboxylic groups; a band at $1,630\text{ cm}^{-1}$ resulting from the C=C vibration of the aromatic groups; band at $1,382\text{ cm}^{-1}$ due to alifatic C-H; band at $1,240\text{ cm}^{-1}$ due to C-O stretching and to OH deformation of carboxylic groups; a band of low intensity around $1,100\text{ cm}^{-1}$ due to C-OH stretching of carbohydrates; and a band at $1,034\text{ cm}^{-1}$ resulting from Si-O vibrations of inorganic material that was not removed by HF treatment. The pattern of the charcoal spectrum was similar to those from SOM, but with less intense bands in the aliphatic ($2,920\text{ cm}^{-1}$) and carboxylic ($1,730\text{ cm}^{-1}$) regions. Concerning this last band, it shows that the applied charcoal contains charged group which confers a hydrophilic character to the material, and that might explain its migration from the upper depth down to 20 cm depth. Furthermore, these carboxylic groups may had also contributed to increase the soil ECEC in T4, as discussed earlier. Lastly, our charcoal FTIR spectra confirms the assumption that the applied material showed a low condensation degree and is similar to that of a charcoal pyrolyzed at $300\text{ }^{\circ}\text{C}$ which was recently presented by Dai et al. (2013).

The aromaticity index $I_{\text{C}=\text{C}}/I_{\text{C}-\text{H}}$ ratio of the SOM from T1, T2 and T3 varied between 2.2 and 2.7 (Table 3). The values observed for T4 were around 3.0, which

indicate the contribution of charcoal to the SOM composition. For the pure charcoal, the $I_{C=C}/I_{C-H}$ index was 10.5 confirming the comparatively higher content of aromatic groups in comparison to that of the endogenous SOM (T1).

3.3 Effect of charcoal application on SOM thermostability

In general, the SOM of all treatments showed the same thermo-decomposition curve pattern (thermograms not shown) and six main temperature intervals were identified.

The weight loss for $T < 105\text{ }^{\circ}\text{C}$ varied between 4.5 and 6.8 % (Table 5) corresponding to the loss of hygroscopic water from organic matter (Critter and Airoidi, 2006). Three temperature intervals were identified within the range of $105\text{ }^{\circ}\text{C} < T < 440\text{ }^{\circ}\text{C}$, in which preferentially the decomposition of more thermolabile structures occurs, such as alkyl and O-alkyl structures, and phenolic and carboxylic groups (Critter and Airoidi 2006). From 105 to $240\text{ }^{\circ}\text{C}$ the weight loss varied between 3.4 and 4.8 %; from 240 to $340\text{ }^{\circ}\text{C}$ the weight loss varied between 14.4 to 20.5 %, and from 340 to $440\text{ }^{\circ}\text{C}$ the weight loss varied between 11 and 17 % (Table 5). In the fifth temperature interval ($440\text{--}520\text{ }^{\circ}\text{C}$) the mass loss corresponds to the decomposition of compounds with higher thermostability, such as aromatic structures (Potes et al. 2012). Here the weight loss ranged between 21 and 34 % (Table 5). The mass loss in the sixth temperature interval ($520\text{--}800\text{ }^{\circ}\text{C}$) was low, varying between 3.3 and 6.6 %, and corresponds to the decomposition of highly thermostable structures, even condensed aromatic structures (Critter and Airoidi 2006).

Despite the HF treatment of soil samples the residual mass for $T > 800\text{ }^{\circ}\text{C}$ was substantially high and varied between 15 and 36 %. This result may be assigned to the presence of quartz, which is not dissolved by the HF treatment and due to neo-precipitated minerals possible formed during the HF treatment (Dick et al. 2008b).

Four temperature intervals were identified in the thermo-decomposition curve of the charcoal and in contrast to the SOM samples the region between 105 °C and 350 °C comprised only one general thermodecomposition event (Table 5). In the third temperature interval (350-535 °C) occurred the greater decomposition of the sample with 70.3 % of weight loss. Such data indicate the predominance of thermostable structures, like aromatic structures, confirming the data obtained with the $I_{C=C}/I_{C-H}$ index (Table 3).

The TGI informs the proportion between the less thermolabile and more thermolabile structures and the larger the value the more thermostable is the analysed material. TGI varied from 0.75 to 1.07 for SOM, while the charcoal presented a higher TGI value of 7.0, as expected (Table 5). The greatest difference of the TGI values was observed between T1 and T4. At the depths 0-5, 5-10 and 10-20 cm, T4 showed values about 25, 16 and 14 % greater than those observed in T1, respectively, indicating an increase of thermostable structures after the incorporation of 40 Mg ha⁻¹ of charcoal. At the 20-30 cm depth no relevant difference of TGI was observed between T1 and T4. These results are in line with the results from $\delta^{13}C$, showing that contribution of charcoal to the SOM composition occurred only within 20 cm depth.

4 Conclusions

The application of charcoal fine residues at a rate of 40 Mg ha⁻¹ improved the soil fertility and the effect was apparent mainly in the upper soil depth. This application increased the SOM content and shifted its composition toward a higher aromaticity degree and thermostability.

However, in the 20 months time span of the experiment, the effects of charcoal fines incorporation on the SOM content and composition were also observed at the 10 -

20 cm depth of the soil profile. This fact indicates that colloidal charcoal fragments can be efficiently transported through the soil profile, most tentatively by leaching. Transport below 20 cm is not evidenced by our data. Either the time span of the experiment was not long enough or the soil chemical and physical parameters at that depth prevented further leaching.

Despite the limited time span of the experiment, the charcoal fines showed a great potential to be used as soil fertilizer and as a source of stable carbon into the soil. Therefore, it would be convenient to start further studies at longer-term scale to investigate the charcoal fines potential mainly as a source of stable carbon.

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References

Abiven S, Hengartner P, Scheneider MPW, Singh N, Schmidt MWI (2011) Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal. *Soil Biol Biochem* 43:1615-1617

483 Angelo LC, Mangrich AS, Mantovani KM, Santos SS (2014) Loading of VO²⁺ and Cu²⁺
 484 to partially oxidized charcoal fines rejected from Brazilian metallurgical industry. J
 485 Soil Sediments 14:353-359

486 Bauer MO, Gomide JA, Silva EAM, Regazzi AJ, Chichorro JF (2008) Anatomical
 487 evaluation and nutritive value of four prevailing forage grasses in natural pasture of
 488 Viçosa-MG. Rev Bras Zootecn 37:9-17

489 Benites VM, Mendonça ES, Schaefer CEGR, Novotny EH, Reis EL, Ker JC (2005)
 490 Properties of black soil humic acids from high altitude rocky complexes in Brazil.
 491 Geoderma 127:104-113

492 Bernoux M, Cerri CC, Neill C, Moraes JFL (1998) The use of stable carbon isotopes for
 493 estimating soil organic matter turnover rates. Geoderma 82:43-48

494 Biederman LA, Harpole WS (2013) Biochar and its effects on plant productivity and
 495 nutrient cycling: a meta-analysis. Global Change Biol Bioe 5:202-214

496 Cambardella CA, Elliot ET (1992) Particulate soil organic matter changes across a
 497 grassland cultivation sequence. Soil Sci Soc Am J 56:777-783

498 Cao X, Harris W (2010) Properties of dairy-manure-derived biochar pertinent to its
 499 potential use in remediation. Bioresource Technol 101:5222-5228

500 Chefetz B, Hatchier PG, Hadar Y, Chen Y (1996) Chemical and biological
 501 characterization of organic matter during composting of municipal solid waste. J
 502 Environ Qual 25:776-785

503 Critter SAM, Airoidi C (2006) Thermal analysis of Brazilian tropical soils originating
 504 from different sources. J Brazil Chem Soc 17:1250-1258

505 Dai Z, Meng J, Muhammad N, Liu X, Wang H, He Y, Brookes PC, Xu J (2013) The
 506 potential feasibility for soil improvement, based on the properties of biochars
 507 pyrolyzed from different feedstocks. J Soil Sediments 13:989-1000

508 De La Rosa JM, Knicker H, López-Capel E, Manning DCA, González-Pérez, JA,
 509 González-Vila FJ (2008) Direct Detection of Black Carbon in Soils by Py-GC/MS,
 510 Carbon-13 NMR Spectroscopy and Thermogravimetric Techniques. *Soil Sci Soc*
 511 *Am J* 72:258-267

512 Dick DP, Martinazzo R, Dalmolin RSD, Jaques AVA, Mielniczuk J, Rosa AS (2008a)
 513 Impacto da queima nos atributos químicos do solo, na composição da matéria
 514 orgânica e na vegetação. *Pesqui Agropecu Bras* 43:633-640

515 Dick DP, Silva LB, Inda AV, Knicker H (2008b) Estudo comparativo da matéria
 516 orgânica de diferentes classes de solos de altitude do Sul do Brasil por técnicas
 517 convencionais e espectroscópicas. *Rev Bras C Solo* 32:2289-2296

518 Dieckow J, Mielniczuk J, Knicker H, Bayer C, Dick DP, Kögel-Knabner I (2005)
 519 Composition of organic matter in a subtropical Acrisol as influenced by land use
 520 cropping and N fertilization, assessed by CPMAS ¹³C NMR spectroscopy. *Eur J*
 521 *Soil Sci* 56:705–715

522 Embrapa (1997) Manual de métodos de análise de solo. Embrapa-CNPQ, Rio de Janeiro

523 Enders A, Hanley K, Whitman T, Joseph S, Lehmann J (2012) Characterization of
 524 biochars to evaluate recalcitrance and agronomic performance. [Bioresource](#)
 525 [Technol](#) 114:644-653

526 FAO (2006) World reference base for soils resources. World Soil No. 103. Rome, Italy

527 Glaser B, Balashov E, Haumaier L, Guggenberger G, Zech W (2000) Black carbon in
 528 density fractions of anthropogenic soils of the Brazilian Amazon region. *Org*
 529 *Geochem* 31:669-678

530 Gonçalves CN, Dalmolin RSD, Dick DP, Knicker H, Klamt E, Kögel-Knabner I (2003)
 531 The effect of 10% HF treatment on the resolution of CPMAS ¹³C NMR spectra
 532 and on the quality of organic matter in Ferrasols. *Geoderma* 116:373-392

533 Henn MR, Chapela IH (2000) Differential C isotope discrimination by fungi during
 534 decomposition of C₃ and C₄-derived sucrose. *Appl Environ Microb* 66:4180-4186
 535 Hilscher A, Knicker H (2011) Degradation of grass-derived pyrogenic organic material,
 536 transport of the residues within a soil column and distribution in soil organic
 537 matter fractions during a 28 month microcosm experiment. *Org Geochem* 42:42-54
 538 Keiluweit M, Nico PS, Johnson MG, Kleber M (2010) Dynamic Molecular Structure of
 539 Plant Biomass-Derived Black Carbon (Biochar). *Environ Sci Technol* 44:1247-
 540 1253
 541 Knicker H, Almendros G, González-Vila FJ, González-Pérez JA, Polvillo O (2006)
 542 Characteristic alterations of quantity and quality of soil organic matter caused by
 543 forest fires in continental Mediterranean ecosystems: a solid-state ¹³C NMR study.
 544 *Eur J Soil Sci* 57:558–569
 545 Knicker H, González-Vila FJ, González-Vásquez R (2013) Biodegradability of organic
 546 matter in fire-affected mineral soils of Southern Spain. *Soil Biol Biochem* 56:31-39
 547 Knicker H, Hilscher A, González-Vila FJ, Almendros G (2008) A new conceptual
 548 model for the structural properties of char produced during vegetation fires. *Org*
 549 *Geochem* 39:935-939
 550 Knicker H, Nikolova R, Dick DP, Dalmolin RSD (2012) Alteration of quality and
 551 stability of organic matter in grassland soils of Southern Brazil highlands after
 552 ceasing biannual burning. *Geoderma* 181-182:11-21
 553 Kuzyakov Y, Bogomolova I, Glaser B (2014) Biochar stability in soil: Decomposition
 554 during eight years and transformation as assessed by compound-specific ¹⁴C
 555 analysis. *Soil Biol Biochem* 70:229-236

556 Laird DA, Chappell MA, Martens DA, Wershaw RL, Thompson M (2008)
 557 Distinguishing black carbon from biogenic humic substances in soil clay fractions.
 558 Geoderma 143:115-122
 559 Leifeld J (2007). Thermal stability of black carbon characterised by oxidative
 560 differential scanning calorimetry. Org Geochem 38:112–127
 561 Liang BJ, Lehmann D, Solomon J, Kinyangi J, Grossman B, O'Neill JO, Skjemstad J,
 562 Thies FJ, Luizão J, Petersen, Neves EG (2006) Black carbon increases cation
 563 exchange capacity in soils. Soil Sci Soc Am J 70:1719-1730
 564 Maia CMBF, Madari BE, Novotny EH (2011) Advances in biochar research in Brazil.
 565 Dyn Soil Dyn Plant 5:53-58
 566 Miltner A, Kopinke FD, Kindler R, Selesi D, Hartmann A, Kastner M (2005) Non-
 567 phototrophic CO₂ fixation by soil microorganisms. Plant Soil 269:193-203
 568 Muccio Z, Jackson JP (2009) Isotope ratio mass spectrometry. Roy Soc Chem.
 569 doi:10.1039/b808232d
 570 Novak JM, Busscher WJ, Laird DL, Ahmedna M, Watts DW, Niandou MAS (2009)
 571 Impact of biochar amendment on fertility of a Southeastern Coastal Plain soil. Soil
 572 Sci 174:105-112
 573 O'Leary MH (1981) Carbon isotope fractionation in plants. Phytochemistry 20:553-567
 574 Pennise DM, Smith KR, Kithinji JP, Rezende ME, Raad TJ, Zhang J, Fan C (2001)
 575 Emissions of greenhouse gases and other airborne pollutants from charcoal making
 576 in Kenya and Brazil. J Geophys Res 106:143-155
 577 Paz-Ferreiro J, Shenglei F, Méndez A, Gascó G (2014) Interactive effects of biochar
 578 and the earthworm *Pontoscolex corethrurus* on plant productivity and soil enzyme
 579 and soil enzyme activities. J Soils Sediments 14:483-494

580 Petter FA, Madari BE, Silva MAS, Carneiro MAC, Carvalho MTM, Marimon BH,
 581 Pacheco LP (2012) Soil fertility and upland rice yield after biochar application in
 582 the Cerrado. *Pesqui Agropecu Bras* 47:699-706
 583 Potes ML, Dick DP, Santana GS, Tomazy M, Bayer C (2012) Soil organic matter in
 584 fire-affected pastures and in an Araucaria forest in South-Brazilian Leptosols.
 585 *Pesqui Agropecu Bras* 47:707-115
 586 Schulze ED, Turner NC, Nicolle D, Schumacher J (2006) Leaf and wood carbon isotope
 587 ratios, specific leaf areas and wood growth of *Eucalyptus* species across a rainfall
 588 gradient in Australia. *Tree Physiol* 26:479-492
 589 Steinbeiss S, Gleixner G, Antonietti M (2009) Effect of biochar amendment on soil
 590 carbon balance and soil microbial activity. *Soil Biol Biochem* 41:1301-1310
 591 Steiner C, Teixeira WG, Lehmann J, Nehls T, Macêdo JLV, Blum WEH, Zech W
 592 (2007) Long term effects of manure, charcoal and mineral fertilization on crop
 593 production and fertility on a highly weathered Central Amazonian upland soil.
 594 *Plant Soil* 291:275-290
 595 Tan KH (2003) Humic matter in soil and the environment principles and controversies.
 596 Marcel Dekker, New York
 597 Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ (1995) Análise de solos,
 598 plantas e outros materiais. Universidade Federal do Rio Grande do Sul, Porto
 599 Alegre
 600 Verheijen FGA, Montanarella L, Bastos, AC (2012) Sustainability, certification, and
 601 regulation of biochar. *Pesqui Agropecu Bras* 47:649-653
 602 Wang X, Liu F, Tan W, Li W, Feng X, Sparks D (2013) Characteristics of Phosphate
 603 Adsorption-Desorption Onto Ferrihydrite: Comparison With Well-Crystalline Fe
 604 (Hydr)Oxides. *Soil Sci* 178:1-11

605 Zanetti M, Cazetta JO, Júnior DM, Carvalho SA (2003) The use of byproducts of
606 vegetal charcoal on growth of 'Rangpur' lime rootstock under screen house. Rev
607 Bras Frutic 25:508-512

608 Zimmerman AR, Gao B, Ahn MY (2011) Positive and negative carbon mineralization
609 priming effects among a variety of biochar-amended soils. Soil Biol Biochem 43:1169-
610 1179

611

612 **Table 1** Characteristics of the charcoal used in this study

Properties	
pH (H ₂ O)	7.59
EC (μS cm ⁻¹) ^a	332
C (%)	46.56
N (%)	1.03
C/N	45.2
Fixed C (%)	7.62
Ash (%)	8.22
Volatile matter (%)	84.16
Ca (g kg ⁻¹) ^b	21.2
Mg (g kg ⁻¹) ^b	4.00
K (g kg ⁻¹) ^b	5.20
P (g kg ⁻¹) ^b	0.73
S (g kg ⁻¹) ^b	0.53
Particles size distribution	
Sieve	Particles of charcoal retained
mm	%
6.30	17.0
4.00	13.5
2.00	24.0
0.212	42.0
0.053	3.5

613 ^aElectrical conductivity

614 ^b Total content

615

Table 2 Soil pH (H₂O), available P, exchangeable K, Ca, Mg and Al, potential acidity (Al+H) and effective cation exchange capacity (ECEC) along a Cambisol profile after different doses of charcoal application

Soil depth	Treatments ^a	pH (H ₂ O)	P	K	Ca	Mg	Al	Al+H	ECEC
cm			-----mg dm ⁻³ -----		-----c mole dm ⁻³ -----				
0 - 5	T1	4.85 b	6.38 b	278.25 b	6.25 b	3.7 a	1.85 a	19.98 a	12.50 b
	T2	5.05 ab	8.33 b	298.25 b	6.93 ab	4.15 a	1.50 ab	15.05 a	13.32 ab
	T3	5.05 ab	7.28 b	338.00 ab	7.20 ab	4.43 a	1.05 ab	13.98 a	13.53 ab
	T4	5.20 a	11.78 a	391.25 a	9.08 a	4.85 a	0.65 b	12.10 a	15.56 a
5 - 10	T1	4.70 a	5.45 a	201.25 a	4.18 a	2.43 a	3.65 a	26.55 a	10.76 a
	T2	4.80 a	5.50 a	206.00 a	4.50 a	2.80 a	2.70 a	21.30 a	10.52 a
	T3	4.80 a	5.95 a	269.25 a	6.05 a	3.70 a	1.95 a	19.60 a	12.37 a
	T4	4.75 a	5.53 a	278.00 a	4.83 a	2.63 a	2.98 a	25.75 a	11.14 a
10 - 20	T1	4.50 a	4.30 a	149.50 a	2.73 a	1.30 a	5.30 a	35.18 a	9.70 a
	T2	4.63 a	4.43 a	150.75 a	2.85 a	1.60 a	4.40 a	32.65 a	9.22 a
	T3	4.63 a	5.20 a	177.50 a	3.63 a	2.08 a	4.00 a	33.00 a	10.15 a
	T4	4.60 a	4.50 a	174.00 a	3.23 a	1.58 a	4.98 a	35.18 a	10.23 a
20 - 30	T1	4.43 a	2.95 a	128.50 a	2.05 a	1.00 a	6.33 a	35.83 a	9.70 a
	T2	4.55 a	3.85 a	136.25 a	1.83 a	1.13 a	6.10 a	35.83 a	9.40 a
	T3	4.53 a	3.50 a	134.75 a	2.38 a	1.43 a	5.58 a	36.23 a	9.72 a
	T4	4.50 a	3.30 a	120.25 a	2.78 a	1.40 a	5.33 a	37.03 a	9.81 a

Means followed by the same letter do not differ between treatments at the same soil depth (Tukey test, $p < 0.10$)

^a T1 = 0 Mg ha⁻¹ (control); T2 = 10 Mg ha⁻¹; T3 = 20 Mg ha⁻¹; T4 = 40 Mg ha⁻¹

Table 3 Soil total carbon (TC), total nitrogen (TN), C and N contents in the particulate fraction ($C_{\text{particulate f.}}$, $N_{\text{particulate f.}}$), C and N contents in the sil+clay size fraction ($C_{\text{silt+clay f.}}$, $N_{\text{silt+clay f.}}$), respective C/N ratios and aromatic index ($I_{\text{C=C}}/I_{\text{C-H}}$) along a Cambisol profile after different doses of charcoal application

Soil depth cm	Treatments ^a	TC g kg ⁻¹	TN g kg ⁻¹	C/N	$C_{\text{particulate f.}}$ g kg ⁻¹	$N_{\text{particulate f.}}$ g kg ⁻¹	C/N	$C_{\text{silt+clay f.}}$ g kg ⁻¹	$N_{\text{silt+clay f.}}$ g kg ⁻¹	C/N	$I_{\text{C=C}}/I_{\text{C-H}}$
0 - 5	T1	41.1 b	3.30 a	12.5 b	12.3 b	0.57 a	22.5 b	28.7 a	2.74 a	10.6 a	2.2
	T2	45.0 b	3.00 a	15.1 ab	18.0 b	0.57 a	31.4 ab	27.0 a	2.43 a	11.3 a	2.4
	T3	46.0 b	3.10 a	15.3 ab	15.9 b	0.47 a	33.4 ab	30.0 a	2.63 a	12.0 a	2.3
	T4	56.6 a	3.48 a	16.5 a	28.0 a	0.75 a	39.7 a	28.6 a	2.73 a	10.6 a	2.6
5 - 10	T1	36.2 a	2.43 a	17.2 a	7.9 a	0.42 a	18.7 a	28.2 a	2.47 a	11.5 a	2.2
	T2	36.9 a	2.45 a	16.0 a	11.7 a	0.52 a	21.5 a	25.2 a	1.93 a	14.3 a	2.3
	T3	38.6 a	2.75 a	14.1 a	11.6 a	0.44 a	26.4 a	27.0 a	2.31 a	11.7 a	2.3
	T4	40.8 a	2.78 a	14.8 a	13.6 a	0.49 a	27.4 a	27.2 a	2.28 a	11.8 a	2.7
10 - 20	T1	29.3 b	2.13 ab	14.2 a	7.0 a	0.45 a	15.8 a	22.2 b	1.67 a	14.6 a	2.3
	T2	30.8 ab	2.13 ab	15.9 a	6.0 a	0.44 a	13.3 a	24.8 ab	1.68 a	17.4 a	2.6
	T3	31.4 ab	2.05 b	17.8 a	8.2 a	0.46 a	19.2 a	23.2 ab	1.60 a	17.8 a	2.6
	T4	36.0 a	2.35 a	16.2 a	8.0 a	0.51 a	15.5 a	28.0 a	1.84 a	16.4 a	2.6
20 - 30	T1	24.8 a	1.93 a	12.9 a	4.5 a	0.33 a	13.6 a	20.2 a	1.60 a	12.7 a	3.0
	T2	28.3 a	1.97 a	14.8 a	6.6 a	0.45 a	14.8 a	21.7 a	1.52 a	14.5 a	3.0
	T3	23.7 a	1.78 a	13.8 a	5.5 a	0.44 a	12.4 a	18.2 a	1.34 a	14.1 a	3.1
	T4	28.8 a	1.95 a	16.6 a	5.9 a	0.38 a	16.1 a	23.0 a	1.57 a	16.4 a	3.0

Means followed by the same letter do not differ between treatments at the same soil depth (Tukey test, $p < 0.10$)

^a T1 = 0 Mg ha⁻¹ (control); T2 = 10 Mg ha⁻¹; T3 = 20 Mg ha⁻¹; T4 = 40 Mg ha⁻¹

Table 4 Total soil carbon $\delta^{13}\text{C}$ signature and contribution of the *Eucalyptus* and of the charcoal to the total soil carbon content along a Cambisol profile after 40 Mg ha⁻¹ of charcoal application

Treatments ^a	Soil depth	$\delta^{13}\text{C}$	Ft _{eucalyptus} ^c	Ft _{charcoal} ^e
	cm	‰	%	%
T1	0 - 5	-19.12 ± 0.27	24.09	-
T1	5 - 10	-19.28 ± 0.70	25.64	-
T1	10 - 20	-17.50 ± 0.32	8.48	-
T1	20 - 30	-16.62 ± 0.18	0.00	-
Ft _{charcoal+eucalyptus} ^d				
			%	
T4	0 - 5	-23.42 ± 0.30	73.16	49.08
T4	5 - 10	-20.21 ± 0.27	39.67	14.03
T4	10 - 20	-18.68 ± 0.38	23.76	15.28
T4	20 - 30	-16.41 ± 0.96	0.00	0.00
Charcoal		-25.99 ± 0.36	-	-
<i>Eucalyptus</i>		-27.00 ^b	-	-

^a T1 = 0 Mg ha⁻¹ (control); T4 = 40 Mg ha⁻¹

^b Schulze et al. (2006)

^c Proportion of the total carbon derived from *Eucalyptus* plants

^d Proportion of the total carbon derived from charcoal + *Eucalyptus* plants

^e Proportion of the total carbon derived from charcoal

Table 5 Mass loss in different temperature intervals, mass residue for T > 800 °C and thermogravimetric index (TGI) of the pure charcoal and of the SOM along a Cambisol profile after different doses of charcoal application

Soil depth	Treatments ^a	Temperature interval (°C)						Mass	TGI ^b
		40 - 105	105-240	240 - 340	340 - 440	440 - 520	520 - 800	residue	
cm		Mass loss (%)						%	
0 - 5	T1	5.2 ± 2.8	4.8 ± 0.2	20.5 ± 1.0	15.3 ± 1.4	25.3 ± 1.0	5.3 ± 0.5	23.6 ± 2.0	0.75
	T2	5.8 ± 3.2	4.8 ± 0.7	20.4 ± 1.2	13.7 ± 0.4	25.5 ± 2.4	6.6 ± 0.0	23.2 ± 0.9	0.82
	T3	6.3 ± 2.4	4.1 ± 0.6	21.4 ± 1.1	15.7 ± 0.9	25.7 ± 2.1	5.1 ± 0.2	21.8 ± 1.0	0.75
	T4	6.4 ± 3.1	4.7 ± 0.6	19.3 ± 0.5	14.4 ± 0.0	32.6 ± 0.3	5.7 ± 0.0	16.9 ± 2.7	1.00
5 - 10	T1	4.5 ± 0.5	4.5 ± 0.4	18.0 ± 0.3	14.0 ± 0.3	28.2 ± 0.5	4.5 ± 0.8	26.3 ± 1.7	0.90
	T2	6.2 ± 6.3	4.3 ± 0.1	20.4 ± 0.8	13.7 ± 0.6	28.7 ± 0.7	4.3 ± 0.5	22.3 ± 4.8	0.86
	T3	6.6 ± 2.7	4.0 ± 0.6	20.3 ± 0.5	15.3 ± 0.6	30.9 ± 2.4	5.8 ± 1.3	17.1 ± 8.0	0.93
	T4	5.4 ± 1.8	4.3 ± 0.8	17.3 ± 1.1	13.4 ± 1.2	33.4 ± 2.4	4.1 ± 0.6	22.0 ± 0.9	1.07
10 - 20	T1	5.9 ± 2.2	3.4 ± 0.2	19.1 ± 1.3	12.7 ± 0.3	25.0 ± 1.7	4.4 ± 0.3	29.5 ± 5.1	0.84
	T2	5.3 ± 2.2	4.5 ± 0.0	20.7 ± 0.9	12.7 ± 0.3	28.4 ± 2.2	5.9 ± 0.3	22.5 ± 5.2	0.90
	T3	6.8 ± 0.5	4.4 ± 0.8	18.0 ± 3.2	16.6 ± 0.1	33.3 ± 2.7	5.1 ± 1.3	15.7 ± 7.1	0.99
	T4	4.9 ± 4.0	4.2 ± 0.3	14.4 ± 1.1	16.4 ± 2.5	31.0 ± 3.0	3.3 ± 0.2	25.7 ± 1.0	0.98
20 - 30	T1	5.6 ± 2.5	3.4 ± 0.7	15.8 ± 0.8	12.4 ± 0.3	26.0 ± 1.5	4.2 ± 1.6	32.6 ± 2.8	0.96
	T2	5.3 ± 2.7	3.6 ± 0.3	17.6 ± 1.6	11.1 ± 0.7	21.9 ± 1.0	4.4 ± 0.3	36.1 ± 0.4	0.81
	T3	6.0 ± 3.8	3.7 ± 0.4	16.3 ± 0.6	12.5 ± 0.1	22.4 ± 1.0	5.0 ± 0.1	34.3 ± 3.9	0.84
	T4	5.6 ± 3.5	3.5 ± 0.2	14.7 ± 1.8	15.1 ± 4.4	26.4 ± 0.7	4.6 ± 1.4	30.2 ± 0.0	0.93
		Temperature Interval (°C)						Mass	TGI ^c
		40 - 105	105 - 350	350 - 535	535 - 800	residue			
		Mass loss (%)						%	
-	Charcoal	4.1 ± 1.1	10.6 ± 0.7	70.3 ± 0.6	3.9 ± 0.3	11.2 ± 0.8	7.0		

^a T1 = 0 Mg ha⁻¹ (control); T2 = 10 Mg ha⁻¹; T3 = 20 Mg ha⁻¹; T4 = 40 Mg ha⁻¹

^b TGI = mass loss (5^a+6^aregions) / mass loss(2^a+3^a+4^a regions)

^c TGI = mass loss (3^a+4^a regions) /mass loss (2^aregion)

Figure captions

Fig. 1 Scanning electron microscopy of the pure charcoal (a) and of the particulate (b) (0-5 cm) and sil+clay size (c) (10-20 cm) fractions of SOM in treatment T4 (40 Mg ha⁻¹). The charcoal fragments (arrows in the Fig. 1b and c) could be identified by energy dispersive X-ray spectroscopy, with a dominant C peak

Fig. 2 FTIR spectra of the SOM along a Cambisol profile after 40 Mg ha⁻¹ of charcoal application. Spectra of T1 (a) and spectra of charcoal and treatment T4 (b) . T1 = 0 Mg ha⁻¹ (control); T4 = 40 Mg ha⁻¹