1 **Mycorrhizal inoculation and treatment with biochar and compost from pruning waste**

2 **improve the qualitative properties of a calcareous soil under wheat cultivation**

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9

10 **Abstract**

11 Most calcareous soils have relatively low levels of organic matter. To address this issue and improve

12 the qualitative properties of calcareous soils, soils can be treated with mycorrhizal fungi and/or

13 exogenous organic material such as biochar or compost derived from tree pruning waste. To evaluate

14 the effect of pruning waste biochar (PWB) and pruning waste compost (PWC) derived from apple and

15 grape trees combined with arbuscular mycorrhizal fungi (AMF) on the biological indices of calcareous

16 soils, a rhizobox study on wheat plants using a completely randomized design was conducted under

17 greenhouse conditions. The studied factors included the source of the type of organic material applied

18 (PWB, PWC, and control), the nature of the microbial inoculation (inoculation with AMF or no

19 inoculation), and the zone to which the treatments were applied (rhizosphere and non-rhizosphere soil).

20 At the end of the plant growth period, organic carbon (OC), microbial biomass carbon (MBC), microbial

21 biomass phosphorous (MBP), microbial respiration (BR), substrate-induced respiration (SIR), alkaline

22 (ALP), acid (ACP) phosphatase enzyme activities in the rhizosphere and non-rhizosphere soils, and root

23 mycorrhizal colonization were determined. Simultaneous application of a source of organic matter and

24 AMF inoculation significantly increased the OC and biological indices of soil relative to those observed

25 when applying organic matter without AMF inoculation. Additionally, MBC, MBP, ACP and ALP -

26 enzymes activities in the rhizosphere zone were significantly higher than in the non-rhizosphere. AMF

27 increased BR and SIR levels in the rhizosphere by 13.06% and 7.95% compared to non-rhizosphere,

28 respectively. It can be concluded that in calcareous soils with low organic carbon contents, organic

29   amendments such as PWC and PWB can improve soil biological properties by increasing microbial

30   activity and changing the properties of the rhizosphere.

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32   **Keywords**: pruning waste, biological Indices, rhizosphere, rhizobox

33

34 1. Introduction

35

36   The arid and semi-arid regions have alkaline calcareous soils with low contents of organic matter,

37   which adversely affects crop yields in these regions [1]. One of the most important ways to improve the

38   organic matter content is to properly manage the use of plant residues such as waste material from the

39   pruning of fruit trees. Tree pruning waste can be converted into biochar or compost, which can then be

40   applied to soils to replenish or increase their nutrient content. This improves the soil's physical and

41   chemical characteristics and is important for supporting the life of soil microorganisms and maintaining

42   a sustainable dynamic equilibrium between the biotic and abiotic components of the soils [2, 3]. Biochar

43   is a carbon-enriched solid generated by the pyrolysis of biomass under conditions with little or no

44   oxygen [4]. The composition and properties of biochar depend on the pyrolysis conditions including the

45   temperature, heating rate, duration, pressure, and input material [5]. Conversely, compost is formed by

46   the decomposition of organic matter mediated by microorganisms under hot, humid, aerobic conditions

47   [6]. Compost is rich in mineral nutrients, some of which are released and made available to plants in

48   soil gradually and continuously [7]. In addition to its physical and chemical properties, soil quality is

49   closely related to its biological characteristics [8]. Biological indicators are thus key descriptors of soil

50   quality, and variation in soil performance should be measured using biochemical parameters and indices

51   (e.g., BR, SIR, MBC, MBP, ACP, and ALP) that represent the diversity of soil microorganisms as well

52   as their distribution and metabolic activity. Soil microbial respiration is essentially a cellular process

53   that involves several biochemical reactions; the rate of microbial respiration is an indicator of both the

54   status and activity of soil microbes but also reflects the rate of organic matter decomposition and cycling

55   of certain nutrients within the soil [9].

56   Enzymatic activity and microbial biomass are the main biological indicators of soil. It seems that

57   among the biochemical parameters, MBC (41%) and the activity of phosphatase (ACP or ALP) enzymes

58   (28%) have been widely used [10].

59   There are contradictory reports about the impact of biochar on soil quality. Liu et al. reported that

60   biochar did not significantly influence soil respiration [11]. However, other studies concluded that the

61   pores of biochar provide protective habitats for microorganisms, leading to increases in the soil's

62   content of various mineral nutrients, energy, and carbon; as a result, biochar administration was found

63   to increase soil biological activity and help maintain soil quality [12, 13]. Another study found that

64   biochar increased microbial biomass and activity and also, it can enhance enzymatic activities in soil;

65   the increase in the activity of enzymes like phosphatases and suggested that the increased activity of

66   microorganisms following biochar administration might improve the availability of nutrients to other

67   microorganisms as a result of increased root exudation [14].

68   Studies on compost have shown that it contains a large population of microorganisms. Therefore,

69   in addition to increasing the organic matter and nutrient content of the soil, compost administration

70   increases and changes its microbial population [15]. Some rhizosphere microorganisms such as AMF

71   can stimulate soil microbial activity and promote increases in activity of phosphatase and soil microbial

72   biomass [16]. Accordingly, multiple studies have found that treatment with biochar and compost

73   promote mycorrhizal root colonization, spore production, and hyphal expansion, thereby improving soil

74   quality [17, 18]. Active root systems continuously release organic compounds into the rhizosphere that

75   promote the growth and activity of the microbial community in the soil as well as the overall health of

76   the soil system [19, 20].

77   The effect of compost and biochar is not confined to the placement of organic matter in and outside

78   the rhizosphere; it also strongly affects microbial activity in the soil outside the rhizosphere [21]. When

79   studying rhizosphere processes, it is helpful to confine root growth to a fixed volume of soil in order to

80   increase root density and accelerate the sampling of the rhizosphere soil. To this end, the concept of the

81   rhizobox was developed; a rhizobox is like a pot except that it hinders direct contact between the roots

82   and the soil without disrupting the mobilization of the soil solution [22, 23].

83   This work investigates the effects of applying biochar and pruning waste compost derived from

84   apple trees and grapevines on some biological characteristics of calcareous soils in the presence of

85   AMF. The combined effects of treatment with biochar and AMF on soil biological processes have not

86   been studied extensively, and most biochar administration studies have focused on acidic soils in

87   tropical and humid climates; consequently, there is little data on their effects in warm and arid regions

88   with non-acidic soils.

89

90 **2. Materials and Methods**

91

92 *2.1. Soil sampling and preparation*

93

94   This study used a randomized factorial complete block design with three replicates. The

95   experiments were conducted under greenhouse condition in rhizoboxes. The factors were the organic

96   matter source (pruning waste biochar [PWB], pruning waste compost [PWC], and control [no added

97   organic matter]), microbial inoculation (AMF and non-inoculation [-AMF]), and the zone to which the

98   treatment was applied (rhizosphere or non-rhizosphere soil). Soil samples were collected from the

99   surface layer (0–30 cm) of a region of non-arable land in Salmas, West Azerbaijan Province, Iran. The

100   samples were air-dried and sieved to pass through a 2 mm mesh, then sterilized in an autoclave at 121°C

101   at 1.5 atm for 2 hours. Before sterilization, some of the soil's physicochemical properties were

102   determined, as shown in Table 1 [24].

103

104   **Table 1.** Physicochemical properties of the studied soil

soil texture	pH	EC (dS m^{-1})	O.C (%)	CaCO$_3$ (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})
Loamy sand	7.53	0.47	0.25	14.25	0.08	7.64	98

105

106 *2.2. Preparation of biochar and compost*

107

108   To prepare biochar, fruit tree pruning waste was collected from orchards in Urmia County in West

109   Azerbaijan Province. The waste was then oven-dried at 65 °C for 48 hours. The dried samples were

110   placed in a reactor (a steel cylinder with a diameter of 7 cm and a height of 31 cm) and then heated to

111   350 °C in an electric furnace to form biochar. The PWC was taken from the research greenhouse of the

112   Soil Science Department in Urmia University. Finally, the PWB and PWC were ground and screened

113   with a 0.5-mm mesh. No ash was observed on the biochar surface, implying that oxygen had been

114   removed and it had been produced correctly. Table 2 summarizes the characteristics of the biochar and

115   compost, which were determined using the analytical methods described by Rajkovich et al. and Alfano

116   et al. [25, 26].

117

118

Table 2. Characteristics of the tested PWC and PWB

Characteristics	unit	Pruning waste biochar (PWB)	Pruning waste compost (PWC)
pH	-	7.29	7.05
EC	dS m^{-1}	0.08	17.87
N	%	0.54	3.72
C	%	67.53	30.02
P	%	0.27	7.54

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2.3. Greenhouse Experiment

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2.3.1.Rhizobox experiment

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124   Lab-built rhizoboxes with dimensions of $20 \times 15 \times 20$ cm (length × width × height) were used in

125   the greenhouse experiments. The inner space of the rhizoboxes was divided into two zones using

126   325 mesh nylon cloth: (i) a 2 cm thick rhizosphere zone, and (ii) a 5.8 cm thick non-rhizosphere zone

127   on either side of the rhizosphere zone (Fig. 1). PWB and PWC derived from apple trees and grape vines

128   was incorporated into the soil so as to provide 1.5% net organic C; each box thus contained 5.80 kg soil

129   complemented with 41.19 g compost kg^{-1} soil (PWC), 22.21 g biochar kg^{-1} soil (PWB), or sterilized

130   inoculated soil (control). In addition, a rock phosphate source providing 80 mg P kg^{-1} soil was placed 5

131   cm below the seeds to act as an insoluble P source.

132   Microbial inoculation was performed using microbial strains from the microbial bank of the Soil

133   Science Department of Urmia University and included the mycorrhizal fungus *Glomus fasciulatum*.

134   The amount of mycorrhiza inoculum was 70 g per box and it was uniformly dispersed under the seeds

135   with a 0.5-cm spacing [27]. After adding the inoculum, seeds of the wheat cultivar 'Pishtaz' (*Triticum*

136   *aestivum* L.) were disinfected with 0.5% sodium hypochlorite and sown in the rhizosphere zones of the

137   rhizoboxes. After the seeds germinated, the seedlings were thinned to provide four plants per rhizobox.

138   During their growth periods, the plants were irrigated with distilled water and fed with P-free Rorison

139   nutrient solution to satisfy their nutrient requirements [28].

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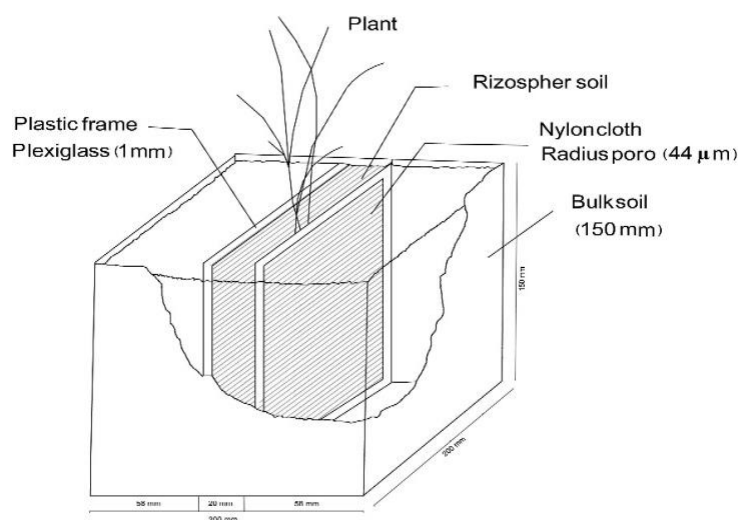
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142   **Figure 1.** A schematic of rhizobox

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144   *2.3.2. Soil analysis*

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146   At the end of the 65-day wheat growth period, two soil samples were collected from the non-

147   rhizosphere zone (specifically, from the 2 cm furthest from the rhizosphere zone) and combined.

148   Rhizosphere soil was then sampled by removing the plexiglass framework of the box and then removing

149   the soil from the box. Main and secondary roots were then carefully removed from the rhizosphere soil

150   and two samples were collected and combined. Two final samples were thus obtained from each

151   rhizobox, one representing the rhizosphere zone and the other representing the non-rhizosphere zone.

152   The OC [24], MBC [29], MBP [30], BR [31], SIR [32], ACP and ALP [33] and arbuscular mycorrhizal

153   fungi colonization percentage [34] were then determined for each sample.

154

155   *2.3.3. Statistical Analysis*

156

157   Statistical analyses including analysis of variance and comparison of data means was performed

158   using Duncan's Multiple Range Test with a significance threshold of $p < 0.05$ using the SAS software

159   package (Version 9.2).

160

161 **3. Results**

162

163   Comparisons of means for the interaction between organic sources and AMF inoculation

164   ($p < 0.001$) revealed that treatments involving AMF inoculation increased organic C and soil biological

165   parameters to a much greater degree than treatments without inoculation. Even without the addition of

166   exogenous organic material, the soil biological parameters after inoculation were substantially higher

167   than under non-inoculated control conditions. However, in the absence of added organic matter, AMF

168   inoculation did not significantly increase the soil's organic C content (Table 3). The highest soil organic

169   C content and biological parameter values were observed for the treatment combining AMF inoculation

170   with compost addition. However, the organic C content did not differ significantly between the compost

171   and biochar treatments under non-inoculation conditions. Biochar treatments yielded higher organic C

172   levels than control treatments under both inoculation and non-inoculation conditions, but the increase

173   in organic C was lower than that caused by adding compost. A comparison of means for the interactive

174   effect of organic source and microbial inoculation on MBC and MBP showed that the addition of

175   organic matter and AMF inoculation had a significant effect ($p < 0.001$) when compared to the control

176   treatment without AMF inoculation. In the compost treatments, the presence of AMF increased MBC

177   and MBP levels 1.66- and 5.24-fold, respectively. Similarly, in the biochar treatments, AMF inoculation

178   increased MBC and MBP levels 1.37- and 1.34-fold, respectively. Under non-inoculation conditions,

179   adding compost increased MBC and MBP to a greater degree than adding biochar (Table 3). Compost

180   treatment combined with AMF inoculation also increased BR and SIR, and this increase was 1.28 times

181   greater in the presence of AMF than without inoculation. Similar trends were observed for biochar

182   treatment.

183

184   **Table 3.** Effects of the organic amendment source and microbial inoculation on OC, MBC, MBP, BR
185   and SIR

Microbial inoculation	Organic sources	OC (%)	MBC (mg kg^{-1})	MBP (mg kg^{-1})	BR (mg CO$_2$ d^{-1} kg^{-1})	SIR (mg CO$_2$ d^{-1} kg^{-1})
+AMF	PWB	1.64[b]	789.3[b]	20.20[b]	53.15[b]	100.5[b]
	PWC	2.0[a]	957.1[a]	85.72[a]	56.17[a]	112.3[a]
	Cont.	0.44[d]	429.0d	7.43[d]	25.97[e]	51.99[e]
-AMF	PWB	0.87c	574.0[c]	15.01[c]	34.86[d]	78.21[d]
	PWC	0.97[c]	573.7[c]	16.33[c]	43.63[c]	87.48[c]
	Cont.	0.32[d]	264.5[e]	3.52[e]	8.61[f]	15.46[f]
LSD$_{0.05}$		0.18	7.31	2.41	2.88	4.34

186   Means followed by the same letters are not significantly different according to Duncan's multiple range test at the
187   p<0.05 level. +AMF, -AMF, PWB, PWC, and Cont denote inoculation with arbuscular mycorrhizal fungi, without
188   micorrhizal inoculation, pruning waste biochar, pruning waste compost, and control (no added organic matter),
189   respectively.
190

191   Adding organic matter (especially compost) to the soil caused the organic matter content of both

192   the rhizosphere zone and the non-rhizosphere zone to differ significantly from that in control treatments

193   ($p < 0.05$; Table 4). The soil organic carbon content was higher in the rhizosphere than in non-

194   rhizosphere in all treatments, but the difference was not significant in all cases. The addition of organic

195   matter to the rhizosphere and non-rhizosphere zones significantly increased their MBC and MBP

196   contents (p <0.01) when compared to controls (Table 4). The highest MBC content was observed when

197   rhizosphere soil was amended with compost; under these conditions, the MBC content of rhizosphere

198   soil was 1.30% higher than that of non-rhizosphere soil. The MBC of the rhizosphere soil was lowest

199   under organic matter-free control conditions; even under these conditions, the MBC of the rhizosphere

200   soil was 3.49% higher than that of the non-rhizosphere soil. The highest MBP was observed in the

201   rhizosphere zone after treatment with compost, which was 5.55% higher than the MBP in the non-

202   rhizosphere. Biochar also improved these biological parameters when compared to the control, albeit

203   to a lesser degree than compost; it increased both BR and SIR in the rhizosphere significantly more

204   ($p < 0.001$) than in the non-rhizosphere zone (Table 4). The BR and SIR in both the rhizosphere and the

205   non-rhizosphere were higher under the organic amendment treatments than under control conditions;

206   the highest BR (5263 mg CO_2 kg^{-1} soil) and SIR (103.3 mg CO_2 kg^{-1} soil) were observed in rhizosphere

207   zone after compost treatment.

209   **Table 4.** OC, MBC, MBP, BR and SIR in different soil zones with different organic amendments

Soil	Organic sources	OC (%)	MBC (mg kg^{-1})	MBP (mg kg^{-1})	BR (mg CO_2 d^{-1} kg^{-1})	SIR (mg CO_2 d^{-1} kg^{-1})
Rhizosphere	PWB	1.29[bc]	687[c]	18.98[c]	46.99[b]	92.61[b]
	PWC	1.52[a]	770.3[a]	52.41[a]	52.63[a]	103.3[a]
	Cont.	0.42[d]	352.7[e]	6.71[e]	20.27[d]	37.13[d]
Non-rhizosphere	PWB	1.22[c]	676.3[d]	16.23[d]	41.03[c]	86.06[c]
	PWC	1.45[ab]	760.4[b]	49.65[b]	47.16[b]	96.51[b]
	Cont.	0.35[d]	340.8[f]	4.24[f]	14.31[e]	30.33[e]
LSD$_{0.05}$		0.18	7.31	2.41	2.88	4.34

210   Means followed by the same letters are not significantly different according to Duncan's multiple range test at
211   the p<0.05 level. PWB, PWC, and Cont. denote pruning waste biochar, pruning waste compost and Control
212   (without organic matter), respectively.

214   AMF inoculation increased organic C in both the rhizosphere and non-rhizosphere zones when

215   compared to the non-inoculated control treatment (Table 5). However, the organic C contents of the

216   rhizosphere and non-rhizosphere did not differ significantly ($p < 0.05$) under either inoculation or non-

217   inoculation conditions. Additionally, the microbial biomass C and P levels in the +AMF treatments

218   were 1.22 and 3.25 times higher, respectively, than in the -AMF treatments (Table 5). On the other

219   hand, MBC and MBP were much higher in the rhizosphere than in the non-rhizosphere ($p < 0.001$).

220   AMF inoculation significantly ($p < 0.001$) increased BR and SIR in both the rhizosphere and non-

221   rhizosphere zones when compared to controls (Table 5). Additionally, AMF inoculation increased BR

222   and SIR in the rhizosphere by 13.06 and 7.95%, respectively, relative to the non-rhizosphere.

224   **Table 5.** OC, MBC, MBP, BR and SIR in rhizosphere and non-rhizosphere soil with and without microbial
225   inoculation

Microbial inoculation	Soil	OC (%)	MBC (mg kg^{-1})	MBP (mg kg^{-1})	BR (mg CO_2 d^{-1} kg^{-1})	SIR (mg CO_2 d^{-1} kg^{-1})
+AMF	Rhizosphere	1.39[a]	730.9[a]	39.08[a]	47.86[a]	91.64[a]
	Non-rhizosphere	1.33[a]	719.4[b]	36.49[b]	42.33[b]	84.89[b]
-AMF	Rhizosphere	0.75[b]	475.7[c]	12.99[c]	32.06[c]	63.73[c]
	Non-rhizosphere	0.69[b]	465.6[d]	10.26[d]	26.01[d]	57.04[d]
LSD$_{0.05}$		0.18	7.31	2.41	2.88	4.34

226   Means followed by the same letters are not significantly different at the p < 0.05 level according to Duncan's test.
227   +AMF and -AMF denote inoculation with arbuscular mycorrhizal fungi and without mycorrhiza, respectively.

229   The highest activity of phosphatase enzymes was measured in compost applied inoculated with AMF.

230   AMF inoculation increased ACP and ALP enzymes by 5.92 and 5.01 times compared to non-inoculated

231   control, respectively. The lowest activity of phosphatase enzymes was observed in non-inoculated

232   control that was not amended with organic matter. Among non-inoculated organic treatments, compost

233   showed higher activity of these enzymes than biochar, although biochar enhanced these enzymes'

234   activity compared to control. Also, its effect under AMF inoculation on the increased activity of the

235   enzymes was in the second rank of magnitude after compost (Figure 2a). Organic treatments increased

236   the activity of phosphatase enzymes in both rhizosphere and non-rhizosphere when compared to control

237   (Figure 2b). According to the results, when PWC was applied, the activity of ACP and ALP enzyme

238   was 1.06 and 1.04 times as great in the rhizosphere as in the non-rhizosphere region, and both regions

239   showed significant differences when they were treated with compost than treated with biochar and

240   control (Figure 2b). According to Figure 2b, it is observed that in all treatments, the activity of the ALP

241   enzyme is greater than that of ACP. In control treatments, too, compost was more effective than biochar

242   in prospering enzymatic activity. AMF inoculation increased the activity of phosphatase enzymes so

243   that the activity of ACP and ALP enzymes under inoculation conditions was 1.30 and 2.07 times as

244   great in the rhizosphere compared to non-rhizosphere region (Figure 2c).

245

a)

b)

c)
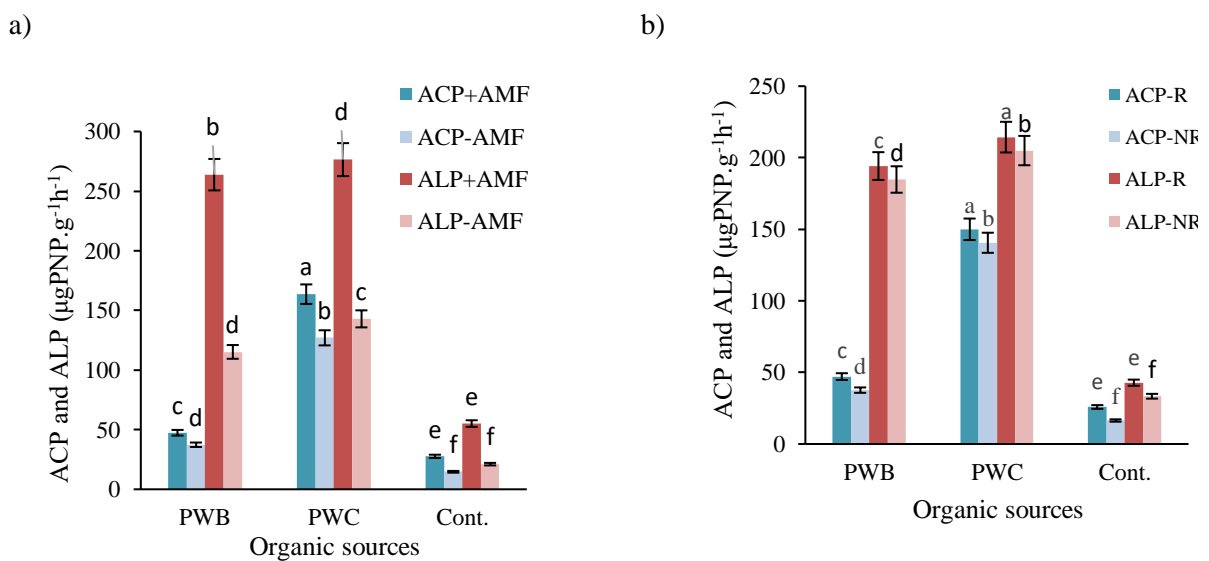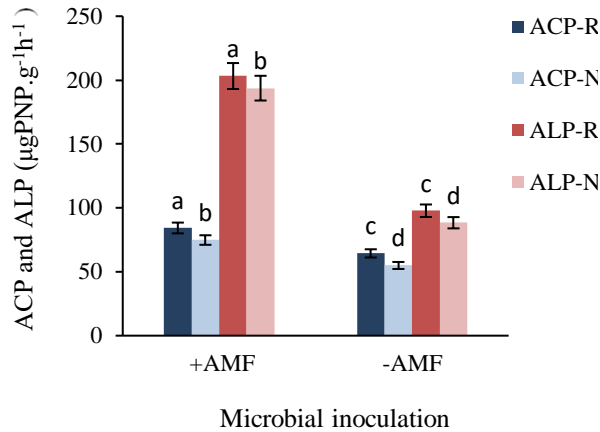
Figure 2. Means comparison of a) organic sources and microbial inoculation (+AMF, -AMF) on the soil ACP and ALP b) organic sources on the soil ACP and ALP, and c) microbial inoculation on the soil ACP and ALP. PWB, PWC, Cont., ACP, ALP, +AMF, -AMF, R and NR are pruning waste biochare, pruning waste compost, control (no organic mater), acid and alkaline phosphatase enzymes, inoculation with arbuscular mycorrhizal fungi, without mycorrhiza, rhizosphere and non-rhizosphere regions, respectively.

246

247 Finally, analysis of variance revealed that organic amendment and AMF significantly increased root

248 mycorrhizal colonization ($p < 0.001$) when compared to controls (Fig. 3). Colonization was highest

249 under the PWC and PWB treatments (51.45 and 41.24% higher than controls, respectively).

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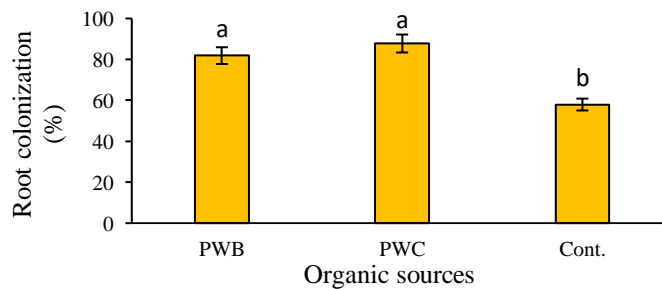
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252 **Figure 3.** The effect of the organic source and AMF on root colonization (%) PWB, PWC and Cont. denote
253 pruning waste biochare, pruning waste compost, and control (no organic mater), respectively.
254

4. Discussion

256 Biochar and compost amendment both increased the soil's organic C content, but when organic

257 amendment was combined with AMF inoculation, amendment with compost increased organic C more

258 than amendment with biochar. This may be due to the stable carbon skeleton of biochar, which is likely

259 to resist microbial decomposition and may thus enable a slow gradual release of organic C into the soil.

260   For example, there is evidence that the lifespan of biochar carbon in soil is between 100 and 1000 years,

261   which is 10-1000 times longer than that of soil organic carbon, making it a valuable long-term carbon

262   source [35]. The increase in MBC following organic amendment may be due to the introduction of

263   beneficial substrates for microorganisms that stimulate their activity and increase soil biological activity

264   [36]. Accordingly, Babalol et al. reported that applying plant residue compost to soil increased its

265   population of microorganisms, leading to an increase in MBP [37]. Amendment with wood biochar can

266   also increase soil microbial activity both by providing a favorable habitat for microorganisms and by

267   serving as a source of moisture, carbon, labile resources, and nutrients. Fig. 4 presents scanning electron

268   microscopy (SEM) images of the apple and grape biochars used in this work, revealing that they consist

269   of irregularly shaped porous particles. The porosity of biochar gives it a high specific area, enabling the

270   adsorption of large quantities of dissolved organic matter, gases, and minerals. Additionally, the

271   diameter of the pores in wood-derived biochars ranges from 2 to 80 µm; this size range is adequate to

272   support mycorrhizal fungi [38]. Consequently, biochar and provides a very favorable habitat for

273   microorganisms, especially AMF; its pores provide shelter against predators and protection from

274   drought while also hosting resources that help satisfy their carbon, energy, and nutrient requirements.

275   Consequently, biochar amendment increases the biological activity of mycorrhizal fungi [13]. Jin

276   reported that the interaction of biochar with AMF increased the population of mycorrhizal fungi and

277   the MBC content of rhizospheric soil to a greater degree than in non-rhizosphere soil [39]. The increase

278   in MBC was more pronounced among mycorrhizal fungi than bacteria, which was attributed to more

279   efficient use of resources by the fungi and more effective carbon assimilation due to the extensive

280   hyphal network of the fungi. Importantly, fungi can establish hyphal bridges between biochar and plant

281   roots.

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(a)   (b)
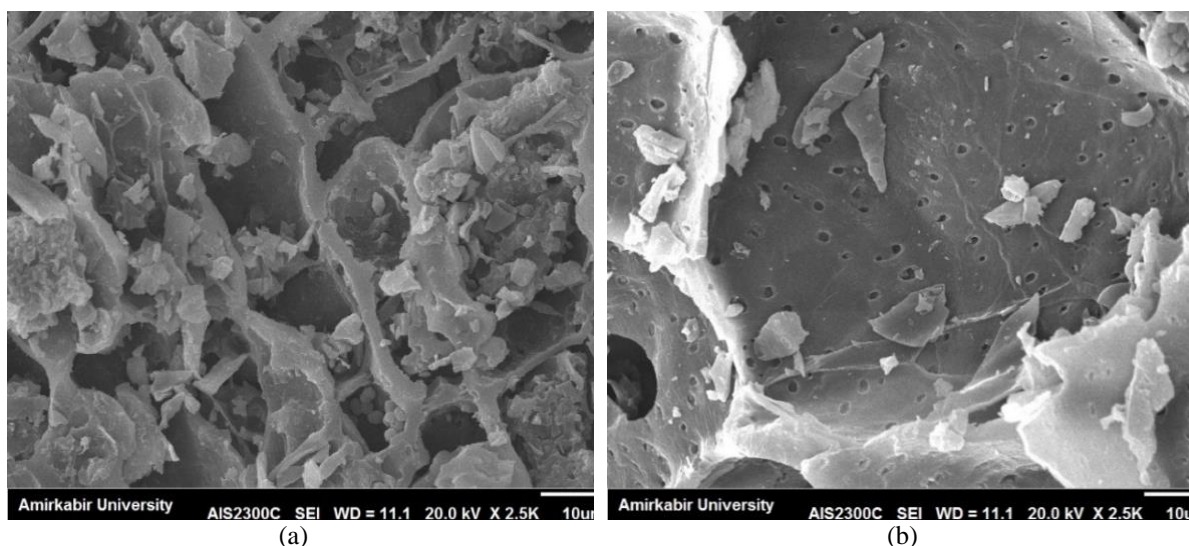
Figure 4. Scanning electron microscope (SEM) imagery of biochar of (a) apple pruning waste and (b) grape pruning waste at pyrolysis temperature of 350°C

Amendment with compost increases the organic C content of the soil as well as both plant growth and microbial activity. This normally leads to increased respiration (both BR and SIR). Compost amendment induced a greater increase in both rates of respiration than amendment with biochar, which may be related to its greater ease of decomposition. Obviously, the availability of adequate nutrients is a key determinant of the activity and growth of microorganisms. The effect of biochar on respiration differs from that of compost due to its structure and long half-life; Liu et al. found that biochars did not significantly influence soil respiration [11], whereas Steiner et al. observed increased microbial activity and growth following biochar amendment [40]. In this work, despite an increase in the microbial population following the addition of glucose to the biochar-treated soil, the rate of soil respiration (SIR) was unchanged. These results imply that the application of nutrient-rich but slowly decomposing biochars can support microbial population growth. Biochars also had some positive impacts on soil biological characteristics; the formation of biochars from wood at low pyrolysis temperatures leads to the retention of diverse compounds that promote microbial growth (e.g., sugars and aldehydes) on the surface of the biochar [41]. The short-term increase in microbial activity and population as well as soil respiration following biochar amendment is probably related to these labile constituents of freshly prepared biochars [42].

In general, the rhizosphere is the main zone in which organic compounds accumulate and decompose. It is thus unsurprising that it contains more organic matter than the non-rhizosphere. It

304 seems that the active carbon of compost is partially decomposed after the compost is applied and that

305 some of this carbon is added to the soil's carbon reserve, increasing the organic carbon content of the

306 soil. Biochar amendment also increased the soil's organic carbon content, but to a lesser degree than

307 treatment with compost. Zaman et al. reported that the application of urban waste compost increased

308 soil MBC by enhancing the soil's content of N, C, and soluble organic C [43]. This is consistent with

309 our finding that the soil's organic carbon content (Table 4) and total absorbed nitrogen (Table 2) were

310 higher after compost amendment than after treatment with other organic materials. Additionally, De

311 Neergaard and Magid found that the application of organic matter improved the MBC content in the

312 rhizosphere of rye to a greater degree than that of the non-rhizosphere zone [44], and a study on the

313 response of the soil microbial community to biochar derived from wheat and willow trees, Watzinger

314 et al. indicated that biochar application increased microbial biomass and changed the microbial

315 community structure [45]. MBP can be either a vital sink for soluble P when microbes compete with

316 plants for P or a crucial source of P that helps partially meet plants' P requirements. Soil microbes can

317 thus help maintain stable levels of labile P in soils with low P availability. Redel et al. incorporated

318 compost into soil and observed a positive association between MBP content and Olsen P (determined

319 by extraction with sodium bicarbonate) [46]. Similarly, in this work, compost amendment increased

320 available P in the soil more than any other treatment (Table 2). Biochar amendment has also been

321 reported to increase MBP, possibly due to its high C content and positive effect on nutrient availability.

322 Compost amendment strongly increased the microbial respiration rate because compost is rich in

323 oxygenated organic compounds such as carbohydrates that are more readily degraded by microbes than

324 aromatic carbon and alkyl carbon chains [47]. However, an earlier study on the effect of biochar on BR

325 under rhizobox conditions [48] found that biochar increased BR, leading to a significantly higher rate

326 of microbial respiration in the rhizosphere than in the non-rhizosphere zone.

327 Microorganisms can be regarded as active organic particles in the soil with charged and catalytically

328 active surfaces and the ability to produce and exude a wide range of organic compounds including

329 carbohydrates, and enzymes [49]. A soil's MBC content is widely interpreted as a measure of its content

330 of soil microbes and their activity. However, it does not distinguish between living and dead microbes;

331 it is likely that AMF cells are included in the soil microbial biomass when their life cycle ends. The

332   increase in MBC following AMF inoculation may thus be partly due to the addition of dead cells to the

333   rhizosphere soil, as suggested in earlier studies on the maize rhizosphere [50]. The beneficial effects of

334   the rhizosphere on the soil biological characteristics have also been highlighted by Zhao et al. [51]. In

335   addition to the increases in rhizospheric MBC due to organic matter amendment and microbial

336   inoculation, plants release organic compounds including sugars, amino acids, and vitamins into the

337   rhizosphere, further reinforcing its differences from the non-rhizosphere zone. These root exudations

338   also increase microbial biomass by making the rhizosphere more hospitable to microbes, which in turn

339   increases plant growth and the production of microbial metabolites [52]. Consequently, the rhizosphere

340   is characterized by elevated microbial activity and has a higher content of organic matter and nutrients

341   than the rest of the soil, allowing it to play a key role in meeting plants' nutrient requirements to support

342   photosynthesis while also supporting a diverse microbial community leading to high levels of soil

343   biological activity (and thus high levels of BR, SIR, MBC, and MBP) [53]. Organic matter application

344   can boost microbial activity to improve enzymatic activities via three ways: (i) the application of organic

345   matter to soil enhances soil microbial activity by microorganism-based energy pathway, resulting in

346   enzyme synthesis; (ii) the enzymes accompanying organic matter reduce energy for the activity of

347   microorganisms of soil for the synthesis of the enzymes, thereby increasing their activity and the rate

348   of organic matter decomposition in soil; (iii) organic matter is partially decomposed in soil and then, it

349   protects the enzymes against enzymatic hydrolysis by adsorbing and physically grasping the enzymes

350   [54]. The enhancement of the activity of soil phosphatase enzymes under the treatments with PWC and

351   PWB may be associated with the increased level of microbial biomass in response to the applied organic

352   matter, soil nutrients, and the improvement of soil (physical, chemical and biological) characteristics,

353   like nutrient and water holding capacity. Also, the higher performance of PWC in enhancing the activity

354   of phosphatase enzymes as compared to PWB is likely to reflect its higher rate of decomposition and

355   the availability of its N content, which stimulates microbial activity. The decline of ACP enzyme

356   activity versus ALP under the application of organic matter, especially biochar, was not surprising

357   because the studied soil, which had alkaline pH and calcareous conditions (Table 1), and the applied

358   biochar, which had alkaline pH (Table 2), were not suitable for the activity of this enzyme. Jin et al.

359   reported that the activity of ACP enzyme was decreased after the application of manure biochar,

360   whereas the activity of ALP enzyme was increased [55]. The loss of ACP enzyme activity was attributed

361   to the increase in soil pH by biochar application. Fungi need extra-cellular enzymes, such as

362   phosphatase, to decompose the substrate of their surrounding medium into smaller molecules and to

363   mobilize these molecules into the cells and also to use them in their metabolic activities. Thus, the

364   medium around a fungus is studied with the exudation of extra-cellular enzymes. Since extra-cellular

365   enzymes decompose promptly, therefore they should be adsorbed to soil particles to maintain their

366   activities. In this respect, specific areas are of particular importance. These areas can be the surface of

367   soil particulates, plant root, or organic matter like compost or biochar. The activity of extra-cellular

368   enzymes depends on the site of the enzyme that is interacting with the surface of biochar particles [56].

369   If the enzyme's active site is not covered and is operational and free to react with environment, its

370   activity will be augmented. Still, if it is blocked, its activity will decline [57]. Therefore, some groups

371   of enzymes become more active by the application of biochar and the other groups lose their activity

372   depending on molecular composition, folding properties, and their adsorption potential [58]. The

373   augmented activity of phosphatase in the rhizosphere versus non-rhizosphere can be associated with the

374   microbial and root activity of the plant. Similarly, in a study on the effect of organic fertilizer on the

375   activities of phosphatase enzymes in the rhizosphere of plants using rhizobox, Balik et al. reported that

376   the action of ACP and ALP enzymes were significantly increased in bulk soil [59].

377   The rate by which microorganisms can synthesize and release phosphatase varies with soil pH. As

378   pH is increased, ALP becomes more stable and active so that its activity is maximized at pH = 11. In

379   addition, ACP is mostly exuded by fungi and the optimal pH for it is acidic to neutral conditions. Still,

380   bacteria predominantly exude ALP and are more active at pH > [60]. Furthermore, ACP is produced

381   mainly by plants and microorganisms, but microorganisms predominantly exude ALP.

382   Organic matter amendment also improves the physical properties of the soil, making it better able

383   to support the development and activity of microorganisms and various bioprocesses [61]. The

384   improvements in soil biochemical properties and nutrient availability induced by PWC or PWB

385   amendment were reinforced by inoculation with AMF, and also enhanced root mycorrhizal

386   colonization. Similarly, Mäder et al. reported that the application of compost to soil increased

387   mycorrhizal colonization by 30-60% in the wheat rhizosphere [62]. Amendment with hardwood biochar

388   (2% w/w) similarly increased root colonization, which was attributed to increased growth of the external

389   hyphae of arbuscular mycorrhiza [17]. Biochar can influence the symbiosis of arbuscular mycorrhiza

390   and plant roots by (i) changing the availability of nutrients affecting the relationships between plants

391   and AMF and/or their physicochemical properties, (ii) stimulating microbial activity and population

392   growth by strengthening mycorrhizal symbiosis, (iii) disturbing chemical signals or detoxifying

393   chemicals that inhibit the activity of mycorrhiza fungi, and (iv) providing physical shelter for

394   mycorrhiza fungi against adverse conditions.

395

396 **5. Conclusion**

397

398   The results presented herein show that combining organic matter amendment with microbial

399   inoculation can have significant positive effects on soil quality parameters. Adding organic matter such

400   as compost or biochar to the soil and inoculation with AMF in the root zone strongly increased soil

401   biological activity, with particularly strong increases in soil quality parameters (BR, SIR, MBC, and

402   MBP) occurring in the rhizosphere. Combined compost amendment and AMF inoculation led to the

403   highest OC, MBC, and MBP values, but the effect of biochar amendment on root mycorrhizal

404   colonization was similar to that of adding compost. In general, amendment with compost had a stronger

405   positive effect on the studied soil biological parameters than treatment with biochar, which may be due

406   to the structure and greater stability of biochar. It should be noted that not all soil quality parameters

407   respond in the same way to microbial inoculation and organic matter amendment, and that the efficiency

408   of plant responses to these treatments will depend on the experimental conditions including the plant

409   type and species, the experiment type (pot or field study), the choice of organic matter, and the nutrient

410   ratios. Consequently, it will be necessary to confirm the results presented herein by studying the

411   combined effects of organic amendment and microbial inoculation in greenhouse and field studies on

412   various plant species and to assess the economic viability of organic amendment, especially with

413   biochar. Nevertheless, the results presented herein show that combining organic matter amendment with

414   AMF inoculation can strongly increase the quality, biological activity, and biodiversity of calcareous

415   alkaline and may represent a very promising alternative to the costly application of chemical fertilizers.

416

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427   **Data Availability Statement:** The data presented in this study are available on request from the

428   corresponding author.

429   **Conflicts of Interest:** The authors declare that they have no conflict of interest.

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