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Charcoal Fine Residues Effects on Soil Organic Matter Humic Substances, Composition, and Biodegradability

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Abstract: Biochar has been shown as a potential mean to enhance carbon sequestration in the soil. In Brazil, approximately 15% of the produced charcoal is discarded as charcoal fines, which are chemically similar to biochar. Therefore, we aimed to test charcoal fines as a strategy to increase soil carbon sequestration. Charcoal fines of hardwood *Mimosa scabrella* were incorporated into a Cambisol down to 10 cm (T1 = 0 and T4 = 40 Mg ha⁻¹) in Southern Brazil. Soil samples were collected (0–30 cm) 20 months after charcoal amendment. Soil organic matter (SOM) acid extract, humic acid, fulvic acid, and humin fractions were separated. Solid-state ¹³C nuclear magnetic resonance (NMR) spectra from charcoal and SOM in T1 and T4 were obtained before and after 165 days of incubation under controlled conditions. Charcoal increased soil carbon as fulvic (10–20 cm) and humic acids (10–30 cm) and, especially, as humin (0–5 cm), which probably occurred due to the hydrophobic character of the charcoal. The ¹³C NMR spectra and mean residence times (MRT) measured from incubation essays indicated that the charred material decomposed relatively fast and MRT of T1 and T4 samples were similar. It follows that the charcoal fines underwent similar decomposition as SOM, despite the high charcoal dose applied to the soil and the high aryl C contribution (78%) to the total ¹³C intensity of the charcoal NMR spectra.

Keywords: field experiment; incubation; ¹³C NMR; mean residence time; slow pool

1. Introduction

In recent years, the literature has reported the potential of biochar as a strategy to mitigate global warming. Basically, biochar can enhance carbon (C) sequestration directly when buried into the soil or indirectly by improving soil quality and crop production, thus, enhancing CO₂ capture from the atmosphere [1,2].

Conceptually, biochar is a C-enriched material intentionally produced via pyrolysis of biomass to be applied to the soil as a means to improve C sequestration, soil quality, and crop yield [1,3]. Therefore, biochar is distinguished from charcoal since energy generation, industrial use, and domestic cooking are the main purposes of charcoal production. Nevertheless, the thermochemical conversion of biomass in pyrogenic C (PyC) is a common process for both biochar and charcoal production [3].

In this way, several authors have reported positive effects of charcoal on soil fertility, crop production, and C sequestration [4–7].

Brazil is one of the world's greatest agricultural producers and consequently generates great amounts of biomass residues [8,9]. Thus, numerous organic residues, such as sugarcane straw and filtercake, rice husk, poultry manure, sawdust, and sewage sludge, have been converted in biochar and applied to agricultural soils, originating promising field and greenhouse experiments [10–14].

In parallel to this scenario, Brazil is the world's greatest charcoal producer with an annual production of 10 million tons of charcoal, supplying mainly the steel industry. However, approximately 15% of this production is lost as charcoal fines, since they are not suitable for industrial use [6,15]. Despite biochar and charcoal own similar composition, minor importance has been directed to the charcoal fine residues in Brazil as a potential soil conditioner and, especially, as a means to sequester C and mitigate global warming. In addition, the utilization of charcoal fines for such purposes concomitantly contributes in reducing environmental liabilities originated by the improper discard of this residue and also in reducing biochar production from organic residues, which can be used for other purposes.

The greater stability of PyC in the soil compared to that of soil organic matter (SOM) has been attributed to the high aromaticity and hydrophobicity of the pyrogenic materials, characteristics supposed to confer high biochemical recalcitrance to this matrix [16,17]. Nevertheless, PyC can undergo transformation and biodegradation in the soil at certain rates, depending on the material source, temperature, and duration of heating, and on oxygen supply during the pyrolysis process [18,19]. Hydrophobicity and oxygenation of the pyrogenic materials in the soil may affect the C distribution in humic fractions. Increments of C as humin (HU) can indicate preservation of PyC in the soil since charcoal is essentially hydrophobic, while increments in humic acid (HA) and fulvic acid (FA) suggest oxidation of aromatic structures, thus, affecting organic matter functions and persistence in the soil [20].

The mean residence time (MRT) of the PyC has been reported to be generally one or two orders of magnitude greater than those of their fresh organic precursors [21–24]. Knicker et al. [25] estimated an MRT of the slow SOM pool to be 3 to 4 times (40 years) longer in soil with charcoal produced via wildfire than in the fire unaffected soil. On the other hand, Schneider et al. [7] did not observe changes in the chemical composition and content of charcoal derived from burned vegetation even after exposure to intense weathering during 100 years in a tropical Humic Nitrosol. These findings are in line with Vasilyeva et al. [26]. The authors evaluated total C (TC) and PyC contents of a fire-affected Chernozem under fallow for 55 years. A smaller loss of PyC stock (6%) in comparison to soil organic C (33%) was observed, and changes in the aromatic condensation degree of PyC were not detected. In general, these findings support the hypothesis that the high aromaticity of the charcoal can lead to longer SOM MRT.

In a previous study, we observed favorable effects of the charcoal fines on soil fertility and consistent increase of SOM thermostability after charcoal incorporation into a subtropical Cambisol at a rate of 40 Mg ha⁻¹ in Southern Brazil [27]. These findings are in line with above-mentioned literature and support the hypothesis that this residue deserves to be tested not only as a soil conditioner but, moreover, as a means to efficiently promote C sequestration in the soil. However, the utilization of charcoal fines as a strategy to sequester C needs elucidation. Additionally, there is a lack of information and ambiguous results in the literature concerning changes in the SOM after charcoal amendments to the soil [28,29].

In this context and based on the high aromaticity of the charcoal fines observed in our previous study, it is hypothesized that such pyrogenic material may alter SOM humic fractions distribution and slow down SOM biodegradability. In order to test this hypothesis, soil samples were collected at field experimental plots with or without charcoal amendment. These samples were incubated under controlled conditions, and SOM composition and degradation were investigated.

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, Brazil, at approximately 855 m above the sea level. The climate is humid subtropical mesothermic-Cfb (Köppen), with frequent and severe frosts during winter (June–September). The annual mean temperature is 17.2 °C, the average rainfall is 194 mm month⁻¹, and the relative humidity is 79.6%. The study was carried out in the *Campus* Irati of the State University of Centro-Oeste (25°27'56" S 50°37'51" W). The relief is undulated and strongly undulated, and the soil is classified as Haplic Cambisol [30]. Sand, silt, and clay contents of the soil at 0–5 cm depth are: 441, 167, and 392 g kg⁻¹, respectively; at 5–10 cm depth: 492, 170, and 338 g kg⁻¹, respectively; at 10–20 cm depth: 467, 162, and 371 g kg⁻¹, respectively; at 20–30 cm depth: 437, 182, and 381 g kg⁻¹, respectively. The experimental area has 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 established in February 2010 when the area was manually and mechanically mowed. About 2.5 Mg ha⁻¹ of dolomitic limestone (85% of the relative power of total neutralization) was applied on the soil surface and subsequently incorporated at 10 cm depth using a light disk harrow.

The charcoal used in this experiment was originated from the pyrolysis of hardwood native Brazilian species, mainly *Mimosa scabrella* Benth. The charcoal fine residues (<6.3 mm) were acquired from a local producer that supplies the Brazilian steel industry. About 45% of the used charcoal particles were smaller than 2 mm. The pyrolysis of the hardwood was performed under artisanal conditions, which might have contributed to its low fixed C (7.6%) and high volatile matter (84%) contents, as discussed in Leal et al. [27]. These data characterize this material as low condensed charcoal produced at low temperatures [31]. The C (46.6%) and the N (1.0%) contents of the charcoal were determined by dry combustion, and its ash content (8.2%) was determined after heating at 750 °C during 4 h. A more exhaustive physicochemical characterization of the charcoal is available in Leal et al. [27].

The study was conducted in a complete randomized block design with four treatments arranged in four blocks: T1 = 0 Mg ha⁻¹ (without charcoal-control); T2 = 10 Mg ha⁻¹; T3 = 20 Mg ha⁻¹; T4 = 40 Mg ha⁻¹. Each field replicate (each block) was composed of three subsamples collected within a 144 m² plot. The treatments were implemented just after soil liming (February 2010). Firstly, the charcoal was applied on the soil surface and, thereafter, it was incorporated up to 10 cm using a light disk harrow. In March 2010, seedlings of *Eucalyptus benthamii* were planted in the experimental plots, and plant height and diameter were monitored up to 210 days after planting. A clear effect of charcoal doses on *Eucalyptus* growth was not observed [32]. Considering that the most outstanding differences regarding the effects of charcoal doses on soil characteristics and C contents were observed between T1 and T4 [27], these treatments were selected for the present study.

The soil samples were collected in September 2012 (20 months after charcoal incorporation in the soil) at 0–5; 5–10; 10–20; 20–30 cm depths. Before analysis, the soil samples were air dried and passed through a 2 mm sieve.

2.2. Total C content and SOM Fractionation

The TC content of the soil samples was determined by dry combustion (975 °C) (Thermo Fisher Scientific-Flash EA1112, Waltham, MA, USA, detection limit = 0.01%, $n = 4$).

The SOM chemical fractionation was performed according to Swift [33], adapted by Dick et al. [34]. In a centrifuge tube, one gram of soil was shaken with 60 mL of 0.5 M HCl for 2 h. The acid extract was separated by centrifugation. This procedure was repeated three times, and the final extraction volume was measured. Hereafter, the material remaining in the tube was shaken with 60 mL of 0.5 M NaOH for 3 h to extract the soluble humic substances (SHS), namely HA and FA. This procedure was repeated until the supernatant became colorless, and the final SHS volume was measured. Aliquots

(5 mL) of the acid and of the SHS extracts were collected for C content determination. The pH of the material remaining in the tube was lowered to 2 with 4 M HCl solution, and the suspension was allowed to settle overnight. Centrifugation was used to separate HA fraction (precipitated) and FA fraction (supernatant). The FA extract volume was measured, and an aliquot (5 mL) was collected for C content determination. The residue of the extraction contained the HU fraction. The C content in the acid (C_{HCl}), SHS (C_{SHS}), and FA (C_{FA}) extracts was quantified by measuring the absorbance at 580 nm (Shimadzu-UV-160A, Kyoto, Japan) after C oxidation with K dichromate in acidic medium at 60 °C during 4 h. For discussion purposes, the concentration of the three fractions (acid extract, SHS, and of FA) was based on the C content allocated in each fraction. Likewise, the C concentration in the HA fraction (C_{HA}) was determined as follows: $C_{HA} = C_{SHS} - C_{FA}$, and the C concentration in the HU fraction (C_{HU}) was determined by the difference: $C_{HU} = TC - C_{HCl} - C_{SHS}$.

2.3. Incubation of Soil Samples and Charcoal

Before incubation, soil samples (10 g) were inoculated with 1 mL of a microbial suspension, which was extracted from a gardening soil after manual shaking with deionized water and subsequent filtering (5 µm pore size). Soil samples (four field replicates) were placed into individual closed incubation vessels (250 mL), and their water content was adjusted to ca. 60% of the maximum soil water holding capacity. In addition to the soil samples, four replicates of charcoal alone (10 g, milled to pass a 2 mm sieve) were also incubated following the same methodology. Considering that part of the charcoal is usually hydrophobic and that it can affect the water distribution within the sample and, therefore, charcoal mineralization rates, the wetted samples were carefully homogenized before incubation.

Soil and charcoal samples were incubated for 165 days at 20 °C under aerobic conditions in a Respicond Apparatus IV (Nordgren Innovations, Alnarp, Sweden). The respiration was measured every three hours by determining changes in the electrical conductivity induced by absorption of CO₂ in a KOH solution (10 mL, 0.6 M), which was allocated inside the incubation vessel [35]. The cumulative C loss was calculated by normalizing the CO₂ production to the C content of each sample and by employing a calibration constant value informed by the producer of the equipment, which takes into account the temperature used during the incubation period. The proportion of remaining C at a given time ($A(t)$) was calculated by subtracting the accumulated C loss from 100%. At the end of the incubation period, data were fitted to a double exponential decay model using Sigmaplot 11.0 according to equation 1. This model separates the decomposition curve in two different compartments, corresponding to the fast and the slow turnover pools, both following the first order kinetics model.

$$A(t) = A_1 \times e^{-k_1 t} + A_2 \times e^{-k_2 t} \quad (1)$$

where $A(t)$ = remaining C (% of TC); A_1 = amount of C relatively labile against mineralization (% of TC); A_2 = amount of C more stable against mineralization (% of TC); t = incubation time; k_1 and k_2 = apparent first order mineralization rate constants for the labile and stable pool (y^{-1}), respectively. The mean residence times of the first-order reactions were $MRT_1 = 1/k_1$ and $MRT_2 = 1/k_2$, whereas the half-life time of A_2 was calculated as $t_{1/2long} = 0.693/k_2$.

Besides the soil and the charcoal samples, one blank (without soil or charcoal) was prepared in order to monitor variations due to temperature changes or background noise. The blank sample showed that no background CO₂ production occurred.

2.4. Solid-State ¹³C Nuclear Magnetic Resonance (NMR) Cross Polarization Magic-Angle Spinning CPMAS Spectroscopy

Since differences between T1 and T4 regarding TC contents were observed only at 0–5 and 10–20 cm depths (Table 1), soil samples from these depths were selected for the ¹³C NMR analysis. Prior to the analysis, composite samples (formed by the four field replicates) were treated with 10%

hydrofluoric acid (HF) solution to concentrate the SOM and to remove paramagnetic materials [36]. Thereafter, samples were washed five times with deionized water and freeze-dried before analysis.

Table 1. Total carbon content (TC), carbon content in the acid extract (C_{HCl}), fulvic acid (C_{FA}), humic acid (C_{HA}), and humin (C_{HU}), and C_{HA}/C_{FA} and $(C_{FA} + C_{HA})/C_{HU}$ ratios of a Cambisol without charcoal (T1) and with charcoal -40 Mg ha^{-1} (T4) application.

Treatments	TC	C_{HCl}	C_{FA}	C_{HA}	C_{HU}	C_{HA}/C_{FA}	$(C_{FA} + C_{HA})/C_{HU}$
g kg ⁻¹							
0–5 cm							
T1	41.1 ***	1.4 ns	7.6 ns	9.2 ns	22.8 **	1.3 ns	0.8 *
T4	56.6	1.4	8.1	11.5	35.6	1.4	0.6
5–10 cm							
T1	36.2 ns	1.2 ns	5.2 ns	8.8 ns	20.9 ns	1.8 ns	0.7 ns
T4	40.8	1.0	6.8	8.1	24.9	1.2	0.6
10–20 cm							
T1	29.3 **	1.8 ns	4.3	5.4 **	17.8 ns	1.2 ns	0.6 *
T4	36.0	1.6	6.7*	8.8	18.9	1.3	0.8
20–30 cm							
T1	24.8 ns	2.2 ns	3.2 ns	5.0 **	14.5 ns	1.7 ns	0.6 *
T4	28.8	1.7	4.3	7.9	14.9	1.9	0.8

Values represent means of four replicates and ns, *, **, and *** indicate *t*-test results of $p > 0.10$, $p < 0.10$, $p < 0.05$, and $p < 0.01$, respectively.

Prior to the ^{13}C NMR analysis, charcoal composite samples (3 g) were placed into centrifuge tubes and treated with 75 mL of 2 M HCl solution for removing soluble ashes and paramagnetic compounds. After 2 h of mechanical shaking and subsequent centrifugation at 2000 g for 10 min, the supernatant was removed and discarded. The material remaining into the containers was washed four times with deionized water and freeze-dried. Aiming to elucidate chemical alterations on soil and charcoal samples resulting from degradation during incubation, these samples were subjected to ^{13}C NMR analysis before and after incubation.

The solid-state ^{13}C NMR spectra were obtained with a Bruker Avance III 600 MHz spectrometer, Billerica, MA, USA from General Services and facilities of the University of Seville (CITIUS, Seville, Spain) operating at a resonance frequency of 150.91 MHz, and CPMAS approach [37] was applied with a spinning speed of 15 kHz. A ramped ^1H pulse was used during the contact time of 1 ms to circumvent spin modulation during the Hartmann-Hahn contact [38,39]. For each sample, about 300–400 mg of finely crushed and homogenized material were packed into 4 mm zirconium rotors. Subsequently, depending on the C content of the samples, about 13,000 to 28,000 scans were accumulated for soil samples (T1 and T4), and 3000 to 8000 scans for charcoal samples with a pulse delay of 300 ms and line broadenings between 50 and 100 Hz. The ^{13}C chemical shifts were calibrated relative to tetramethylsilane (0 ppm) with glycine (COOH at 176.08 ppm). The contributions of the various C groups to the total ^{13}C intensity were calculated using the MestreNova 8.1 software. Firstly, the integration of the signal intensity of all C groups was performed. Subsequently, the area of each chemical shift region was divided by the total area, and this result was multiplied by 100. The calculation was carried out by taking into account the spinning sideband disturbance [40]. The chemical shift assignments of the CPMAS ^{13}C NMR spectra were performed as follows: 0–45 ppm, alkyl C; 45–60 ppm, N-alkyl C; 60–110 ppm, O-alkyl C; 110–160 ppm, aryl C; 160–220 ppm, carboxyl C [25].

In order to calculate the loss of each C group resulting from the microbial activity during the incubation experiment, the intensities of each chemical region in the spectra of samples, after incubation, were multiplied by the percentage of remaining C (% of the initial TC) in the sample at the end of the incubation [25]. Therefore, for samples after incubation, the difference between 100% and the sum

of ^{13}C intensities corresponds to the C loss occurring during incubation. Finally, these values were subtracted from the intensities obtained for the spectra of the samples before the incubation.

2.5. Statistical Analysis

Paired *t*-tests were used to compare the means of T1 and T4 variables within soil depth. All statistical analyses were performed using the software R [41].

3. Results and Discussion

3.1. Total C Content and Humic Substances

The application of 40 Mg ha^{-1} of charcoal (T4) increased TC contents at 0–5 and 10–20 cm depths by 38 and 23%, respectively, in comparison to T1. At 0–5 cm depth, the greater concentration of C found in T4 could be attributed to the considerable amount of charcoal particles remaining near the soil surface (even after charcoal incorporation up to 10 cm depth), as evidenced, in a previous study, by scanning electron microscopy (SEM) and by the higher C content in the particulate SOM fraction [27]. The higher TC content observed in T4 at 10–20 cm depth was probably associated with the vertical transport of smaller charcoal particles.

In general, the charcoal application did not affect C_{HCl} contents, which varied from 1.0 to 2.2 g kg^{-1} (Table 1). The contribution of C_{HCl} to the TC content ranged from 2 to 9% (Figure 1). The C_{HCl} fraction is mainly composed of organic compounds that are originated from the microbial activity and from root exudations. Such chemical structures are smaller and more labile than that of the HA and FA, and due to their fast turnover, C_{HCl} is usually found in low proportions in subtropical soils [42].

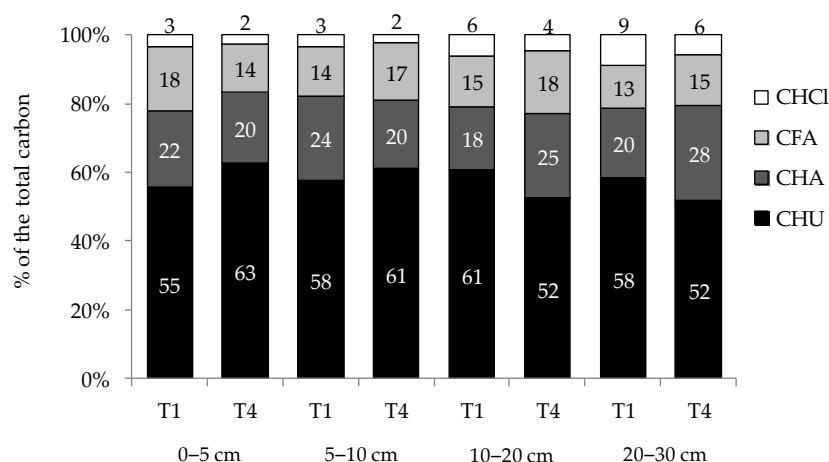


Figure 1. Contribution of C content in the acid extract (C_{HCl}), fulvic acid (C_{FA}), humic acid (C_{HA}), and humin (C_{HU}) fractions to the total carbon content of a Cambisol without charcoal (T1) and with charcoal -40 Mg ha^{-1} (T4) application.

Different from C_{HCl} contents, the C distribution in the other SOM chemical fractions was considerably affected by the charcoal application. The C_{FA} contents varied from 3.2 to 8.1 g kg^{-1} (Table 1) and represented 13 to 18% of TC (Figure 1). Compared to T1, C_{FA} content in T4 was 55% higher at 10–20 cm depth (Table 1). Significant differences were not observed in other depths.

The C_{HA} contribution to TC ranged from 18 to 28% (Figure 1). Interestingly, the increments in C_{HA} contents in response to the charcoal application were observed not near to soil surface, where charcoal particles were concentrated, but at 10–20 and 20–30 cm depths, where C_{HA} contents in T4 were 64% and 59% higher than in T1, respectively. According to the $\delta^{13}\text{C}$ isotopic ratio data of the soil samples presented in a previous study [27], after incorporation, most of the charcoal particles remained at the 0–5 cm depth and a lower proportion of them was moved to 10–20 cm depth. The presence at 10–20 cm depth of humified compounds derived from the charcoal material, might explain the higher

C_{FA} and C_{HA} contents noticed at this depth. The mild conditions of the charcoal production along with the weathering of the charcoal during the time span of the field experiment until soil sampling (20 months) might have contributed to its partial oxidation, originating carboxylic groups directly linked to aromatic structures [43–45]. Possibly, these organic compounds have migrated downward from upper depths with a preferential accumulation in the alkaline extractable fraction (SHS). In fact, literature has reported that aging of charcoal can start very quickly after entering the soil, enhancing the number of functional groups (mostly carboxyl) in the charcoal structure, leading to an increase of polar sorptive sites and, thus, facilitating the movement of charcoal compounds downward the soil profile [46–48]. The leaching of charcoal particles in soils has been also evidenced by the increase of SOM aromaticity degree in deeper depths, as a result of functionalization of aromatic charcoal structures [49–51]. At 20–30 cm depth, the greater content of C_{HA} and C_{FA} in T4 compared to T1 might be associated to an indirect effect of the charcoal on the endogenous SOM dynamics of the upper depths, promoting its functionalization and, thus, an increase of the SHS fraction, since $\delta^{13}C$ isotopic ratio data of the soil samples did not indicate charcoal presence at 20–30 cm depth [27].

The C_{HU} contents varied from 14.5 to 35.6 g kg^{−1} (Table 1), and regardless of treatment and soil depth, the contribution of C_{HU} to TC was higher than 50% (Figure 1). Similarly, to C_{FA} and C_{HA} , charcoal incremented C_{HU} contents in the soil. At 0–5 cm depth, C_{HU} content in T4 was 56% higher than in T1. This result is probably related to the concentration of charcoal particles in the soil surface even after its incorporation and to the hydrophobic character and particulate size of the charcoal, leading this material to accumulate in the non-alkaline extractable fraction, HU [20].

The relative enrichment of C as HU at 0–5 cm depth after charcoal incorporation was evidenced by the lower $(C_{FA} + C_{HA})/C_{HU}$ ratio in T4 compared to T1 (Table 1). At 10–20 and 20–30 cm depths, where charcoal did not affect C_{HU} contents, higher $(C_{FA} + C_{HA})/C_{HU}$ ratios were observed in T4 in comparison to T1 due to the increment of C_{HA} and/or C_{FA} content. The intensification of the humification process, particularly HA formation, in soils with PyC (e.g., “Terra Preta” in Amazon) has been reported in the literature [52–54] and can be attributed to the increase of N, P, and Ca contents in the soil due to ashes addition and/or to the increase of the effective cation exchange capacity (ECEC) of the soil as a consequence of PyC amendments. In this sense, higher P and Ca contents, as well as ECEC in T4 in comparison to T1, especially at 0–5 cm depth, were observed in our previous study [27] and might support such interpretation.

3.2. SOM and Charcoal Biodegradability

Figure 2 shows the curves of remaining C (% of the initial C) versus the incubation time (165 days expressed in hours) for T1 and T4 samples at each soil depth and for charcoal alone. In order to facilitate graphs visualization, only every tenth measured data is presented. All the coefficients of determination (R^2) were greater than 0.96 (Table 2).

During the incubation experiment, the data acquisition was interrupted for a few days (gaps between symbols in the graphs, Figure 2) due to electric power cuts and consequent computer instabilities. However, such interruption did not interfere in the amount of CO₂ accumulated in the KOH solution and, thus, the cumulative C loss measurement was not affected.

The C loss during the incubation of the soil samples ranged from 3.8 to 6.5% of the initial C and tended to decrease with soil depth regardless of treatment (Table 2). These findings suggest that SOM at the soil surface was biochemically more labile than that at deeper depths. Stabilization of SOM at deeper depths occurs mainly via organo-mineral interactions, thus, hindering its biodegradability. In the present study, the chemical SOM composition seemed to have a minor role in the SOM stabilization, since the more easily degradable functional group, i.e., O-alkyl C, decreased with depth, neither in T1 nor in T4 (Table 3).

Differences between T1 and T4 regarding C loss during incubation were not observed (Table 2) regardless of the soil depth. Despite the accumulation of charcoal particles and the substantially higher

C_{HU} content in T4 compared to T1 at 0–5 cm depth, the SOM biodegradability remained unaffected in terms of organic matter conversion to CO_2 .

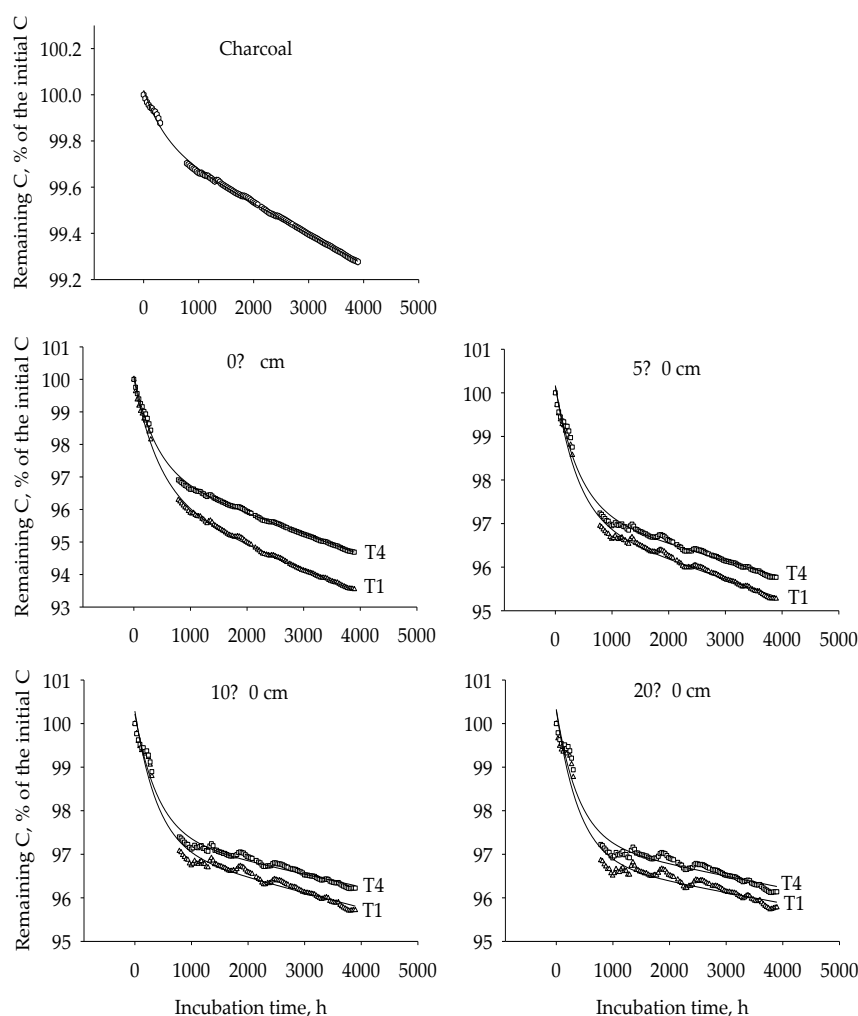


Figure 2. Remaining carbon (C) as a function of incubation time for a Cambisol without charcoal (T1) and with charcoal -40 Mg ha^{-1} (T4) and for charcoal alone (not mixed with soil).

Mineralization of charcoal (alone) observed in the present study (0.73% of the initial C) was in the range of values reported by Hamer et al. [55] (from 0.3 to 1.2%) for charred maize and rye straw (thermally altered at 350°C) and wood (thermally altered at 800°C) incubated for 60 days. Slower mineralization ($<0.25\%$) for biochars, derived from wheat and eucalyptus shoot incubated for 74 days, was reported by Farrel et al. [56], and faster mineralization of hardwood derived charcoal pyrolyzed at 500°C (2.8%) after 84 days of incubation was reported by Zavalloni et al. [57]. These comparisons suggest that the potential of the charcoal fine residues for C sequestration is comparable to that presented by biochars, including biochars produced at high temperatures and derived from hardwood, which are usually considered as highly resistant against degradation. However, it is important to highlight that when charcoal was mixed with soil and subjected to weathering under field conditions (20 months) and posterior incubation, it did not alter SOM biodegradability. The TC contents of the Cambisol (without charcoal) were considerable, 24.8 up to 41.1 g kg^{-1} (Table 1), and mainly composed of easily decomposable organic compounds (as O-alkyl C) (Table 3), which might suggest that availability of labile organic compounds possibly stimulated microorganisms to degrade assumed less labile compounds, like that from charcoal. Acceleration of PyC (charred maize and rye residues

and oak wood) mineralization due to the presence of labile organic material (glucose) in a 60-day incubation experiment (air temperature of 20 °C) was already reported in the literature [55].

Table 2. Cumulative carbon (C) loss after 165 days incubation experiment, proportion of the fast (A_1) and slow (A_2) organic matter pools, their respective constant mineralization rates (k_1 and k_2) and mean residence times (MRT_1 and MRT_2), and half-life time of the slow organic matter pool ($t_{1/2 \text{ long}}$) of the charcoal alone (not mixed with soil) and of a Cambisol without charcoal (T1) and with charcoal –40 Mg ha^{−1} (T4) application.

Treatments	C loss % of TC	A_1 % of TC	k_1 year ^{−1}	MRT_1 years	A_2 % of TC	k_2 year ^{−1}	MRT_2 years	$t_{1/2 \text{ long}}$ years	R^2 *
0–5 cm									
T1	6.5 ^{ns}	3.7 ^{ns}	19.5 ^{ns}	0.05 ^{ns}	96.3 ^{ns}	0.066 ^{ns}	15.7 ^{ns}	10.9 ^{ns}	0.998
T4	5.2	2.9	22.7	0.04	97.3	0.058	17.4	12.1	0.998
5–10 cm									
T1	4.7 ^{ns}	3.1 ^{ns}	22.6 ^{ns}	0.05 ^{ns}	97.1 ^{ns}	0.041 ^{ns}	25.2 ^{ns}	17.5 ^{ns}	0.991
T4	4.3	3.0	21.2	0.05	97.2	0.034	29.7	20.6	0.990
10–20 cm									
T1	4.2 ^{ns}	3.1 ^{ns}	23.3 ^{ns}	0.04 ^{ns}	97.2 ^{ns}	0.030 ^{ns}	34.3 ^{ns}	23.8 ^{ns}	0.979
T4	3.8	2.8	22.1	0.05	97.4	0.027	37.8	26.2	0.982
20–30 cm									
T1	4.3 ^{ns}	3.6 ^{ns}	21.5 ^{ns}	0.05 ^{ns}	96.9 ^{ns}	0.025 ^{ns}	42.1 ^{ns}	29.2 ^{ns}	0.965
T4	3.9	3.1	20.6	0.05	97.3	0.024	41.9	29.1	0.967
Charcoal									
	0.73 (0.09) ¹	0.23 (0.04)	21.9 (0.00)	0.05 (0.00)	99.8 (0.05)	0.012 (0.00)	87.0 (8.19)	60.3 (5.68)	0.998

Values represent means of four replicates, and ns indicates t-test results of $p > 0.10$. ¹ Values within parentheses refer to the standard deviation of the charcoal data. * Coefficient of determination of the fit correlating the cumulative C loss versus time according to a double exponential decay model. TC: total carbon.

Table 3. Comparison of the relative intensity distribution (%) of Solid-State ¹³C NMR spectra, before and after 165 days of incubation, of the charcoal and of the Cambisol samples collected at 0–5 and 10–20 cm depth after charcoal application.

Treatments ¹	Incubation	Carboxyl C	Aryl C	O-Alkyl C	N-Alkyl C	Alkyl C
		220–160	160–110	110–60	60–45	45–0
%						
0–5 cm						
T1	Before	6.5	12.3	38.3	12.9	30.0
	After	8.4	12.7	35.2	9.0	28.3
	Before-after	–2	0	3	4	2
T4	Before	8.7	22.9	33.1	8.7	24.8
	After	8.8	25.4	31.6	8.3	24.0
	Before-after	0	–2.5	1.5	0	1
10–20 cm						
T1	Before	6.5	13.4	37.0	12.4	30.7
	After	9.5	13.4	33.8	8.7	30.5
	Before-after	–3	0	3	4	0
T4	Before	7.5	15.4	34.8	11.3	30.9
	After	9.2	15.5	32.0	8.4	31.1
	Before-after	–2	0	3	3	0
Charcoal						
	Before	6.1	78.3	8.0	2.7	4.9
	After	6.7	66.6	11.6	3.8	10.5
	Before-after	–1	12	–4	–1	–6

¹ T1 = 0 Mg ha^{−1} (control); T4 = 40 Mg ha^{−1}.

The lower mineralization of the charcoal (0.73%) in comparison to that of soil samples (from 3.9 to 6.5%) could be assigned to the higher aryl C and lower O-alkyl and N-alkyl C proportions in the charcoal compared to the SOM (Table 3) assuming that aryl C compounds are more resistant to biodegradation than O-alkyl and N-alkyl C [58–60].

The proportion of the fast SOM pool (A_1) to the TC ranged from 3.1 to 3.7% in T1 and from 2.8 to 3.1% in T4 (Table 2), and charcoal did not affect this SOM compartment. The charcoal is mainly composed of less labile organic compounds, as aryl C compounds, which accounted for 78% of total ^{13}C intensity in the charcoal NMR spectrum (before incubation) (Table 3). Labile compounds are less representative in charcoal materials, and possibly these compounds were degraded within the 20 months of field experiment and exposition of charcoal to weathering. In fact, relatively rapid oxidation of charcoal after entering the soil has been reported in the literature, especially for those materials produced under mild temperatures [25,43,47], as in the case of the charcoal used in this study [27].

The mineralization rate of the fast SOM pool (k_1) for T1 and T4 ranged from 19.5 to 23.3 year^{-1} and for charcoal alone was 21.9 year^{-1} (Table 2). Differences between T1 and T4 k_1 values were not observed regardless of the soil depth (Table 2). In this way, T1 and T4 did not differ with respect to the mean residence time of the fast SOM pool (MRT_1). MRT_1 for soil samples varied from 0.04 to 0.05 years and were comparable to the MRT_1 of the charcoal alone (0.05 years), reinforcing that labile charcoal-derived compounds undergo similar decomposition as labile SOM compounds. Similar MRT_1 values (0.03 to 0.05 years) were reported by authors evaluating the biodegradability of the SOM in fire-affected and unaffected soils from Southern Spain [25].

In charcoal samples, the amount of C more stable against mineralization (A_2) accounted for 99.8% of the TC (Table 2). In the soil samples, regardless of the treatment, values were high as well, varying between 96 and 97% (Table 2). Similar A_2 values (91 to 96%) were reported by other researchers after 206 days incubation of fire-affected and unaffected soil samples [25].

The k_2 observed for pure charcoal (0.012 year^{-1}) was about 2 to 5-fold lower than that observed for soil samples (from 0.024 to 0.066 year^{-1}), indicating that for this SOM pool, the chemical composition of the C source is relevant. However, when applied to the soil, even at a rate of 40 Mg ha^{-1} , which is a considerably high amount of charcoal application to the soil, the charcoal did not promote higher A_2 and k_2 values in T4 compared to T1 (Table 2). More remarkable effect in A_2 values due to charcoal application was expected due to a coupled effect resulting from: i) charcoal particles concentration at 0–5 cm [27] and ii) the aromatic character of the charcoal (78% aryl C contribution, Table 3), which is supposed to confer slow biodegradability to this material. Mean residence time of the slow pool (MRT_2) for pure charcoal was of 87 years, about 2 to 6-fold higher than those observed for T1 and T4, from 15.7 to 42.1 years (Table 2). However, charcoal addition did not increase MRT_2 in T4 compared to T1 despite the high aryl C intensity in the charcoal ^{13}C NMR spectra (Table 2). Yet, based on the MRT_2 of the charcoal alone, a more relevant increase of MRT_2 for T4 samples was expected. As discussed before, labile organic compounds from SOM possibly stimulated soil microorganisms to degrade charcoal [31,55]. Although not statistically significant, MRT_2 at 0–5, 5–10, and 10–20 cm depths tended to be higher in T4 than in T1, suggesting the influence of the added charcoal on the greater MRT_2 values. At 20–30 cm depth, MRT_2 values observed for T1 and T4 were similar. In fact, charcoal particles were mainly concentrated within 0–20 cm depth and not evidenced at 20–30 cm depth [27], justifying why such tendency was restricted to the first 20 cm depth. One alternative to increase SOM MRT_2 more consistently aiming C sequestration into the soil through charcoal fines addition would be to increase charcoal doses applied to the soil, i.e., higher than 40 Mg ha^{-1} in this case. However, it is important to consider that applying higher doses of charred material to the soil, on the other side, could be prejudicial to soil biota, plant growth, and food chain, a part of the uncertain effects of these materials to the environment in the long-term. Also, improper deposition of such materials could occur as a result of water erosion in charcoal or biochar-amended soils [61–64].

The $t_{1/2 \text{ long}}$ of the charcoal alone (60.3 years) was 2 to 6-fold higher than those of soil samples, which ranged from 10.9 to 29.2 years (Table 2). Regardless of the soil depth, $t_{1/2 \text{ long}}$ values did not differ between T1 and T4 (Table 2). Similar to MRT_2 , $t_{1/2 \text{ long}}$ values tended to be greater in T4 within 0–20 cm depth, most probably due to the concentration of charcoal particles up to 20 cm depth, as discussed previously. At 20–30 cm, $t_{1/2 \text{ long}}$ values were quite similar in T1 and T4, 29.2 and 29.1 years, respectively, corroborating suppositions that large charcoal fragments, which are less prone to fast degradation and leaching [65], were not relevantly transported to depths below 20 cm [27].

3.3. Alteration of SOM and Charcoal Chemical Composition after Incubation

The ^{13}C NMR spectrum of the charcoal alone before the incubation experiment was dominated by the ^{13}C intensity band corresponding to the aryl C region, which accounted for 78% of the total ^{13}C intensity (Table 3). The other signals found in the charcoal ^{13}C NMR spectrum, and their contribution to the total ^{13}C intensity were: O-alkyl C –8%, carboxyl C –6%, alkyl C –5%, which can be assigned to short alkyl chains from lignin that remained in the charcoal after the mild charring process [40], and N-alkyl C –3% (Table 3). The low signal in the 100–60 ppm region (O-alkyl C) can be assigned to aromatic C compounds, which may have caused resonance lines mainly in the 110–90 ppm region or to the few easily decomposable structures, as carbohydrates from *Mimosa scabrella* trees, which may have resisted to the charring [40,59]. After 165 days of incubation, the ^{13}C NMR spectrum of the charcoal exhibited the same pattern as prior to incubation (Figure 3). However, the contributions of the chemical groups to the total ^{13}C intensity were altered, despite the low mineralization of the charcoal during the incubation experiment (0.73% of C loss of the initial TC). The aryl C contribution after the incubation period decreased to 67%, suggesting that some microorganisms were able to use the aromatic compounds as a C source. This event might have been magnified when charcoal was mixed with soil (in the field), at least partially explaining why charcoal did not increment SOM resistance against biodegradability, especially MRT_2 . Consequently, after incubation, the relative contribution of the alkyl C increased from 5% to 11% and that of O-alkyl C from 8% to 12% (Table 3).

The ^{13}C NMR spectra of T1 and T4 samples, before and after incubation experiment, presented the same pattern and differed markedly from that of charcoal (Figure 3). Soil samples spectra were dominated by the ^{13}C intensity in the O-alkyl C chemical shift region (30 to 38% of the total ^{13}C intensity), which is assigned mainly to carbohydrates from the microbial biomass and plant residues [59,66]. Due to the aromaticity of the charcoal and its concentration near to the soil surface, higher aryl C ^{13}C intensity in T4 compared to T1 was expected, but difference of aryl C contribution to the total ^{13}C intensity in T4 and T1 was only 2.5% (Table 3), which can more likely be attributed to instrumental variations and samples heterogeneity. These results suggest that despite the aromaticity and thermostability of the charcoal fines [27], once they were applied to the Cambisol (40 Mg ha^{−1}), they were not able to increment aryl C contribution to the SOM composition, probably due to the susceptibility of this material to biodegradation. At 10–20 cm depth, relevant differences in ^{13}C NMR spectra between treatments, before incubation experiment, were not evidenced as well. Decrease of aryl C contribution to the total ^{13}C intensity from 46% to 23%, when PyC amended A-horizon samples were collected four weeks and 7 years after forest fire in Southern Spain, was recently reported in the literature and attributed to the microbial decomposition, leaching, or erosion of such material [67]. These findings reinforce that PyC can suffer rapid microbial decomposition into the soil, and moreover, that the assumption of PyC as a stable C compartment in the soil should be taken carefully.

After 165 days of incubation, the ^{13}C NMR spectra of T1 and T4 samples were not altered compared to the spectra before incubation, regardless of the soil depth, and ^{13}C intensity variations of up to 4% within chemical shift regions (Table 3) were attributed to instrumental aspects. In general, these results indicate that preferential preservation of charcoal derived-aromatic structures against degradation did not occur as could be expected when assuming charcoal as a highly recalcitrant material. Moreover, aryl C ^{13}C intensity enrichment in T4 after incubation was not observed, supporting why charcoal did not increment MRT_2 , as discussed before.

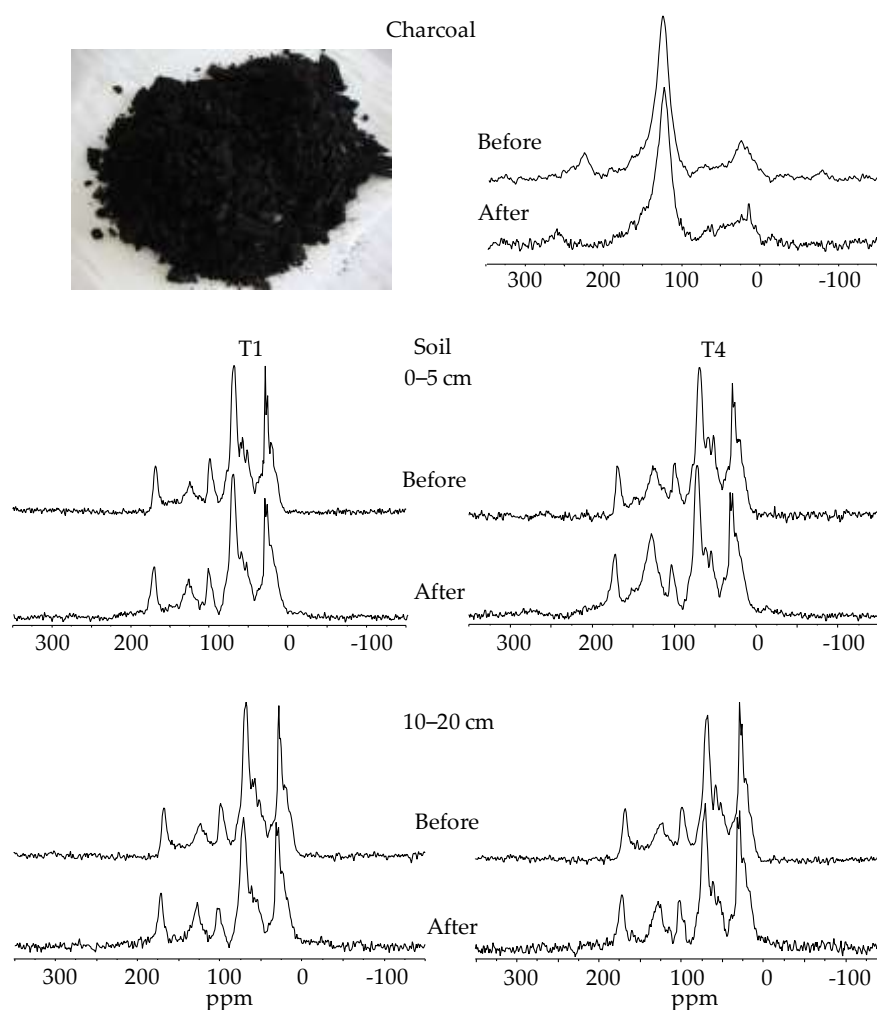


Figure 3. Charcoal illustration and Solid-state ^{13}C NMR spectra of the HCl-treated charcoal alone (not mixed with soil) and of HF-treated samples of a Cambisol without charcoal (T1) and with charcoal -40 Mg ha^{-1} (T4) application, before and after 165 incubation days, at 0–5 and 10–20 cm depth.

4. Conclusions

The charcoal fragments located at 0–5 cm depth were preferentially accumulated in the humin fraction, most probably due to the charcoal hydrophobic character. The greater content of humic acids and fulvic acids in the charcoal amended soil was related to charcoal oxidation and to the effect of the charcoal on endogenous SOM humification dynamics.

Despite the concentration of charcoal particles at 0–5 cm depth, leading to carbon content increase (as humin) and the aromaticity of the charcoal verified by ^{13}C NMR, charcoal did not increment the slow soil organic matter pool. Apparently, the availability of labile organic compounds from SOM stimulated the biodegradation of charcoal aromatics when charcoal was applied to the soil and suffered weathering. Preferential preservation of aromatic structures in the charcoal-amended soil after incubation was not evident, supporting such interpretations.

Overall, our findings suggest that incorporation of charcoal fine residues at a rate of 40 Mg ha^{-1} to a subtropical Cambisol was not an efficient strategy to promote carbon sequestration in the soil and that this material could be preferentially used as a means to improve soil chemical agronomical attributes.

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