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2 **Fate of soil organic carbon and polycyclic aromatic hydrocarbons in a vineyard soil treated**
3 **with biochar**
4

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16 **Abstract**

17 The effect of biochar addition on the levels of black carbon (BC) and polycyclic aromatic
18 hydrocarbons (PAHs) in a vineyard soil in central Italy was investigated within a two year period.
19 Hydropyrolysis (HyPy) was used to determine the contents of BC (BC_{HyPy}) in the amended and
20 control soils while the hydrocarbon composition of the semi-labile (non-BC_{HyPy}) fraction released
21 by HyPy was determined by gas chromatography-mass spectrometry, together with the solvent-
22 extractable PAHs. The concentrations of these three polycyclic aromatic carbon reservoirs, changed
23 and impacted differently on the soil organic carbon over the period of the trial. The addition of
24 biochar (33 ton dry biochar ha⁻¹) gave rise to a sharp increase in soil organic carbon which could be
25 accounted for by an increase of BC_{HyPy}. Over time, the concentration of BC_{HyPy} decreased
26 significantly from 36 to 23 mg g⁻¹, and as a carbon percentage from 79% to 61%. No clear time
27 trends were observed for the non-BC_{HyPy} PAHs varying from 39 to 34 µg g⁻¹ in treated soils, not
28 significantly different from control soils. However, the concentrations of extractable PAHs
29 increased markedly in the amended soils, and decreased with time from 153 to 78 ng g⁻¹ remaining
30 always higher than those in untreated soil. The extent of the BC_{HyPy} loss was more compatible with
31 physical rather than chemical processes.

32 **Introduction**

33 Biochar is the carbonaceous residue from biomass pyrolysis that has been proposed as an
34 amendment in agricultural practices to increase soil organic carbon (SOC) and to restrain the
35 growth of atmospheric CO₂ through soil carbon sequestration.¹⁻⁴ The real or possible benefits and
36 drawbacks of using biochar in agro-environmental management are being debated as experimental
37 data emerge from field studies.⁵⁻⁷ The knowledge of recalcitrance of carbon that can be sequestered
38 in soil and the potential contamination from polycyclic aromatic hydrocarbons (PAHs) are crucial
39 issues for evaluating the environmental impact of biochar and its role on SOC dynamics. For
40 characterising SOC, the refractory fraction of biochar can be described as black carbon (BC), the
41 pyrogenic carbon produced from the partial combustion of organic materials, including biomass and
42 fossil fuels. In analogy to BC, the biochar matrix comprises complex assemblage of polyaromatic
43 structures along with heteroaromatic components and alkyl moieties from thermally degraded
44 biomacromolecules.⁸⁻¹⁰ Although the inherent complexity of biochar makes comprehensive
45 characterization extremely challenging, the degree of aromaticity and ring condensation have been
46 considered to be key structural factors affecting thermal resistance and environmental
47 recalcitrance.¹¹⁻¹² It is often assumed that environmental recalcitrance is intimately connected with
48 thermal stability associated with these structural factors (aromaticity and ring condensation)¹³⁻¹⁵,
49 thus thermo/chemical parameters have been proposed to classify the carbon sequestering potential
50 of different biochars by a number of techniques, including thermogravimetry,^{11,16} flash
51 pyrolysis,^{17,18} and hydrolysis (HyPy).¹⁹⁻²¹

52 Amongst these techniques, the characterization of biochar by HyPy is of interest because as well as
53 quantifying BC in soils accurately,²² it defines BC in terms of the smallest polyaromatic structures
54 present. In HyPy, the sample mixed with a suitable catalyst is pyrolysed in the presence of
55 hydrogen at high pressure to promote the reductive removal of thermally labile organic matter
56 leaving a refractory carbonaceous residue. This residue, named as BC_{HyPy}²² or stable polycyclic
57 aromatic carbon (SPAC),¹⁹ comprises polyaromatic units larger than 7 rings²⁰ and has an atomic

58 H/C ratio below 0.5.²² The organic fraction that is evolved by thermal reductive cleavage, denoted
59 as non-BC_{HyPy}, revealed the presence of PAHs comprising ≤ 7 rings.²¹ These non-BC_{HyPy} PAHs are
60 supposed to be less permanent (“semi-labile”) compared to larger PAH structures that constitute the
61 BC_{HyPy} macromolecular network.¹⁹ It is hypothesized that the semi-labile fraction comprises PAHs
62 occurring in a variety of different interactions with the amorphous organic matter (e.g. biopolymers
63 and humic substances) and the carbonaceous materials (e.g. BC) occurring in soil. The level of
64 individual PAHs in biochar and soil of interest in environmental quality guidelines can be
65 determined by extraction with appropriate solvents and analysis by gas chromatography-mass
66 spectrometry (GC-MS). However, due to the strong interaction of PAHs with the carbonaceous
67 matrix²³, only a minor fraction of extractable PAHs in biochar was found to be bioavailable.²⁴

68 Clearly, the incorporation of biochar into soil systems represents an input of PAHs.²⁵ Besides of
69 being a source, biochar may also act as sink for PAHs via adsorption.²⁶ Therefore, biochar could
70 affect the persistence of PAHs in soils probably by reducing their bioavailability.²⁷

71 Several laboratory incubation experiments have demonstrated the degradability of biochar and
72 BC in soils or model systems and provided valuable information on the extent of mineralization in
73 relation to char types^{13,14,18,28} and load,²⁹ environmental conditions³⁰⁻³² and soil characteristics.³³⁻³⁵

74 The few studies in the literature reporting on the fate of biochar in field experiments have shed
75 light on its role on SOC dynamics and highlighted the importance of the changes to soil properties
76 that occur after biochar incorporation as well as the chemical and physical changes that biochar
77 undergoes after soil incubation³⁶⁻³⁹. However, more investigations in field and with different
78 techniques are needed to increase our knowledge on the impact of biochar on organic carbon pools
79 in treated agricultural soils.

80 This study reports for the first time the combined application of HyPy and solvent extraction to
81 soil samples deriving from a multiannual time-scale biochar incubation experiment of a cultivated
82 soil^{40,41} to track the fate of stable (BC_{HyPy}) and semi-labile (non-BC_{HyPy} and solvent extractable

83 PAHs) fractions in the amended and control plots to understand the effect of biochar addition on
84 SOC.

85 **Experimental**

86 **Field experiment**

87 The field experiment performed in a vineyard at the “Marchesi Antinori - La Braccasca Estate”,
88 Montepulciano, Tuscany, Italy (43°10'15" N, 11°57'43" E) was previously described.^{40,41} Overall,
89 the experiment consisted of 15 plots, each 7.5 m in width and 30 m in length, including 4 vineyard
90 rows and 3 inter-rows. Specifically, 10 plots were investigated as depicted in Figure 1. We have
91 analysed two treatments (five replicates plots each): with biochar (amended; two applications, in
92 2009 and 2010 at the same rate, with a total application corresponding to 33 t ha⁻¹ of dry biochar
93 (considering an incorporation depth of 0.3 m and a soil density of 1.2, the application used in the
94 experiment corresponded to 0.91% in weight)) and without biochar as a control. The agricultural
95 soil was classified as sandy-clay-loam⁴² textured with 70% sand, 15% silt and 15% clay. The soil
96 characteristics were as follows: pH 5.37, total C 0.77%, total N 0.24%, total H 0.43%, and a cation
97 exchange capacity of 12.1 meq 100 g⁻¹.^{40, 41}

98 Soil was sampled four times from 2011 to 2013 (August 2011, December 2011, May 2012 and
99 May 2013). For the 10 replicates (5 replicates X 2 treatments) soil was sampled in 5 randomly
100 chosen points in the inter-row space of each replicate by means of a soil core sampler at 0-30 cm;
101 from these sub-samples an average sample for each replicate was obtained (5 replicates x 2
102 treatments x 4 sampling dates = 40 samples). Each sample was air-dried, sieved (mesh size: 2 mm)
103 in order to obtain homogeneous samples free of stones, larger roots and other coarse fragments, and
104 stored at - 20 °C before analysis.

105 The biochar used in the experiment was a commercial charcoal provided by “Romagna Carbone
106 s.n.c.” (Italy) obtained from dried (10% humidity) orchard pruning biomass (*Pirus communis*,
107 *Malus domestica*, *Persica vulgaris*, *Vitis vinifera*) through a slow pyrolysis process with a
108 transportable ring kiln of 2.2 m in diameter and holding around 2 t of feedstock. A peak temperature

109 of 500 °C was hold for 2.5 hours, the average heating rate before reaching the peak temperature was
110 15-18 °C/min.⁴⁰

111 **Analysis**

112 Biochar analyses were performed on a sub-sample obtained by mixing three individual specimens
113 (about 5 g each) withdrawn in different places from the original biochar sample (1 Kg), then
114 thoroughly homogenized by grinding with an agate mortar and pestle, sieved (mesh size: 2 mm),
115 oven dried at 40 °C for 72 h, and stored at - 20 °C prior to analysis.

116 The carbon, nitrogen, hydrogen and sulfur (C,H,N,S) contents of the biochar and carbon content in
117 soil samples were determined by combustion using a Thermo Scientific FLASH 2000 Series
118 CHNS/O Elemental Analyzer (Thermo Fisher Scientific, Waltham, US). About 2-4 mg of biochar
119 or soil test samples were analysed and compared by calibration with the Reference Material 2,5-
120 bis(5-tert-butyl-2-benzo-oxazol-2-yl)thiophene.

121 The content of carbonate in soils and the possible contribution of inorganic carbon from biochar ash
122 was negligible (< 0.1%) as confirmed by comparing total organic carbon measured after
123 hydrochloric acid (HCl) treatment and total carbon on a set of treated and amended soil samples.
124 Therefore, the total carbon determined in soil corresponded to the total organic carbon and was
125 termed SOC for uniformity.

126 The ash content of the biochar was measured by heating samples in a muffle at 550 °C for 6 hours.

127 The oxygen content of biochar was calculated by difference from the mass balance.

128 Analyses of extractable PAHs in biochar and soils were conducted using the method described
129 in more detail elsewhere.⁴³ Briefly, 5 g of sample spiked with perdeuterated PAHs (acenaphthene-
130 d₁₀, phenanthrene-d₁₀, chrysene-d₁₂) were extracted with 160 ml acetone/cyclohexane (1:1 v/v)
131 mixture for 36 hours. After addition of 1 ml of *n*-nonane, the solvent was evaporated, the mixture
132 cleaned-up by silica gel solid phase extraction and analysed by GC–MS. Occasionally, the
133 procedure was tested analyzing the soil certified reference material ERM–CC013a (manufactured
134 by the Federal Institute for Materials Research and Testing; Berlin, Germany). No significant

135 differences were observed between measured and certified values (relative errors in the -5% +11%
136 range, details in Table S1). Calibrations were performed in the 0.005-2.5 mg/L concentration range
137 for each PAH (serial dilutions of the PAH-Calibration Mix Supelco). Results from calibration and
138 blank analyses (limit of detection and quantitation, R^2) are reported in Table S2. Recoveries of
139 surrogate PAHs (mean values \pm s.d. n=40) were 75% \pm 22%, 82% \pm 21 and 83% \pm 23 for acenaphthene-
140 d₁₀, phenanthrene-d₁₀, chrysene-d₁₂, respectively.

141 **Hydropyrolysis**

142 Hydropyrolysis (HyPy) was performed using the procedure described in detail by Meredith *et*
143 *al.*^{21,22} Briefly, 50-100 mg of biochar sample and 3-4 g of biochar amended soil were loaded with a
144 sulphided molybdenum (Mo) catalyst using an aqueous/methanol 0.2 M solution of ammonium
145 dioxodithiomolybdate [(NH₄)₂MoO₂S₂]. Catalyst weight was ~ 5% of the sample weight for soils, ~
146 10% for biochar. Analyses were performed under H₂ at 15 MPa from 50 to 250°C at 300°C min⁻¹,
147 then from 250°C to 550°C for 2 min at 8 °C min⁻¹. The evolved products (non-BC_{HyPy}) were
148 quickly removed from the reactor vessel by a hydrogen sweep flow and trapped in a silica gel-filled
149 trap cooled by dry ice; the silica gel was eluted with *n*-hexane and dichloromethane, the organic
150 solutions concentrated, added with 100 µl internal standard solutions (*n*-hexatriacontane and tri-
151 *tert*-butylbenzene, 100 mg l⁻¹ each) and analysed by GC-MS (see below).

152 The weight of the solid residue in the reaction vessel (BC_{HyPy}) was calculated by difference,
153 after weighing the catalyst loaded samples prior to and after each HyPy analysis.

154 Elemental compositions (C,H,N,S) of the samples before and after HyPy were determined by
155 combustion as described above. The presence of catalyst in the samples (5 %) did not affect
156 significantly the calculation of % BC_{HyPy}. From elemental analysis and weight determinations, the
157 soil concentrations of BC_{HyPy} (mgC g_{soil}⁻¹) and its content relative to SOC (%BC_{HyPy}), on a carbon
158 base, were calculated:

$$\%BC_{HyPy} \left(\frac{\text{mgC}}{100\text{mgSOC}} \right) = \frac{\text{weight of residue (mg)} * C (\%)}{\text{initial weight of soil (mg)} * SOC(\%)} * 100$$

$$\text{BCHyPy} \left(\frac{\text{mgC}}{\text{gsoil}} \right) = \frac{\% \text{BCHyPy} * \text{SOC}}{10}$$

159 GC–MS analyses of the non-BC_{HyPy} fractions were performed on a 6850 Agilent HP gas
160 chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer (Agilent
161 technologies Inc. Santa Clara, CA, USA) operating by electron ionization at 70 eV, and full scan
162 mode in the 35–650 *m/z* range. Analytes were separated with a HP-5MS fused silica capillary
163 column (stationary phase poly[5% diphenyl/95%dimethyl]siloxane, 30 m × 0.25 mm i.d., 0.25 μm
164 film thickness), using helium as the carrier gas. Individual *n*-alkanes were quantified from the *m/z*
165 57 mass chromatograms and by comparison with the added internal standard *n*-hexatriacontane
166 assuming equal response factors for each compound. PAHs were quantified from the mass
167 chromatograms of the molecular ion for each compound and by comparison with added 1,3,5-tri-
168 *tert*-butylbenzene using the relative response factors determined by single calibration point (PAH-
169 Calibration Mix Supelco at 10 mg L⁻¹ for each PAH). Procedural blank analyses showed absence of
170 contamination.

171 Quantitative data are presented as mean values ± standard deviation (s.d., five replicates). An
172 analysis of variance (ANOVA) test was conducted with The “R Foundation for Statistical
173 Computing” R software version 3.1.2 (2014-10-31; <http://www.r-project.org>) to was to evaluate
174 significant difference between control and biochar amended soils, and between sampling periods. A
175 difference was considered statistically significant at level of *p* < 0.05.

176 **Results**

177 **Stable fraction (BC_{HyPy})**

178 The utilised biochar obtained by slow pyrolysis was highly carbonized with 71% C content, and
179 atomic H/C and O/C ratios of 0.26 and 0.11, respectively (Table 1), consistent with a high degree of
180 aromaticity.¹² However, the ash content was rather high (about 20%, Table 1), thus the evaluation
181 of the aromaticity from the O content measured by difference could be inadequate.¹² The impact of
182 this biochar in the amended soils was clearly demonstrated by the higher values of carbon (4.8-3.5

183 %) in comparison to control soil (0.7-0.9%, Table 2). Considering that the content of inorganic
184 carbon was negligible in these acidic soils, the total content of carbon corresponded to that of SOC
185 (see experimental). The addition of biochar increased significantly the total pool of SOC by over six
186 times (Table 2). An apparent reduction (statistically significant only in the last sampling, Table S3)
187 of SOC in biochar treated soils was measured with time and three years after the biochar application
188 in the vineyard, the SOC in amended soil was 3.8 times higher than that of control soil.

189 Recalcitrant black carbon (BC) accounted for the bulk of the additional SOC. This finding can
190 be observed in Figure 2 showing the percentage of the SOC occurring in a stable form as %BC_{HyPy}.
191 The %BC_{HyPy} in the untreated soil was on average $6 \pm 1\%$ (n=20) of the SOC, a value within the
192 range reported in the literature (BC/SOC 2-13% quartile range),²³ and did not exhibit any trend with
193 time. Hence, the majority of SOC in the control soil occurred in a semi-labile form, probably
194 derived from a combination of lignocellulosic debris, humic acids, microbial biomass including
195 thermally labile charcoal. Amending the soil with biochar created the opposite situation, with the
196 majority of the SOC pool in the form of recalcitrant carbon, with BC_{HyPy} levels of 70 % of SOC on
197 average. Specifically, the soil was amended with biochar two times during two years, then 15
198 months after the end of the second treatment, the %BC_{HyPy} was $79\% \pm 4\%$ (first soil sampling,
199 Figure 2). This value was very similar to that of original biochar (83%, Table 1). Obviously, the
200 effect of biochar addition in soils on the level of BC depended on the level of stable polycyclic
201 aromatic carbon (SPAC) in the original biochar which in turn is governed by the nature of the
202 feedstock and pyrolysis conditions as described by McBeath *et al.*¹⁹ The high percentage of BC_{HyPy}
203 characterizing the biochar (Table 1) is consistent with the SPAC values reported for aromatized
204 biochars produced at high temperatures,¹⁹ indicative of resistance to degradation in soil.
205 Nevertheless, a gradual decrease of the %BC_{HyPy} was observed with time down to $61\% \pm 2\%$ after
206 36 months following the end of the biochar application (Figure 2), a difference statistically
207 significant (Table S3). The absolute soil concentration of BC_{HyPy} also decreased significantly with

208 time as depicted in figure 3A from $36 \pm 5 \text{ mgC g}_{\text{soil}}^{-1}$ at the first sampling to $23 \pm 5 \text{ mgC g}_{\text{soil}}^{-1}$ after
209 21 months, representing a loss of 36% (statistical results in Table S3).

210 The total loss of BC_{HyPy} could be assigned to a variety of factors, including microbial degradation,³⁰
211 chemical weathering^{13,32} and physical disturbance^{37,38} as discussed in the final section.

212 **Semi-labile fraction (non- BC_{HyPy})**

213 Exemplar mass chromatograms of the hydropyrollysates for the non- BC_{HyPy} fraction in control and
214 amended soils are shown in Figures 4B and 4C and these are characterized by the presence of
215 aliphatic hydrocarbons, alkylbenzenes, diphenyls and PAHs. The composition of hydropyrollysates
216 of the treated and untreated soils was similar in terms of tentatively identified compounds,
217 suggesting that the incorporation of biochar did not markedly change the chemical nature of the
218 main hydrocarbons, but rather their relative amount.

219 Aliphatic hydrocarbons are characterised by *n*-alkanes in the range C_{13} to C_{27} with a distribution
220 centred at C_{16} and C_{18} , and an even carbon number predominance (Tables S4-S5 in supporting
221 information). Iso- and anteiso C_{15} and C_{17} were also identified. Fatty acids are a more probable
222 source of these alkanes as they exhibited an even over odd preference, and typically include a high
223 abundance of C_{16} hexadecanoic (palmitic) acid and C_{18} octadecanoic (stearic) acid.⁴⁴ It is known
224 that under HyPy conditions saturated fatty acids are converted into the corresponding alkanes
225 preserving the number of carbon atoms, while cracking into smaller hydrocarbons could occur with
226 unsaturated fatty acids.⁴⁵ The skeleton backbone is also preserved so therefore the iso and anteiso
227 hydrocarbons can be assigned to the presence of the corresponding C_{15} and C_{17} acids of bacterial
228 origin.⁴⁶ Fatty acids were probably covalently bond as acyl to lipids or macromolecular matrix and
229 make up the aliphatic polymethylene network of the semi-labile BC. These aliphatic compounds
230 were not evident in the non- BC_{HyPy} fraction from the original biochar (Figure 4A).

231 The non- BC_{HyPy} aromatic fraction of soils comprised monoaromatic rings represented by
232 alkylated benzenes and diphenyls and a PAH pattern dominated by pyrene (Figures 4B and 4C).
233 The ring size distribution was similar to that generated by HyPy from the organic soil component

234 remaining after dichromate oxidation which was dominated by pyrene and minor levels of fluorene,
235 phenanthrene, chrysene, and benzo[ghi]perylene among those identified in our study.²¹ A similar
236 pattern was observed in the original biochar (Figure 4A) providing evidence of a common
237 polyaromatic backbone featured by specific alternant PAHs (phenanthrene, pyrene, chrysene;
238 benzo[ghi]perylene was not detected). Methylated naphthalenes, phenanthrenes and pyrenes were
239 detected as well. It is worth noting that partially hydrogenated PAHs were tentatively identified in
240 the MS-hydropyrollysates suggesting that a degree extent of hydrogenation does occur with signal a
241 peak assigned to dihydropyrene (m/z 204) being observed close to that for fluoranthene.

242 Distinctive differences between the amended and control soils were observed in the mean
243 concentrations of aliphatic and aromatic hydrocarbon constituents (Tables S4-S7 in supporting
244 information). The total concentrations of *n*-alkanes were higher in the control (around 100 $\mu\text{g g}^{-1}$,
245 Table S4) than in the amended soils (about 40 $\mu\text{g g}^{-1}$, Table S5). The mean concentrations of non-
246 BC_{HyPy} PAHs were higher in the amended than in the control soil (about 40 $\mu\text{g g}^{-1}$ compared to 20
247 $\mu\text{g g}^{-1}$, Tables S6 and S7, see also Figure 3B). However, these differences could not be proved to be
248 statistically significant (with the exception of control vs. treated soils after 9 months from the initial
249 sampling) due to the high dispersion of values in the amended soils caused by the inhomogeneous
250 distribution of biochar particles in the samples withdrawn from the same parcel and possible
251 fluctuations due to seasonal changes in SOC source and decay. Besides, it was shown that PAHs
252 could be heterogeneously distributed in the biochar samples.⁴⁷ However, a systematic higher content
253 of non-BC_{HyPy} PAHs in the amended soils was observed across the whole sampling period (figure
254 3B). Four years after the addition of biochar to soil, semi-labile PAHs apparently were not
255 degraded.

256 **Extractable PAHs**

257 The concentrations of solvent extractable PAHs in amended and control soils are presented in
258 Figure 3C. Almost one year after the last biochar application, the total PAH concentrations in the
259 amended soils ($153 \pm 38 \text{ ng g}^{-1}$) were significantly higher than those in the control soil ($24 \pm 3 \text{ ng g}^{-1}$)

260 ¹, n = 5, Figure 3C and Tables S8-S9). The level of PAHs in the amended soils decreased
261 significantly after 35 months down to $78 \pm 20 \text{ ng g}^{-1}$, while that of control remained almost constant
262 at $23 \pm 3 \text{ ng g}^{-1}$ (average in the whole period, n=20). The diminution in concentration of PAHs in
263 the treated soils involved principally the two-four ring PAHs (> 40% loss), and less for the five-six
264 ring PAHs (loss < 40%, Tables S8-S9).

265 Discussion

266 The pool of polycyclic aromatic carbon structures comprising the SOC have been operationally
267 differentiated into three fractions: distinctive (GC analyzable) PAH compounds released by solvent
268 extraction^{24,43} and HyPy (semi-labile fraction, non-BC_{HyPy}),^{19,21} and the undefined large ring
269 polycyclic aromatic carbon matrix resistant to HyPy (stable fraction, BC_{HyPy} or SPAC).^{19,21,22} The
270 fate of these fractions in a vineyard soil treated with biochar has been investigated over a time span
271 of about two years and compared to that of a control soil. In both soils, the concentrations of these
272 polyaromatic reservoirs spanned six orders of magnitude: ng g^{-1} (extractable, Figure 3C), $\mu\text{g g}^{-1}$
273 (semi-labile, Figure 3B) and mgC g^{-1} (stable, Figure 3A). Their relative abundances were also
274 vastly different: on average the extractable PAH fraction accounted for 0.1 % (control)-0.3%
275 (treated) of the total semi-labile PAH pool, which in turn represented 4% (control)-0.1% (treated) of
276 the stable fraction.

277 The solvent extractable fraction is of importance for the assessment of environmental quality in
278 regulatory procedures. However, exhaustive solvent extraction tends to over-estimate the fraction of
279 bioavailable and bioaccessible PAHs that may pose a threat to living organisms and other
280 methodologies should be used to determine this fraction.^{24,48-50} Solvent extractable PAHs increased
281 remarkably (five times on average) after biochar treatment and remained significantly higher than
282 that in the control soil after almost two years (Figure 3C). Similar results were obtained by Quilliam
283 *et al.*²⁷ who observed a significant increase of PAHs following biochar addition in two different
284 agricultural soils. Although the application of biochar occurred with an inevitable addition of
285 extractable PAHs, the levels in the amended soils remained within the range reported for

286 background soils (<1 to 7,840 ng g⁻¹).⁵¹ Obviously, the level of soil contamination will be
287 determined by the degree of contamination of biochar which is dependent on feedstock materials
288 and process conditions.^{24,43,52-54} These data supported the view that when the PAH concentrations in
289 the biochar fulfill the threshold levels proposed by the IBI⁵⁵ and EBC,⁵⁶ as for the biochar utilised in
290 this study (Table 1, Table S10), the impact to soil is expected to be minimal. However, the large
291 pool of BC_{HyPy} may influence the persistence of mobile PAHs by lowering the microbial
292 activity.^{15,27} Alternatively, it may act as a reservoir for PAHs¹⁵ or favoring the sorption of
293 endogenous and environmental PAHs²⁶ given the relatively high biochar-water partitioning
294 coefficients.²⁴

295 The non-BC_{HyPy} PAH fractions in treated and control soils were not significantly different
296 (Figure 3B) and did not change significantly (statistical results in Table S3 for treated soil) with
297 time suggesting that naturally occurring non-BC_{HyPy} PAHs are dominating this pool turnover. The
298 content of non-BC_{HyPy} PAHs in the original biochar was around 1 mg g⁻¹ (Table 1, individual PAHs
299 in Table S10), probably not sufficient to impact markedly the SOC. These PAHs were probably
300 covalently linked²¹ or strongly sorbed onto aromatic surfaces, nanopores or occluded sites of the
301 BC_{HyPy} matrix, and therefore not prone to decomposition. On the contrary, biochar amendment
302 increased massively the stable BC_{HyPy} reservoir in comparison to the untreated soil (sixty times,
303 Figure 3A) and ten times on average its proportion to SOC (% BC_{HyPy}, Figure 2). Noteworthy is that
304 its absolute concentration decreased with time with a 36% carbon loss in 21 months (Figure 3A). It
305 is known that biochar can be mineralized by both abiotic (oxidation) and biotic processes in
306 laboratory incubation, however, the reported losses were much lower than those observed in this
307 study (e.g. < 3%,^{30,35} < 5%,¹⁸ <12%²⁸ in days/months, 0.5%-8.9% in five years,¹⁴ 6% after eight
308 years³³). Small losses (3%) due to respiration were reported in field studies,³⁸ while significant
309 decomposition of BC (up to 70%) was reported to occur in topsoil in the first 30 years due to
310 physical processes (vertical and lateral export) in addition to chemical mineralisation.³⁶ Therefore,
311 the decreasing trends of BC_{HyPy} (Figure 3A) could be explained by physical redistribution of

312 biochar particles rather than mineralization, even though the latter can be important in the first
313 period due to priming effects.⁵⁷

314 The depletion of the relative contribution of BC_{HyPy} (Figure 2) confirmed that the aromatic
315 recalcitrant fraction was lost preferentially in comparison to other SOC components. This finding is
316 in accordance with the studies by Rumpel *et al.* 2009 with rain simulators.³⁷ These authors found
317 that the lateral and vertical (infiltration) removal of SOC in agricultural soils by water erosion can
318 be significant (up to 55%) and more pronounced for the carbon-rich (less dense) recalcitrant
319 (chemically resistant) BC fraction. The vertical infiltration of pyrogenic carbon was found in soil
320 microcosms.³⁴ The type of prevailing physical distribution could be governed by the different water
321 regime in tropical and temperate zones, with low-intensity rainfall favoring vertical transport and
322 splash erosion horizontal transport.³⁷ In our study, erosion could be favored by the gentle slope of
323 the vineyard which may induce a preferential loss of BC as observed for steep slopes with high
324 erosion rates.⁵⁸ Based on the results from carbon isotope analysis, Major *et al.*³⁸ supposed that water
325 runoff was the principal process capable to explain the loss of biochar in a treated soil after two
326 years, being the loss by respiration and vertical transport minimal.

327 The absolute and relative concentration of BC_{HyPy} did not change significantly in the last two
328 sampling campaigns (April 2012 and May 2013) while the cumulated rainfall increased,⁴¹
329 indicating that a substantial part of carbon from biochar is resistant to migration processes. In fact,
330 the grape productivity increased (up to 66%) in all the harvests following biochar amendment in the
331 2010-2014 period, even though the fruit quality remained unaffected. The increased yields were
332 likely to be due to the enhanced soil water content and plant available water in the treated soils in
333 comparison to the control soil.^{40,41} Other field studies on vineyards with a slope gradient reported
334 subtle effects on productivity and quality, but the fate of biochar was not investigated.⁵⁹ The
335 persistence of BC demonstrated by HyPy analysis supports the role played by biochar in regulating
336 water availability, but potential losses due to SOC dynamics should be carefully evaluated
337 especially in long-term field experiments where abiotic and biotic components drive the carbon

338 stability rather than the inherent biochar recalcitrance.⁶⁰ This study demonstrated the usefulness of
339 HyPy to shed light on the characteristics of BC put into the environment when biochar is applied in
340 soil systems.

341 **Associated content**

342 **Supporting Information Available**

343 Supporting information contains the results from the analysis of PAHs in CRM soil (Table S1),
344 PAH calibration (Table S2), statistical analysis of treated soils (Table S3), analysis of individual
345 non-BC_{HyPy} alkanes (Tables S4 and S5), analysis of individual non-BC_{HyPy} PAHs (Tables S6 and
346 S7), analysis of individual solvent extractable PAHs (Tables S8 and S9), analysis of individual
347 extractable and non-BC_{HyPy} PAHs in biochar (Table S10). This information is available free of
348 charge via the Internet at <http://pubs.acs.org>.

349 **Author information**

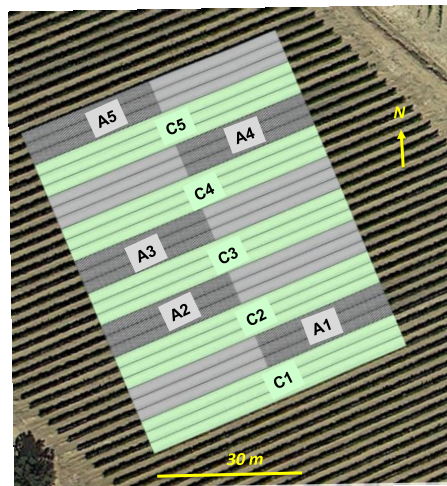
350 **Corresponding author:*** E-mail: dani.fabbri@unibo.it; phone: ++39-0544-937344; fax: ++39-
351 0544-937411.

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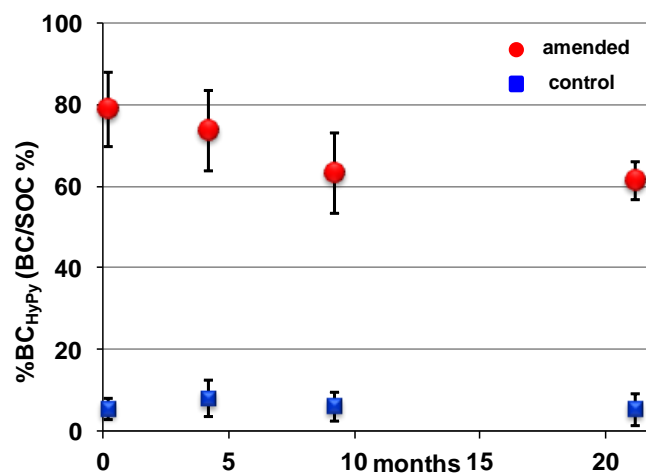
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364 **Figure 1.** Experimental layout in the vineyard indicating the location of the five plots amended
365 with biochar (A) under the same conditions and the nearby control (C) plots. Photo from
366 GoogleEarth.

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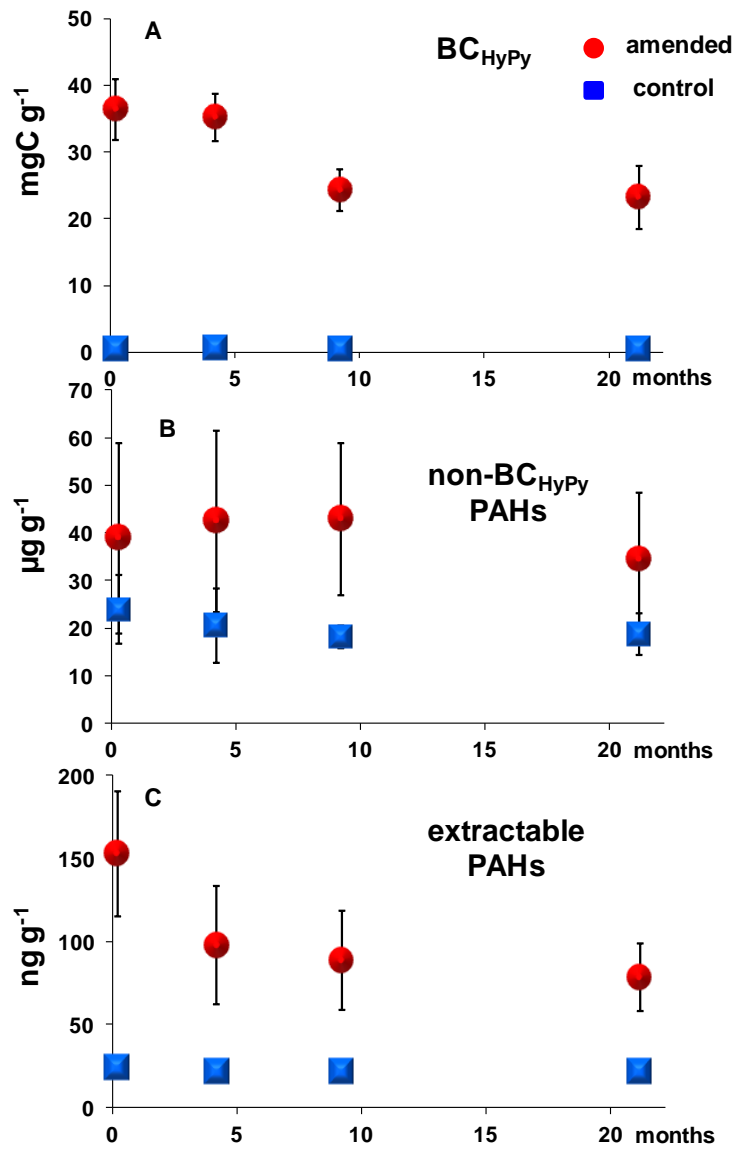


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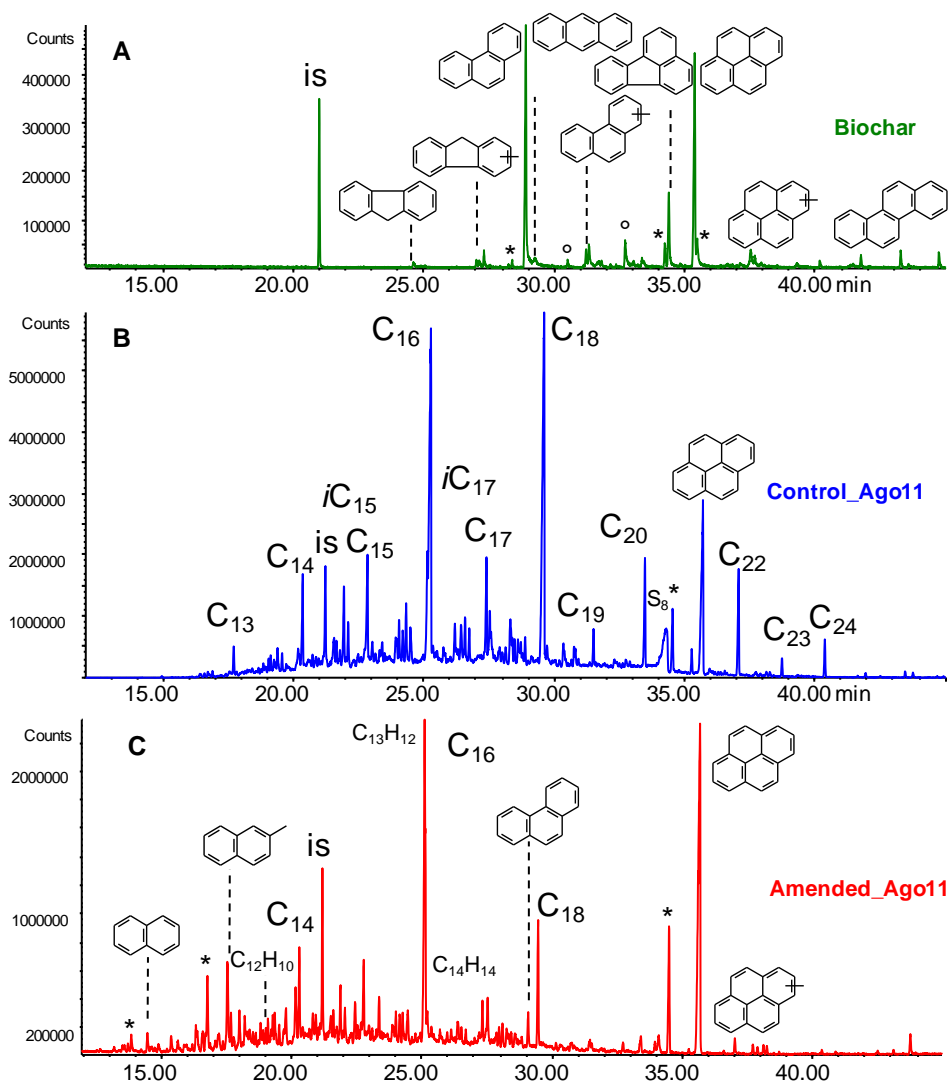
370

371 **Figure 2.** Black carbon from HyPy (%BC_{HyPy}) as percentage of soil organic carbon in the biochar
372 amended (red circles) and control (blue squares) soils vs. sampling time (months from the first
373 sampling). Mean values ± 2 *s.d. (n=5).



374

375 **Figure 3.** Concentration vs. sampling time (months from the first sampling) of biochar amended
 376 (red circles) and control soils (blue squares) of different polycyclic aromatic fractions. (A) stable
 377 BC_{HyPy}, (B) non-BC_{HyPy} PAHs, and (C) solvent extractable PAHs. Mean values ± s.d. (n=5, error
 378 bars not visible when smaller than square size).



379

380

381 **Figure 4.** Examples of total ion chromatograms for the hydroxyrolysates (non-BC_{HyPy} fraction).

382 From top to bottom: (A) biochar, (B) control soil and (C) soil amended with biochar sampled in

383 August 2011. C_x: n-alkanes with x carbon atoms, i: iso/anteiso, C_xH_y: biphenyls, °:

384 phenylanthracenes (tentative), *: probably hydrogenated PAHs.

385

386 **Table 1.** Elemental analysis (oxygen by difference), atomic H/C and O/C ratios, ash content,
 387 solvent extractable PAHs, non-BC_{HyPy} PAHs and %BC_{HyPy} (%BC_{HyPy}/SOC) of the biochar applied
 388 in the field experiment (mean values \pm standard deviation s.d., n=3 on a dry basis).

389

| parameter | units | mean value \pm s.d. |
|-----------------------------------|----------------------|---|
| C | % | 71.4 \pm 1.2 % |
| H | % | 1.54 \pm 0.11 |
| N | % | 0.72 \pm 0.05 |
| S | % | 0.59 \pm 0.05 |
| O | % | 5.9 \pm 0.7 |
| Ash | % | 19.9 \pm 1.5 |
| H/C | atomic | 0.26 |
| O/C | atomic | 0.11 |
| extractable PAHs | $\mu\text{g g}^{-1}$ | 3.8 \pm 0.8 |
| non-BC_{HyPy} PAHs | mg g^{-1} | 1.1 \pm 0.2 |
| %BC_{HyPy} | % | 83 \pm 3 |

390

391 **Table 2.** Soil organic carbon (SOC) of the soil treated with biochar and untreated soil (control) in
 392 different sampling periods (months elapsed after the last biochar application). Values are mean
 393 values \pm s.d., n=5, % on a dry basis.

| | Aug 2011 | Dec 2011 | May 2012 | May 2013 |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|
| SOC% | months 0 | 4 | 9 | 21 |
| Control soil | 0.76 \pm 0.21 | 0.76 \pm 0.21 | 0.83 \pm 0.21 | 0.91 \pm 0.12 |
| Biochar amended soil | 4.79 \pm 0.58 | 4.30 \pm 0.83 | 3.97 \pm 0.75 | 3.49 \pm 0.29 |

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