

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Effect of biochar amendment on organic matter and dissolved organic matter composition of agricultural soils from a two-year field experiment

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Effect of biochar amendment on organic matter and dissolved organic matter composition of agricultural soils from a two-year field experiment / Rombola A.G.; Torri C.; Vassura I.; Venturini E.; Reggiani R.; Fabbri D.. - In: SCIENCE OF THE TOTAL ENVIRONMENT. - ISSN 0048-9697. - STAMPA. - 812:(2022), pp. 151422.1-151422.11. [10.1016/j.scitotenv.2021.151422]

Availability:

This version is available at: <https://hdl.handle.net/11585/860333> since: 2022-02-17

Published:

DOI: <http://doi.org/10.1016/j.scitotenv.2021.151422>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Rombolà, A.G., Torri, C., Vassura, I., Venturini, E., Reggiani, R., Fabbri, D., 2022. Effect of biochar amendment on organic matter and dissolved organic matter composition of agricultural soils from a two-year field experiment. Science of The Total Environment 812, 151422.

The final published version is available online at:
<https://doi.org/10.1016/j.scitotenv.2021.151422>.

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

1 **Effect of biochar amendment on organic matter and dissolved organic matter composition of**
2 **agricultural soils from a two-year field experiment**

3

4 Alessandro G. Rombolà^a, Cristian Torri^b, Ivano Vassura^c, Elisa Venturini^c, Roberto Reggiani^d,
5 Daniele Fabbri^a

6

7 *^aDepartment of Chemistry “Giacomo Ciamician” and C.I.R.I. MAM Tecnopolo di Rimini, University*
8 *of Bologna, Via Dario Campana 71, 47192, Rimini, Italy*

9 *^bDepartment of Chemistry “Giacomo Ciamician”, University of Bologna, Campus di Ravenna, via*
10 *Sant’Alberto 163, 48123, Ravenna, Italy*

11 *^cDepartment of Industrial Chemistry “Toso-Montanari” and C.I.R.I. FRAME, University of Bologna,*
12 *Campus di Rimini, via Dario Campana 71, 47922, Rimini, Italy*

13 *^dExperimental Farm Stuard SCRL, Strada Madonna dell’Aiuto 7/a, 43126, San Pancrazio (Parma),*
14 *Italy*

15

16 * Corresponding author: alessandro.rombola@unibo.it (A.G. Rombolà)

17

18 **Abstract**

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

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

35

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

37

38 **1. Introduction**

39 Biochar soil amendment continues to receive worldwide interests for integrated
40 agricultural/environmental strategies to build soil, enhance water quality, and increase agricultural
41 productivity while sequestering C and thus mitigating global climate change (Lehmann and Joseph,
42 2015; Woolf et al., 2016; Purakayastha et al., 2019; Majumder et al., 2019; Giagnoni et al., 2019).

43 The agronomic and environmental impacts of biochar, and especially its potential as a C sequestration
44 strategy, require a full comprehension of its effects on native soil organic matter (SOM). However,
45 while there can be no doubt that the application of biochar to soils increases the recalcitrant fraction
46 of soil organic carbon (SOC), little is known about how biochar addition affects SOM composition,
47 especially dissolved organic matter (DOM). Moreover, understanding the real or possible benefits
48 and drawbacks of using biochar in agroenvironmental management requires knowledge of quantity
49 of biochar remaining in soil (Koide et al., 2011; Dong et al., 2017). A variety of thermal and chemical
50 soil analysis methods have been used for biochar quantification (Raya-Moreno et al., 2017), and the
51 most suitable methods for its assessment are still under debate (Nakhli et al., 2019).

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

61 The incorporation of biochar into soil systems represents an input of biochar-derived DOM that could
62 play an important role in the carbon dynamics and microbial communities in soil. Once applied in the
63 field, biochar could change the content and composition of soil DOM (Smebye et al., 2016; Liu et
64 al., 2019; Feng et al., 2021). In recent years, biochar addition effects on DOM have attracted
65 considerable attention of researchers. However, the results seem to be contradictory. For instance,
66 Smebye et al. (2016) in a batch experiment found that biochar could increase the leaching of DOM
67 from soil, as well as change the DOM composition towards molecules with a larger size and higher
68 aromaticity by sorbing smaller aliphatic species to its micropores. Such effect was also observed in
69 field experiments (Zhang et al., 2017; Liu et al., 2019). In these studies, DOM released from biochar
70 and biochar induced increase of soil pH and hydraulic conductivity were presented as possible
71 mechanisms responsible for the increase in DOM content. On the other hand an opposite effect due
72 to biochar amendment has also been reported. Eykelbosh et al. (2015) in a column experiment found
73 that biochar amended soil attenuated DOM leaching and the biochar preferentially retained high-
74 molecular weight, humic-like DOM species. Dong et al. (2019), in a long-term field experiment,
75 proposed that biochar had little effect on soil DOM content. Differences in the results among these
76 studies are primarily attributed to the largely variable properties of different biochars and soils. In
77 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,

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

114

115 **2. Materials and Methods**

116 **2.1. Field experiment**

117 The field experiment was conducted over two consecutive growing seasons (2016/2017 and
118 2017/2018) at the "Ganazzoli Filippo" farm (Parma, Emilia-Romagna, Italy) on an agricultural soil
119 (named as GA) classified as clay (USDA, 2005) textured with 7.7% sand, 37.1% silt and 55.2% clay.
120 The soil characteristics were as follows: pH 8.14 ± 0.01 , total C $3.64 \pm 0.09\%$, total N $0.19 \pm 0.02\%$, total
121 H $0.99 \pm 0.02\%$, and a cation exchange capacity of $37.2 \text{ mequiv } 100 \text{ g}^{-1}$. The amount of total organic
122 carbon (TOC) present in the agriculture soil is of 2%, in the typical range of common SOC
123 concentrations.

124 The biochar employed (named as PSR) in the amendment of agricultural soils is a commercially
125 available biochar derived by pyrogasification of forest wood and brushwood waste (Borgo Val di
126 Taro, Italy). The experimental design consisted of a randomized complete block with plots of 7.5 m^2
127 ($5 \times 1.5 \text{ m}$), considering ten treatments: two controls without biochar (GA17-T sampled in 2017 and
128 GA18-T sampled in 2018) and soil treated with different amount of biochar PSR: 15 t ha^{-1} (GA17-
129 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

130 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-
131 120) of biochar applied two times, in 2016 and 2017 at the same rate and sampled in 2018. There
132 were four plots per treatment, resulting in a total area of 30 m² for each. The biochar was incorporated
133 manually into the top 20 cm of soil immediately, using a hand hoe, to leave an apparently uniform
134 distribution.

135 Control and soil treated by six levels of biochar amendment were sampled for analysis in December
136 2017 and May 2018 in five randomly chosen points in the space of each replicate by means of soil
137 core sampler at 0-30 cm. A total of forty samples were collected from soil depths of up to 30 cm.
138 Forty subsamples (one for each plot, ~ 50 g) were prepared and examined, each subsample was dried
139 at 40 °C, sieved (mesh size: 2 mm) to obtain homogeneous samples free of stones, larger roots, wood
140 sticks and other coarse fragments, and stored at – 20.

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

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

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

158 The ash content of the biochars was determined as the residual mass left after exposure at 600 °C for
159 5 h. The oxygen content of the biochars was calculated from the mass balance: $\text{Oxygen (\%)} = 100 -$
160 $\text{Ash content (\%)} - \text{C (\%)} - \text{H (\%)} - \text{N (\%)} - \text{S (\%)}$. The pH of the biochar and soil samples was
161 measured at room temperature with a digital pH meter (VWR pH100, VWR International) in a 1:10
162 and 1:2.5 soil:water suspension, respectively. Additional details about the properties of the PSR can
163 be found in Marmiroli et al. 2018.

164

165 **2.2. Development of the method for quantitative analysis of biochar in soil**

166 **2.2.1. Samples**

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

176 Biochar samples were thoroughly homogenized and oven-dried at 40 °C for 72 h and stored at – 20
177 °C prior to analysis. Proximate analysis was performed according to ASTM D7582 method (ASTM,
178 2015) with slight modifications with a thermogravimetric analyzer (Mettler Toledo TGA /SDTA
179 851e).

180 Soil samples were provided by “Azienda Agricola Sperimentale Stuard”. The soils were sampled
181 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 Quorzola 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 ($\geq 99.5\%$ w/w),
189 Sigma Aldrich; Calcium carbonate ($\geq 99\%$), 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 μ L 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 $^{\circ}\text{C min}^{-1}$ to 600 $^{\circ}\text{C}$ for organic material (OM)
199 determination, then they were ramped at 25 $^{\circ}\text{C min}^{-1}$ to 850 $^{\circ}\text{C}$ for carbonate determination (Kasozi
200 et al., 2009). The heating rates of 10 $^{\circ}\text{C min}^{-1}$ and 25 $^{\circ}\text{C min}^{-1}$ 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

208 varying amount of biochar. In particular, calibration standards containing 0.20, 0.50, 1.0, 2.5 and
209 5.0% (w/w) of biochar each were prepared by adding different amount of biochar PSR to control GA
210 soil composite sample. The mix was homogenized in a mortar before the analysis. GA control
211 composite sample represents the level 0% of biochar concentration in the calibration curve.
212 In addition to the five concentrations of biochar in soil (above), duplicate samples of 100% soil (no
213 biochar) and 100% biochar (no soil) were also prepared in order to determine the proportion of control
214 soil and of biochar that is lost in the temperature range of recalcitrant OM (390-600 °C).

215

216 **2.3. Characterization of organic matter in soil and biochar amended soil**

217 **2.3.1. Quantitative analysis of carbon content**

218 The ability of two different dry combustion methods to determine carbon C content was evaluated in
219 model soil/biochar systems. Specifically, TOC analyser (method 1) and HCN analyser (method 2)
220 were applied to determine total carbon (TC), TOC and TIC in agriculture soils, with low (T) and high
221 (Q) content of IC, mixed with biochar at 1.00 % wt. level. Detailed descriptions of each method are
222 provided in Supplementary Materials. The method 1 were selected for the determination biochar
223 influence on TOC levels in agricultural soil of field experiment. Briefly, TOC content was determined
224 with a TOC analyzer (mod. SSM 5000A, Shimadzu, Japan) and calculated as the difference between
225 TC and TIC. Sample was placed in a ceramic boat and introduced in the combustion tube using IR
226 detection and calibration with D-(+)-glucose and Na₂CO₃ for TC and TIC, respectively. Carbonate-
227 rich soils (TIC > 20%) showed slow combustion rates or thermal decomposition reactions causing
228 underestimated TC values, because the time limit of instrument analysis is shorter than combustion
229 time. Therefore, these samples were mixed with V₂O₅ catalyst in order to accelerate the combustion
230 rate.

231

232 **2.3.2. Molecular characterization of organic matter by analytical pyrolysis**

233 The molecular composition of SOM in agricultural soil treated with different concentrations of
234 biochar was assessed with pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). Py-GC-
235 MS was performed using an EGA/PY-3030D micro-furnace pyrolyser (Frontier Laboratories Ltd.,
236 Japan) coupled with a 7890 Agilent HP gas chromatograph (GC) connected to a 5977 Agilent HP
237 quadrupole mass spectrometer (MS) (Agilent Technologies, USA). Unaltered ground samples
238 (25 ± 0.1 mg) were placed in small crucible capsules and introduced into the furnace, which was
239 preheated at 500 or 900 °C for 1 min using helium as carrier gas (1 mL min^{-1}) and an interface
240 temperature of 280 °C. The evolved gases were then directly injected into the GC-MS for analysis.
241 The GC injector was operated in split mode with a 10:1 ratio at 280 °C. Pyrolysis products were
242 separated by a HP-5MS fused silica capillary column (stationary phase poly[5% diphenyl/95%
243 dimethyl]siloxane, $30\text{m} \times 0.25\text{mm i.d.}$, 0.25mm film thickness, Agilent Technologies, USA) with the
244 following temperature program: 45 °C to 300°C at $10 \text{ }^\circ\text{C min}^{-1}$, then a hold for 5 min at 300 °C, using
245 helium as carrier gas (1 mL min^{-1}). The MS was operated in EI positive mode (70 eV, scanning 29-
246 600 m/z) with transfer line temperature 250 °C, ion source temperature 230 °C and quadrupole
247 temperature 150 °C. The relative abundance of each pyrolysis product was calculated as the
248 percentage of their signal relative to the 18 most abundant peaks, using the main ions (m/z) of each
249 product.

250

251 **2.4. Characterization of DOM in soil and biochar amended soil**

252 **2.4.1. Extraction of DOM**

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

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

264

265 **2.4.2. Quantitative analysis of DOC and TN**

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

272

$$273 \quad (1) \text{ WSOC } \left(\frac{\text{mg DOC}}{\text{g SOC}} \right) = \frac{V \text{ (L)} * C \left(\frac{\text{mg}}{\text{L}} \right)}{\text{TOC} \left(\frac{\text{g}}{\text{Kg}} \right) * M \text{ (Kg)}}$$

274

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

278

279 **2.4.3. Spectroscopic analysis of DOM**

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

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

284

$$285 \quad (2) \text{SUVA}_{254} = \frac{a_{254}}{\text{DOC}}$$

286

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

289

$$290 \quad (3) a_{254} (\text{m}^{-1}) = \frac{UV_{254} \times 2.303}{l (\text{m})}$$

291

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

294

295 **2.4.4. Analytical pyrolysis of DOM**

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

307 600 m/z) with transfer line temperature 250 °C, ion source temperature 230 °C and quadrupole
308 temperature 150 °C. The relative proportions of each pyrolysis product were calculated as the
309 percentage of the sum of all peak areas (total quantified peak area, TQPA). Quantification of each
310 pyrolysis product was based on the peak area of specific m/z fragments.

311

312 **2.5. Statistical analysis**

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

319

320 **3. Results and discussion**

321 **3.1. Soil and biochar characterization**

322 The characteristics of the biochar used for soil amendment are reported in Table 1. The molar H/C
323 and O/C ratios of 0.10 and 0.08, respectively, indicate a high degree of carbonization and aromaticity
324 (Conti et al., 2014). The C content of fresh biochar decreases to 56% with an aging period of 1 year
325 in the field (Table 1). Therefore, through the aging process, biochar C content significantly decreases
326 by 4% ($p < 0.05$). Similarly, N and ash content of aged biochar were significantly lower than fresh
327 biochar, whereas H content of aged biochar (0.94%) was significantly higher than fresh biochar
328 (0.50%).

329

330 *Table 1.* Elemental analysis (oxygen by difference), ash content, molar ratios and pH of field soil,
331 fresh and aged biochar applied in the field experimental from samples GA17-45 and GA17-60 (mean
332 values \pm 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

333

334 The chemical and physical characteristics of the control soil and biochar amended soils (15, 30, 45,
335 60, 90 and 120 t ha⁻¹) during the two years since the first application of biochar are reported in Tables
336 1 and 2. The soil pH has been slightly modified by the amendments in both years (soil 2017 and
337 2018). The pH of the soil in the first year after application increased with the concentration of biochar
338 amendment, from 8.14 in the control soil to 8.37 in the biochar amended GA17-45 and in the second
339 year from 8.24 in the control soil to 8.54 in the biochar amended GA18-120 (Table 2). The increase
340 in soil pH after the application of biochar is attributed to the alkaline substances in biochar with high
341 degree of carbonization (Conti et al., 2014). Several studies showed that biochar increases the soil
342 pH (Ding et al., 2016; Rombolà et al., 2019). However, the application of biochar with lower pH than
343 the targeted soils might have the potential to decrease soil pH, especially with higher biochar
344 application rates. In these cases, acidic materials produced by the oxidation of biochar and organic
345 matters may have caused the pH decrease (Liu et al., 2012).

346

347 *Table 2.* Chemical characteristics of the control soil and biochar amended soils (15, 30, 45, 60, 90
348 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

349

350 **3.2. Determination of biochar content in field experiment**

351 The TGA method was applied to determine the concentration of biochar in soil samples withdrawn
352 from field experiments (GA) amended with different amount of biochar PSR. Mass loss at different
353 temperature intervals relative to labile OM (220-390 °C), recalcitrant OM (390-600 °C) and
354 carbonates (600-800 °C) are reported in Table 3. The soils treated with different biochar loads
355 presented similar content of labile OM (about 3 % mass loss) indicating that biochar did not affect
356 this component. Instead, the content of recalcitrant OM of field amended soils was higher than control
357 due to the contribution of biochar to this OM component. To the purpose of determining the
358 concentration of biochar, a calibration curve was generated utilizing a control soil sample mixed with
359 different amount of biochar PSR. The obtained data fitted the equation $y = 0.688x + 3.93$ (n=6) where
360 y is the mass loss and x the biochar concentration in soil (% weight). The mass loss of PSR biochar
361 occurred in the temperature range of recalcitrant OM (390-600 °C). A linear regression between
362 weight loss and biochar content in soil was observed, with a significant coefficient of determination
363 ($R^2 = 0.997$). The slope was similar to that obtained with a soil rich in inorganic carbon (soil Q) in
364 accordance with the important mass loss in the thermal region of carbonate decomposition (Table 3).
365 This calibration curve was used to determine the content of biochar in the real sample. The measured
366 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
-----------------	-----------	-----------	---------	-----------	------

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

388

389 **3.3. Organic matter characterization in soils untreated and treated with biochar**

390 **3.3.1. Quantification of total and organic carbon**

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

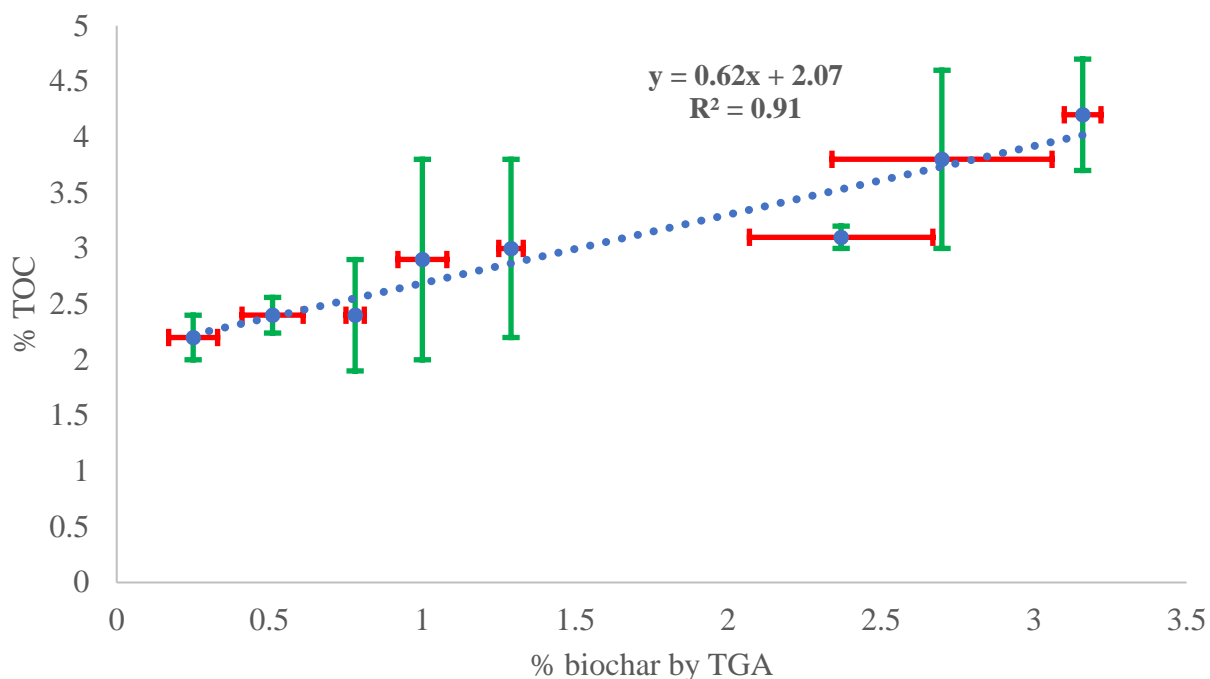
398 Figure 1 shows the relationship between the TOC contents measured with the TOC analyzer and the
399 biochar concentration determined by the TGA method in the field soils treated with biochar. Each
400 data point represents the average value from four replicate measurements. Linear regression analysis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

407

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

410

411



412
413
414

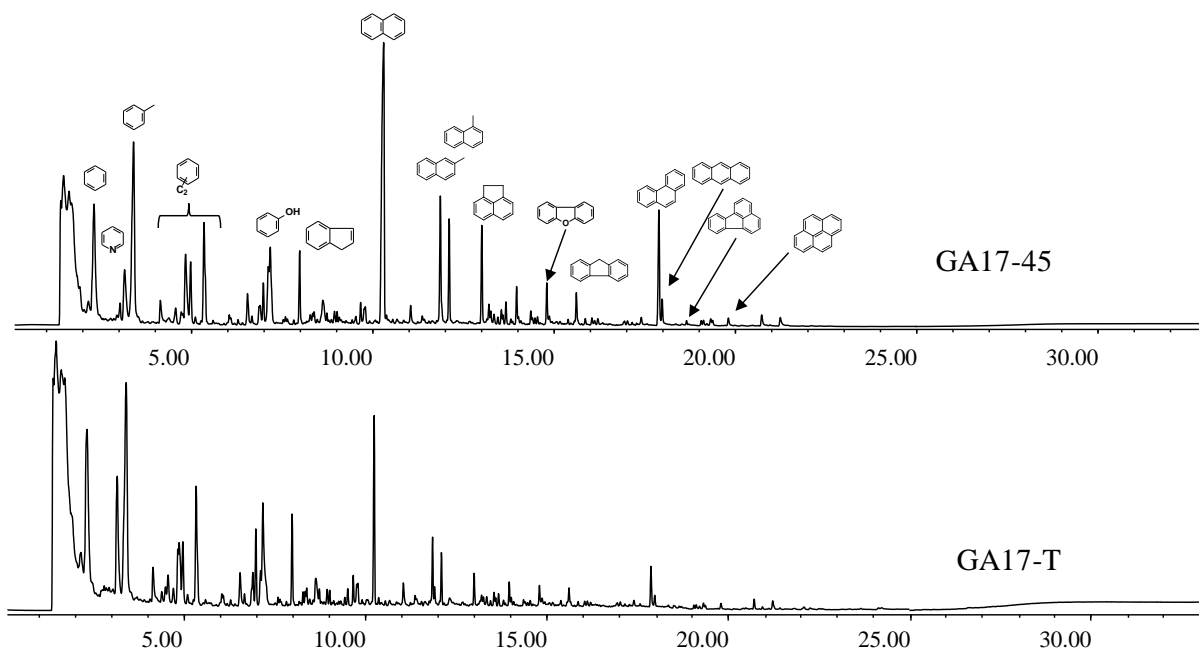
415 3.3.2. Organic matter characterization by analytical pyrolysis

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

429 °C). The appearance of PAH has traditionally been identified as a product of the analytical pyrolysis
430 of charred materials (González-Pérez et al., 2007; Girona-García et al., 2019). In analogy to the
431 pyrogenic carbon produced from the partial combustion of organic materials, including biomass and
432 fossil fuels, the biochar matrix comprises a complex assemblage of polyaromatic structures along
433 with heteroaromatic components and alkyl moieties from thermally degraded biomacromolecules
434 (Rombolà et al., 2016). However, in this study, the results of fresh and aged biochar Py-GC-MS
435 analysis showed that the increased levels of PAHs in amended soils in comparison to control soil are
436 not caused by analytical pyrolysis of biochar present in the soil. Moreover, the results (see paragraph
437 3.6.) showed that added biochar into soil may enhance the microbial activity, probably accelerating
438 the degradation of soil organic matter (Mitchell et al., 2015) and influencing the persistence of PAHs.
439 Rising microbial activity initially promotes the decomposition of labile compounds, and later
440 promotes the degradation of more recalcitrant substances. Moreover, Yang et al., (2019) reported that
441 high temperature pyrolyzed biochars preferentially retained high-molecular-weight humic-like DOC
442 species within soil.

443

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



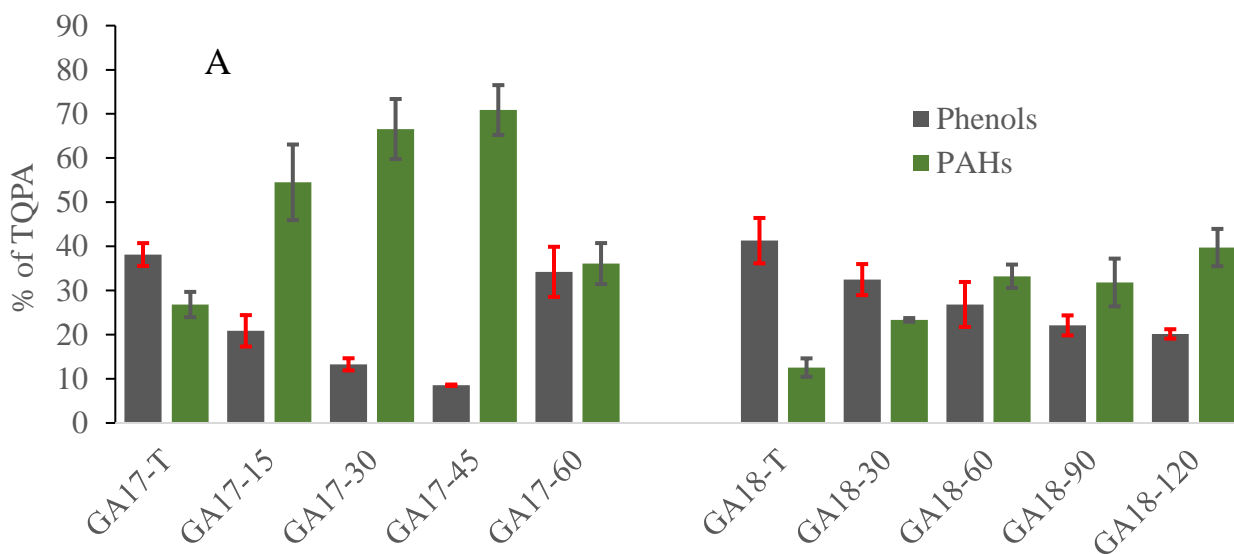
446

447

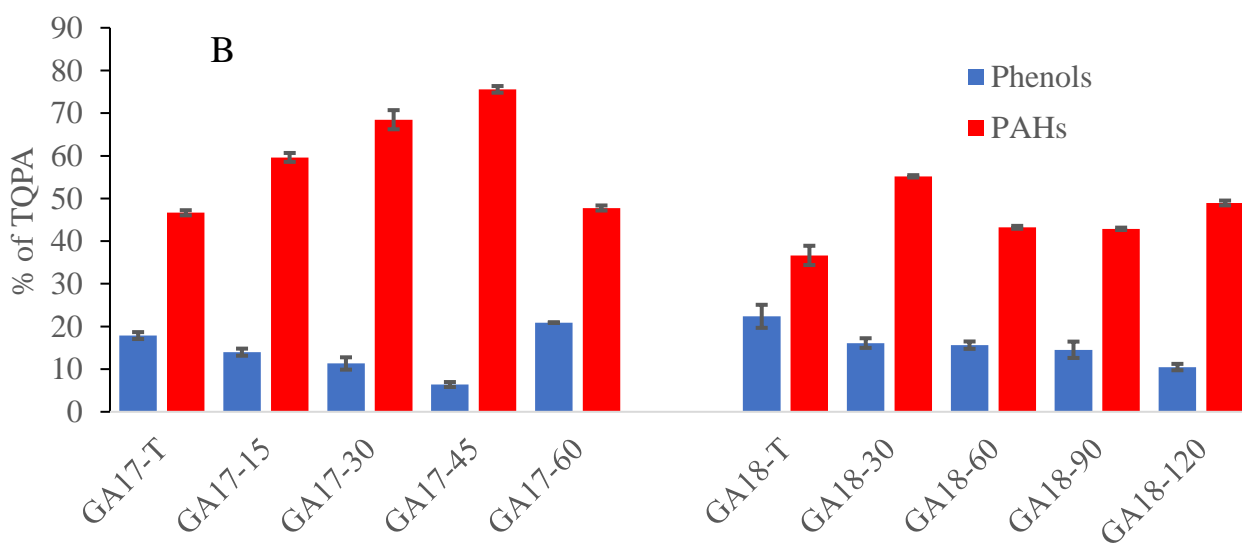
448 Other pyrolytic products were phenol and C1 alkyl phenols, which can be originated from any
 449 phenolic precursor including lignin, tannin, proteinaceous biomass, weakly charred BC and
 450 carbohydrates (Stuczynski et al., 1997; Vancampenhout et al., 2009). However, since phenols are
 451 minor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

458

459 *Figure 3.* Relative proportions (% of total quantified peak area, sum 100%) of the Py-GC-MS
 460 analyses performed at 500 °C (A) and at 900 °C (B) of control soils and biochar amended soils (15,
 461 30, 45, 60, 90 and 120 t ha⁻¹) from field experiment at different sampling dates. Mean values ± s.d.
 462 (n = 3).



463



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.

471 Analytical pyrolysis has been proposed as a fast alternative approach for the analysis of PAHs
472 included in environmental matrices (i.e., soil and sediments), as pollutants, constituents (i.e., coals
473 and black carbon) or pyrogenic (i.e., chars and charred materials from forest fires) (González-Pérez
474 et al., 2014; Biache et al., 2017). The methyl/parent PAH ratios of selected PAHs, for instance
475 methylnaphthalene/naphthalene (MeNAP/NAP), have been proposed as suitable indices to evaluate
476 the biochar carbonization degree (Calvelo Pereira et al. 2011; Rombolà et al., 2016). However, little
477 is known about how biochar addition in soil affects the methyl/parent PAH ratios. Therefore, the
478 degree of alkylation in control soils and in biochar amended soils was studied by Py-GC-MS. In
479 addition, in order to assess the biochar impact on soil PAHs, the PAH isomeric ratios determined by
480 Py-GC-MS were reported. Frequent routine methodological approaches to study PAHs and PAH
481 isomeric ratios from environmental samples are based on analyses of solvent extracts and further
482 chromatographic separation. Rombolà et al. (2019) reported that solvent extractable naphthalene
483 NAP, phenanthrene PHE, fluoranthene FLA and their isomers anthracene ANT and pyrene PYR in
484 biochar amended soils could be considered as potential candidates for tracking the PAH imprinting
485 of biochar by means of diagnostic ratios. For solvent extractable PAHs, the isomeric ratios
486 $ANT/(ANT+PHE)$ and $FLA/(FLA+PYR)$ are frequently used for source apportionment (Yunker et
487 al., 2015). In addition, in order to assess sources of PAHs in soils, the non-isomeric ratio
488 $NAP/(NAP+PHE)$ was used for soil treated with biochar (Rombolà et al., 2019). The few studies in
489 the literature reporting on the PAH isomeric ratios determine by Py-GC-MS have shed light on a fast
490 alternative approach to detect PAHs in environmental samples (González-Pérez et al., 2014).

491 The PAH ratios determined by Py-GC-MS at 500 °C of control soils and biochar amended soils are
492 reported in Table SM8. The MeNAP/NAP ratios were lower in the biochar amended soil decreasing
493 significantly with the concentration of biochar amendment. In particular, in the first year after biochar
494 application, MeNAP/NAP decreased from 0.66 in the control soil to 0.33 in the biochar amended soil
495 GA17-60 with 0.78% of biochar. In the second year, from 0.87 in control soil to 0.23 in GA17-120
496 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

523 DOC. Soil-derived DOC concentrations in the soils treated with biochar (15-32 mg L⁻¹) were lower
524 than in the soils without biochar (31-35 mg L⁻¹; Table 4). Specifically, the DOC of the soil in the first
525 year after application significantly decreased with the concentration of biochar amendment, from
526 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%
527 of biochar and 26.4±1.0 mg L⁻¹ in the GA17-45 with 1.3% of biochar, representing a loss of 26%.
528 In the second year after application, the DOC values showed a more marked difference between the
529 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
530 particular, DOC concentration decreased significantly with biochar concentration from 30.7± 2.4 mg
531 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
532 of 50%. Similarly, Feng et al., (2021) showed that high temperature pyrolyzed biochars (> 700 °C,
533 as PSR biochar used in our field experiment) decrease soil DOC concentration up to 50% and the
534 decrement increases with the increase of biochar amount. The potential of biochar to decrease of
535 DOC in field experiment was evidenced by Liu et al., (2019), who observed that biochar amendment
536 causes a significant increase of macropores and thus the enhanced infiltration of soil water. Such
537 changes could lead to increased flow discharge, which in turn results in elevated leaching of organic
538 carbon during rainfalls. In this study, moreover, more marked differences are observed between
539 amended and control soils in the dissolved fraction of total SOC (WSOC = mg DOC g⁻¹ TOC). The
540 values of WSOC decreased after biochar amendments in both years and in all treatments (Table 4),
541 in proportion to the TGA and TOC values. Almost two years after the first biochar application, the
542 amount of WSOC in the GA18-120 amended soils (3.4 mg g⁻¹ TOC, biochar 3.16%) were significantly
543 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
544 results of this study suggested that biochar reduces the DOC, and this effect increased with time and
545 biochar amount over the first two years after application. This could be due to several phenomena,
546 such as adsorption and microorganism growth, which can be enhanced with time and aging of
547 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 SUVA₂₅₄ 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 SUVA₂₅₄ 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, SUVA₂₅₄ 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 SUVA₂₅₄ value, and all treatments
572 showed higher SUVA₂₅₄ values. The soil SUVA₂₅₄ value increased from 1.38 L mg⁻¹m⁻¹ in control
573 soil to 2.24 L mg⁻¹m⁻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⁻¹m⁻¹ in control soil to 3.55 L mg⁻¹m⁻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 SUVA₂₅₄ significantly increased in the extracted DOM of biochar-amended soils relative to the
578 control (Li et al., 2018; Zhang et 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 SUVA₂₅₄ 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 SUVA₂₅₄ values was not strong indicating that SUVA₂₅₄ 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

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

595 Some studies have been conducted in various types of soil on the molecular composition of DOM by
596 Py-GC-MS, and different dominant compounds have been identified (Rosa et al., 2015; Jiang et al.,
597 2017). Contrary to previous observations of soil-derived DOM analyzed by Py-GC-MS (Jiang et al.,
598 2017), pyrolysis products that can be traced back to relatively intact plant-derived polysaccharides,
599 e.g., pyrans and anhydrosugars (Pouwels et al., 1989), were not detected. In the present study, only
600 3-hydroxy-2-methyl-4H-pyran-4-one was identified. Other carbohydrate products, i.e., furans,
601 furfurals, and cyclopentenones can be produced by pyrolysis of plant-derived, microbial and
602 planktonic carbohydrates.

603 In the first year after application, the MAHs account for $6.1 \pm 0.9\%$ in the control soil (GA17-T), 3.4
604 $\pm 0.5\%$ (GA17-30) and $3.8 \pm 0.5\%$ (GA17-45) for biochar amended soil with 1% and 1.3% of biochar,
605 respectively. Benzene is the most abundant product, followed by toluene and styrene (Table SM10).
606 However, the MAHs determined by Py-GC-MS in the soils GA18 indicated no significant difference
607 between the amended and control soils, except for GA18-30 vs. GA18-T. The MAHs are not
608 diagnostic of any source and are probably related to microbial proteinaceous material (e.g., toluene).
609 PAHs are associated with polycondensation of aromatic moieties in pyrogenic organic matter, e.g.
610 charcoal or soot (Kaal et al., 2016). Clearly, the incorporation of biochar into soil systems represents
611 an input of PAHs (Rombolà et al., 2019). However, several studies have shown a low bioavailability
612 of PAHs in biochars, probably due to their strong interaction with the carbonaceous matrix of biochar
613 (Hale et al., 2012; Tomczyk et al., 2020). Therefore, biochar application could increase or decrease
614 the aromatic hydrocarbon components of DOM, influencing the release of PAHs by pyrolysis. This
615 aromatic fraction is affected by different degrees cording to the complex effects of biochar on DOM
616 and on mobility, persistence and degradation in soil. For instance, biochar may increase or decrease
617 the extent and rate of degradation of aromatic hydrocarbons depending on cell density, the microbial
618 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 $4.2 \pm 0.7\%$ and $6.5 \pm 1.2\%$ 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 ($2.4 \pm 0.8\%$ GA17-45, $2.3 \pm 0.3\%$ GA18-30 and $2.3 \pm 0.6\%$ 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 $0.69 \pm 0.10\%$ in
643 control soil GA17-T to $0.14 \pm 0.02\%$ in soil GA17-45 with biochar and from $0.80 \pm 0.04\%$ in control
644 soil GA18-T to $0.21 \pm 0.02\%$ in soil GA18-30 with biochar. Decrease of phenols in Py-GC-MS of

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

647 In the first year after application, the compounds with N (NCOMPs) in their structure accounted for
648 $51 \pm 0.2\%$ in the control soil (GA17-T), $63 \pm 2.3\%$ (GA17-30) and $66 \pm 11\%$ (GA17-45) for biochar
649 amended soil with 1% and 1.3% of biochar, respectively (Table 5). The NCOMPs in the second year
650 after application accounted for $44 \pm 3.3\%$ in the control soil (GA18-T), $67.8 \pm 0.09\%$ (GA18-30) and
651 $77 \pm 6.2\%$ (GA18-90) for biochar amended soil, respectively. Therefore, the NCOMPs levels
652 increased in all soil treatments with biochar, except for GA17-60 and GA18-120.

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

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

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 \pm s.d. (n = 2).*

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

698 ^aAbbreviations: 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

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

722

723 **Acknowledgements**

724 Study partly conducted within the project N. 5005053 RIFASA of the Programma di Sviluppo Rurale
725 (PSR 2014-2020) della Regione Emilia Romagna Tipo operazione 16.1.01. We owe our special
726 thanks to "Ganazzoli Filippo" farm where the experimental trial was conducted.

727

728 **References**

- 729 Bachmann, H. J., Bucheli, T. D., Dieguez-Alonso, A., Fabbri, D., Knicker, H., Schmidt, H. P.,
730 Ulbricht, A., Becker, R., Buscaroli, A., Buerge, D., Cross, A., Dickinson, D., Enders, A., Esteves,
731 V. I., Evangelou, M. W. H., Fellet, G., Friedrich, K., Gasco Guerrero, G., Glaser, B., Hanke, U.M.,
732 Hanley, K., Hilber, I., Kalderis, D., Leifeld, J., Masek, O., Mumme, J., Paneque, M., Calvelo
733 Pereira, R., Rees, F., Rombolà, A.G., De la Rosa, J.M., Sakrabani, R., Sohi, S. Soja, G., Valagussa,
734 M., Verheijen, F., Zehetner, F., 2016. Toward the Standardization of Biochar Analysis: The COST
735 Action TD1107 Interlaboratory Comparison. *J. Agric. Food Chem.*, 64, 513-527.
736 <https://doi.org/10.1021/acs.jafc.5b05055>
737
738 Bi, Y., Kuzyakov, Y., Cai, S., Zhao, X., 2021. Accumulation of organic compounds in paddy soils
739 after biochar application is controlled by iron hydroxides. *Sci. Total Environ.*, 764, Article 144300.
740 <https://doi.org/10.1016/j.scitotenv.2020.144300>
741
742 Biache, C., Lorgeoux, C., Saada, A., Colombano, S., Faure, P., 2017. Fast method to quantify PAHs
743 in contaminated soils by direct thermodesorption using analytical pyrolysis. *Talanta*, 166, 241-248.
744 <https://doi.org/10.1016/j.talanta.2017.01.055>
745
746 Buurman, P., Roscoe, R., 2011. Different chemical composition of free light, occluded light and
747 extractable SOM fractions in soils of Cerrado and tilled and untilled fields, Minas Gerais, Brazil: A
748 pyrolysis-GC/MS study. *Eur. J. Soil Sci.*, 62(2), 253-266. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2389.2010.01327.x)
749 [2389.2010.01327.x](https://doi.org/10.1111/j.1365-2389.2010.01327.x)
750
751 Calvelo Pereira, R., Kaal, J., Camps Arbestain, M., Pardo Lorenzo, R., Aitkenhead, W., Hedley, M.,
752 Macías, F., Hindmarsh, J., Maciá-Agulló, J. A., 2011. Contribution to characterisation of biochar to

753 estimate the labile fraction of carbon. *Org. Geochem.*, 42(11), 1331-1342.
754 <https://doi.org/10.1016/j.orggeochem.2011.09.002>
755

756 Conti, R., Rombolà, A. G., Modelli, A., Torri, C., Fabbri, D., 2014. Evaluation of the thermal and
757 environmental stability of switchgrass biochars by Py-GC-MS. *J. Anal. Appl. Pyrolysis*, 110(1),
758 239-247. <https://doi.org/10.1016/j.jaap.2014.09.010>
759

760 Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., Zheng, B., 2016. Biochar
761 to improve soil fertility. A review. *Agron. Sustain. Dev.*, 36 (2), 36. [https://doi.org/10.1007/s13593-](https://doi.org/10.1007/s13593-016-0372-z)
762 [016-0372-z](https://doi.org/10.1007/s13593-016-0372-z)
763

764 Dong, X., Li, G., Lin, Q., Zhao, X., 2017. Quantity and quality changes of biochar aged for 5 years
765 in soil under field conditions. *Catena*, 159, 136-143. <https://doi.org/10.1016/j.catena.2017.08.008>
766

767 Dong, X., Ma, L. Q., Gress, J., Harris, W., Li, Y., 2014. Enhanced Cr(VI) reduction and As(III)
768 oxidation in ice phase: Important role of dissolved organic matter from biochar. *J. Hazard Mater.*
769 267, 62-70. <https://doi.org/10.1016/j.jhazmat.2013.12.027>
770

771 Dong, X., Singh, B. P., Li, G., Lin, Q., Zhao, X., 2019. Biochar has little effect on soil dissolved
772 organic carbon pool 5 years after biochar application under field condition. *Soil Use Manag.*, 35(3),
773 466-477. <https://doi.org/10.1111/sum.12474>
774

775 El-Naggar, A., Lee, S.S., Rinklebe, J., Farooq, M., Song, H., Sarmah, A.K., Zimmerman, A.R.,
776 Ahmad, M., Shaheen, S.M., Ok, Y.S., 2019. Biochar application to low fertility soils: a review of
777 current status, and future prospects. *Geoderma*, 337, 536-554.
778 <https://doi.org/10.1016/J.GEODERMA.2018.09.034>

779

780 Eykelbosh, A. J., Johnson, M. S., & Couto, E. G., 2015. Biochar decreases dissolved organic carbon
781 but not nitrate leaching in relation to vinasse application in a Brazilian sugarcane soil. *J. Environ.*
782 *Manag.*, 149, 9-16. <https://doi.org/10.1016/j.jenvman.2014.09.033>

783

784 Fan, Q., Sun, J., Quan, G., Yan, J., Gao, J., Zou, X., Cui, L., 2020. Insights into the effects of long-
785 term biochar loading on water-soluble organic matter in soil: Implications for the vertical co-
786 migration of heavy metals. *Environ. Int.*, 136, Article 1054392.
787 <https://doi.org/10.1016/j.envint.2019.105439>

788

789 Feng, Z., Fan, Z., Song, H., Li, K., Lu, H., Liu, Y., Cheng, F., 2021. Biochar induced changes of
790 soil dissolved organic matter: The release and adsorption of dissolved organic matter by biochar
791 and soil. *Sci. Total Environ.*, 783, Article 1470912. <https://doi.org/10.1016/j.scitotenv.2021.147091>

792

793 Ghidotti, M., Fabbri, D., Mašek, O., Mackay, C. L., Montalti, M., Hornung, A., 2017. Source and
794 Biological Response of Biochar Organic Compounds Released into Water; Relationships with Bio-
795 Oil Composition and Carbonization Degree. *Environ. Sci. Technol.*, 51(11), 6580-6589.
796 <https://doi.org/10.1021/acs.est.7b00520>

797

798 Giagnoni, L., Maienza, A., Baronti, S., Vaccari, F. P., Genesio, L., Taiti, C., Martellini, T.,
799 Scodellini, R., Cincinelli, A., Costa, C., Mancuso, S., Renella, G., 2019. Long-term soil biological
800 fertility, volatile organic compounds and chemical properties in a vineyard soil after biochar
801 amendment. *Geoderma*, 344, 127-136. <https://doi.org/10.1016/j.geoderma.2019.03.011>

802

803 Gmach, M. R., Cherubin, M. R., Kaiser, K., Cerri, C. E. P., 2020. Processes that influence dissolved
804 organic matter in the soil: A review. *Sci. Agric.*, 77, Article e20180164.
805 <https://doi.org/10.1590/1678-992x-2018-0164>
806

807 González-Pérez, J. A., Almendros, G., de La Rosa, J. M., González-Vila, F. J., 2014. Appraisal of
808 polycyclic aromatic hydrocarbons (PAHs) in environmental matrices by analytical pyrolysis (Py-
809 GC/MS). *J. Anal. Appl. Pyrolysis*, 109, 1-8. <https://doi.org/10.1016/j.jaap.2014.07.005>
810

811 Green, S. A., Blough, N. V., 1994. Optical absorption and fluorescence properties of chromophoric
812 dissolved organic matter in natural waters. *Limnol. Oceanogr.*, 39, 1903-1916.
813 <https://doi.org/10.4319/lo.1994.39.8.1903>
814

815 Greenwood, P. F., Berwick, L. J., Croué, J. P., 2012. Molecular characterisation of the dissolved
816 organic matter of wastewater effluents by MSSV pyrolysis GC-MS and search for source markers.
817 *Chemosphere*, 87(5), 504-512. <https://doi.org/10.1016/j.chemosphere.2011.12.051>
818

819 Hale, S. E., Lehmann, J., Rutherford, D., Zimmerman, A. R., Bachmann, R. T., Shitumbanuma, V.,
820 O'Toole, A., Sundqvist, K. L., Arp, H. P. H., Cornelissen, G., 2012. Quantifying the total and
821 bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars. *Environ. Sci. Technol.*,
822 46(5), 2830-2838. <https://doi.org/10.1021/es203984k>
823

824 Huang, M., Li, Z., Luo, N., Yang, R., Wen, J., Huang, B., Zeng, G., 2019. Application potential of
825 biochar in environment: Insight from degradation of biochar-derived DOM and complexation of
826 DOM with heavy metals. *Sci. Total Environ.*, 646, 220-228.
827 <https://doi.org/10.1016/j.scitotenv.2018.07.282>
828

829 Inamdar, S., Finger, N., Singh, S., Mitchell, M., Levia, D., Bais, H., Scott, D., McHale, P., 2012.
830 Dissolved organic matter (DOM) concentration and quality in a forested mid-Atlantic watershed,
831 USA. *Biogeochemistry*, 108(1-3), 55-76. <https://doi.org/10.1007/s10533-011-9572-4>
832

833 Jaffé, R., Ding, Y., Niggemann, J., Vähätalo, A. v., Stubbins, A., Spencer, R. G. M., Campbell, J.,
834 & Dittmar, T., 2013. Global charcoal mobilization from soils via dissolution and riverine transport
835 to the oceans. *Science*, 340(6130), 345-347. <https://doi.org/10.1126/science.1231476>
836

837 Jiang, T., Kaal, J., Liang, J., Zhang, Y., Wei, S., Wang, D., Green, N. W., 2017. Composition of
838 dissolved organic matter (DOM) from periodically submerged soils in the Three Gorges Reservoir
839 areas as determined by elemental and optical analysis, infrared spectroscopy, pyrolysis-GC–MS and
840 thermally assisted hydrolysis and methylation. *Sci. Total Environ.*, 603-604, 461-471.
841 <https://doi.org/10.1016/j.scitotenv.2017.06.114>
842

843 Kaal, J., Cortizas, A. M., & Biester, H., 2017. Downstream changes in molecular composition of
844 DOM along a headwater stream in the Harz mountains (Central Germany) as determined by FTIR,
845 Pyrolysis-GC–MS and THM-GC–MS. *J. Anal. Appl. Pyrolysis*, 126, 50-61.
846 <https://doi.org/10.1016/j.jaap.2017.06.025>
847

848 Kaal, J., Martínez-Cortizas, A., Nierop, K. G. J., Buurman, P., 2008. A detailed pyrolysis-GC/MS
849 analysis of a black carbon-rich acidic colluvial soil (Atlantic ranker) from NW Spain. *Appl.*
850 *Geochem.*, 23(8), 2395-2405. <https://doi.org/10.1016/j.apgeochem.2008.02.026>
851

852 Kaal, J., Wagner, S., Jaffé, R., 2016. Molecular properties of ultrafiltered dissolved organic matter
853 and dissolved black carbon in headwater streams as determined by pyrolysis-GC-MS. *J. Anal. Appl.*
854 *Pyrolysis*, 118, 181-191. <https://doi.org/10.1016/j.jaap.2016.02.003>

855

856 Koide, R. T., Petprakob, K., Peoples, M., 2011. Quantitative analysis of biochar in field soil. *Soil*
857 *Biol. Biochem.*, 43(7), 1563-1568. <https://doi.org/10.1016/j.soilbio.2011.04.006>

858

859 Laghari, M., Naidu, R., Xiao, B., Hu, Z., Mirjat, M.S., Hu, M., Kandhro, M.N., Chen, Z., Guo, D.,
860 Jogi, Q., Abudi, Z.N., 2016. Recent developments in biochar as an effective tool for agricultural soil
861 management: a review. *J. Sci. Food Agric.*, 96, 4840-4849. <https://doi.org/10.1002/jsfa.7753>.

862

863 Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar
864 effects on soil biota – a review. *Soil Biol. Biochem.* 43, 1812-1836.
865 <https://doi.org/10.1016/j.soilbio.2011.04.022>.

866

867 Lehmann, J., Joseph, S., 2015. Biochar for environmental management: an introduction. In:
868 Lehmann, J., Joseph, S. (Eds.), *Biochar for Environmental Management: Science, Technology and*
869 *Implementation*. Taylor and Francis, London, 1-13.

870

871 Li, G., Khan, S., Ibrahim, M., Sun, T. R., Tang, J. F., Cotner, J. B., Xu, Y. Y., 2018. Biochars
872 induced modification of dissolved organic matter (DOM) in soil and its impact on mobility and
873 bioaccumulation of arsenic and cadmium. *J. Hazard Mater.*, 348, 100-108.
874 <https://doi.org/10.1016/j.jhazmat.2018.01.031>

875

876 Liu, C., Wang, H., Li, P., Xian, Q., Tang, X., 2019. Biochar's impact on dissolved organic matter
877 (DOM) export from a cropland soil during natural rainfalls. *Sci. Total Environ.*, 650, 1988-1995.
878 <https://doi.org/10.1016/j.scitotenv.2018.09.356>

879

880 Liu, X.-H., Zhang, X.-C., 2012. Effect of Biochar on pH of Alkaline Soils in the Loess Plateau:
881 Results from Incubation Experiments. *J. Agric. Biol.*, 14, 745-750. <http://www.fspublishers.org>
882

883 Lutsenko, T. N., Chernova, E. N., Lysenko, E. V., Ryzhakov, D. S., 2015. Organic Matter in the
884 Small Lakes of the Sikhote-Alin Biosphere Reserve. *Achiev. Life Sci.*, 9(1), 32-36.
885 <https://doi.org/10.1016/j.als.2015.05.005>
886

887 Majumder, S., Neogi, S., Dutta, T., Powel, M. A., Banik, P., 2019. The impact of biochar on soil
888 carbon sequestration: Meta-analytical approach to evaluating environmental and economic
889 advantages. *J. Environ. Manag.*, 250, 1-11. <https://doi.org/10.1016/j.jenvman.2019.109466>
890

891 Mitchell, P.J., Simpson, A.J., Soong, R., Simpson M.J., 2015. Shifts in microbial community and
892 water-extractable organic matter composition with biochar amendment in a temperate forest soil.
893 *Soil Biol. Biochem.*, 81, 244-254. <http://dx.doi.org/10.1016/j.soilbio.2014.11.017>
894

895 Nakhli, S. A. A., Panta, S., Brown, J. D., Tian, J., Imhoff, P. T., 2019. Quantifying biochar content
896 in a field soil with varying organic matter content using a two-temperature loss on ignition method.
897 *Sci. Total Environ.*, 658, 1106-1116. <https://doi.org/10.1016/j.scitotenv.2018.12.174>
898

899 Nebbioso, A., Piccolo, A., 2013. Molecular characterization of dissolved organic matter (DOM): A
900 critical review. *Anal. Bioanal. Chem.*, 405 (1), 109-124. <https://doi.org/10.1007/s00216-012-6363-2>
901

902 Neff, J. C., Finlay, J. C., Zimov, S. A., Davydov, S. P., Carrasco, J. J., Schuur, E. A. G., Davydova,
903 A. I., 2006. Seasonal changes in the age and structure of dissolved organic carbon in Siberian rivers
904 and streams. *Geophys. Res. Lett.*, 33 (23). <https://doi.org/10.1029/2006GL028222>
905

906 Pan, Q., Zhuo, X., He, C., Zhang, Y., Shi, Q., 2020. Validation and Evaluation of High-Resolution
907 Orbitrap Mass Spectrometry on Molecular Characterization of Dissolved Organic Matter. ACS
908 Omega, 5(10), 5372-5379. <https://doi.org/10.1021/acsomega.9b04411>
909

910 Pouwels, A. D., Eijkel, G. B., Boon, J. J., 1989. Curie-point pyrolysis–capillary gas
911 chromatography–high-resolution mass spectrometry of microcrystalline cellulose. J. Anal. Appl.
912 Pyrolysis, 14, 237-280.
913 [https://doi.org/10.1016/0165-2370\(89\)80003-8](https://doi.org/10.1016/0165-2370(89)80003-8)
914

915 Purakayastha, T. J., Bera, T., Bhaduri, D., Sarkar, B., Mandal, S., Wade, P., Kumari, S., Biswas, S.,
916 Menon, M., Pathak, H., Tsang, D. C. W., 2019. A review on biochar modulated soil condition
917 improvements and nutrient dynamics concerning crop yields: Pathways to climate change
918 mitigation and global food security. Chemosphere, 227, 345-365.
919 <https://doi.org/10.1016/j.chemosphere.2019.03.170>
920

921 Raya-Moreno, I., Cañizares, R., Domene, X., Carabassa, V., Alcañiz, J. M., 2017. Comparing
922 current chemical methods to assess biochar organic carbon in a Mediterranean agricultural soil
923 amended with two different biochars. Sci. Total Environ., 598, 604-618.
924 <https://doi.org/10.1016/j.scitotenv.2017.03.168>
925

926 Rombolà, A. G., Fabbri, D., Baronti, S., Vaccari, F. P., Genesio, L., Miglietta, F., 2019. Changes in
927 the pattern of polycyclic aromatic hydrocarbons in soil treated with biochar from a multiyear field
928 experiment. Chemosphere, 219, 662-670. <https://doi.org/10.1016/j.chemosphere.2018.11.178>
929

930 Rombolà, A. G., Fabbri, D., Meredith, W., Snape, C. E., Dieguez-Alonso, A., 2016. Molecular
931 characterization of the thermally labile fraction of biochar by hydropyrolysis and pyrolysis-GC/MS.
932 J. Anal. Appl. Pyrolysis, 121, 230-239. <https://doi.org/10.1016/j.jaap.2016.08.003>
933

934 Rombolà, A. G., Meredith, W., Snape, C. E., Baronti, S., Genesio, L., Vaccari, F. P., Miglietta, F.,
935 Fabbri, D., 2015. Fate of Soil Organic Carbon and Polycyclic Aromatic Hydrocarbons in a
936 Vineyard Soil Treated with Biochar. Environ. Sci. Technol., 49(18), 11037-11044.
937 <https://doi.org/10.1021/acs.est.5b02562>
938

939 Rosa, E., Dębska, B., Banach-Szott, M., Tobiasova, E., 2015. Use of HPLC, Py-GCMS, FTIR
940 methods in the studies of the composition of soil dissolved organic matter. Pol. J. Soil Sci., 48(1),
941 101-110. <https://doi.org/10.17951/pjss/2015.48.1.101>
942

943 Sharma, V.K., Anquandah, G.A.K., Yngard, R.A., Kim, H., Fekete, J., Bouzek, K., Ray, A.K.,
944 Golovko, D., 2009. Nonylphenol, octylphenol, and bisphenol-A in the aquatic environment: a
945 review on occurrence, fate, and treatment. J. Environ. Sci. Health, 44 (5), 423-442.
946 <https://doi.org/10.1080/10934520902719704>
947

948 Smebye, A., Alling, V., Vogt, R. D., Gadmar, T. C., Mulder, J., Cornelissen, G., Hale, S. E., 2016.
949 Biochar amendment to soil changes dissolved organic matter content and composition.
950 Chemosphere, 142, 100-105. <https://doi.org/10.1016/j.chemosphere.2015.04.087>
951

952 Song, C., Shan, S., Yang, C., Zhang, C., Zhou, X., Ma, Q., Yrjälä, K., Zheng, H., Cao, Y., 2020.
953 The comparison of dissolved organic matter in hydrochars and biochars from pig manure. Sci. Total
954 Environ., 720, Article 137423. <https://doi.org/10.1016/j.scitotenv.2020.137423>
955

956

957 Song, Y., Kong, Y., Wang, J., Ruan, Y., Huang, Q., Ling, N., Shen, Q., 2018. Identification of the
958 produced volatile organic compounds and the involved soil bacteria during decomposition of
959 watermelon plant residues in a *Fusarium*-infested soil. *Geoderma*, 315, 178-187.

960 <https://doi.org/10.1016/j.geoderma.2017.11.021>

961

962

963 Taherymoosavi, S., Joseph, S., Munroe, P., 2016. Characterization of organic compounds in a
964 mixed feedstock biochar generated from Australian agricultural residues. *J. Anal. Appl. Pyrolysis*,
965 120, 441-449. <https://doi.org/10.1016/j.jaap.2016.06.017>

966

967 Templier, J., Miserque, F., Barré, N., Mercier, F., Croué, J. P., Derenne, S., 2012. Is nitrogen
968 functionality responsible for contrasted responses of riverine dissolved organic matter in pyrolysis?
969 *J. Anal. Appl. Pyrolysis*, 97, 62-72. <https://doi.org/10.1016/j.jaap.2012.05.002>

970

971 Tomczyk, B., Siatecka, A., Jędruchiewicz, K., Sochacka, A., Bogusz, A., Oleszczuk, P., 2020.
972 Polycyclic aromatic hydrocarbons (PAHs) persistence, bioavailability and toxicity in sewage
973 sludge- or sewage sludge-derived biochar-amended soil. *Sci. Total Environ.*, 747, Article 141123.

974 <https://doi.org/10.1016/j.scitotenv.2020.141123>

975

976 Vancampenhout, K., Wouters, K., de Vos, B., Buurman, P., Swennen, R., Deckers, J., 2009.
977 Differences in chemical composition of soil organic matter in natural ecosystems from different
978 climatic regions - A pyrolysis-GC/MS study. *Soil Biol. Biochem.*, 41(3), 568-579.

979 <https://doi.org/10.1016/j.soilbio.2008.12.023>

980

981 Woolf, D., Lehmann, J., Lee, D. R., 2016. Optimal bioenergy power generation for climate change
982 mitigation with or without carbon sequestration. *Nat. Commun.*, 7, Article 13160.
983 <https://doi.org/10.1038/ncomms13160>
984

985 Yang, X.Y., Chang, K.H., Kim, Y.J., Zhang, J., Yoo, G., 2019. Effects of different biochar
986 amendments on carbon loss and leachate characterization from an agricultural soil. *Chemosphere*,
987 226, 625-635. <https://doi.org/10.1016/j.chemosphere.2019.03.085>
988

989 Yunker, M. B., Macdonald, R. W., Ross, P. S., Johannessen, S. C., Dangerfield, N., 2015. Alkane
990 and PAH provenance and potential bioavailability in coastal marine sediments subject to a gradient
991 of anthropogenic sources in British Columbia, Canada. *Org. Geochem.*, 89-90, 80-116.
992 <https://doi.org/10.1016/j.orggeochem.2015.10.002>
993

994 Zavalloni, C., Alberti, G., Biasiol, S., Vedove, G. D., Fornasier, F., Liu, J., Peressotti, A., 2011.
995 Microbial mineralization of biochar and wheat straw mixture in soil: A short-term study. *Soil*
996 *Ecol.*, 50(1), 45-51. <https://doi.org/10.1016/j.apsoil.2011.07.012>
997

998 Zhang, A., Zhou, X., Li, M., Wu, H., 2017. Impacts of biochar addition on soil dissolved organic
999 matter characteristics in a wheat-maize rotation system in Loess Plateau of China. *Chemosphere*,
1000 186, 986-993. <https://doi.org/10.1016/j.chemosphere.2017.08.074>
1001

1002 Zhang, P., Huang, P., Xu, X., Sun, H., Jiang, B., & Liao, Y., 2020. Spectroscopic and molecular
1003 characterization of biochar-derived dissolved organic matter and the associations with soil microbial
1004 responses. *Sci. Total Environ.*, 708, Article 134619. <https://doi.org/10.1016/j.scitotenv.2019.134619>
1005

1006 Zhu, X., Chen, B., Zhu, L., Xing, B., 2017. Effects and mechanisms of biochar-microbe interactions
1007 in soil improvement and pollution remediation: a review. *Environ. Pollut.*, 227, 98-115.
1008 <https://doi.org/10.1016/j.envpol.2017.04.032>