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Abstract

Dissolved organic matter (DOM) is an important organic matter fraction that plays a key role in many biological and chemical processes in soil. The effect of biochar addition on the content and composition of soil organic matter (SOM) and DOM in an agricultural soil in central Italy was investigated within a two-year period. UV-Vis spectroscopy and analytical pyrolysis have been applied to study complex components in DOM soil samples. Additionally, analytical pyrolysis was used to provide qualitative information of SOM at molecular level and the properties of biochar before and one year after amendment. A method was developed to quantify biochar levels by thermogravimetric analysis that enabled to identify deviations from the amendment rate. The water-

soluble organic carbon (WSOC) in the amended soils were significantly lower than those in the control soils, indicating that biochar decreased the leaching of DOM. DOM in treated soils was characterized by a higher aromatic character according to analytical pyrolysis and UV-Vis spectroscopy. Moreover, a relatively high abundance of compounds with N was observed in pyrolyzed of treated soils, suggesting that biochar increased the proportion of microbial DOM. The results from thermal and spectroscopy techniques are consistent in highlighting significant changes in DOM levels and composition due to biochar application with important effects on soil carbon storage and cycling.

Keywords: Biochar; Dissolved organic carbon; Py-GC-MS; Soil amendment; PAH

1. Introduction

Biochar soil amendment continues to receive worldwide interests for integrated agricultural/environmental strategies to build soil, enhance water quality, and increase agricultural productivity while sequestering C and thus mitigating global climate change (Lehmann and Joseph, 2015; Woolf et al., 2016; Purakayastha et al., 2019; Majumder et al., 2019; Giagnoni et al., 2019). The agronomic and environmental impacts of biochar, and especially its potential as a C sequestration strategy, require a full comprehension of its effects on native soil organic matter (SOM). However, while there can be no doubt that the application of biochar to soils increases the recalcitrant fraction of soil organic carbon (SOC), little is known about how biochar addition affects SOM composition, especially dissolved organic matter (DOM). Moreover, understanding the real or possible benefits and drawbacks of using biochar in agroenvironmental management requires knowledge of quantity of biochar remaining in soil (Koide et al., 2011; Dong et al., 2017). A variety of thermal and chemical soil analysis methods have been used for biochar quantification (Raya-Moreno et al., 2017), and the most suitable methods for its assessment are still under debate (Nakhli et al., 2019).

DOM, the more mobile and bioavailable fraction of organic matter in soil, is commonly defined as a continuum of organic molecules of different sizes and structures that pass through a filter of 0.45 μm pore size, including dissolved organic carbon (DOC), dissolved organic nitrogenous and dissolved organic phosphorus compounds (Song et al., 2020). DOM is a major form of organic matter and is made up of a small amount of organic acids, sugars, amino acids and humic substances (Nebbioso et al., 2013; Kalbitz et al., 2000). DOM plays a key role in soil aggregation (formation of organometallic complexes), energy source for microorganisms, as well as C storage, cycling, and provision of plant-available nutrients. The change of DOM contents is affected by many anthropogenic and natural factors. Especially DOM is dynamically balanced with complex processes in farmland soil.

The incorporation of biochar into soil systems represents an input of biochar-derived DOM that could play an important role in the carbon dynamics and microbial communities in soil. Once applied in the field, biochar could change the content and composition of soil DOM (Smebye et al., 2016; Liu et al., 2019; Feng et al., 2021). In recent years, biochar addition effects on DOM have attracted considerable attention of researchers. However, the results seem to be contradictory. For instance, Smebye et al. (2016) in a batch experiment found that biochar could increase the leaching of DOM from soil, as well as change the DOM composition towards molecules with a larger size and higher aromaticity by sorbing smaller aliphatic species to its micropores. Such effect was also observed in field experiments (Zhang et al., 2017; Liu et al., 2019). In these studies, DOM released from biochar and biochar induced increase of soil pH and hydraulic conductivity were presented as possible mechanisms responsible for the increase in DOM content. On the other hand an opposite effect due to biochar amendment has also been reported. Eykelbosh et al. (2015) in a column experiment found that biochar amended soil attenuated DOM leaching and the biochar preferentially retained high-molecular weight, humic-like DOM species. Dong et al. (2019), in a long-term field experiment, proposed that biochar had little effect on soil DOM content. Differences in the results among these studies are primarily attributed to the largely variable properties of different biochars and soils. In fact, some findings have confirmed that the content, composition and characteristics of biochar DOM

78 are related to the biochar source, its preparation process and extraction and used analysis methods (Li
79 et al., 2017; Liu et al., 2019; Huang et al., 2019). For instance, the pyrolysis temperature is a critical
80 factor affecting the balance of release and adsorption, with the biochars produced at lower
81 temperatures (<400°C) increasing DOM content mainly by releasing indigenous DOM, while
82 biochars produced at higher temperatures (>700°C) decrease DOM content mainly by adsorbing soil
83 DOM (Feng et al, 2021). Feedstock type seemed to be a less important factor according to the results
84 of this study, though it could affect the release and adsorption of DOM by biochar to a certain extent.
85 The biochar impact on soil DOM is a multi-factor problem related to biochar characteristics, soil
86 properties as well as experimental conditions. Given the complexity of DOM several advanced
87 techniques were used to provide information about relevant changes due to biochar application:
88 namely UV-Vis spectroscopy (Zhang et al., 2020), fluorescent excitation-emission matrices (EEMs)
89 with parallel factor (PARAFAC) analysis for UV-fluorescence spectroscopy (Fan et al., 2020),
90 Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (Zhang et al., 2020), Liquid
91 Chromatography-Organic Carbon Detection (LC-OCD), Gas Chromatography-Mass Spectrometer
92 (GC–MS) (Taherymoosavi et al., 2016), high-resolution Orbitrap mass spectrometer (Orbitrap MS)
93 (Pan et al., 2020) and Nuclear Magnetic Resonance (NMR) (Bi et al., 2021).
94 Among available techniques, Py-GC-MS is a useful tool that allows a direct investigation of DOM,
95 providing information on molecular structures thereof, and was previously used for the study of
96 biochar-derived DOM. Nonetheless, Py-GC-MS was never used to investigate the structure of soil
97 DOM consequential to biochar application experiments.
98 The objective of the present work is to increase the present knowledge on the impacts of biochar
99 additions on the organic carbon pool in treated agricultural soils by an array of different analytical
100 techniques. The changes of organic carbon (total and recalcitrant) in agricultural soils with repeated
101 treatments have been previously investigated in a field experiment previously (Rombolà et al., 2015;
102 Rombolà et al., 2019). These studies have not addressed the effects on the chemistry of the more
103 mobile and bioavailable fraction of soil organic matter, as the water-soluble fraction. For this purpose,

this study investigated the SOM and DOM evolution in agricultural soils amended with two different doses of biochar obtained by a two-year field experiment, studying quantity and quality characteristics by a multi-methodological approach. In order to evaluate the real effect of the biochar content on OM and DOM composition of the soil, a quick, accurate and robust method was developed to quantify the biochar in amended soils by thermogravimetric analysis (TGA). This study reports for the first time the combined application of Py-GC-MS and spectroscopic analysis to soil samples deriving from a multi-annual time-scale biochar incubation experiment of a cultivated soil to assess the effect of biochar amendment on the characteristics of soil DOM. Additionally, Py-GC-MS was used to provide qualitative information on SOM and to study the properties of biochar before and one year after amendment.

2. Materials and Methods

2.1. Field experiment

The field experiment was conducted over two consecutive growing seasons (2016/2017 and 2017/2018) at the "Ganazzoli Filippo" farm (Parma, Emilia-Romagna, Italy) on an agricultural soil (named as GA) classified as clay (USDA, 2005) textured with 7.7% sand, 37.1% silt and 55.2% clay. The soil characteristics were as follows: pH 8.14±0.01, total C 3.64±0.09%, total N 0.19±0.02%, total H 0.99±0.02%, and a cation exchange capacity of 37.2 mequiv 100 g^{-1}. The amount of total organic carbon (TOC) present in the agriculture soil is of 2%, in the typical range of common SOC concentrations.

The biochar employed (named as PSR) in the amendment of agricultural soils is a commercially available biochar derived by pyrogasification of forest wood and brushwood waste (Borgo Val di Taro, Italy). The experimental design consisted of a randomized complete block with plots of 7.5 m^2 (5 × 1.5 m), considering ten treatments: two controls without biochar (GA17-T sampled in 2017 and GA18-T sampled in 2018) and soil treated with different amount of biochar PSR: 15 t ha^{-1} (GA17-15), 30 t ha^{-1} (GA17-30), 45 t ha^{-1} (GA17-45) and 60 t ha^{-1} (GA17-60) of biochar applied in 2016 and

sampled in 2017; 30 t ha^{-1} (GA18-30), 60 t ha^{-1} (GA18-60), 90 t ha^{-1} (GA18-90) and 120 t ha^{-1} (GA18-120) of biochar applied two times, in 2016 and 2017 at the same rate and sampled in 2018. There were four plots per treatment, resulting in a total area of 30 m^2 for each. The biochar was incorporated manually into the top 20 cm of soil immediately, using a hand hoe, to leave an apparently uniform distribution.

Control and soil treated by six levels of biochar amendment were sampled for analysis in December 2017 and May 2018 in five randomly chosen points in the space of each replicate by means of soil core sampler at 0-30 cm. A total of forty samples were collected from soil depths of up to 30 cm. Forty subsamples (one for each plot, ~ 50 g) were prepared and examined, each subsample was dried at 40 °C, sieved (mesh size: 2 mm) to obtain homogeneous samples free of stones, larger roots, wood sticks and other coarse fragments, and stored at – 20.

Composite samples were prepared (about 4 g) for each different amount of biochar added to soil, by joining about 1 g of subsamples taken from the different batches. The composite sample was homogenized in a mortar prior to analysis. After the aging period, the aged biochar (BC_{aged}) in soil was extracted by the method described in Dong et al., 2017. Biochar particles with diameter > 2 mm from samples GA17-45 and GA17-60 were separated by forceps until no visible biochar particles were present in soil samples. The recovered biochar particles were further separated from the soil by rinsing with deionized water at a ratio of 1:10 (w/v) and shaken slightly to remove adhering soil particles. This procedure was repeated four times and then the biochar dried at 60 °C. Both fresh and aged BC were ground to <2 mm prior use, to aid in homogenization.

The TOC content in soil treated and untreated was analyzed by a TOC analyzer (mod. SSM 5000A, Shimadzu) and calculated as the difference between total carbon (TC) and total inorganic carbon (TIC). The carbon, hydrogen, nitrogen and sulfur (C, H, N and S) contents of the biochars and soils were determined by combustion using a Thermo Scientific FLASH 2000 Series CHNS/O Elemental Analyzer (Thermo Fisher Scientific, Waltham, U.S.A.) (Rombolà et al., 2015). The biochar samples were acid tested for the presence of carbonates as described in Rombolà et al., 2016. The carbonate

content of each biochar was determined on triplicate samples by comparing TOC measured after hydrochloric acid (HCl) treatment and total carbon.

The ash content of the biochars was determined as the residual mass left after exposure at 600 °C for 5 h. The oxygen content of the biochars was calculated from the mass balance: Oxygen (%) = 100 − Ash content (%) − C (%) − H (%) − N (%) − S (%). The pH of the biochar and soil samples was measured at room temperature with a digital pH meter (VWR pH100, VWR International) in a 1:10 and 1:2.5 soil:water suspension, respectively. Additional details about the properties of the PSR can be found in Marmiroli et al. 2018.

2.2. Development of the method for quantitative analysis of biochar in soil

2.2.1. Samples

An internal reference biochar sample (MSP) was utilized for optimization of the method for biochar determination in soil by TGA. The MSP biochar is a standard biochar purchased from the UK Biochar Center, University of Edinburgh. MSP was obtained from pyrolysis of Miscanthus at 700 °C (UK Biochar Research Centre, 2018). Other two available biochars already characterized were used in the tests (Table 1). The first one (RB) is a standard biochar obtained by a pilot up-draft gasification plant from commercial wood pellet (700-800°C) developed in the framework of the research project RIFASA (Regione Emilia-Romagna); temperature was between 700 and 800 °C. The second one is a biochar highly characterized (BC1) (Bachmann et al., 2016) obtained from pyrolysis of shavings from wood chip production at 620 °C.

Biochar samples were thoroughly homogenized and oven-dried at 40 °C for 72 h and stored at − 20 °C prior to analysis. Proximate analysis was performed according to ASTM D7582 method (ASTM, 2015) with slight modifications with a thermogravimetric analyzer (Mettler Toledo TGA /SDTA 851e).

Soil samples were provided by “Azienda Agricola Sperimentale Stuard”. The soils were sampled from agricultural soils of the “Azienda Agricola Querzola” (Parma, Emilia-Romagna, Italy) and of

182 the " Azienda Agricola Sperimentale Tadini" (Podenzano, Emilia-Romagna, Italy) by means of soil
183 core sampler at 0-30 cm. Specifically, two different types of agricultural soil were used, a carbonate-
184 rich (Q) and a carbonate-poor soil (T) from Querzola and Tadini Farm, respectively. They were
185 prepared as indicated in CEN EN 16179 method (European Standards, 2012). Briefly, samples were
186 air dried and extraneous materials were removed (e.g., wood sticks and stones). Then, samples were
187 homogenized and a subsample was crushed to obtain a smaller granulometry.
188 Additionally, some standards were analyzed for comparison purpose: D-(+)-Glucose (≥ 99.5% w/w),
189 Sigma Aldrich; Calcium carbonate (≥ 99%), Merck and, soot, which was generated in our laboratory
190 by incomplete combustion of acetylene.
191
192 **2.2.2. Analysis of biochar in soil by TGA**
193 Calibration standards containing 1.0, 2.0, 5.0 and 10% of MSP biochar were prepared by adding
194 different amount of MSP to soil samples. The mix was homogenized in a mortar before the analysis.
195 Thermogravimetric analysis was conducted by introducing sample aliquots ranging from 10 to 15 mg
196 in a 70 µL alumina crucible. The incremental mass changes (resolution of 0.0001 mg) recorded over
197 this program were processed using STARe software version 9.10. Samples were ramped under an
198 oxidant atmosphere (air, 90 mL min^{-1}) at 10 °C min^{-1} to 600 °C for organic material (OM)
199 determination, then they were ramped at 25 °C min^{-1} to 850 °C for carbonate determination (Kasozi
200 et al., 2009). The heating rates of 10 °C min^{-1} and 25 °C min^{-1} were chosen because they provided
201 the optimal resolution in soils.
202 In order to better interpret the obtained peaks, MSP and PSR biochars were also analyzed with the
203 same thermal program.
204
205 **2.2.3. Testing the method by quantitative analysis of biochar in field soil**
206 The method developed for biochar determination in soil was tested by comparing actual and
207 calculated biochar contents of approximately 10 g samples of field soil (GA) to which we added

varying amount of biochar. In particular, calibration standards containing 0.20, 0.50, 1.0, 2.5 and 5.0% (w/w) of biochar each were prepared by adding different amount of biochar PSR to control GA soil composite sample. The mix was homogenized in a mortar before the analysis. GA control composite sample represents the level 0% of biochar concentration in the calibration curve.

In addition to the five concentrations of biochar in soil (above), duplicate samples of 100% soil (no biochar) and 100% biochar (no soil) were also prepared in order to determine the proportion of control soil and of biochar that is lost in the temperature range of recalcitrant OM (390-600 °C).

2.3. Characterization of organic matter in soil and biochar amended soil

2.3.1. Quantitative analysis of carbon content

The ability of two different dry combustion methods to determine carbon C content was evaluated in model soil/biochar systems. Specifically, TOC analyser (method 1) and HCN analyser (method 2) were applied to determine total carbon (TC), TOC and TIC in agriculture soils, with low (T) and high (Q) content of IC, mixed with biochar at 1.00 % wt. level. Detailed descriptions of each method are provided in Supplementary Materials. The method 1 were selected for the determination biochar influence on TOC levels in agricultural soil of field experiment. Briefly, TOC content was determined with a TOC analyzer (mod. SSM 5000A, Shimadzu, Japan) and calculated as the difference between TC and TIC. Sample was placed in a ceramic boat and introduced in the combustion tube using IR detection and calibration with D-(+)-glucose and Na_2CO_3 for TC and TIC, respectively. Carbonate-rich soils (TIC > 20%) showed slow combustion rates or thermal decomposition reactions causing underestimated TC values, because the time limit of instrument analysis is shorter than combustion time. Therefore, these samples were mixed with V_2O_5 catalyst in order to accelerate the combustion rate.

2.3.2. Molecular characterization of organic matter by analytical pyrolysis

The molecular composition of SOM in agricultural soil treated with different concentrations of biochar was assessed with pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). Py-GC-MS was performed using an EGA/PY-3030D micro-furnace pyrolyser (Frontier Laboratories Ltd., Japan) coupled with a 7890 Agilent HP gas chromatograph (GC) connected to a 5977 Agilent HP quadrupole mass spectrometer (MS) (Agilent Technologies, USA). Unaltered ground samples (25±0.1 mg) were placed in small crucible capsules and introduced into the furnace, which was preheated at 500 or 900 °C for 1 min using helium as carrier gas (1 mL min^{-1}) and an interface temperature of 280 °C. The evolved gases were then directly injected into the GC-MS for analysis. The GC injector was operated in split mode with a 10:1 ratio at 280 °C. Pyrolysis products were separated by a HP-5MS fused silica capillary column (stationary phase poly[5% diphenyl/95% dimethyl]siloxane, 30m × 0.25mm i.d., 0.25mm film thickness, Agilent Technologies, USA) with the following temperature program: 45 °C to 300°C at 10 °C min^{-1}, then a hold for 5 min at 300 °C, using helium as carrier gas (1 mL min^{-1}). The MS was operated in EI positive mode (70 eV, scanning 29-600 m/z) with transfer line temperature 250 °C, ion source temperature 230 °C and quadrupole temperature 150 °C. The relative abundance of each pyrolysis product was calculated as the percentage of their signal relative to the 18 most abundant peaks, using the main ions (m/z) of each product.

2.4. Characterization of DOM in soil and biochar amended soil

2.4.1. Extraction of DOM

Water-soluble organic matter (WSOM) was extracted by a water extraction method previously described in Ghidotti et al., 2017. Briefly, soils were air-dried and ground to pass through a 2 mm mesh before WSOM extraction. A soil/water ratio of 1:10 (g mL^{-1}) was used to extract soil WSOM by ultrapure water from a Millipore Direct-Q 5 UV system (18.2 Ω·cm, Merck KGaA, Darmstadt, Germany). The WSOM was extracted by shaking at 150 rpm and 25°C for 72 h. Afterward, the resulting solutions were centrifuged at 4000 rpm for 8 min, and the supernatant was filtered using a

pre-washed PTFE syringe filter 0.45 μm (Whatman Inc., Maidstone, UK). The filtered solution was stored at 4 °C until the dissolved organic carbon (DOC) and the total nitrogen (TN) concentrations of the WSOM were measured with a Shimadzu TOC-L analyzer. For molecular characterization by analytical pyrolysis, solid DOM samples were prepared by freezing a subset of WSOM solution (20 mL) overnight followed by freeze-drying.

2.4.2. Quantitative analysis of DOC and TN

Concentrations of DOC and TN were determined with a Shimadzu TOC-L series analyzer coupled with the TN-module (Shimadzu Corp., Kyoto, Japan). Quantification of each analysis is presented here as mean of three to four injections of 50 μL, where the coefficient of variance for the replicate injections was < 2%. The WSOC (mg $g^{-1\ TOC}$) contents of the control and the biochar amended soil were determined as the proportion of the total SOC pool that was extractable by the water extraction method and they were calculated using the following equation (1):

$$(1)\ WSOC\left(\frac{mg\ DOC}{g\ SOC}\right) = \frac{V\ (L) * C\ (\frac{mg}{L})}{TOC\ \left(\frac{g}{Kg}\right) * M\ (Kg)}$$

where *V* is the volume of water (L) in each extraction procedure, *C* is the DOC concentration (mg L^{-1}) in the sample, TOC is the organic carbon concentration (mg Kg^{-1}) in soil sample and *M* is the mass of soil sample (Kg) in each extraction procedure.

2.4.3. Spectroscopic analysis of DOM

The absorbance of DOM was determined within a spectrum of 200–600 nm using a Cary 300 UV-Visible Spectrophotometer (Agilent Technologies). Quartz cuvettes (1 cm) were used for this purpose

and properly cleaned before each use. Ultrapure water (18.2 Ω·cm) was used as a reference. The values of $SUVA_{254}$ (L mg^{-1} m^{-1}) were measured using Eq. (2).

(2) $SUVA_{254} = \frac{a_{254}}{DOC}$

where a_{254} is the absorption coefficient at wavelength 254 nm. The a_{254} indexes the DOM aromaticity and was calculated using the Eq. (3).

(3) $a_{254}\ (m^{-1}) = \frac{UV_{254} \times 2.303}{l\ (m)}$

In the above equation, UV_{254} is the UV/Vis absorbance at a wavelength of 254 nm, 2.303 is the transform coefficient, and *l* is the cell pathlength in meters (Green et al., 1994; Li et al., 2018).

2.4.4. Analytical pyrolysis of DOM

Analytical pyrolysis (Py-GC-MS) of DOM was performed using an EGA/PY-3030D micro-furnace pyrolyser (Frontier Laboratories Ltd., Japan) coupled with a 7890 Agilent HP gas chromatograph (GC) connected to a 5977 Agilent HP quadrupole mass spectrometer (MS) (Agilent Technologies, USA). About 1 mg of DOM sample was placed into a pyrolysis stainless-steel cup and inserted into the microfurnace. Analyses were performed with a pyrolysis temperature of 600 °C and a pyrolysis time of 1 min using helium as carrier gas (1 mL min^{-1}) and an interface temperature of 280 °C. The GC injector was operated in split mode with a 10:1 ratio at 280 °C. Pyrolysis products were separated by a HP-5MS fused silica capillary column (stationary phase poly[5% diphenyl/95% dimethyl]siloxane, 30m × 0.25mm i.d., 0.25mm film thickness, Agilent Technologies, USA) with the following temperature program: 45 °C to 300°C at 10 °C min^{-1}, then a hold for 5 min at 300 °C, using helium as carrier gas (1 mL min^{-1}). The MS was operated in EI positive mode (70 eV, scanning 29-

600 m/z) with transfer line temperature 250 °C, ion source temperature 230 °C and quadrupole temperature 150 °C. The relative proportions of each pyrolysis product were calculated as the percentage of the sum of all peak areas (total quantified peak area, TQPA). Quantification of each pyrolysis product was based on the peak area of specific m/z fragments.

2.5. Statistical analysis

Mean and standard deviation of three replicates were used to compare results of soils and biochar amended soils. All statistical analyses were done in the statistical environment R using analysis of variance (ANOVA) conducted with R software version 4.0.5 (2021-03-31) followed by Tukey's post hoc tests to evaluate statistically significant differences between control and biochar amended soils and between sampling periods. The difference between the treated soils and the control was evaluated with Dunnett's test ($p < 0.05$).

3. Results and discussion

3.1. Soil and biochar characterization

The characteristics of the biochar used for soil amendment are reported in Table 1. The molar H/C and O/C ratios of 0.10 and 0.08, respectively, indicate a high degree of carbonization and aromaticity (Conti et al., 2014). The C content of fresh biochar decreases to 56% with an aging period of 1 year in the field (Table 1). Therefore, through the aging process, biochar C content significantly decreases by 4% ($p < 0.05$). Similarly, N and ash content of aged biochar were significantly lower than fresh biochar, whereas H content of aged biochar (0.94%) was significantly higher than fresh biochar (0.50%).

Table 1. Elemental analysis (oxygen by difference), ash content, molar ratios and pH of field soil, fresh and aged biochar applied in the field experimental from samples GA17-45 and GA17-60 (mean values ± sd, n = 3).

Parameters	Units	Fresh biochar PSR	Aged biochar PSR	Control soil GA
TC	%	60±1.5	56.3±0.4	3.20±0.09
TOC	%	58±1.1	54.8±0.8	2.0±0.2
TIC	%	1.8±0.4	1.5±0.8	1.2±0.2
N	%	0.21±0.02	0.161±0.002	0.19±0.03
H	%	0.50±0.01	0.94±0.08	0.99±0.10
S	%	n.d.	n.d.	n.d.
O	%	6.1±1.9	15±0.7	-
Ash	%	33.2±1.2	27.6±0.5	-
H/C	atomic	0.10	0.24	3.3
O/C	atomic	0.08	0.20	-
pH		9.95±0.01	-	8.14±0.01

The chemical and physical characteristics of the control soil and biochar amended soils (15, 30, 45, 60, 90 and 120 t ha^{-1}) during the two years since the first application of biochar are reported in Tables 1 and 2. The soil pH has been slightly modified by the amendments in both years (soil 2017 and 2018). The pH of the soil in the first year after application increased with the concentration of biochar amendment, from 8.14 in the control soil to 8.37 in the biochar amended GA17-45 and in the second year from 8.24 in the control soil to 8.54 in the biochar amended GA18-120 (Table 2). The increase in soil pH after the application of biochar is attributed to the alkaline substances in biochar with high degree of carbonization (Conti et al., 2014). Several studies showed that biochar increases the soil pH (Ding et al., 2016; Rombolà et al., 2019). However, the application of biochar with lower pH than the targeted soils might have the potential to decrease soil pH, especially with higher biochar application rates. In these cases, acidic materials produced by the oxidation of biochar and organic matters may have caused the pH decrease (Liu et al., 2012).

Table 2. Chemical characteristics of the control soil and biochar amended soils (15, 30, 45, 60, 90 and 120 t ha^{-1}) in the field experiment at different sampling dates. Mean values ± s.d. (n = 3).

Samples	Load (%)	N (%)	H (%)	TOC (%)	C/N	pH

GA17-T	0.0	0.245±0.004	1.05±0.03	1.9±0.2	13.0	8.14±0.01
GA17-15	0.42	0.256±0.007	1.02±0.04	2.2±0.2	13.3	8.20±0.01
GA17-30	0.84	0.261±0.006	1.00±0.04	2.9±0.9	16.1	8.30±0.01
GA17-45	1.26	0.260±0.004	1.06±0.04	3.0±0.8	16.5	8.37±0.02
GA17-60	1.68	0.246±0.003	1.02±0.07	2.4±0.5	15.5	8.29±0.01
GA18-T	0.0	0.230±0.001	1.05±0.06	2.0±0.1	15.3	8.24±0.01
GA18-30	0.84	0.221±0.007	1.00±0.07	2.4±0.2	18.5	8.28±0.01
GA18-60	1.68	0.254±0.019	1.07±0.05	3.8±0.8	20.9	8.40±0.01
GA18-90	2.52	0.229±0.014	0.98±0.09	3.1±0.1	20.5	8.30±0.01
GA18-120	3.36	0.219±0.025	0.94±0.06	4.2±0.5	27.9	8.54±0.01

3.2. Determination of biochar content in field experiment

The TGA method was applied to determine the concentration of biochar in soil samples withdrawn from field experiments (GA) amended with different amount of biochar PSR. Mass loss at different temperature intervals relative to labile OM (220-390 °C), recalcitrant OM (390-600 °C) and carbonates (600-800 °C) are reported in Table 3. The soils treated with different biochar loads presented similar content of labile OM (about 3 % mass loss) indicating that biochar did not affect this component. Instead, the content of recalcitrant OM of field amended soils was higher than control due to the contribution of biochar to this OM component. To the purpose of determining the concentration of biochar, a calibration curve was generated utilizing a control soil sample mixed with different amount of biochar PSR. The obtained data fitted the equation $y = 0.688x + 3.93$ (n=6) where y is the mass loss and x the biochar concentration in soil (% weight). The mass loss of PSR biochar occurred in the temperature range of recalcitrant OM (390-600 °C). A linear regression between weight loss and biochar content in soil was observed, with a significant coefficient of determination ($R^2 = 0.997$). The slope was similar to that obtained with a soil rich in inorganic carbon (soil Q) in accordance with the important mass loss in the thermal region of carbonate decomposition (Table 3). This calibration curve was used to determine the content of biochar in the real sample. The measured biochar content versus biochar expected content is shown in Table 3. The concentration of biochar

367 calculated with the regression curve increased with the increasing load of biochar in the soils 2017
368 from 15 to 45 t ha^{-1}.
369 The soil treated with 60 t ha^{-1} exhibited a biochar concentration determined 57% lower than expected
370 according to the values of biochar concentration calculated from the quantity of applied biochar
371 (Table 3). The trend resulting from TGA was confirmed by TOC that showed in the soils 2017 values
372 increasing from 15 to 45 t ha^{-1} and lower value at 60 t ha^{-1} (Table SM3). In the second year after
373 application, the results of the soils 2018 also showed marked differences between the biochar content
374 and biochar expected for soils treated with 30 and 60 t ha^{-1}. In fact, GA18-30 and GA18-60 exhibited
375 a concentration of biochar determine 40 and 61% lower and higher, respectively, than expected values
376 of biochar concentration calculated. The 2018 soil data also show that the biochar contents
377 determined by TGA were concordant with TOC values including discrepancies with expected values
378 (see also paragraph 3.3.1.). This finding would indicate that the discrepancy between calculated and
379 measured biochar concentration was not due to analytical errors, but inherent to the field experiment.
380 For instance, a decrease of biochar content in soil with time probably associated with physical loss
381 has been documented (Rombola et al., 2015).
382
383 *Table 3.* Data from TGA of agricultural soil samples amended with different load (t/ha) of biochar
384 PSR; concentration of biochar expected and determined by the TGA method.
385

Samples	**220 - 390 C° weight loss (%)**	**390 - 600 C° weight loss (%)**	**600 - 800 C° weight loss (%)**	**biochar by TGA (%)**	**biochar calculated* (%)**
GA17-T	3.01±0.02	3.94±0.02	6.20±0.07	-	0.0
GA17-15	3.00±0.07	4.10±0.08	6.07±0.03	0.25±0.08	0.42
GA17-30	3.0±0.1	4.6±0.3	6.19±0.03	1.0±0.08	0.84
GA17-45	2.96±0.01	4.82±0.04	6.3±0.1	1.29±0.04	1.26
GA17-60	2.82±0.04	4.47±0.03	6.40±0.08	0.78±0.03	1.68
GA18-T	2.77±0.04	3.95±0.12	7.06±0.3	-	0.0
GA18-30	2.97±0.12	4.28±0.07	7.74±0.7	0.51±0.1	0.84
GA18-60	3.52±0.20	5.80±0.25	8.43±0.11	2.7±0.4	1.68
GA18-90	2.84±0.18	5.56±0.20	8.05±0.01	2.4±0.3	2.52

GA18-120	2.76±0.19	6.54±0.16	9.2±0.8	3.16±0.06	3.36

*The values are obtained by considering a soil bulk density of 1.2 g cm^{-3} and a soil depth of 0.3 m (Zavalloni et al., 2011).

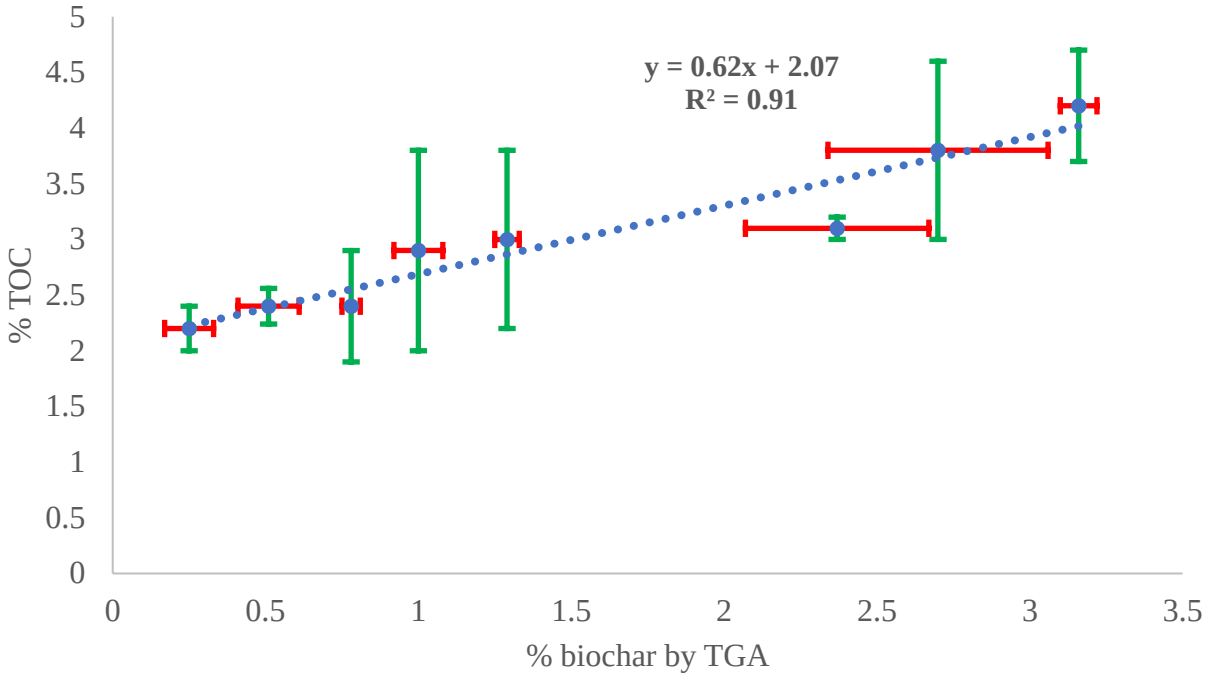
3.3. Organic matter characterization in soils untreated and treated with biochar

3.3.1. Quantification of total and organic carbon

The results determined by TOC analyser show that the biochar amendment caused a consistent increase in TC and TOC with respect to control soil in the soils with biochar (Table SM3). Specifically, the TOC of the GA17 soils in the first year after application significantly increased with the concentration of biochar amendment, from 1.9±0.2% in the control soil to 2.9±0.9% in the soil GA17-30 with 1% of biochar and 3.0±0.8% in the GA17-45 with 1.3% of biochar. In the second year after application, the TOC of GA18 soils increased from 2.0±0.1% in the control to 4.2±0.5% in the soil GA18-120 with 3.16 % of biochar.

Figure 1 shows the relationship between the TOC contents measured with the TOC analyzer and the biochar concentration determined by the TGA method in the field soils treated with biochar. Each data point represents the average value from four replicate measurements. Linear regression analysis showed an excellent correlation (R^2 = 0.91) between the TOC contents measured and biochar determined in the soils. The intercept of the relationship was 2.0% corresponding to the total content of organic carbon in control (1.9-2.0%) and the slope of the correlation is equal to 0.62, which is similar to the TOC of biochar (0.58 gC/gbiochar). Such data confirm that the increase of soil TOC is directly due to biochar addition, and TOC variability among biochar treated soil is mainly attributed to biochar variable biochar content, due to losses and translocation of thereof.

Figure 1. TOC % vs. biochar % by TGA in biochar amended soils (15, 30, 45, 60, 90 and 120 t ha^{-1}) from field experiment at different sampling dates. Mean values ± s.d. (n = 4).



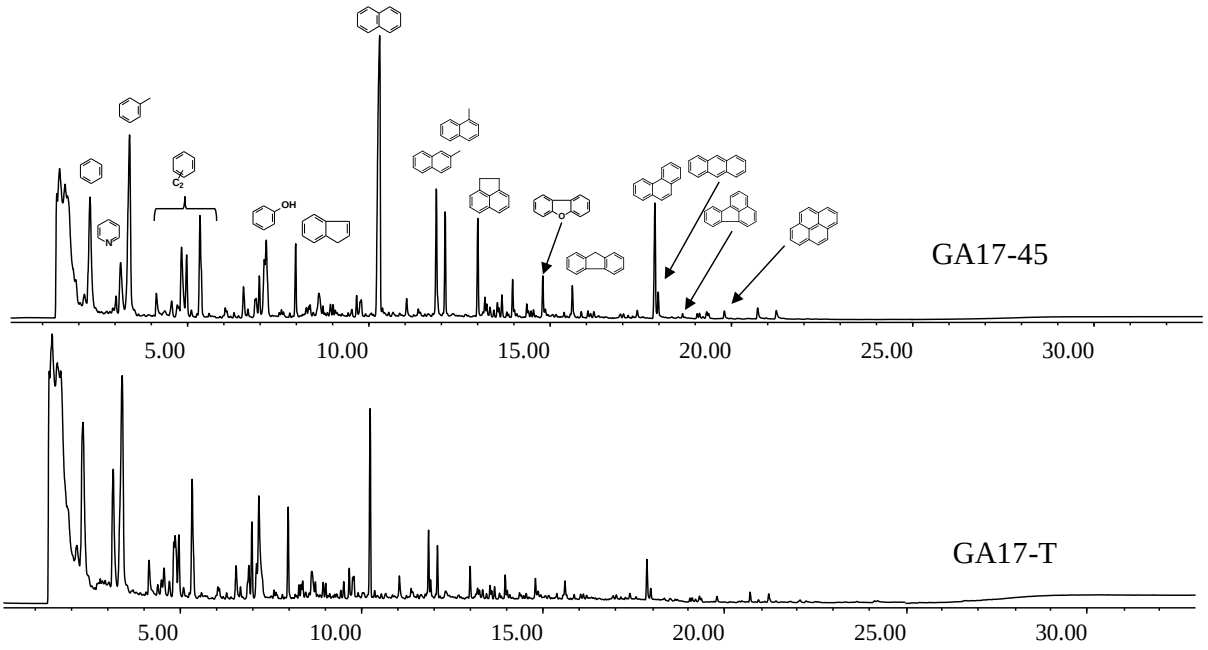
412
413
414

415 **3.3.2. Organic matter characterization by analytical pyrolysis**

416 Product lists and relative proportions (% TQPA) of the Py-GC-MS analyses performed at 500 and
417 900 °C are shown in Appendix Tables SM4-7. Exemplar TIC pyrograms of the control and amended
418 soils obtained at 500°C are shown in Figure 2. These are characterized by the presence of phenols,
419 PAHs and MAHs. The pyrolytic pattern of the treated and untreated soils was similar in terms of
420 identified compounds, suggesting that the incorporation of biochar did not markedly change the
421 chemical nature of organic matter. Analytical pyrolysis TIC at 900 °C investigates the most stable
422 organic component, mainly BC-like material, but no major differences were observed between the
423 Py-GC-MS analyses performed at 500 °C and 900 °C. The main biochar-induced effects observed in
424 TIC pyrograms at 500 and 900 °C were an increase in the peak areas of pyrolysis products and a
425 relative enrichment in aromatic compounds. According to the TGA data, the pyrolysis temperature at
426 500 °C should ensure each sample decomposed completely. The relative abundance of PAHs in the
427 soil produced by pyrolysis at 500°C and 900 °C was higher in the soil treated with biochar than in
428 the control soil, 23-71% vs. 13-25% (Fig. 3A, Py 500 °C) and 43-76% vs. 37-47% (Fig. 3B, Py 900

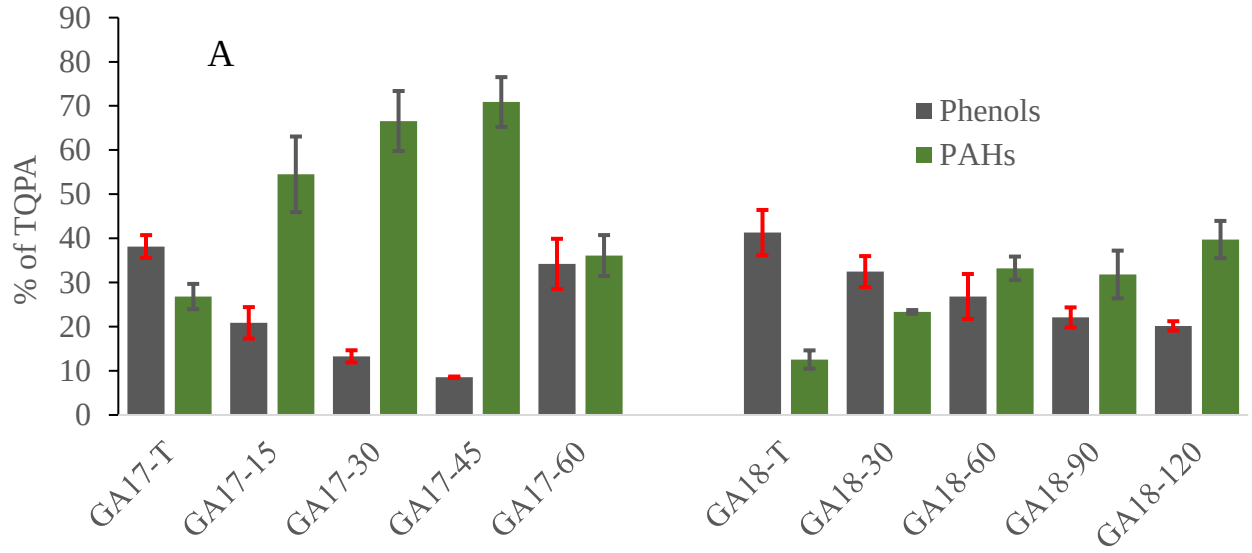
°C). The appearance of PAH has traditionally been identified as a product of the analytical pyrolysis of charred materials (González-Pérez et al., 2007; Girona-García et al., 2019). In analogy to the pyrogenic carbon produced from the partial combustion of organic materials, including biomass and fossil fuels, the biochar matrix comprises a complex assemblage of polyaromatic structures along with heteroaromatic components and alkyl moieties from thermally degraded biomacromolecules (Rombolà et al., 2016). However, in this study, the results of fresh and aged biochar Py-GC-MS analysis showed that the increased levels of PAHs in amended soils in comparison to control soil are not caused by analytical pyrolysis of biochar present in the soil. Moreover, the results (see paragraph 3.6.) showed that added biochar into soil may enhance the microbial activity, probably accelerating the degradation of soil organic matter (Mitchell et al., 2015) and influencing the persistence of PAHs. Rising microbial activity initially promotes the decomposition of labile compounds, and later promotes the degradation of more recalcitrant substances. Moreover, Yang et al., (2019) reported that high temperature pyrolyzed biochars preferentially retained high-molecular-weight humic-like DOC species within soil.

Figure 2. Total ion chromatograms of the Py-GC-MS analyses performed at 500 °C of 25±0.1 mg of control soil (GA17-T) and biochar amended soil (GA17-45) samples from field experiment.



Other pyrolytic products were phenol and C1 alkyl phenols, which can be originated from any phenolic precursor including lignin, tannin, proteinaceous biomass, weakly charred BC and carbohydrates (Stuczynski et al., 1997; Vancampenhout et al., 2009). However, since phenols are minor compounds of the pyrograms of proteins and polycarboxylic acids, the large proportion of phenols in the pyrolyzate of soil samples may be explained by the abundance of lignin. Phenols were not revealed in the Py-GC-MS analysis of fresh and aged biochar, thus phenols and methylphenols are of little diagnostic value with respect to highly pyrolysed lignin. The relative abundance of phenol compounds decreased significantly in the soil treated with biochar, while the absolute abundance did not change significantly in the treated and control soils, suggesting that the phenols originate from soil lignin rather than biochar treatment.

Figure 3. Relative proportions (% of total quantified peak area, sum 100%) of the Py-GC-MS analyses performed at 500 °C (A) and at 900 °C (B) of control soils and biochar amended soils (15, 30, 45, 60, 90 and 120 t ha^{-1}) from field experiment at different sampling dates. Mean values ± s.d. (n = 3).



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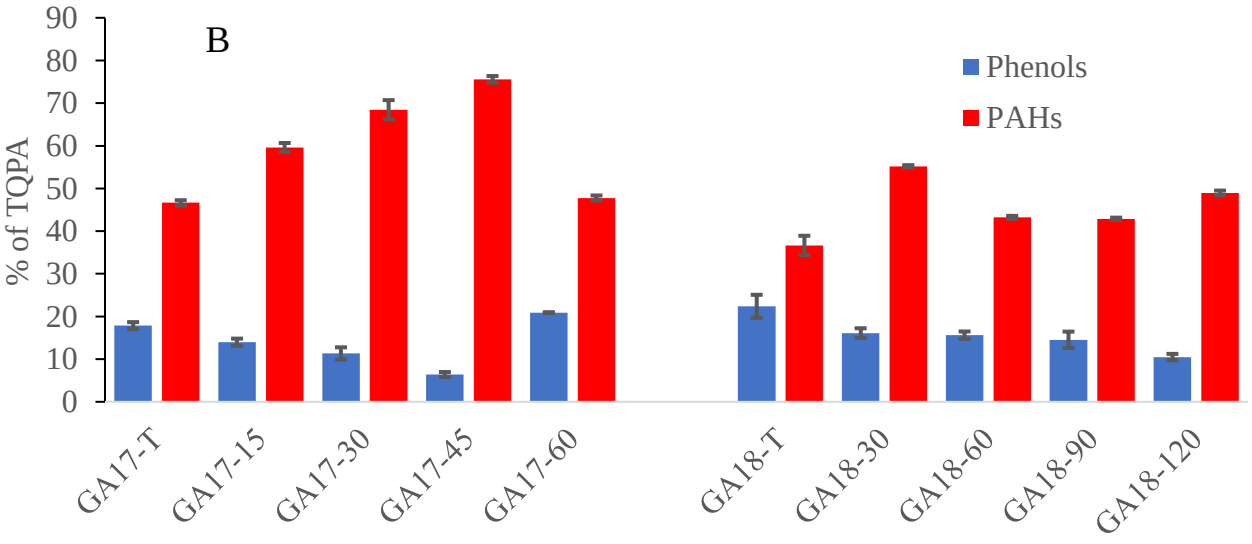

464

465 The individual levels of PAHs in control soils and in soils amended with one and two consecutive
466 applications of biochar are presented in supplementary materials (Tables SM4-7). Dibenzofuran,
467 which is heterocyclic analogues of PAHs, was grouped with the PAHs. The PAHs with 2 and 3 rings
468 composed the majority of PAHs in control soil and in amended soil samples. Naphthalene was always
469 the most abundant PAH in control soils and in amended soils. However, soil treated with biochar
470 contains 2 to 4 times more naphthalene than untreated soil.

Analytical pyrolysis has been proposed as a fast alternative approach for the analysis of PAHs included in environmental matrices (i.e., soil and sediments), as pollutants, constituents (i.e., coals and black carbon) or pyrogenic (i.e., chars and charred materials from forest fires) (González-Pérez et al., 2014; Biache et al., 2017). The methyl/parent PAH ratios of selected PAHs, for instance methylnaphthalene/naphthalene (MeNAP/NAP), have been proposed as suitable indices to evaluate the biochar carbonization degree (Calvelo Pereira et al. 2011; Rombolà et al., 2016). However, little is known about how biochar addition in soil affects the methyl/parent PAH ratios. Therefore, the degree of alkylation in control soils and in biochar amended soils was studied by Py-GC-MS. In addition, in order to assess the biochar impact on soil PAHs, the PAH isomeric ratios determined by Py-GC-MS were reported. Frequent routine methodological approaches to study PAHs and PAH isomeric ratios from environmental samples are based on analyses of solvent extracts and further chromatographic separation. Rombolà et al. (2019) reported that solvent extractable naphthalene NAP, phenanthrene PHE, fluoranthene FLA and their isomers anthracene ANT and pyrene PYR in biochar amended soils could be considered as potential candidates for tracking the PAH imprinting of biochar by means of diagnostic ratios. For solvent extractable PAHs, the isomeric ratios ANT/(ANT+PHE) and FLA/(FLA+PYR) are frequently used for source apportionment (Yunker et al., 2015). In addition, in order to assess sources of PAHs in soils, the non-isomeric ratio NAP/(NAP+PHE) was used for soil treated with biochar (Rombolà et al., 2019). The few studies in the literature reporting on the PAH isomeric ratios determine by Py-GC-MS have shed light on a fast alternative approach to detect PAHs in environmental samples (González-Pérez et al., 2014).

The PAH ratios determined by Py-GC-MS at 500 °C of control soils and biochar amended soils are reported in Table SM8. The MeNAP/NAP ratios were lower in the biochar amended soil decreasing significantly with the concentration of biochar amendment. In particular, in the first year after biochar application, MeNAP/NAP decreased from 0.66 in the control soil to 0.33 in the biochar amended soil GA17-60 with 0.78% of biochar. In the second year, from 0.87 in control soil to 0.23 in GA17-120 with 3.4% of biochar. The utilized biochar obtained by pyrogasification was highly carbonized with

497 atomic H/C and O/C ratios of 0.10 and 0.08, respectively (Table 1), and MeNAP/NAP ratios of 0.02
498 for fresh and aged biochar (Table SM8), consistent with a high degree of aromaticity. Therefore, the
499 PAHs in the biochar applied in the field experiment, in accordance with dealkylation processes
500 occurring at high pyrolysis temperatures, were largely de-alkylated. The impact of biochar was
501 clearly demonstrated by the decreased levels of alkylated PAHs in amended soils in comparison to
502 control soil.
503 The values of ANT/[ANT+PHE] ratios in control soils (0.30) were slightly higher than those of
504 amended soils (0.28-0.25). The FLA/[FLA+PYR] ratios ranged from 0.53 to 0.59 and did not exhibit
505 significant changes between control soils and biochar amended soils. Thus, this isomeric ratio is not
506 relevant to track the impact of biochar in the treated soils. The non-isomeric ratio (NAP/[NAP+PHE])
507 ratio in fresh and aged biochar was the same (0.98) and it was markedly higher than that of control
508 soils (0.75). Consistently amended soil presented higher values in soil with biochar determine by
509 TGA > 0.5%. The ratio in amended soils increased from 0.75 in soil with 0.4% of biochar (GA17-15)
510 to 0.91 in soil with 2.4% of biochar (GA18-90). The trend can be better visualized in the form of the
511 so-called cross plots as depicted in Figure SM3, where the NAP/(NAP+PHE) is plotted vs. the
512 ANT/[ANT+PHE]. Moreover, the actual biochar concentrations determined plotted vs. the
513 NAP/(NAP+PHE) ratio showed that the biochar signature of PAHs can be visualized in amended
514 soils. Therefore, analytical pyrolysis can also be used to investigate PAH isomeric ratios. These ratios
515 can be an efficient supporting tool in studying the persistence of PAHs in soils with biochar and their
516 cross plots of the PAH diagnostic allowed to differentiate between soil with and without biochar.
517 However, the usefulness of the PAH diagnostic ratios to track the biochar in soils cannot be
518 generalized, as they are related to the specific pattern of biochar and soil.
519
520 **3.4. DOM yield and elemental analysis**
521 Significant differences (ANOVA, $p<0.05$) were observed in DOC and in WSOC when the soil was
522 treated with biochar (Table 4), indicating that the biochar addition gave rise to a sharp decrease in

DOC. Soil-derived DOC concentrations in the soils treated with biochar (15-32 mg L^{-1}) were lower than in the soils without biochar (31-35 mg L^{-1}; Table 4). Specifically, the DOC of the soil in the first year after application significantly decreased with the concentration of biochar amendment, from 34.7±2.0 mg L^{-1} in the control soil to 25.5±0.9 mg L^{-1} in the biochar amended soil GA17-30 with 1% of biochar and 26.4±1.0 mg L^{-1} in the GA17-45 with 1.3% of biochar, representing a loss of 26%. In the second year after application, the DOC values showed a more marked difference between the amended and control soils: 30.7 mg L^{-1} and 18.5-14.7 mg L^{-1} in the soil without and with biochar. In particular, DOC concentration decreased significantly with biochar concentration from 30.7± 2.4 mg L^{-1} in control soil to 14.7±0.6 mg L^{-1} in the GA18-120 with 3.16 % of biochar, representing a decrease of 50%. Similarly, Feng et al., (2021) showed that high temperature pyrolyzed biochars (> 700 °C, as PSR biochar used in our field experiment) decrease soil DOC concentration up to 50% and the decrement increases with the increase of biochar amount. The potential of biochar to decrease of DOC in field experiment was evidenced by Liu et al., (2019), who observed that biochar amendment causes a significant increase of macropores and thus the enhanced infiltration of soil water. Such changes could lead to increased flow discharge, which in turn results in elevated leaching of organic carbon during rainfalls. In this study, moreover, more marked differences are observed between amended and control soils in the dissolved fraction of total SOC (WSOC = mg DOC $g^{-1\ TOC}$). The values of WSOC decreased after biochar amendments in both years and in all treatments (Table 4), in proportion to the TGA and TOC values. Almost two years after the first biochar application, the amount of WSOC in the GA18-120 amended soils (3.4 mg $g^{-1\ TOC}$, biochar 3.16%) were significantly lower than those in the control soil (18.3 ± 1.1 mg $g^{-1\ TOC}$ in 2017, 15.4 ± 1.2 mg $g^{-1\ TOC}$ in 2018). The results of this study suggested that biochar reduces the DOC, and this effect increased with time and biochar amount over the first two years after application. This could be due to several phenomena, such as adsorption and microorganism growth, which can be enhanced with time and aging of biochar/soil mixture, and/or due to the increase of soil macropores. The environmental consequences

548 of this are presently unknown but may be reflected in the reduction of DOC bioavailability and
549 associated effects on soil aggregation (formation of organometallic complexes), energy source for
550 microorganisms, as well as C storage, cycling, and provision of plant-available nutrients (Gmach et
551 al., 2018), microbial loop dynamics and aquatic food webs (Jaffé et al., 2013).
552 Yang et al., (2019) reported that the biochar amendment caused a consistent decrease in the TN levels
553 of the WSOM due to the higher micropore surface area of higher-temperature biochar, which can
554 retain more inorganic N. On the contrary, the results shown in Table 4 indicated that the TN
555 concentrations in the WSOM were slightly higher in the biochar amended soils. These results are
556 compatible with the hypothesis that biochar treatment is responsible for the observed increase of soil
557 microbial nitrogen.
558
559 *Table 4*. Total nitrogen (TN) in water-soluble matter, dissolved organic carbon (DOC) and water-
560 soluble organic carbon (WSOC) concentration of control soils and biochar amended soils (15, 30, 45,
561 60, 90 and 120 t ha^{-1}) from field experiment at different sampling dates. Mean values ± s.d. (n = 3).

Samples	TN (mg L^{-1})	DOC (mg L^{-1})	WSOC (mg $g^{-1\ TOC}$)
GA17-T	4.43 ± 0.1	34.7 ± 2.0	18.3 ± 1.1
GA17-15	4.50 ± 0.2	28.8 ± 1.1	13.4 ± 0.5
GA17-30	4.53 ± 0.1	25.5 ± 0.9	8.9 ± 0.3
GA17-45	4.91 ± 0.3	26.4 ± 1.0	8.9 ± 0.3
GA17-60	5.26 ± 0.1	30.6 ± 0.5	12.6 ± 0.2
GA18-T	3.69 ± 0.1	30.7 ± 2.4	15.4 ± 1.2
GA18-30	3.69 ± 0.2	16.3 ± 0.7	6.8 ± 0.3
GA18-60	4.15 ± 0.4	16.8 ± 1.6	4.4 ± 0.4
GA18-90	4.39 ± 0.3	18.5 ± 0.7	6.0 ± 0.2
GA18-120	5.10 ± 0.1	14.7 ± 0.6	3.4 ± 0.1

562
563 **3.5. DOM UV–vis absorption**
564 The $SUVA_{254}$ of control soils and biochar amended soils are presented in Supporting information
565 (Table SM9). The UV–vis absorption is a commonly used technique to characterize the DOM
566 structure and composition in soil solution or other aqueous media (Nebbioso et al., 2013). The

567 $SUVA_{254}$ index indicated the presence of aromatic substances such as humic acids and high molecular
568 weight of DOM (Weishaar et al., 2003; Inamdar et al., 2012). In addition, $SUVA_{254}$ values are
569 correlated with C=O and C=C bonds present in aromatic compounds and humic-like substances
570 (Dong et al., 2014).
571 The findings revealed that biochar application increased the soil $SUVA_{254}$ value, and all treatments
572 showed higher $SUVA_{254}$ values. The soil $SUVA_{254}$ value increased from 1.38 L $mg^{-1}m^{-1}$ in control
573 soil to 2.24 L $mg^{-1}m^{-1}$ in GA17-60 with 0.78% of biochar, in the first year after biochar application.
574 In the second year, from 1.65 L $mg^{-1}m^{-1}$ in control soil to 3.55 L $mg^{-1}m^{-1}$ in GA18-30 with 0.51% of
575 biochar, demonstrating the higher aromaticity of the WSOM in the biochar amended soil.
576 Other field studies have examined the biochar impact on DOM of soil reported that the values of
577 $SUVA_{254}$ significantly increased in the extracted DOM of biochar-amended soils relative to the
578 control (Li et al., 2018; Zhang al., 2020). Fan et al. (2020) reported that, during the aging process, a
579 significant amount of aromatics were released from the biochar into the soil, which increased the
580 DOC and the aromaticity of the WSOM. Li et al. (2017) supposed that more aromatics dissolved out
581 from biochar and led to an increase in $SUVA_{254}$ because smaller aliphatic DOM molecules were more
582 strongly sorbed on the biochar surface. In fact, strong/weak adsorption on biochar was partly
583 attributed to the hydrophobicity/hydrophilicity of aliphatics/aromatics. In this study, the correlation
584 between DOC and $SUVA_{254}$ values was not strong indicating that $SUVA_{254}$ index did not provide a
585 persuasive evidence of high C present in aromatic DOM-pools or humic-like substances (Inamdar et
586 al., 2012).
587
588 **3.6. Pyrolysis-GC-MS of DOM**
589 The relative proportions of the main compound groups are shown in Table 5. A typical chromatogram
590 is presented in Figure SM4. The peaks were categorized into nine components: carbohydrate products
591 (CARB), lignin products (LG), monocyclic aromatic hydrocarbons (MAHs), polycyclic aromatic
592 hydrocarbons (PAHs), phenols (PHENs), nitrogen-containing compounds (NCOMPs), aldehydes

(ALDs), methylene chain compounds (MCCs), and others. For the details on proportions of individual pyrolysis products, see Supporting information (Tables SM10 and SM11).

Some studies have been conducted in various types of soil on the molecular composition of DOM by Py-GC-MS, and different dominant compounds have been identified (Rosa et al., 2015; Jiang et al., 2017). Contrary to previous observations of soil-derived DOM analyzed by Py-GC-MS (Jiang et al., 2017), pyrolysis products that can be traced back to relatively intact plant-derived polysaccharides, e.g., pyrans and anhydrosugars (Pouwels et al., 1989), were not detected. In the present study, only 3-hydroxy-2-methyl-4H-pyran-4-one was identified. Other carbohydrate products, i.e., furans, furfurals, and cyclopentenones can be produced by pyrolysis of plant-derived, microbial and planktonic carbohydrates.

In the first year after application, the MAHs account for 6.1 ± 0.9% in the control soil (GA17-T), 3.4 ± 0.5% (GA17-30) and 3.8 ± 0.5% (GA17-45) for biochar amended soil with 1% and 1.3% of biochar, respectively. Benzene is the most abundant product, followed by toluene and styrene (Table SM10). However, the MAHs determined by Py-GC-MS in the soils GA18 indicated no significant difference between the amended and control soils, except for GA18-30 vs. GA18-T. The MAHs are not diagnostic of any source and are probably related to microbial proteinaceous material (e.g., toluene). PAHs are associated with polycondensation of aromatic moieties in pyrogenic organic matter, e.g. charcoal or soot (Kaal et al., 2016). Clearly, the incorporation of biochar into soil systems represents an input of PAHs (Rombolà et al., 2019). However, several studies have shown a low bioavailability of PAHs in biochars, probably due to their strong interaction with the carbonaceous matrix of biochar (Hale et al., 2012; Tomczyk et al., 2020). Therefore, biochar application could increase or decrease the aromatic hydrocarbon components of DOM, influencing the release of PAHs by pyrolysis. This aromatic fraction is affected by different degrees cording to the complex effects of biochar on DOM and on mobility, persistence and degradation in soil. For instance, biochar may increase or decrease the extent and rate of degradation of aromatic hydrocarbons depending on cell density, the microbial species, the soil and/or biochar type and concentration. Naphthalene and acenaphthene were detected

619 in all DOM samples from soil with and without biochar. The biochar amendment caused a significant
620 increase in the PAH levels in the pyrolysates DOM with respect to control soil in all soils with biochar
621 (Table 5). In the first year, the PAH levels detected with Py-GC-MS increased from 0.6% relative
622 area in control soil to 1.8% in GA17-30 with 1.0% of biochar. In the second year, a similar increase
623 was observed, namely from 0.9% in control soil to 1.6% in GA18-60 and GA18-90 with 2.7 and 2.4%
624 of biochar, respectively. Therefore, the increase of PAH levels was less pronounced in soil 2018 and
625 not evident trends with biochar levels were observed. These results suggest that the PAH levels in
626 DOM are probably related to the condensed pyrogenic organic matter from biochar, whose spike
627 disappears as time after application increases. This agrees with the higher aromaticity of the WSOM
628 in the biochar amended soil determined by $SUVA_{254}$.
629 Phenols accounted for an average of 4.2 ± 0.7% and 6.5 ± 1.2% of TQPA among the control soil
630 samples, in the soils GA2017 and GA2018, respectively (Table 5). In the biochar amended soils
631 phenols are less abundant (2.4 ± 0.8% GA17-45, 2.3 ± 0.3% GA18-30 and 2.3 ± 0.6% GA18-120).
632 Therefore, the phenol levels determined by Py-GC-MS in DOM indicated significant difference
633 between the amended and control soils, except for GA17-60. These phenols are major pyrolysis
634 products of degraded lignin and proteinaceous biomass and have been previously reported as being
635 abundant in soil DOM pyrolysates (Kaal et al., 2016). Lignin is an important marker of terrigenous
636 sources, mainly originating from vascular plants. They produce methoxyphenols (guaiacols, and
637 syringols) upon pyrolysis. High methoxyphenol yields in DOM have been reported previously and
638 are considered to be indicative of degradation products of lignin-derived DOM in aquatic ecosystems
639 (Neff et al., 2006).
640 In the present study, 4-methylguaiacol was detected in all DOM samples from Py-GC-MS of soils
641 with and without biochar. The biochar treatment impacts on the levels of 4-methylguaiacol in soil
642 DOM products (Table 5). The lignin-derived DOM products value decreased from 0.69±0.10% in
643 control soil GA17-T to 0.14±0.02% in soil GA17-45 with biochar and from 0.80±0.04% in control
644 soil GA18-T to 0.21±0.02% in soil GA18-30 with biochar. Decrease of phenols in Py-GC-MS of

DOM, by a factor five, could explain a significant portion of reduction in DOM concentration observed over a two-year time.

In the first year after application, the compounds with N (NCOMPs) in their structure accounted for 51 ± 0.2% in the control soil (GA17-T), 63 ± 2.3% (GA17-30) and 66 ± 11% (GA17-45) for biochar amended soil with 1% and 1.3% of biochar, respectively (Table 5). The NCOMPs in the second year after application accounted for 44 ± 3.3% in the control soil (GA18-T), 67.8 ± 0.09% (GA18-30) and 77 ± 6.2% (GA18-90) for biochar amended soil, respectively. Therefore, the NCOMPs levels increased in all soil treatments with biochar, except for GA17-60 and GA18-120.

This suggests an increase in the proportion of microbial DOM in biochar amended soils. The fact that NCOMPs are enriched in biochar amended soils whereas phenols and methylphenols products are not suggests that the phenols originate from polyphenols rather than peptidic DOM. Moreover, the predominance of N-containing products pyridine, pyrrole, acetamide, indole, and benzonitrile is in agreement with the abundance of degraded and microbial carbohydrates (furfural, cyclopentenone and aldehydes; Table 5) which reflect primarily microbial DOM. Specifically, acetamide was found to be abundant in all soil DOM with and without biochar (25-30% in soil GA17 and 7.6-20% in soil GA18). Acetamide is a marker of chitin-derived organic matter, which originates from fungal cell walls or arthropod exoskeleta (Kaal et al., 2017), and has been used as a marker of intense organic matter mineralization conditions in DOM (Templier et al., 2012). Moreover, its presence suggests that the pyrroles and pyridines are also of microbial origin. The detection of indole and benzyl nitrile is indicative of relatively intact proteinaceous material in DOM (Buurman et al., 2011). Benzonitrile is associated with N-containing structures in the dissolved Black Carbon (Kaal et al., 2008). Furthermore, a series of compounds with dominant m/z 59 and 72 were identified as C_{16}-, C_{18}- and C_{22}- alkylamides.

Compounds based on a polymethylene chain (MCC) such as n-alkenes and C_{16}-fatty acid methyl ester are indicative of aliphatic components, principally lipids and account for only 0.42 ± 0.02% (GA17-T) and 0.30 ± 0.16% (GA18-T) in control soil, and between 0.49 ± 0.03% (GA17-15) and 0.06 ±

671 0.01% (GA18-90) in the soil with biochar. The remarkably low abundance of these aliphatic products,
672 especially in comparison with the pyrolyzates of SOM, has been reported earlier for DOM
673 pyrolyzates (Kaal et al., 2017).
674 Other compounds that are grouped in "Other" include 4-octadecyl-morpholine from tomato cultivars,
675 4-tert-octylphenol from industrial non-ionic alkylphenolpolyethoxylate surfactants (Greenwood et al.
676 2012), often detected in wastewaters and landfill leachates (Sharma et al., 2009), Benzene, 1,4-
677 dichloro from plastics contamination and unidentified compounds. In particular, 4-octadecyl-
678 morpholine was found to be abundant in all soil DOM with and without biochar. This compound is a
679 metabolite identified in the tomato volatile metabolomic composition (Song et al., 2018) and its
680 presence is due to the fact that tomato plants were grown on the soils of the field experiment in the
681 growing season 2016/2017.
682 In summary, biochar amendment caused a significant change in Py-GC-MS of the soil DOM. The
683 PAHs are increased during the first year after biochar application, whereas DOM derived phenols
684 showed a marked decrease irrespective of the year. The control and treated soils yielded relatively
685 high abundances of nitrogen containing pyrolysates with a significant increase in the soil treated with
686 biochar, evidencing an increase in the proportion of microbial DOM in biochar amended soils
687 compared to control. This is in accordance with several studies that demonstrated the influence of
688 biochar on soil microbial community (Michell et al., 2015; El-Naggar et al., 2019), providing suitable
689 habitat for useful soil microbes (e.g., by enhancing soil aeration, increasing water content, mitigating
690 soil compaction, etc.) (Laghari et al., 2016), supplying nutrients for their growth (Zhu et al., 2017),
691 and stimulating their activity (Lehmann et al., 2011; Zhu et al., 2017). Therefore, the results of this
692 study showed that biochar might change soil microbial communities, and thereby influence soil
693 nutrient cycling with significant effects on the environment.
694

695 *Table 5.* Relative percentages (%) of the main groups of compounds identified by DOM Py-GC-MS[a]
696 of control soils and biochar amended soils (15, 30, 45, 60, 90 and 120 t ha^{-1}) from field experiment
697 at different sampling dates. Mean values ± s.d. (n = 2).

Samples	MAHs	NCOMPs	CARB	ALDs	PHEs	PAHs	LIG	MCCs	Other
GA17-T	6.1±0.9	51.0±0.2	7.0±0.3	4.0±0.6	4.2±0.7	0.61±0.01	0.69±0.10	1.66±0.07	27±1.1
GA17-15	3.6±0.1	55±4.9	2.9±0.2	5.0±0.7	3.7±0.6	1.18±0.07	0.15±0.02	1.15±0.07	32±3.8
GA17-30	3.4±0.5	63±2.3	4.1±0.3	5.3±0.5	3.4±0.8	1.8±0.4	0.15±0.05	1.09±0.01	24±2.2
GA17-45	3.8±0.1	66±11	4.6±0.4	4.9±0.3	2.4±0.8	1.71±0.09	0.14±0.02	1.4±0.3	21±8.5
GA17-60	4.0±0.9	49±1.1	8.6±0.9	4.2±0.5	4.3±0.4	1.44±0.02	0.88±0.2	0.6±0.1	28±2.2
GA18-T	7.4±0.7	44±3.3	13.2±0.6	5.1±0.7	6.5±1.2	0.41±0.03	0.80±0.04	1.8±0.6	25±3.0
GA18-30	5.6±1.7	67.8±0.09	2.7±0.3	4.0±1.2	2.3±0.3	0.91±0.18	0.21±0.07	1.50±0.03	24±4.2
GA18-60	8.0±1.3	61±4.1	2.5±0.2	4.6±0.3	3.6±0.2	1.6±0.4	0.30±0.18	1.55±0.01	27±1.1
GA18-90	6.2±0.5	77±6.2	2.9±0.6	4.8±0.3	3.3±0.5	1.6±0.2	0.48±0.14	1.1±0.1	11±1.1
GA18-120	7.1±0.1	35±6.8	8.6±1.5	2.1±0.6	2.3±0.6	1.13±0.09	0.7±0.1	1.9±0.6	42±7.9

698 [a]Abbreviations: MAHs = monocyclic aromatic hydrocarbons, NCOMPs = nitrogen-containing
699 compounds, CARB = carbohydrate products, ALDs =aldehydes, PHENs = phenols, PAHs =
700 polycyclic aromatic hydrocarbons, LIG = lignin products and MCCs =methylene chain compounds.
701

702 **4. Conclusions**

703 This study provides new insight into the effects of biochar on SOM in cultivated agricultural soils,
704 particularly with regard to the quantity and quality characteristics of DOM leached after one- and
705 two-years field experiment. Py-GC-MS and spectroscopic analysis provided qualitative information
706 on DOM. The results of SOM and DOM characterization by molecular analysis were compared with
707 the real biochar concentration determined by a novel TGA method developed to quantify the biochar
708 in amended soils. Biochar determined by TGA fitted with TOC values and in general were in
709 accordance with amended rates. The biochar application in soil systems at different load significantly
710 decreased the values of DOC and modified the soil properties (TOC, pH) in both years and in all
711 treatments, in proportion to the TGA and TOC values. Considering that DOC is vital for many soil
712 processes, the reduction of DOC caused by biochar application could lead to important environmental
713 consequences. We hypothesized that this decrease may have been caused by biochar impact on soil
714 microbial community and soil macropores. Analytical pyrolysis results of soil with and without

biochar showed a higher abundance of nitrogen-containing compounds in the soil treated with biochar to the control soil without biochar, evidencing an increase in the proportion of microbial DOM in biochar amended soils. These results demonstrated that added biochar into the soil may enhance the microbial activity, probably accelerating the degradation of soil organic matter and influencing the composition of DOM with the initial decomposition of labile compounds, and later degradation of the more recalcitrant substances like PAHs. The increase of the SUVA254 values and the PAH levels of pyrolysates in biochar amended soil DOM confirmed this hypothesis.

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