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1 **Short-term effects of biochar on grapevine fine root dynamics and arbuscular mycorrhizae**
2 **production**
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20 **Abstract**
21 Application of biochar to the soil is globally recognised as a means to improve soil structure and
22 fertility, increase carbon sequestration, enhance crop production and mitigate climate change.
23 However, although the fine root system is fundamental for plant growth, crop productivity, carbon
24 and nutrient cycling, little is known about the effect of biochar on plant fine roots. This study,
25 conducted in a Montepulciano (*Vitis vinifera* L.) vineyard, was aimed at investigating the impact of
26 biochar application (at the rate of 10 t ha^{-1}) on soil chemical and physical properties, fine root

27 dynamics and arbuscular mycorrhizal fungi (AMF) production during a one-year sampling period.
28 To this aim, seasonal variation of fine root mass, length and diameter was measured by the
29 sequential coring technique, whereas fine root annual production was calculated by minimum-
30 maximum procedure and turnover rate of live roots by maximum standing biomass. For AMF
31 annual production, in-growth mesh bags were used to measure glomalin as quantitative indicator of
32 mycorrhizae presence. Results showed that biochar significantly increased organic carbon (20.7 %),
33 available ammonium (84.4 %), and available water content of the soil (11.8%), while it also
34 promoted the formation of the large fraction of macro aggregates (ø > 2 mm; 3.1% control; 5.5%
35 treated). Cation exchange capacity, pH, total nitrogen content, and total and available phosphorus
36 content remained unaffected. Immediately after biochar soil amendment, while fine root length
37 remained unchanged, a significant increase in fine root biomass was measured resulting in a higher
38 mean annual biomass (8.56 g m^{-2} control; 13.34 g m^{-2} treated), annual production (8.71 g m^{-2}
39 control; 12.7 g m^{-2} treated) and lifespan (as evidenced by a lower turnover rate; 1.02 yr^{-1}control;
40 0.95 yr^{-1} treated). Moreover, the increase of fine root biomass resulted to be associated with radial
41 growth since mean fine root diameter was significantly higher in biochar-treated plants (0.56 mm)
42 than in control plants (0.46 mm). Biochar had no significant effect on the annual production of
43 AMF. The results of the present study show that the improvements of soil chemical and physical
44 features due to biochar application have an immediate effect on fine root dynamics and
45 morphology. Furthermore, the increase of fine root biomass is mainly due to radial growth and
46 occurs during the water shortage period, supporting fruit setting and ripening in grapevine plants.

47

48 Keywords: biochar, *Vitis vinifera* L., fine root dynamics, fine root diameter, arbuscular mycorrhiza

49

50 Introduction

51

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52 Biochar, a charcoal produced by controlled pyrolysis, has been widely recognised for its potential
53 use to improve soil fertility, sequester carbon (C), mitigate climate change (Lehmann et al., 2006;
54 Lehmann, 2007a; Laird, 2008; Sohi et al., 2010) and enhance phytostabilisation of contaminated
55 soils (Brennan et al., 2014; Lomaglio et al., 2016). Indeed, the positive effects of biochar on
56 agricultural productivity have been attributed to: i) the reduction of soil acidity (Yuan et al., 2011;
57 Pereira et al., 2015); ii) the improvement of cation-exchange capacity (CEC) and nutrient
58 availability; iii) the dissolution of organic carbon in low-pH acidic soils (Mukherjee and
59 Zimmerman, 2013); iv) the increase of water retention capacity (Downie, 2011; Baronti et al.,
60 2014); and v) the availability of plant water content (Tammeorg et al., 2014). Despite the growing
61 amount of data reported in the literature on the positive effects of biochar on agricultural
62 productivity, plant responses to biochar soil amendments have largely focused on above-ground
63 biomass and crop yields. Biochar has been reported to increase rooting during germination
64 (Vookova and Kormutak, 2001), and to enhance root biomass (Lehmann et al., 2003; Joseph et al.,
65 2010; Makoto et al., 2010) and length (Noguera et al., 2010). However, in most of these cases the
66 analysis of root response to biochar amendment was limited to biomass measurements (Lehmann et
67 al., 2003; Noguera et al., 2010; Prendergast-Miller et al., 2011) and, therefore, the mechanisms
68 controlling root–biochar interactions still remain poorly understood (Lehmann et al., 2011). Roots
69 have important functions in plants, including nutrient and water uptake, anchorage, and mechanical
70 support, and are the first organs to be affected by biochar (Prendergast-Miller et al., 2013).
71 Furthermore, within the plant root system, fine roots are the principal structures involved in water
72 and nutrient acquisition (Mainero et al. 2009; Montagnoli et al. 2012a, 2012b, 2014; McCormack et
73 al. 2015; Terzaghi et al. 2016). Fine root lifespan has important implications for individual plant
74 growth, crop productivity, plant-environment interactions, and belowground carbon (C) and nutrient
75 cycling (Godbold et al. 2003; Montagnoli et al. 2010; Di Iorio et al. 2013; Madhu and Hatfield
76 2013; Terzaghi et al. 2013; McCormack and Guo 2014). Indeed, Jackson et al. (1997) estimated that
77 as much as 33% of global annual net primary productivity in terrestrial ecosystems is devoted to

78 fine root production, and the growth and maintenance of fine roots may use up to 50% of the daily-
79 produced photosynthate in crop plants (Lambers, 1987).
80 It has been proposed that biochar may affect root growth and plant performance through two
81 mechanisms: i) as a direct nutrient source and ii) by enhancing nutrient availability (Lehmann et al.,
82 2011). In a recent investigation, Prendergast-Miller et al. (2013) showed that biochar controls plant
83 root nutrient acquisition in rhizobox-grown spring barley (*Hordeum vulgare* L.), both directly as a
84 nutrient source and indirectly by altering soil nutrient content. Similarly, again in a rhizobox
85 experiment, Reibe and co-workers (2015) found that nutrients released from different kinds of
86 biochar might affect root morphology of spring wheat. Furthermore, different types of chars had
87 different effects on root and shoot growth and soil changes, depending on the feedstock, the
88 production process and the amount of biochar applied (Bhattacharjya et al., 2015).
89 Through its effect on nutrient cycles (Steiner et al., 2008) or soil structure (Mummey and Rillig,
90 2006), biochar has also been shown to create a habitat for beneficial soil microorganisms (Rillig and
91 Thies, 2009), which in turn may improve plant growth (Warnock et al., 2007). However, the effects
92 of biochar on soil biota abundance and composition may differ for different groups of
93 microorganisms (reviewed in Lehmann et al., 2011). Living in symbiosis with plant roots,
94 arbuscular mycorrhizal fungi (AMF) develop an extensive extraradical hyphal network, which plays
95 an important role in plant nutrient uptake (Harrison and van Buuren, 1995; Read and Smith, 1997;
96 Avio and Giovannetti, 2002) promoting plant growth (Schwartz et al., 2006; Compant et al., 2010).
97 In fact, AMF provide their host plants with mineral nutrients receiving photosynthetically-derived
98 carbohydrates in return (Read and Smith, 2008). Thus, the presence of AMF is particularly
99 important in marginal soils, where their contribution to nutrient uptake may be more critical to the
100 plant (Bücking et al., 2014). Glomalin is a wall protein of the AMF mycelium with concentrations
101 in the soil generally ranging from 2 to 14 mg g^{-1} (Pikul et al., 2002) and, therefore, commonly used
102 as a quantitative indicator (Upadhyaya and Wright, 1996; Lovelock et al., 2004). The presence of
103 biochar in the soil seems to have a general positive effect on mycorrhizal fungi (reviewed in

104 Warnock et al., 2007), although negative results have also been reported (Birk et al., 2010; Warnock
105 et al., 2010).
106 Due to the economic importance of grapevine, over the last years much attention has been paid to
107 the effect of biochar on amended groves. Recent studies revealed that the positive effects of biochar
108 on grape yield and quality are mainly due to: i) the attenuation of water stress (Baronti et al., 2014;
109 Genesio et al., 2015); ii) the improvement of soil chemical and biological fertility and nutrient
110 supply to plants (Glaser et al., 2002; Sohi et al., 2010, Vaccari et al., 2011, Schulz et al., 2013); iii)
111 the enhancement of plant growth and yield (Lehmann and Rondon, 2005; Chan et al., 2007; Major
112 et al., 2010b); and iv) the reduction of greenhouse gas emissions through C sequestration (Van
113 Zwieten et al., 2010; Ippolito et al., 2012; Zhang et al., 2012). Once again, in these studies, the
114 effects of biochar were investigated mainly in terms of changes in soil physical and chemical
115 characteristics, plant yield and biomass production. Indeed, to our knowledge, the influence of
116 biochar on fine root lifespan and on the mutualistic interaction between grapevine roots and AMF
117 (Groot-Obbink and Possingham, 1971; Deal et al., 1972; Menge et al., 1983; Nappi et al., 1985) has
118 not been investigated yet. Studying the impact of biochar on fine root dynamics of perennials plants
119 such as grapevine is fundamental for understanding plant-soil interactions and their consequences
120 for plant growth. Given the above-mentioned effects that biochar can have on soil nutrient and
121 water availability, we hypothesised that changes in resource supply play an immediate role in root
122 dynamics and AMF colonization, thereby further affecting crop production and yield. To test this
123 hypothesis, after assessing the effects of biochar on soil physical-chemical properties, fine root
124 dynamics and AMF production in a vineyard were investigated in a short-term (one-year) time
125 course experiment. The identification of possible relationships between any alterations of soil
126 physical-chemical properties, fine root dynamics and AMF production may further contribute to
127 elucidating the mechanisms of biochar actions.

128 **2. Material and methods**

129

130 *2.1 Experimental site and set up*

131 The field experiment was carried out in a vineyard of the *Valerio Vini* estate (41°32'19.8"N
132 14°09'34.9"E; 270 m a.s.l.) in the municipality of Monteroduni (Molise, Central Italy). The
133 vineyard (Montepulciano wine grape variety) consists of 24 north-west oriented plant rows (2.5 m
134 spacing), each containing 15-year-old plants (80 cm spacing), not irrigated. During the study period,
135 from May 2014 to May 2015, total rainfall was approximately 1310 mm with an average air
136 temperature of 14 °C (data from the Fornelli (IS) weather station, supplied by the Regione Molise).
137 Soil-milling operations (20 cm depth) were carried out at the beginning of April 2014 as usual
138 management practice. At the beginning of May 2014, biochar was applied at a rate of 10 t ha^{-1} (Van
139 Zwieten et al., 2008; Brandstaka et al., 2010; Ndor et al., 2015). In order to obtain a homogeneous
140 soil application, the biochar was crushed into smaller particles, sieved at 2 mm size and
141 homogeneously broadcasted by hand (Major, 2010a), between plants and within the whole plot area
142 (4 m^2). To avoid biochar loss by wind or water erosion, immediately after spreading biochar on the
143 soil surface, moisture was applied with a Verdigris sprayer (Karer et al., 2013) and biochar was
144 incorporated into the soil with a hand-powered rotary hoe at low rotation speed (10 cm depth; Karer
145 et al. 2013). Finally, another inter-row soil milling was carried out one year later (April 2015),
146 before the last sampling point. Measurements were carried out in eight plots (four control and four
147 biochar-treated) of 4 m^2 in size, each including three plants displaced on the same row (Figure 1).

148

149 *2.2. Biochar characterization*

150 Biochar used in this study was produced by *Romagna Carbone s.n.c*. (Italy) from orchard pruning
151 biomass through a slow pyrolysis process with an average residence time of 3 hours at 500 °C in a
152 kiln of 2.2 m in diameter and holding around 2 ton of feedstock. pH measurements were carried out
153 by potentiometry (pH meter Eutech Instruments pH 700, 2013) according to IBI standards (2014).
154 The electrical conductivity (EC) value was obtained by direct instrumental determination in 1:20
155 soil:water (w/v) extracts, according to IBI standards (2014). Cation exchange capacity (CEC) was

156 assessed according to Mehlich (1938) using $BaCl_2$. Moisture content was calculated according to
157 the Black method (1965) as the difference in sample weight before and after oven drying at 105 °C
158 to constant weight.

159 Several parameters can be used to assess carbon stability in biochar. Calvelo Pereira et al. (2011)
160 used the thermo-labile fraction and the oxidation efficiency with potassium permanganate and
161 potassium dichromate, while Enders et al. (2012) used a combination of volatile matter and H:C
162 ratios corrected for inorganic C. In the present work, we referred to IBI standards (2014), which
163 define carbon stability as the molar ratio of hydrogen to organic carbon (maximum 0.7).

164 Total nitrogen (N_{tot}), total carbon (C_{tot}), organic carbon (C_{org}) and hydrogen (H) contents were
165 determined by dry combustion (Dumas 1831) using a CHN elemental analyser (Carlo Erba
166 Instruments, Mod 1500, series 2). In the case of C_{org}, combustion was carried out after the complete
167 removal of inorganic C with acid. Available nitrogen (N_{av}) was determined by a modified Kjeldahl
168 procedure using Devarda's alloy (Liao, 1981) as reducing agent to convert $(NO_3)^-$ and $(NO_2)^-$ into
169 $(NH_4)^+$ and subsequent Kjeldahl digestion. Total phosphorus (P_{tot}) was detected by
170 spectrophotometry (UV-1601 Shimadzu) according to the test method described by Bowman
171 (1988). Available phosphorus (P_{av}) was extracted by a $NaHCO_3$ solution at pH 8.5 and evaluated by
172 spectrophotometry according to the Olsen test method (1954). Alkalinity of samples with a pH
173 value greater than 7.0 was determined by titrimetry according to the Higginson and Rayment
174 method (1992). Particle size distribution (hereafter also named soil texture) was quantified by
175 hydrometer analysis through a modification of the Bouyoucos method (1962) (according to Beretta
176 et al. 2014), on samples previously dry-sieved at 2 mm. The fraction <2mm was treated with H_2O_2
177 and wet sieved at 200 µm, 50 µm and 20 µm. Measurements of density were carried out by a
178 hydrometer on samples smaller than 20 µm previously dispersed with sodium hexametaphosphate
179 solution. Moreover, in order to quantify the large fraction of macro aggregates, particles that did not
180 pass through the 2 mm sieve were treated with sodium hexametaphosphate solution to disrupt

181 aggregates and, subsequently, the difference in weight before and after wet sieving at 2 mm was
182 measured (Kemper and Koch 1966).
183
184 *2.3 Soil characterization*
185 To assess soil chemical-physical properties and the effects of biochar on these characteristics, four
186 soil samples for each plot were collected at two time points (T0, May 2014 and T1, February 2015)
187 (see details in Figure 1), i.e. before and after treatment with biochar, respectively. Sampling points
188 were located at approximately 40 cm distance from the plants, thus, reasonably far enough to be
189 considered bulk soil even though a few weed roots were found. Soil samples, once freed from roots,
190 were mixed together in one bulk sample, air dried until constant weight, passed through a 2 mm
191 sieve and stored at room temperature in a closed container until analysis.
192 Methods for the characterization of moisture, CEC, P_{tot} and P_{av}, N_{tot}, alkalinity (only for soil
193 samples with pH>7.0) and particle size distribution were as described in the previous paragraph.
194 The pH was determined by potentiometry (pH meter Eutech Instruments pH 700, 2013) according
195 to Conyers and Davey (1988). EC was measured by direct instrumental determination according to
196 Rhoades (1996). The different forms of available mineral nitrogen were determined by ion selective
197 electrodes (Greenberg et al., 1985) on soil samples dissolved in deionized water. Organic carbon
198 (C_{org}) was assessed according to the Black and Walkley (1934) test method based on carbon
199 incomplete oxidation. Available water content (AWC) was measured for soil samples collected in
200 February 2015, within each plot, at 5 and 15 cm soil depth. Samples belonging to the same
201 treatment and soil depth were mixed and homogenized for a total of 8 samples (2 for each treatment
202 at each soil depth). Soil water retention curves were obtained according to the method described in
203 Cresswell et al. (2008). Values of AWC (m^3 m^{-3}) were calculated as the difference between water
204 content measured at field capacity (pressure -0.33 MPa) and at wilting point (-15 MPa) multiplied
205 by the gravimetrically determined bulk density.
206

207 *2.4 Mycorrhiza measurements*

208 For arbuscular-mycorrhizal fungi (AMF) analysis, in-growth nylon mesh bags were prepared
209 (Wallander et al., 2001; Lovelock et al., 2004) with 40 μm pore size and 25 cc capacity. Soil was
210 collected from biochar-treated and control plots, air dried, sieved (<1 mm) to eliminate plant
211 material and fine roots, sterilized by autoclaving and filled into mesh bags. Within each plot four
212 mesh bags were placed in the ground at 20 cm depth on 10 June 2014 and harvested on 30 June
213 2015 (according to the sampling scheme reported in Figure 1). Due to its AM fungus specificity,
214 glomalin was used as a quantitative indicator of mycorrhizae presence (Upadhyaya and Wright,
215 1996; Lovelock et al., 2004). Glomalin is composed of two fractions, which differ in the ease of
216 extraction: easily extractable glomalin (EEG) and total glomalin (TG), which requires more drastic
217 extraction conditions. Because the distinction between these two fractions is beyond the scope of
218 this study, we proceeded directly to the quantification of TG. Following the procedure described by
219 Upadhyaya and Wright (1996), the extraction was carried out in 50 mM sodium citrate, pH 8.0 at
220 121 °C with five extraction cycles of 90 min each. The supernatant of each extraction cycle was
221 collected in a single pool and the protein content was determined by spectrophotometric reading
222 (UV-1601 Shimadzu) according to the Bradford method using bovine serum albumin as a standard.

223

224 *2.5 Fine root measurements*

225 The soil core sampling method (Persson and Vogt 1991) was used to quantify fine root mass (<2
226 mm in diameter) during the 2014-2015 growing season. Within each of the 8 plots (4 control and 4
227 biochar-treated) at each sampling date, two soil cores (4 cm diameter, 40 cm deep) were collected
228 using a motor-driven portable core sampler (adapted from Alley and Ponder, 1997) (Figure 1). To
229 investigate the kinetics of biomass and necromass, soil samples were collected on five dates
230 between 21 May 2014 and 29 May 2015. Samples were stored in plastic bags at 4°C until further
231 processed. Each soil sample was placed in a nylon bag (300-μm mesh), contained in a plastic
232 cylinder (6-mm mesh), and washed automatically using a washing machine (adapted from

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233 Benjamin and Nielsen, 2004). Fine roots were examined under the microscope and divided into two
234 groups: grapevine and other species. Fine roots from grapevine were classified *live* [hereafter
235 termed fine root biomass (FRB)] or *dead* [hereafter termed fine root necromass (FRN)] depending
236 on their colour, texture and shape (Persson and Vogt, 1991). After selection, grapevine root
237 fragments were first roughly grouped by calliper method in half-millimetre-diameter classes and
238 scanned at a resolution of 400 dpi with a calibrated flatbed scanner coupled to a lighting system for
239 image acquisition (Epson Expression 10000 XL). Successively, images were analysed by WinRhizo
240 Pro V. 2007d (Regent Instruments Inc. Quebec). After fine root length (FRL) measurement,
241 grapevine live and dead fine root samples were oven-dried separately and weighed. Annual fine
242 root production was estimated using the minimum–maximum method procedure. This method
243 calculates, and sums in case of a multimodal seasonal pattern, only significant differences between
244 seasonal minimum and maximum fine root dry mass (live mass plus necromass) (Edwards and
245 Harris, 1977; Mc Claugherty et al., 1982; Hertel and Leuschner, 2002). Fine root turnover rates of
246 FRB were calculated as annual root production divided by maximum standing biomass (Gill and
247 Jackson, 2000; Godbold et al., 2003). The following fine root traits were determined: (1) mean live
248 dry mass (FRB - g m^{-2}) and dead dry mass (FRN - g m^{-2}); (2) mean live length (FRL - m m^{-2}) and
249 dead length (nFRL - m m^{-2}); (3) dry mass density (FRMD - mg cm^{-3}) and length density (FRLD –
250 cm cm^{-3}) at different soil depths; (4) seasonal pattern of the above mentioned traits; and (5) annual
251 production and turnover rate of live mass.

252 FRB = Fine root biomass (live; gm^{-2})

253 FRN = Fine root necromass (dead; gm^{-2})

254 FRL = Fine root length (live; cm m^{-2})

255 nFRL = Fine root length of necromass (dead; cm m^{-2})

256 FRP= Fine root annual production (g m^{-2})

257 FRTR= Fine root turnover rate (yr^{-1})

258

259 *2.6 Statistical analysis*

260 To evaluate significant differences between soil chemical-physical properties of biochar-treated and
261 control plots, a randomized design with four replicates (one at each of four plots) was set up. Data
262 obtained were analysed with a two-tailed T-test with a significance level of 95% ($p<0.05$). The
263 same applies to glomalin data for which 12 replicates (four at each of the three treated and control
264 plots) were carried out. The root data did not meet a normal distribution, neither when square-root
265 or log-transformed. Therefore, non-parametric statistics were applied. The Kruskal-Wallis multiple-
266 comparison test was used to compare root biomass, root length and live root diameter
267 measurements among sampling dates. The Mann-Whitney U test was applied as post hoc test for
268 pairwise comparison among sampling dates. It was also applied to compare control and biochar-
269 treated plots for sampling dates and for mean annual values. Analyses of non-parametric methods
270 were applied at a 95% significance level. Statistical analysis was carried out using statistical
271 software package SPSS 17.0 (SPSS Inc, Chicago, IL, USA).

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274 **3. Results**

275

276 *3.1 Biochar characteristics*

277 The biochar tested was found to meet European Biochar Certificate (EBC, 2012) and IBI-Standard
278 (2014) requirements with regard to C_{tot} and C_{org} content, respectively. Its C:H value, close to 0.7,
279 ensures a good stability to the organic carbon. The conductivity value showed that the biochar used
280 has a higher salt content than soil. Moreover, available phosphorus and nitrogen represented 17.7%
281 and 0.3% of total phosphorus and nitrogen, respectively (Table 1). Particles larger than 2 mm
282 accounted for 11.9% of the total mass. Particles smaller than 2 mm were distributed as follows:
283 16.1% between 2 mm and 200 µm, 10.1% between 200 µm and 50 µm, 52.7% between 50 µm and
284 20 µm, 17.4% between 20 µm and 2 µm and 3.7% smaller than 2 µm (Table 1).

285

286 *3.2 Soil characteristics*

287 The soil samples analysed had the typical characteristics of a non-saline soil, with a clay texture as
288 obtained by the USDA texture calculator[1], neutral pH (USDA, 2005) and insufficient organic
289 carbon content in relation to clay content (Soltner, 1988). Both total nitrogen and ammonium-
290 nitrogen concentration values were characteristic of a medium soil (Horneck et al. 2011; Giardini,
291 2002). No interpretation was given to the nitrate value, due to its high variability, influenced by
292 seasonal meteorological conditions and fertilization practices (Table 2). Available phosphorus,
293 considered in relation to the cation exchange capacity, was below the threshold of sufficiency (see
294 CONTROL column in Table 2). Results obtained from the analysis of biochar-treated soil revealed
295 that pH value, CEC, total nitrogen, available nitrogen in the nitrate form $(NO_3)^-$-N, total phosphorus
296 and available phosphorus remained unaffected. Differently, C_{org} content and

297 [1]http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/?cid=nrcs142p2_0541

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298 available nitrogen in the ammonium form, $(NH_4)^+$-N, significantly increased (20.7%, $p<0.05$ and
299 84.4% $p<0.05$, respectively) in treated plots when compared to untreated control plots (see
300 BIOCHAR column in Table 2).
301 In both treated and control plots, AWC did not show significant differences between the two soil
302 depths (0-10 cm and 10-20 cm) (Figure 2). However, when the AWC values of amended and
303 control plots were compared (Figure 2), a significant difference was detected (11%; $p<0.05$)
304 independently of the soil depth. Finally, a significant increase (77.4 %; $p<0.05$) in the particle size
305 fraction greater than 2 mm was observed in treated plots compared to the control (Table 2).
306
307 *3.3 Arbuscular mycorrhizal fungi (AMF) and fine root characterisation*
308 Total extractable glomalin (TG) analysis showed that, over a time period of one year, no significant
309 difference in the amount of AMF was found between biochar-treated and control plots (Figure 3),
310 which showed values of 0.9 and 1.0 mg g^{-1}, respectively.
311 Fine root biomass (FRB) of treated plots showed an increase 112 days after biochar application (10
312 September 2014; Figure 4a). At the same sampling point, fine roots of control plots showed an
313 opposite trend with a slight decrease in FRB. At the third sampling point (24 November 2014), FRB
314 showed a slight increase in both control and treated plots (Figure 4a). Afterwards, FRB of control
315 plots continued to increase (26 February 2015), while fine roots of treated plots showed a slight but
316 not significant decrease (Figure 4a). At the last sampling point (29 May 2015), right after the
317 milling operation, FRB significantly decreased in both control and treated grapevine plots (Figure
318 4a). Fine root necromass (FRN) showed the highest values at the first and last sampling points, right
319 after the milling operations. Throughout the sampling period from September 2014 to February
320 2015, FRN remained very low compared to FRB and did not show any significant differences
321 between control and treated plots (Figure 4b). Unlike FRB, the value of fine root length (FRL) did
322 not show any significant differences throughout the experiment or between control and treated plots
323 (Figure 4c). Fine root length of necromass (nFRL) values, as in the case of FRN, were highest at the

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324 first and last sampling points, right after the milling operations (Figure 4d). At the sampling points
325 between September 2014 to February 2015, nFRL remained very low while FRL showed the
326 highest values (Figure 4d). Mean diameter of the control fine root population (Figure 5) showed
327 similar values, ranging between 0.40 and 0.50 mm, throughout the whole experiment. In the case of
328 treated plots, mean fine root diameter was significantly higher at both the 10 September and 24
329 November 2014 sampling points (Figure 5).

330 Mean annual fine root biomass (FRB; Table 3) in treated plots (13.34 g m^{-2}) was significantly
331 higher ($p=0.049$) than in control plots (8.56 g m^{-2}), whereas mean annual fine root length (FRL;
332 Table 3) did not show significant differences ($p=0.676$). FRD was significantly higher ($p=0.037$) in
333 treated plants (0.56 mm) than in control plants (0.46 mm). Similarly, fine root annual production
334 (FRP) in treated plots (12.7 g m^{-2}) resulted higher than those measured in control plots (8.71 g m^{-2}).
335 Finally, fine root lifespan resulted almost one year for control plots (1.02 yr^{-1}) and slightly longer
336 for treated plots (0.95 yr^{-1}).

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337 **Discussion**

338 An increasing amount of literature has reported studies focused on the effects of biochar
339 amendment on physical and chemical properties of various soils (reviewed by Ding et al., 2016).
340 All these studies show that the effects of biochar depend on the physical-chemical properties of
341 biochar itself and on the characteristics of the soil to which it is applied. The vineyard soil of the
342 experimental plots in the present study was characterised by neutral pH, clay texture, and low C_{org},
343 C_{av} and P_{tot}. Our results show that amendment of this soil with an alkaline biochar (pH 9.2),
344 characterised by total nitrogen and CEC values much higher than those of excessive endowed (5 g
345 kg^{-1}) and high-value (>20 cmol kg^{-1}) agricultural soils, respectively (Giardini, 2002), had no effect
346 on pH value, CEC, and total nitrogen and phosphorus content (both total and available). The lack of
347 a biochar effect on the pH of vineyard soil may be attributed to the soil buffering capacity that
348 counteracts pH change and is in line with data reported in the literature showing that biochar
349 amendment caused a significant increase in pH of acidic soil while not altering the pH of neutral or
350 alkaline soil (Atkinson et al., 2010; Biederman and Harpole, 2013; Macdonald et al. 2014).
351 However, biochar significantly increased AWC, C_{org} and the particle size fraction greater than 2
352 mm in amended soil. The total porosity of biochar may retain water in small pores, thereby
353 increasing AWC (Asai et al., 2009; Baronti et al., 2014). In addition, biochar influences soil
354 aggregation due to its interaction with soil organic matter, minerals and microorganisms (Verheijen
355 et al., 2010). Furthermore, it is well established that plant roots, through different mechanisms,
356 contribute to improving soil aggregation (Ola et al., 2015 and references therein). Several authors
357 suggested that the increase in soil organic matter content might lead to improved soil aggregation
358 (Kong et al., 2005; Domingo-Olivé et al., 2016; Ma et al., 2016) and in turn higher soil water
359 retention (Verheijen et al., 2010; Abel et al., 2013). Thus, in biochar-amended soil the marked
360 increase in organic carbon content and in fine root biomass reported in the present work may
361 contribute to the higher fraction of macro aggregates (larger than 2 mm) and be responsible for the
362 improvement of AWC as well as macro and micro pore formation. Previous work, conducted in a

363 vineyard in central Italy, reported similar soil–water relation results after biochar application
364 (Baronti et al., 2014).
365 In the present study, biochar did not affect phosphorus availability, whose value remained under the
366 limit of detection both in amended and untreated soil. The low value of available phosphorus with
367 respect to CEC measured can be attributed to soil adsorption phenomena and, therefore, a negative
368 effect of biochar on nutrient availability as reported in previous experiments on calcareous soil
369 (Chintala et al., 2014) may be excluded. Indeed, on the contrary, nitrogen availability was affected
370 by biochar amendment as evidenced by an increase in the ammonium form, $(NH_4)^+$-N. This
371 increment might be due to biochar's potential to adsorb ammonium through its high carbon content
372 and, therefore, negative charge (Takaya et al. 2016). In addition, while the value of total N
373 remained unchanged, the biochar-induced increase in carbon content leads to a 10% increase in C/N
374 ratio. Despite this increase, the C/N ratio remained within the optimal range for a balance between
375 decomposition and humification processes (Tan, 2005).
376 Plant nutrient availability is a key factor in the establishment of arbuscular mycorrhizal interactions
377 in grape roots (Trouvelot et al. 2015). In both biochar-amended and untreated soil, glomalin values
378 where lower than those reported in the literature (Wright et al. 1996; Wright & Upadhyaya 1998;
379 Pikul et. al. 2002), indicating that no or little symbiosis occurred (Nicolas and Miller, 2003;
380 Lovelock et al. 2004; Nichols and Write, 2004; Rilling 2004; Saidi et al., 2014), which may be
381 attributed to the low level of phosphorus. Indeed, in the AM symbiosis, phosphate is transferred
382 from fungus to plant that constitutes a signal for the transfer of photosynthate from plant to fungus
383 (Harrison et al., 2002). Fitter (2006) suggested that the interruption of that signal, due to the failure
384 of phosphorus transfer from fungus to plant, leads to the end of such symbiosis. An additional
385 factor that may have contributed to the lack of symbiosis is probably related to the soil-milling
386 operation as reported by Nichols and Wright, 2004. Indeed, similarly to what was previously found
387 by these authors, the soil-milling operations during our study, performed as standard agronomic

388 practice, may have damaged the mycorrhizal network, which explains the low values of glomalin
389 observed.
390 Biochar-induced changes in grapevine fine root characteristics are evident soon after the soil
391 amendment. In fact, after almost four months, FRB had significantly increased in biochar-amended
392 plants. Furthermore, this increase occurred during the summer season (May-September), coinciding
393 with the phase of fruit set and ripening, and remained unchanged until the end of February
394 (dormancy period). According to Bates et al. (2002) and Comas et al. (2005), grapevine fine roots
395 mainly grow during the postharvest period, as was observed in the present study in untreated
396 grapevine plants. These findings highlight that biochar changes the grapevine fine roots seasonal
397 pattern, stimulating fine root growth when plants are most in need of water and nutrient supply. On
398 the other hand, the general FRB decrease observed at the end of the experiment may be attributed to
399 the soil-milling operations.
400 The increase in fine root biomass (FRB) in biochar-treated plants seems to be determined by the
401 stimulation of radial growth (root diameter FRD) rather than elongation (FRL). The morphological
402 plasticity of roots has been reported in previous studies, especially for the fine root fraction (Bjork
403 et al., 2007; Makita et al., 2011). In a recent work on turkey oak, Montagnoli et al. (2012a) showed
404 that root plasticity is a plant mechanism to overcome drought periods by enhancing soil exploitation
405 for water and nutrients. Indeed, in trees subject to natural water shortage, carbon is invested in
406 lengthening rather than in enlarging fine roots. Recent investigations by Baronti et al. (2014) on
407 grapevine under drought conditions showed a reduction in plant water stress and an increase in
408 photosynthetic activity and soil water content, after two seasons of biochar treatment. However,
409 despite these improvements, the quality of grape production remained unaffected. In the light of this
410 knowledge, we may assert that grapevine plants, when growing in biochar-amended soil
411 characterised by a higher soil water content, optimize the investment of carbon by increasing the
412 mean diameter rather than the length of very fine roots. In a milestone meta-analysis, Gill and
413 Jackson (2000) found that fine root turnover rate decreases with increasing diameter class. In fact,

414 in biochar-treated plants we found a lower turnover rate compared to control plants. Along with
415 these direct and indirect effects, our results draw attention to the biochar-induced improvement of
416 carbon sequestration through the extension of fine root life span. To evaluate the multiple impacts
417 of biochar on plants, soil, and ecosystem services, many authors recommend future studies focused
418 on the comparison of biochar effects in short- and long-term field experiments (Jones et al., 2012;
419 Zhang et al., 2014).

420

421 **Conclusion**

422 In conclusion, the results presented here highlight that biochar, by improving soil characteristics in
423 terms of water availability, organic carbon and available nitrogen, has an immediate effect on fine
424 root seasonal pattern and lifespan. Furthermore, these effects seem to occur when the plant needs to
425 optimize water and nutrient uptake, i.e. during fruit set and ripening. Our findings stress the need to
426 take into consideration the phenology of perennial plants when studying the effects of biochar.

427

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435

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759

760 **Figure captions**

761 **Figure 1.** Sampling scheme for chemical-physical analysis (T0), fine-root soil coring (1) and
762 mesh bags ( ) indicating sampling dates.
763

764 **Figure 2.** Available Water Content (AWC) (open box ☐) and biochar-treated (filled box ■) plots
765 at two soil depths (0-10 and 10-20 cm). Soil moisture data are means (n = 8) ± 1 SE. Letters *a* and *b*
766 indicate significant differences ($p<0.05$) between the two soil depths within control and treatment.
767 Letters *x* and *y* indicate significant differences ($p<0.05$) between control and treatment within the
768 soil depth.

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770 **Figure 3:** Mean values of total glomalin (TG; mg g^{-1}) in control (open box ☐) and biochar-treated
771 (filled box ■) plots. Each value represents the mean (*n* = 8) ± 1 SE. Means with different letters
772 are significantly different ($p<0.05$).

773

774 **Figure 4.** Seasonal variation of live (FRB; a) and dead (FRN; b) fine root mass (g m^{-2}), and live
775 (FRL; c) and dead (nFRL; d) fine root length (m m^{-2}) in control (open box ☐) and biochar-treated
776 (filled box ■) plots. Data refer to each sampling date represented as mean (*n* = 8) ± 1 SE. Means
777 with different letters are significantly different ($p<0.05$) between sampling points within the same
778 treatment: letters *a* and *b* for control plots, letters *x* and *y* for treated plots. Asterisks (*) indicates
779 significant differences ($p<0.05$) between control and treated plots at the same sampling point.

780

781 **Figure 5.** Seasonal variation of mean diameter size (mm) for fine root biomass in control (open box
782 ☐) and biochar-treated (filled box ■) plots. Each value represents the mean (*n* = 8) ± 1 SE. Means
783 with different letters are significantly different ($p<0.05$) between sampling points within the same
784 treatment: letters *a* and *b* for control plots, letters *x* and *y* for treated plots. Asterisks (*) indicates
785 significant differences ($p<0.05$) between control and biochar-treated plots at the same sampling
786 point.

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Tables
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1 **Tables**

2 Table 1. Biochar chemical-physical characteristics. Each value represents the mean (n = 8) ± 1 SE.

PARAMETER	UNIT	VALUE
pH	-	9.7±0.1
EC	dS m^{-1}	7.5±0.4
CEC	cmol kg^{-1}	21.3±0.3
Moisture	g kg^{-1}	62.4±1.2
N_{tot}	g kg^{-1}	9.1±0.2
N_{av}	mg kg^{-1}	30±0.4
P_{tot}	mg kg^{-1}	1221.9±21.3
P_{av}	mg kg^{-1}	217± 3.0
C_{tot}	g kg^{-1}	778.1±0.1
C_{org}	g kg^{-1}	705.6±0.1
H	g kg^{-1}	45.3±0.2
H/C_{org}		0,76
Alkalinity	% $CaCO_3$	18.2±0.6
~~Texture~~Particle size distribution:		
ø < 2 µm	%	3.7±0.7
2 < ø < 20 µm	%	17.4±1.3
20 < ø < 50 µm	%	52.7±4.1
50 < ø < 200 µm	%	10.1±0.1
200 µm < ø < 2mm	%	16.1±1.3
ø > 2 mm	%	11.9±1.0

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Table 2. Chemical-physical analysis performed on soil samples of control and biochar-treated plots at T1. Each value represents the mean (n = 8) ± 1 SE. Means in bold are significantly different ($p<0.05$).

PARAMETER	UNIT	CONTROL	BIOCHAR
pH	pH	7.0±0.04	7.1±0.1
EC	ds m^{-1}	0.6±0.2	0.6±0.2
Moisture	g kg^{-1}	42.4±4.4	39.3±0.6
Ashes	%	90.1±0.5	90.0±0.3
N_{tot}	g kg^{-1}	1.1±0.03	1.2±0.2
N_{av} NH_4^+-N	mg kg^{-1}	**9.0±0.6**	**16.6±0.8**
N_{av} NO_3^--N	mg kg^{-1}	7.3±0.3	7.5±0.2
P_{tot}	mg kg^{-1}	212.6±11.0	233.5±6.5
P_{av}	mg kg^{-1}	<12	<12
C_{org}	g kg^{-1}	**11.6±0.3**	**14.0±0.6**
CEC	cmol kg^{-1}	16.6±0.2	16.5±0.3
Alkalinity	% $CaCO_3$		7.9±0.7
Particle size distribution:			
ø < 2 µm	%	48.1±0.7	47.4±0.5
2 µm < ø < 20 µm	%	16.3±1.0	16.3±1.4
20 µm < ø < 50 µm	%	22.5±1.3	21.7±1.9
50 µm < ø < 200 µm	%	5.8±0.4	6.8±0.1
200 µm < ø < 2 mm	%	7.3±0.7	7.8±0.3
ø > 2 mm	%	**3.1±0.3**	**5.5±0.2**

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14 Table 3. Annual mean fine root traits, irrespective of diameter class (d<0.2 mm)

PARAMETER	UNIT	CONTROL	BIOCHAR	*P*
FRB	g m^{-2}	8.56±1.46	13.34±2.35	**0.049**
FRL	cm m^{-2}	177±28	176±25	0.676
FRD	mm	0.46±0.02	0.56±0.03	**0.037**
FRP	g m^{-2}	8.71	12.70	
FRTR	yr^{-1}	1.02	0.95	

15 Values are the mean (*n* = 40) ± 1SE
16 FRB (fine root standing biomass), FRL (fine root length), FRD (fine root diameter), FRP (fine root
17 annual production), FRTR (fine root turnover rate). Boldface *P* values are significant at a
18 probability level of *P*<0.05 (Mann-Whitney U test)
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Figure 1

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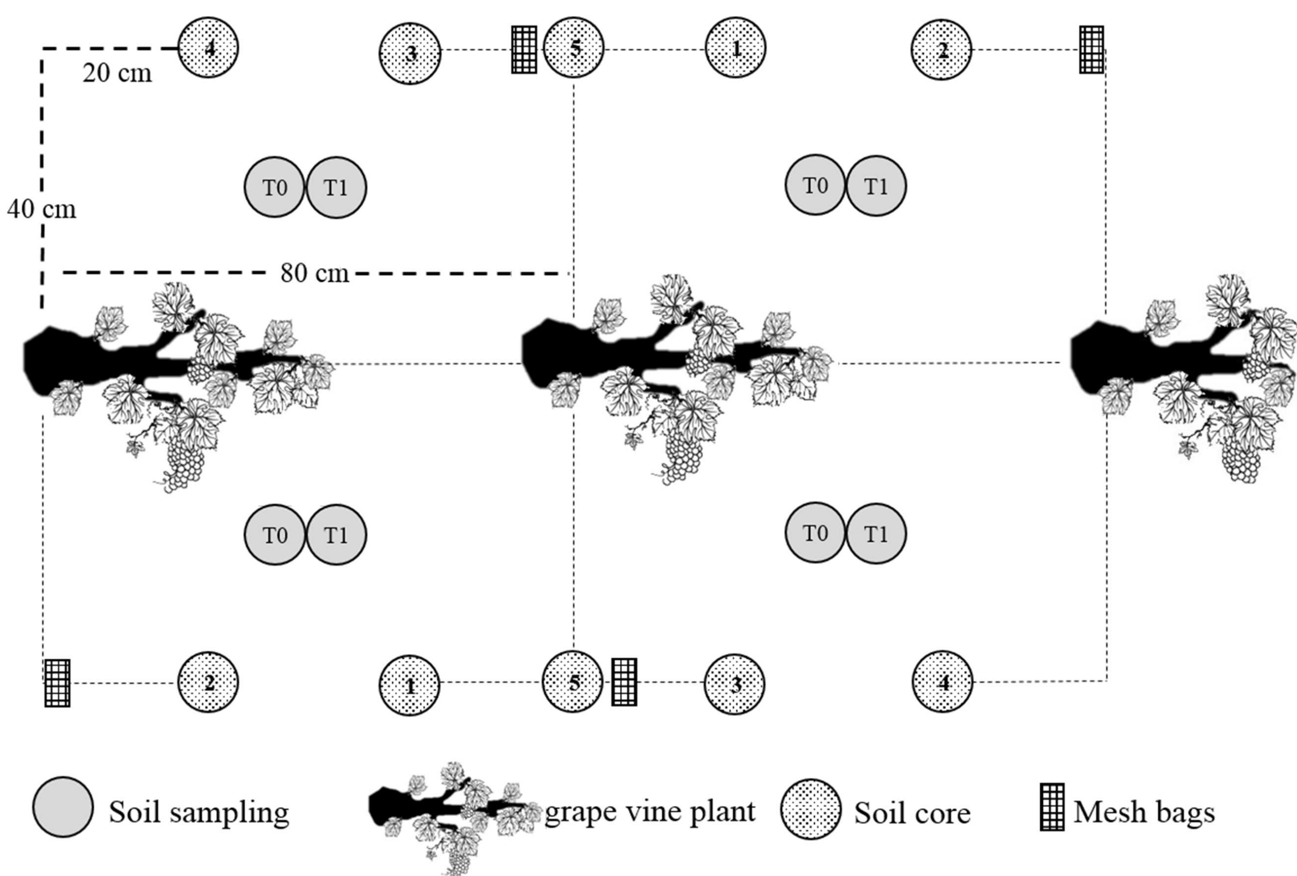

Figure 4

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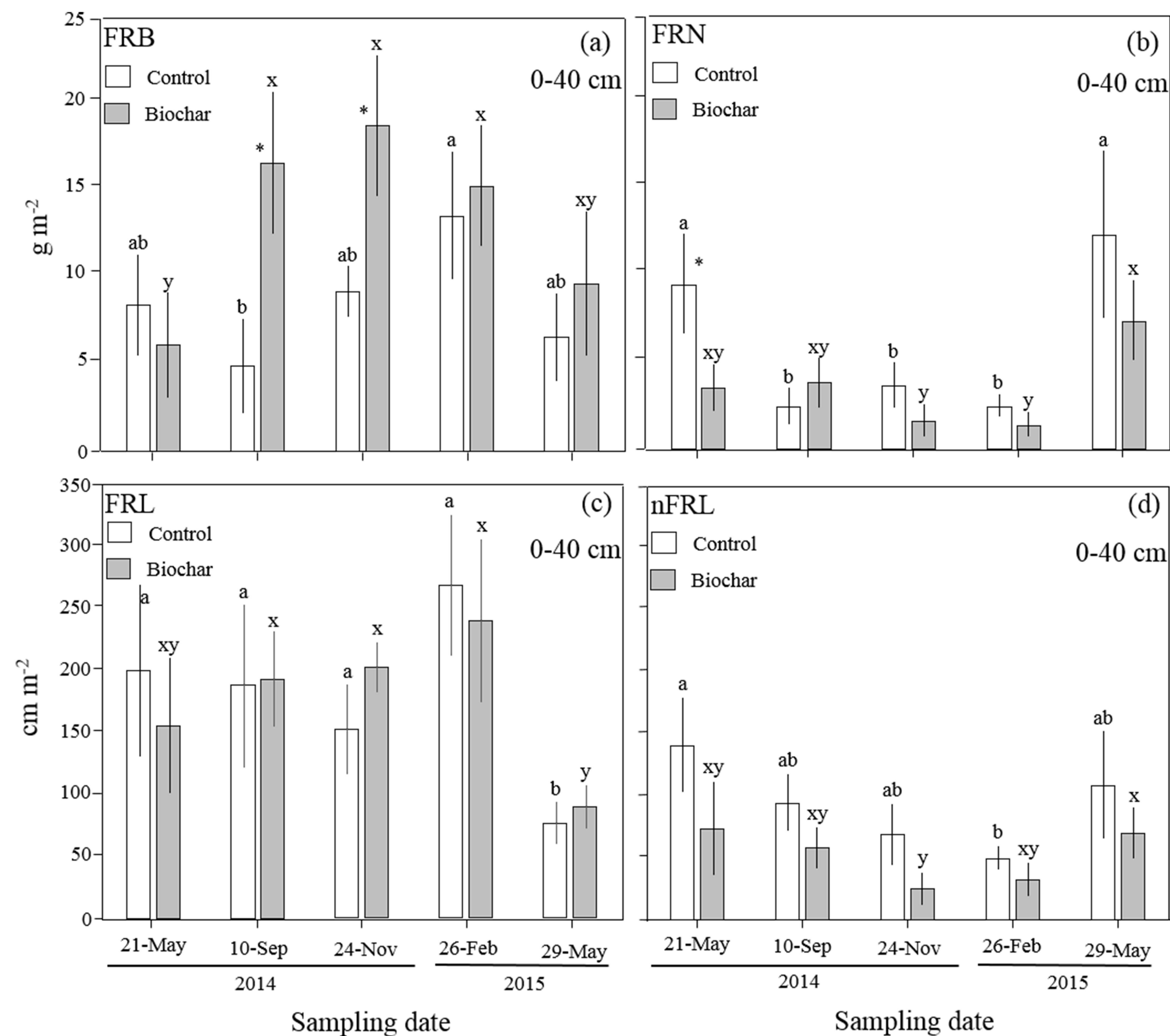

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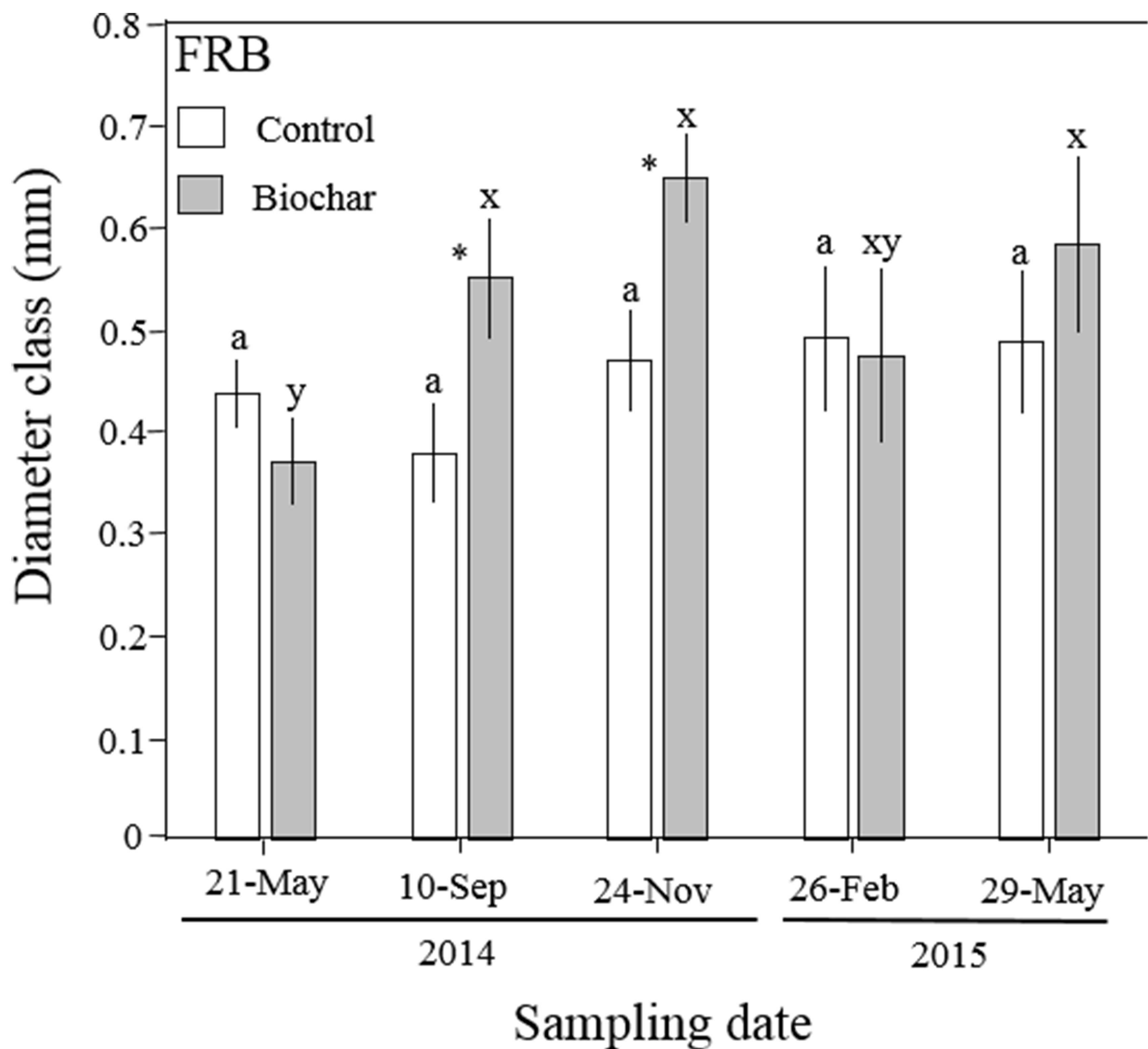

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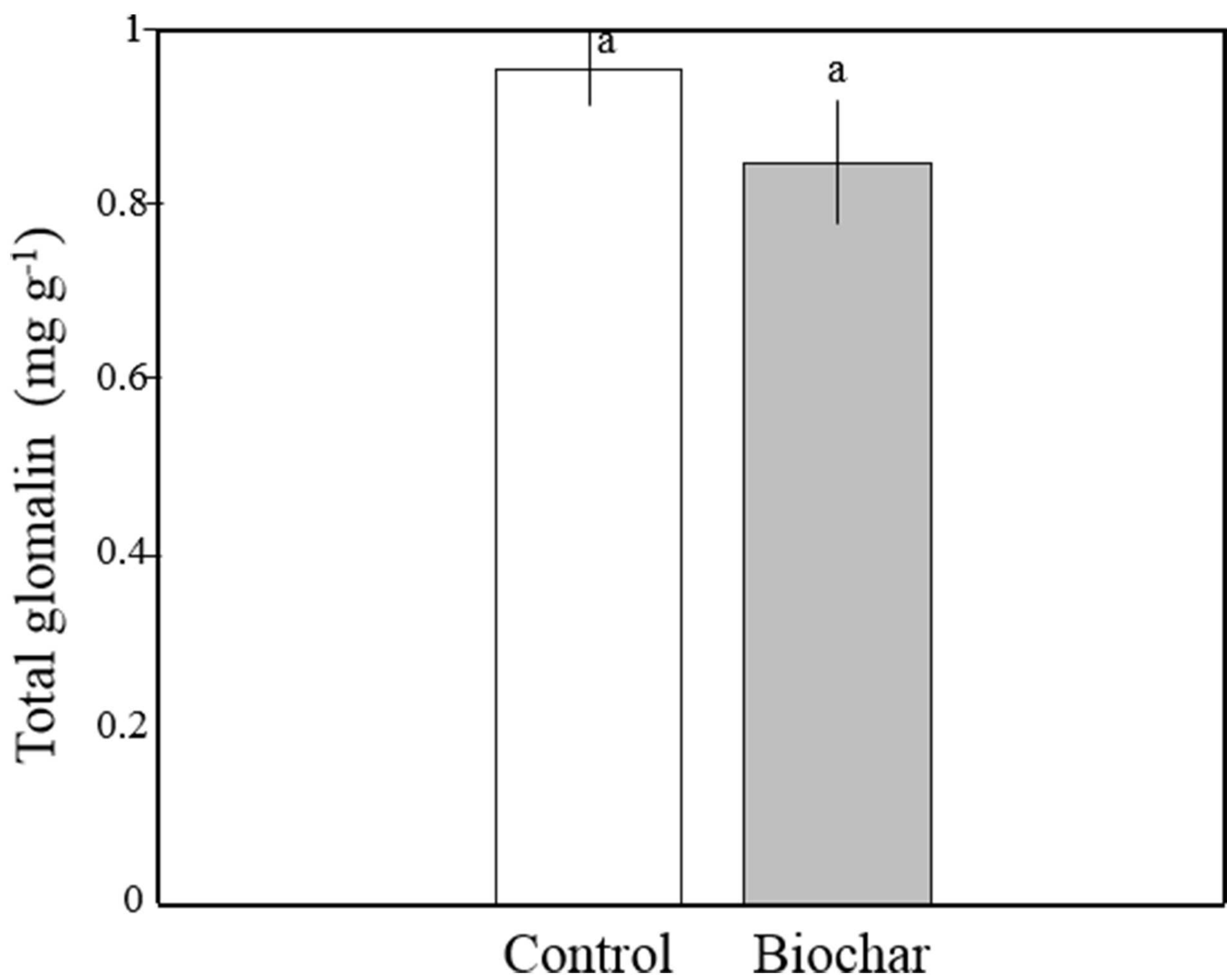

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