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1 **The older, the better: ageing improves the efficiency of biochar-compost**
2 **mixture to alleviate drought stress in plant and soil.**

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21

22

23

24 **Abstract**

25 Due to increased drought frequency following climate change, practices improving water use
26 efficiency and reducing water-stress are needed. The efficiency of organic amendments to
27 improve plant growth conditions under drought is poorly known. Our aim was to investigate if
28 organic amendments can attenuate plant water-stress due to their effect on the plant-soil system
29 and if this effect may increase upon ageing. To this end we determined plant and soil responses
30 to water shortage and organic amendments added to soil. We compared fresh biochar/compost
31 mixtures to similar amendments after ageing in soil.

32 Results indicated that amendment application induced few plant physiological responses under
33 water-stress. The reduction of leaf gas exchange under watershortage was alleviated when
34 plants were grown with biochar and compost amendments: stomatal conductance was least
35 reduced with aged mixture aged mixture (-79 % compared to -87% in control), similarly to
36 transpiration (-69 % in control and not affected with aged mixture). Belowground biomass
37 production (0.25 times) and nodules formation (6.5 times) were enhanced under water-stress
38 by amendment addition. This effect was improved when grown on soil containing the aged as
39 compared to fresh amendments. Plants grown with aged mixtures also showed reduced leaf
40 proline concentrations (two to five times) compared to fresh mixtures indicating stress
41 reduction. Soil enzyme activities were less affected by water-stress in soil with aged
42 amendments.

43 We conclude that the application of biochar-compost mixtures may be a solution to reduce the
44 effect of water-stress to plants. Our findings revealed that this beneficial effect is expected to
45 increase with aged mixtures, leading to a better water-stress resistance over time. However,
46 while being beneficial for plant growth under water-stress, the use of amendments may not be
47 suited to increase water use efficiency.

48

49 **Keywords:** Organic amendment, Biochar-compost mixtures, Ageing, Water stress, Water use
50 efficiency, Soil-plant functioning, Plant stress

51

52 **1. Introduction**

53

54 Water availability is affecting the activity of all living organisms. Under the current
55 climate change, the hydrological cycle will change dramatically leading to increased frequency
56 of extreme events such as flooding and drought (IPCC, 2021). Under the current climate
57 scenarios, water shortage is predicted to be one of the main threats to agricultural production
58 (FAO, 2013). Indeed, about two third of the worldwide cultivated area is affected by drought,
59 and this proportion is expected to increase due to global warming (Farooqi et al., 2020; Ullah
60 et al., 2021). In addition, drought events will be not only more frequent, but also longer and
61 more severe (Liu et al., 2018; Hari et al., 2020).

62 Drought may affect plant growth directly as well as indirectly. Under water deficit, plants
63 generally show reduced growth, water content and nutritious value (Kramer and Boyer, 1995;
64 Zia et al., 2021). This may be related to physiological drought effects, such as decreased
65 photosynthetic activity following stomata closure to prevent transpiration (Tani et al., 2019; Zia
66 et al., 2021), increased instantaneous water use efficiency (Wang et al., 2018; Zhang et al.,
67 2018), and reduced nutrient uptake. It may also be related to less available soil water inducing
68 a reduction in soil organic matter decomposition and nutrient availability (Xue et al., 2017).
69 Both phenomena have been demonstrated for various crops (Król et al., 2014; Mouradi et al.,
70 2016; Nadeem et al., 2017; Luo et al., 2019; Yang et al., 2020; Khan et al., 2021).

71 One possibility to overcome water deficits is irrigation. However, irrigation has not only an
72 additional cost for the farmers, but it also alters environmental quality by, for instance,
73 promoting soil salinization or increasing soil organic matter mineralisation and thus greenhouse

74 gas emissions (Maljanen et al., 2010; Liu et al., 2019). Moreover, water resources are far from
75 being available for irrigation everywhere, therefore, there is a need to find a more sustainable
76 way to overcome negative impacts of drought. A recent study indicated that the drought-related
77 yield gaps may be closed by increasing soil organic matter stocks (Iizumi and Wagai, 2019).
78 Thus, adaptation of agricultural systems to climate change and in particular extreme drought
79 may rely on organic matter management and fostering soil organic carbon (SOC) storage
80 (Kopittke et al., 2022). In intensive agricultural systems, organic amendments may be used for
81 this purpose because (1) they can retain water through physicochemical processes (Ajayi et al.,
82 2016; Głab et al., 2020) and (2) increase the soil's capacity to retain water by improving its
83 structure and porosity (Sisouvanh et al., 2021). In addition to the beneficial effects on soil water
84 content, organic amendments can improve soil fertility through increasing organic matter
85 content, enhancing nutrient contents and availabilities (Diacono and Montemurro, 2010).
86 Finally, many organic amendments are derived from waste materials and their use for soil
87 application is thus a promising option to recycle and divert them from the waste stream thus
88 contributing to the circular economy.

89 Among the possible organic amendments, biochar and compost have shown potential as soil
90 improvers, able to reduce drought stress (Kammann et al., 2011; Tanure et al., 2019;
91 Abdelrasheed et al., 2021; Ullah et al., 2021). Biochars are solid materials resulting from the
92 pyrolysis of biomass (Paz-Ferreiro et al., 2014). Their application to soil has been proven to
93 have agronomic interest mainly because of their beneficial effects on soil physical and chemical
94 properties (Cha et al., 2016). They are particularly interesting because of their high porous
95 structure and their high cation exchange capacity that allow them to bind and stabilize water
96 and various elements in soil, in addition to acting as microhabitat for microorganisms
97 (Agegnehu et al., 2017). Considering their very high stability and carbon content, biochars may
98 allow to contribute to soil carbon sequestration (Wang et al., 2016; Smith, 2016). In contrast,

99 composts contain a large amount of labile organic matter, which stimulates soil biota, influences
100 microbial community composition, promotes plant growth and, through its effect on microbial
101 activity, improves soil physical and chemical properties (Fischer and Glaser, 2012; Eden et al.,
102 2017). Some studies unraveled the potential mechanisms of compost and biochar to improve
103 the capacity of plants to sustain drought: (i) improvement of soil moisture (Wang et al., 2019)
104 through water binding on the surface (Jačka et al., 2018) and amelioration of soil structure
105 (Wang et al., 2019) allowing a better development and penetration of plant roots in soil
106 (Nadeem et al., 2017), and (ii) improvement of soil nutrient availability (Tanure et al., 2019),
107 especially nitrogen, which is essential for CO₂ assimilation and thus plant growth.

108 The combined use of both materials may induce synergistic effects on SOC storage and plant
109 growth (Agegnehu et al., 2016). For example, Nadeem et al. (2017), studying the use of biochar
110 and compost mixtures, showed that they allow in combination with rhizobacteria inoculation to
111 enhance cucumber growth under water-stress conditions better than biochar or compost alone.
112 Obadi et al. (2020) also showed that biochar and compost applied together can alleviate
113 negative effects of water shortage on pepper growth, yield and plant water use efficiency.

114 However, following field exposure, properties of organic amendments are changing due to
115 ageing processes. For instance, physical weathering of biochar compost mixtures may alter their
116 material properties, especially through labile compound leaching (Aubertin et al., 2021). The
117 altered mixtures may improve plant biomass production due to increased compost
118 mineralisation when mixed with biochar most probably due to greater ability of aged biochars
119 for nutrient adsorption (Chang et al., 2019). Amendment application and their ageing can affect
120 soil, plant and microorganism properties (Zhao et al., 2010; Sun et al., 2017; Paetsch et al.,
121 2018; Rahman et al., 2018). However, up to now, mechanistic studies on amendment effects on
122 the plant-soil continuum are scarce because plant and soil compartments are usually studied
123 separately. To the best of our knowledge, no studies have been carried out to investigate the

124 effect of aged biochar-compost mixtures on plant physiology and soil microbial functioning,
125 although both compartments may be crucial to understand the long-term benefits of this option.
126 In this study, we carried out a greenhouse experiment with alfalfa plants and a silt-loamy
127 temperate agricultural soil and investigated the effect of biochar-compost mixtures and their
128 natural ageing on the plant-soil system. We aimed to investigate (1) if organic amendment
129 application attenuates negative effects of water stress on plant development and microbial soil
130 functioning and (2) if this effect is changing upon their ageing during field exposure.

131 To this end, we monitored plant parameters, such as growth, aboveground and
132 belowground biomass, photosynthetic activities, plant stress indicators and their stem water
133 potential during water stress. To examine the contribution of soil processes and plant
134 physiology to water stress responses, we compared plant indicators and soil enzyme activities.
135 We hypothesised that biochar-compost mixtures affect plant physiological responses to water
136 stress because of their effect on soil water conditions and microbial functioning. We also
137 hypothesised that ageing of biochar-compost mixtures will modify plant responses under water
138 stress due to enhancement of their soil effects.

139

140 **2. Materials and Methods**

141 *2.1. Amendments and soil*

142 Biochar and compost materials originated from a compost platform of the association
143 ‘agriculteurs composteurs’ in France. At this platform, green waste compost (Fertivert, Seine-
144 Maritime, France) was sampled after green waste composting for six months (four months of
145 fermentation and two months of maturation). To take advantage of non-compostable residues
146 (composed of poplar and conifers branches), with the objective to re-use them to improve

147 compost amendments, they were transformed into biochar with a medium-scale pyrolizer
148 developed by ETIA (Oise, France), by pyrolysis at 450 °C for 10 min.

149 We sampled the first 5 cm depth of an agricultural soil with amendment mixtures in Beauvais
150 (Oise, France) at the UniLaSalle campus (GPS coordinates 49°25'49''N, 2°04'51''E). This soil
151 is part of a field experiment implemented in 2018, where the effects of long-term amendment
152 application are assessed under a temperate cropping system. The study site is representative of
153 the agricultural region of Northern France. This region is increasingly impacted by climate
154 change and particularly its effects on the water supply of crops. Among others, this includes:
155 drier springs and summers, milder winters but with an increased risk of late spring frosts, more
156 recurrent episodes of high heat and drought, a more random winter recharge of groundwater,
157 which is the source of the majority of irrigation water. In this region, annual precipitations are
158 669 mm.y⁻¹ on average and average temperatures range from 6.5 to 14.9 °C. The soil was
159 classified as a silt loam Haplic Luvisol, and its physico-chemical characteristics and those of
160 amendments are given in Table 1. Soil was sieved at 5 mm before use.

161

162 *2.2. Experimental design*

163 As the purpose of this study was to evaluate the effect of amendment ageing, we had three
164 treatments: (i) the non-amended soil sampled from our field experiment (control -C), (ii) the
165 same soil as the control amended with fresh biochar-compost mixture (F), composed of biochar
166 applied at 0.39 kg.m⁻² dry weight (DW) and compost applied at 0.78 kg.m⁻² (DW) respectively
167 (equivalent to 3.9 t.ha⁻¹ and 8.8 t.ha⁻¹, respectively), and (iii) the soil amended with the same
168 biochar-compost amendment dose (incorporated at 5 cm depth with a rotative harrow), which
169 had been subjected to field ageing for two years (A) and collected on the site at the same time
170 as the non-amended soil. The rate of application of biochar-compost mixtures was based on a
171 previous experiment (unpublished data) and was considered as realistic and economically

172 viable by local farmers. The unamended control and two-year amended soils had been subjected
173 to similar agricultural treatments. The F and A soils, showed similar physicochemical
174 properties, confirming that fresh and aged organic amendments were present in F and A soils
175 in similar amounts (Supplementary Materials, S1, Figure 1).

176 The soils were placed in 0.9 L pots at a bulk density of 1.3 g.cm^{-3} , with ten replicates per
177 treatment. After two weeks of acclimatisation at 70 % field capacity (FC), *i.e.* 25 g H₂O for 100
178 g dry soil, 20 seeds of *Medicago sativa L.* were sown. Nine days after sowing, the number of
179 plants was reduced to three.

180 Plants were grown for 64 days under greenhouse conditions (23 °C, natural light with the
181 deployment of a shadow if superior to 70 cd.sr.m⁻², Supplementary Materials, S1, Figure 2).

182 During the first 41 days, the pots were weighted daily at the end of the day and tap water was
183 added to maintain the treatments at 70 % FC. At the 42nd day and until the end of the experiment,
184 *i.e.* during three weeks (22 days), water stress was implemented in half of the pots, randomly
185 selected: the non-stressed pots were still watered at 70 % FC (5 replicates), whereas the stressed
186 pots were watered at 40 % FC *i.e.* 14 g H₂O for 100 g dry soil (5 replicates). The amount of
187 water corresponding to 70 % FC was based on the FC of the control treatment, so that all
188 treatments had the same volumetric water content throughout the experiment. Daily amount of
189 added water was used to calculate the cumulated water supply over the experimental period.

190

191 After nine weeks of growth, plants were harvested. Each plant was removed from the pots and
192 soil was gently separated from the roots. Soils were homogenized and an aliquot sample (half)
193 was air-dried, while plants were subjected to root exudate collection followed by separation of
194 shoots and roots. Fresh roots of each plant were photographed and image processed with ImageJ
195 1.52a (Wayne Rasband, National institutes of Health, USA) and Fiji (ImageJ 1.52i, Wayne
196 Rasband, National institutes of Health, USA) (supplementary materials, S1, Figure 3) in order

197 to analyse root branching (i.e. the number of image detected roots segments). Root nodules
198 were counted. An aliquot of fresh soil was used for enzyme analyses. After three days of drying
199 (50°C), dry mass of plant shoots and roots were recorded.

200

201 *2.3. Plant development*

202 In order to monitor plant growth, we took a photo twice a week at a fixed time during the whole
203 experiment, between 10 am – 11 am. Photos were taken at the same place on a table each time
204 in front of a white wall on which was attached a black disk of a known surface. The camera
205 was set on a first fixed distance and height from the pot until they grew too much to fit in the
206 frame. We then replaced it by a new distance until the end of monitoring without changing the
207 height. Three photos were taken per plant with a lateral rotation of 90° each in order to limit the
208 effect of the spatial variability of each plant. After image processing, the average plant surface
209 on these three photos was saved for each plant and expressed in cm². Image processing was
210 performed with the software ImageJ 1.52a (Wayne Rasband, National institutes of Health, USA)
211 by determining the plant surface comparing the number of plant pixels to the number of pixels
212 identified on the disk (Supplementary Materials, S2, Figure 4).

213

214 *2.4. Stem water potential*

215 A Schölander pressure chamber (PMS instrument, model 600) was used to measure the stem
216 water potential in the first and third week of water stress (Ritchie and Hinckley, 1975). In order
217 to allow the leaves to equilibrate with the stem, each leaf was sealed with aluminium foil to be
218 in the dark during 2h prior to measurement (del Pozo et al., 2017). Data were recorded at solar
219 noon by cutting the leaf from the plant, removing the leaf from the aluminium and placing it in
220 the chamber in which an increasing pressure was applied. From the moment bubbles were
221 observed out of the peduncles of the leave, sap pressure was recorded.

222

223 2.5. Gas exchange

224 In the first and last week of the water stress, leaf gas exchanges (net assimilation rate nAR
225 ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), stomatal conductance g_s ($\text{mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), transpiration rate E (mmol
226 $\text{H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and the resulting instantaneous water use efficiency WUE_i , ($\text{mmol CO}_2 \cdot\text{mol}^{-1}$
227 H_2O) were measured using a Li-6400 Portable Photosynthesis System (Li-Cor Biosciences Inc.,
228 Lincoln, NA, USA). Measurements were performed between 10.00 am and 1.00 pm local time,
229 on the 5th leaf from the top of the plant which is considered as fully developed leaf, on one plant
230 randomly selected in each pot. Inside the measured chamber, temperature was set to 21 °C, and
231 vapour pressure deficit was kept between 0.8 and 1.5 kPa, CO_2 concentration in the air (C_a)
232 was regulated at 400 ppm and photosynthetically active radiation (PAR) was $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.
233 ¹. The CO_2 concentration value set up to simulate the average CO_2 concentration in our
234 atmosphere. The PAR value was decided after realisation of light curves, before induction of
235 the stress, which showed maximum CO_2 assimilation at $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The measure was
236 taken after 20 s of equilibration. The measured leaf was photographed in order to measure leaf
237 area, using ImageJ 1.52a (Wayne Rasband, National institutes of Health, USA), and express
238 the parameter on a leaf area basis (supplementary materials, S3, Figure 5).

239

240 2.6. Stress indicators

241 To assess the degree of plant stress following water shortage, we determined the proline
242 content of plant leaves and the carboxylates exudation of plant roots. For proline measurements,
243 we sampled one representative plant leaf, in the first and last week of water shortage. It was
244 immediately frozen in liquid nitrogen and stored frozen until the extraction. Extraction was
245 done based on the method described in Bates et al. (1973). Briefly, 30-40 mg of frozen material

246 was crushed in liquid nitrogen and extracted with 3 % sulfosalicylic acid followed by the
247 addition of ninhydrin buffer and toluene to extract coloration. Then, absorbance at 520 nm was
248 measured using a NanoDrop (NP80, Implen, Germany). Proline concentration was calculated
249 using a seven-point proline standard curve, and expressed in mmoles per kilogram of fresh
250 weight material.

251 For carboxylates measurements, after whole plant sampling (see section 2.7), its roots
252 were immersed in a CaCl_2 solution at 0.2 mol.m^{-3} for an hour in order to collect plant root
253 exudates. Thereafter, the solution was frozen until it could be filtered and analysed using a
254 HPLC system (Thermo Fisher, San Jose, CA, USA) reverse phase Luna $5\mu\text{m}$ C18 column
255 $250\text{mm} \times 4 \text{ mm}$ (Phenomenex, 147 Le Pecq, France) as described by Yacoumas et al., (2020).
256 We quantified seven carboxylates, i.e. malate, malonate, lactate, acetate, citrate and fumarate
257 (supplementary materials, Figure 6) and calculated their sum as an indicator of total carboxylic
258 acid exudation.

259

260 *2.7. Soil analyses.*

261 Enzyme activities were measured on 0.004 kg of fresh soil. We determined β -glucosidase
262 (GLU), urease (URE), β -galactosidase (GAL) and arylamidase (ARN) following the methods
263 developed by Browman and Tabatabai (1978), Sinsabaugh et al. (2000) and Acosta-Martínez
264 and Tabatabai, (2000). Cellulase activity (CEL) was measured following the protocole of
265 Deshpande et al., (1984) and xylanase (XYL) with a method established by Taguchi et al.
266 (1996). Potential nitrification (NIT) was measured by ammonium oxidation according to the
267 ISO 15685:2012 certification. The measure of the nitrate reductase (NITRED) was carried out
268 with the method developed in Abdelmagid and Tabatabai (1987). The reaction occurs with
269 MUF substrates placed in a 96 wells micro-plates (PS, Nunc, VWR) and a microplate reader

270 (Génius 2 Lector, Safas). Enzyme activities are expressed in mU (nmol of release per
271 minutes).g⁻¹ of dry soil (DW).

272

273 *2.10. Statistics*

274 Normality of the datasets and the homogeneity of the variances were evaluated with the
275 Shapiro-Wilk test ($P > 0.05$) and Bartlett test ($P > 0.05$), respectively. If normality and
276 homoscedasticity tests were satisfied, an Analysis of Variance (ANOVA) as well as a Tukey
277 multiple comparison test (with a confidence interval of 5%) were performed. If normality was
278 not achieved, a non-parametric Kruskal-Wallis tests followed by Dunn multiple comparison
279 tests (with a confidence interval of 5%) were carried out. We used principal component analyses
280 (PCA) to analyse whether plant and soil parameters of the different treatments could be
281 distinguished according to their water status. Statistics were performed with RStudio version
282 1.4.1717.

283

284 **3. Results**

285 *3.1. Cumulative water supply*

286 During the first 41 days of the experiment, when all the pots were watered at 70 % FC,
287 cumulated water supply reached 2.04 L (+/- 0.13) for the control treatment without
288 amendments, 2.04 L (+/- 0.09) for the F treatment with fresh biochar-compost mixtures and
289 2.21 L (+/- 0.10) for the A treatment with altered biochar-compost mixtures. The pots of the A
290 treatment required 9 % more water than the control pots, while cumulated water supply was not
291 different between the control and F (supplementary materials, Figure 7).

292 During water stress, cumulated water supply was lower in the stressed condition than the non-
293 stressed pots (Fig. 1). In both cases, the highest cumulated water supply was observed in the A
294 treatment, which received 5.19 L (+/- 0.26) under non-stressed conditions and 3.40 L (+/- 0.17)
295 under water shortage, while the lowest water supply was measured in F condition, with 4.66 L
296 (+/- 0.26) and 2.86 L (+/- 0.09) in the non-stressed and stressed conditions, respectively.
297 Cumulated water supply was not different compared to the control both in the treatment
298 receiving fresh amendments and in the treatment receiving aged amendments (F and A), in non-
299 stressed and stressed conditions. However, cumulated water supply was higher in the A
300 treatment compared to the F treatment, by 11 % and 19 % in the non-stressed and stressed
301 conditions, respectively.

302

303 *3.2. Plant development*

304 Five days after seeding, plants grown on soil amended with aged compost-biochar mixtures
305 (A) showed a significantly higher germination rate than the two other treatments
306 (supplementary materials, Figure 8). Before water stress, plant surface linearly increased to
307 reach 258.5 (+/- 43.3) cm² for controls (C), 224.1 (+/- 31.0) cm² for fresh compost-biochar
308 mixtures (F) and 210.5 (+/- 71.8) cm² for aged compost-biochar mixtures (A) (Fig. 2a). Plants
309 of A and F treatments showed a higher plant surface area compared to the control (C) until
310 day 38 (non-significant differences). At 42 days of growth, *i.e.* the day before water stress
311 application, the control plants show a significant higher surface than F and A (Fig. 2a).

312 In treatments without water stress (NS), plant surface continued to increase until 52 days after
313 the start of the experiment, thereafter they stabilised around 404.3 (+/- 74.3) cm² for NS-C,
314 380.0 (+/- 104.6) cm² for NS-A and 340.2 (+/- 56.0) cm² for NS-F (Fig. 2b). NS-C treatments
315 continued to show a higher surface than those amended with mixtures until the end of the
316 experiment (63 days) and plants grown in NS-A treatments showed higher development than

317 those of F-NS treatments, even if the differences were not significant.

318 Under water stress, plant surface stopped growing from the first day of water stress and a
319 decrease was even observed between 52 and 58 days due to the fall of dead leaves. At the end
320 of the experiment the plants subjected to water stress (S) reached a surface of 215.4 (+/- 46.7)
321 cm² for S-C, 167.8 (+/- 27.8) cm² for S-F and 191.2 (+/- 28.8) cm² for S-A, with no significant
322 differences (Fig. 2b).

323 Shoot dry masses obtained at the end of the experiment ranged between 3.5 (+/-0.3) and 7.7
324 (+/-0.5) g and showed a trend following the order C > A > F for both stressed and non-
325 stressed treatments (Fig. 2c). Root dry masses ranged between 3.3 (+/- 0.5) and 5.0 (+/-0.4) g
326 following the order C > A > F in well-watered conditions and the order A > C > F in stressed
327 conditions (Fig. 2c). However, the differences among treatments were not significant. In
328 general shoot biomass was significantly higher than root biomass in non-stressed treatments,
329 (p-value < 0.001), while similar root and shoot biomass was recorded under water stress for
330 all treatment. As consequence shoot:root ratio was significantly decreased after water
331 shortage in all treatment (respectively -25, -36 and -43 % in C, F and A) but not significantly
332 different between treatments neither in well-watered or stress conditions.

333 Several root-related parameters showed significant changes under water stress compared to
334 well watered conditions (Fig. 3): (1) the number of root branching was significantly lower for
335 the C (-61%) and F (-57%) treatments but the difference was not significant in the case of A (-
336 30%), (2) the amount of branches was significantly higher for A than C and F treatments, (3)
337 nodosities were found in significantly lower quantity for C (-85%) and F (-88%) treatments
338 but not for A (-30%), and (4) the amount of nodules was significantly higher in A (49.4 +/-
339 22.8) than C (7.6 +/- 7.4) and F (9.0 +/- 10.2) treatments.

340

341 *3.3. Stem water potential measurements*

342 During the first week after the implementation of the water stress, significantly more negative
343 stem water potential was found for well watered than water stress conditions for the C, F and
344 A treatments with the latter not being significant (Fig. 4). Considering the same watering
345 conditions, no significant differences were noted among the different amendments (C, F and
346 A). After three weeks of water stress, the F treatment showed statistically significant lower
347 stem water potential than under well watered conditions.

348

349 *3.4. Leaf gas exchange*

350 During the first week of water stress, net assimilation rate (nAR) was significantly lower in
351 stressed than in non-stressed plants, except for the A treatment A (Fig. 5a). In the C treatment,
352 nAR was $20.5 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (± 5.9) and dropped by 58 % under water stress. Plants in the
353 F treatment had a nAR value of $19.2 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (± 8.1) under regular watering, and this
354 value was reduced by 66 % under stress. However, the nAR was not different between the
355 control, F and A treatments. After three weeks of stress, nAR was $17.3 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (\pm
356 3.0) in the non-stressed control and was significantly lower in the stressed control treatment
357 ($9.4 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ± 1.9). In the two amended treatments, there was contrasting decrease
358 in nAR values between the non-stressed and stressed plants, by 79 % in F and 35 % in A.
359 Moreover, in the non-stressed conditions, nAR values did not differ between the control, F and
360 A while under stress, nAR was significantly higher in A ($13.2 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ± 3.7)
361 compared to F ($4.7 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ± 2.6) treatments, while none of the amended conditions
362 differed from the control.

363 During the first week of stress, the leaf stomatal conductance (gs) of plants grown on the control
364 was $0.56 \text{ mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the non-stressed condition, and was reduced by 87 % when water
365 supply was reduced (Fig. 5b). Similarly, gs was reduced in the stressed compared to non-
366 stressed plant in the amended treatments F ($0.04 \text{ mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ± 0.01 compared to 0.52 mol

367 $\text{H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ +/- 0.21) and A ($0.12 \text{ mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ +/- 0.05 compared to $0.71 \text{ mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
368 +/- 0.21). The highest reduction in gs was measured in F (92 %), followed by the C (87 %) and
369 A (79 %) treatments. There was no amendment effect on gs in the first week, *i.e.* no difference
370 between the C, F and A regardless the water stress conditions. During the third week of stress,
371 when plants were watered at 70 % FC, gs was $0.56 \text{ mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (+/- 0.20) in the well-
372 watered C treatment, and reduced by 78 % under stress ($0.09 \text{ mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ +/- 0.03). A
373 significant reduction in gs between non-stressed and stressed plants was also observed for the
374 two other treatments F and A. This reduction was more important in the case of F (89 %
375 reduction) than A (only 65 % reduction). Similarly to what was measured during the first week,
376 no amendment effect on gs value was found.

377 Transpiration rate (E) did not differ among the treatments in the non-stressed and stressed
378 plants, respectively, in the first week of stress (Fig. 5c), which all showed significantly reduced
379 E under water stress. More precisely, E was $4.34 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (+/- 1.03) in non-stressed
380 control and reduced to $0.87 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (+/- 0.41) under stress. In F treatment, the E value
381 was $4.16 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (+/- 1.80) in non-stressed plants and dropped to $0.58 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
382 (+/- 0.19) under stress, while in A treatment, E was $5.07 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (+/- 1.05) and
383 $1.31 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (+/- 0.46) in non-stressed and stressed plants, respectively. The highest
384 reduction of E under stress was measured in plants of the F treatment (85 %) followed by those
385 of C (80 %) and A (74 %) treatments. After three weeks of water stress, E was significantly
386 reduced in the C and F treatments by 69 % and 87 %, whereas E of plants of A treatments was
387 unaffected.

388 As a result, after a week of water shortage, instantaneous water use efficiency (WUE_i) was
389 increased under water stress in the C (from 4.7 to 10.0 %), F (from 4.6 to 11.1 %) and A (from
390 3.9 to 7.4 %) treatments but only significantly for C and F. After the third week of water

391 shortage, WUEi was still increased under water stress in the C (from 5.8 to 9.7 %), F (from 5.3
392 to 8.1 %) and A (from 4.9 to 7.0 %) treatments significantly only for C.

393 Finally, internal CO₂ concentrations (Ci) were not affected by amendment application in the
394 first and third weeks of stress (supplementary material, Figure 9). However, Ci was reduced
395 under water shortage. Specifically, in the first week of water stress, Ci values were 308 (+/- 21)
396 μmol CO₂.mol⁻¹, 313 (+/- 10) μmol CO₂.mol⁻¹ and 327 (+/- 10) μmol CO₂.mol⁻¹ in plants grown
397 of C, F and A treatments, respectively, which was reduced by 40%, 52% and 24% when water
398 stress was applied. In the third week under water stress, Ci was 294 (+/- 27) μmol CO₂.mol⁻¹
399 for non-stressed control and 199 (+/- 46) μmol CO₂.mol⁻¹ for the stressed control, corresponding
400 to a 32% reduction in Ci, whereas for F and A treatments, Ci was 291 (+/- 16) μmol CO₂.mol⁻¹
401 ¹ and 306 (+/- 14) μmol CO₂.mol⁻¹ in non-stressed condition, and was unaffected by water
402 limitation.

403

404 *3.5. Stress indicators*

405 *3.5.1 Proline*

406 Two days after the induction of water stress, in well watered C treatments, leaf proline
407 concentration was 0.7 (+/- 0.04) mmol.kg⁻¹ fresh weight (FW) (Fig. 6). In the A treatment, a
408 reduction of leaf proline concentration down to 0.3 (+/- 0.02) mmol.kg⁻¹ FW was noted. When
409 plants were subjected to water stress, leaf proline concentration of C and F treatments showed
410 a strong proline increase ranging between 3.8-fold and 3.9-fold, while it was not affected in the
411 A treatment. In the third week after stress application, plants from all treatments grown in the
412 well-watered conditions showed a similar leaf proline concentrations of 0.6 (+/- 0.07) mmol.kg⁻¹
413 ¹ FW. Higher values were recorded under water stress conditions for all treatments, ranging
414 between 1.2 (+/- 0.08) mmol.kg⁻¹ FW and 2.0 (+/- 0.09) mmol.kg⁻¹ FW. Compared to values

415 found in plants of C treatments, lower proline concentrations were measured for plants grown
416 on the two amended substrates (F and A treatments) (Fig. 6).

417

418 *3.5.2. Root exudation*

419 Total root exudation ranged between 2.4 and 8.7 mmol.kg⁻¹ of dry roots and was not
420 significantly affected by organic amendment addition or water stress treatment (Fig. 7a).
421 Exudate amounts tended to be higher under water shortage as compared to well-watered
422 treatments. We observed significant increases for malate, which was almost twice as high when
423 released from plant roots grown of the F treatment under water stress as compared to the well-
424 watered plants (Fig. 7b), while the other two treatments showed no effect (supplementary
425 materials Figure 6).

426

427 *3.6. Enzymes activities*

428 Water limitation induced a change in soil enzyme activities (Fig. 8). Highest enzyme activities
429 were recorded in C treatments with significant differences between water stressed and well
430 watered conditions (β -glucosidase, arylamidase, cellulase and β -galactosidase, all significantly
431 lower under water stress). On the other hand, only one enzyme showed significant differences
432 between well-watered and stressed conditions for F treatments: nitrate reductase which was
433 significantly higher under water stress, and for A treatments: cellulase, which was significantly
434 lower under water stress. Separately most soil enzymes activities were reduced under water
435 stress, but if considering the sum of all enzymes (mU.g⁻¹ dry soil (DW)) water shortage
436 increased soil enzyme activities mainly because of the significantly higher nitrate reductase
437 activity. In soils without water shortage, the two amended treatments F and A showed
438 significant differences in arylamidase, β -galactosidase, potential nitrification and xylanase

439 activities, which were all significantly higher in A than F treatments. In well-watered
440 conditions, no clear difference with the control treatments occurred except for two enzymes:
441 potential nitrification and arylamidase, which showed significantly lower values compared to
442 the control for F (32 % lower) and higher values for A treatment (14 % higher)

443

444 *3.7. Combining plant and soils parameters to analyse responses to water stress*

445 Principal component analyses (PCA) were carried out to investigate whether plant and soil
446 parameters showed contrasting responses to water stress and if this response is influenced by
447 amendment application.

448 When all soil enzyme activities were analysed together, the first two components of the PCA
449 analysis explained 76% of data variability (Fig. 9). In the PCA plan showing the results for well
450 watered conditions, the amended treatments A and F were not clearly separated from the control
451 treatment C, while A was separated from F along the first axis. Under water stress, the first two
452 components explained 71% of the data variability., All treatments were clearly separated in the
453 PCA plan. Treatment A was distinguished along the first axis from the other two treatments
454 indicating that this treatment showed an overall higher soil enzyme activity than the other two.

455

456 PCA analysis of all plant parameters (Fig. 10), indicated that the first two components explained
457 between 47 and 52% of the data variability for well-watered and water stress conditions. In the
458 PCA plan for well watered conditions, the three treatments were separated along the first axis
459 correlated to nodule number, net assimilation rate and proline content at the end of the
460 experiment. These parameters may be increased following mixture addition. In stressed
461 conditions the A treatment was separated from the two others along the first axes correlated to
462 plant biomass, water supply and assimilation. Moreover, treatments were differentiated

463 according to proline concentration and nodulation, being opposed in the correlation plot for
464 water stress conditions.

465

466 **4. Discussion**

467 *4.1 Plant and soil response to compost-biochar mixture application*

468 Amendment application improved germination rate and early biomass production of Alfalfa,
469 but did not show any effect on shoot and root biomass at the end of the experiment, although
470 we observed more nodules in F and A treatments. Moreover, we observed a decreased proline
471 content in amended soils. These results were similar to those of other authors, who showed that
472 biochar and compost might increase germination rate and root penetration by influencing soil
473 physical properties, in particular by reducing soil bulk density (Nada et al., 2012; Kranz et al.,
474 2020). Biochar-compost mixtures were sometimes found to increase plant biomass and yield
475 (Doan et al., 2015; Agegnehu et al., 2016; Naeem et al., 2018), but this effect is most probably
476 limited to tropical soils. Absence of plant growth effects in our treatment may be explained by
477 the low amendment application rate and by the nutrient-rich temperate soil, which often does
478 not show plant growth effects after soil amendment (Seehausen et al., 2017; Jien, 2019;
479 Aubertin et al., 2021). In addition, most of studies applied higher amendment rates, which could
480 also explain the lack of effect of the compost-biochar mixture on plant biomass in our study.
481 On the contrary, biochar-compost mixtures increased the number of nodules on plant roots,
482 which was consistent with previous studies (Thies and Rillig, 2009; Agegnehu et al., 2015;
483 Hashem et al., 2019; Phares et al., 2020). These findings reflected the overall good plant activity
484 as nodule formation highly depends on carbohydrate and photosynthetic activity (Aranjuelo et
485 al., 2014). Indeed, decreased plant proline contents demonstrate that the amendment mixtures
486 reduced plant stress (Farooqi et al., 2020).

487 Amendment addition resulted in a rise in carbon and nitrogen contents in the amended soil, as
488 observed in previous studies (Agegnehu et al., 2016; Mierzwa-Hersztek, 2016; Azeem et al.,
489 2020). This could lead to changes of the arylamidase synthesis, which catalyses the release of
490 amino acids from SOM (Acosta-Martínez and Tabatabai, 2000, 2001). Potential nitrification,
491 considered as the maximum capacity of soil microorganisms to transform NH_4^+ into NO_3^- , was
492 sometimes reduced by the addition of biochar-compost mixture, which could be due to the NH_4^+
493 sorption capacity of biochar (Abujabhah et al., 2016). However, most enzyme activities were
494 positively affected by amendment application.

495

496 *4.2 Effect of water stress on plant growth and physiological parameters*

497 The monitoring of plants during the water shortage period showed that a reduction of water
498 supply reduced plant growth and biomass production, impaired plant physiological activity
499 (reduction of leaf gas exchange and water potential) and increased oxidative stress inside the
500 plant (increase of proline content in the leaves).

501 We observed reduced plant growth and biomass production under water stress, which had
502 already been demonstrated previously (Mouradi et al., 2018). Similarly, water stress reduced
503 the stem water potential, which results from a higher transpiration associated to lower water
504 uptakes, leading to higher tension in the xylem. This was particularly observed in the first days
505 following the induction of water stress. With time, leaf water potential was less negative, which
506 can be related to the reduction in the transpiration. Moreover, a more negative leaf water
507 potential under water stress demonstrates that plants needed more strength to extract additional
508 water from the soil (Bell et al., 2007). Finally, as often observed under water shortage
509 conditions, proline content of leaves and malate exudation of roots increased with water
510 shortage (Sicher et al., 2012). Malate being known to act as a signal for microbial attraction

511 under various stress conditions (Ortíz-Castro et al., 2009; Blouin, 2018), might have in turn
512 increased some of the soil enzyme activities (Sanaullah et al., 2011).

513 These effects of water shortage may be explained by a reduction of photosynthetic activity
514 resulting from a reduced leaf gas exchange indicated by lower transpiration rates (assimilation,
515 stomatal conductance, and transpiration) (Ahmed et al., 2018; Tani et al., 2019; Baccari et al.,
516 2020), stomata closure to prevent water loss (Tani et al., 2019; Baccari et al., 2020; Zia et al.,
517 2021) and increased WUE_i. Moreover, reduction of photosynthesis can also be attributed to a
518 reduction of carboxylation efficiency, since internal C_i concentration decreased concomitantly
519 with assimilation (supplementary materials, Figure 9) (Xu et al., 2020). Also, the increased
520 WUE_i under water deficit is commonly found (Naidoo and Naidoo, 2018; Guo et al., 2021),
521 and is an adaptive response to water stress, in order to improve resource use efficiency under
522 stress (Naidoo and Naidoo, 2018). More precisely, plants can produce more assimilates while
523 consuming lower amounts of water (Swann, 2018). The decrease of nodules can be related to
524 lower photosynthesis activity and thus carbohydrate production by the plant (Aranjuelo et al.,
525 2014). In accordance with a reduction of their physiological functions, leaf proline content was
526 found to be increased, (Liu et al., 2015; Luo et al., 2019; Mouradi et al., 2018; Kiani et al.,
527 2020). Such accumulation of proline helps prevent cell damage, stabilizes molecules, maintains
528 osmotic potential and stores carbon and nitrogen for a potential use after stress removal
529 (Szabados and Saviouré, 2010; Ben Rejeb et al., 2014; Luo et al., 2019; Kiani et al., 2020; Khan
530 et al., 2021). In the rhizosphere, overall reduction of microbial enzyme activities under water
531 stress was already observed in previous studies (Sanaullah et al., 2011; Zaheer et al., 2021).

532

533 Decreasing the water supply increased plant stress expression, resulting in a decrease of the
534 plant activity toward an improvement of their water use efficiency. In consequence, shoot and
535 root development was stopped. Soil enzyme activities were also affected by water shortage

536 most probably due to stress induced modification of exudate release by plants, indicating the
537 importance to study both plant and soil functioning simultaneously.

538

539 *4.3. Can water shortage effects be alleviated by soil amendment with biochar compost*
540 *mixtures?*

541 Few significant amendment effects were noted for plant physiological parameters. However,
542 amendment addition affected the overall plant response to (water) stress as shown by PCA
543 analysis (Fig. 10). Indeed, plant stress seems to be reduced by amendment addition as indicated
544 by the significantly lower proline contents in plant leaves of amended treatments. In addition,
545 even if the differences between well-watered and stressed plants were not significant, organic
546 acid exudation increased more between well-watered and stressed plants under fresh mixture
547 application mainly because of malate production which is involved, among others, in plant
548 nutrition and N₂ fixation through nodule activity (Irigoyen et al., 1992; Schulze et al., 2002;
549 Aranjuelo et al., 2014). This could have participated to the improvement of plant resistance to
550 water stress and is in accordance to the lower level of proline found in treatments with fresh
551 mixture addition.

552 Lower proline content after biochar or compost amendment was already observed in previous
553 studies (Kammann et al., 2011; Abdelrasheed et al., 2021; Hafez et al., 2021; Khan et al., 2021).
554 These results could be explained by a higher nutrient content and availability in amended soils
555 (Din et al., 2018.; Hafez et al., 2021). Our data showed that these improvements affected plant
556 physiology without altering plant growth.

557 In water-stressed soil, PCA analysis showed that enzymes activity differed between the
558 control and F treatments (Fig. 9). The use of fresh mixture (F) allowed alleviating the negative
559 effect of water shortage on β -glucosidase, arylamidase, cellulase and β -galactosidase enzyme
560 activities and increased nitrate reductase activity, an enzymatic complex able to catalyse the

561 nitrate reduction during denitrification.

562

563 *4.4. Does field ageing alter amendment effects on plant responses to water stress?*

564 Compared to fresh mixtures, the aged biochar-compost mixture increased root branch
565 numbers and nodule formation under water stress. In addition, the internal CO₂ concentration
566 and transpiration rates were maintained with aged amendments despite the negative impacts
567 of water shortage on these parameters. Leaf proline content also increased less during water
568 stress with aged mixtures. In agreement with the maintenance of the assimilation rate in
569 treatment with aged amendments, this might indicate an improvement of the plants' water
570 stress resistance.

571 Mixture ageing may also have a positive effect on soil functioning under water stress, as
572 indicated by increased enzyme activities. Under well-watered conditions, the nitrification
573 potential activity decreased by the application of the fresh biochar-compost mixture, but the
574 activity was recovered to its initial level, with the ageing of the mixture. Under water shortage
575 conditions the β -galactosidase decreased in F and C treatments, and increased only in A
576 treatments with the aged mixtures. PCA analysis of soil enzyme activities under water shortage
577 (Fig. 9) illustrated that, soil enzyme activity is favoured when soil is amended with aged
578 mixtures. Plants favoured root development over shoot development under water stress (Fig.
579 10), to prevent water loss through transpiration and allowing to expand the volume of soil where
580 they can reach water and nutrients. Thereby plants of A treatments were able to increase soil
581 water consumption together with photosynthetic activity leading to a decrease of their WUE_i.
582 This shows that water shortage had a lower impact on plant functioning in A treatments. WUE_i
583 has been shown to be related to root development as observed in our study (Li et al., 2011).
584
585 Effects of amendments ageing might be explained by alterations of their material properties.

586 Indeed, with maturation in soil, the surface of biochar may become more hydrophilic, their
587 specific surface area may decrease, its CEC may increase, and the minerals on the biochar
588 surface may dissolve, and provide nutrients for microorganisms and plants (Mia et al., 2017;
589 Wang et al., 2020; Liu and Chen, 2022), while compost when applied alone mineralizes once
590 in the soil, leading to the release and leaching of nutrients (Aubertin et al., 2021). During co-
591 composting, biochar ageing can be accelerated when brought with fresh organic matter in result
592 of an enhanced microbial activity induce by the microhabitat provided by biochar, the co-
593 mineralisation of biochar and biochar surface adsorbed organic matter (Mia et al., 2017). In
594 their review, Gorovtsov et al. (2020) stated that around biochar particles, microorganism's
595 mycelia could favour the formation of aggregates; which could further enhance microbial
596 activities, especially those related to water, nutrient and organic matter cycling (Agegnehu et
597 al., 2017; Gorovtsov et al., 2020). The development of such properties is occurring after
598 prolonged field exposure (Mukherjee et al., 2014) and one year of soil ageing of biochar was
599 found to lead to preservation of microbial activities under water stress (Paetsch et al., 2018). In
600 the same line, our results indicate that ageing of organic amendments containing biochar is
601 beneficial not only for maintaining soil microbial activity but also to ameliorate plant
602 functioning under water shortage.

603 Finally, our experiment highlighted that if plant resistance to water limitation was improved by
604 amendment ageing in soil, WUE_i of plants decreased and their water consumption was not
605 reduced. We can thus consider that aged mixtures allowed improved plant functioning under
606 low water contents, without reducing their water requirements. To investigate the use of organic
607 amendments to reduce water requirements and plant survival under water stress, no water
608 additions should have been made. However, soil water content was maintained at a low but
609 stable level during the duration of the experiment. We suggest that that to investigate the effects
610 of organic amendments, future experiments should consider two different types of water stress

611 experiments: (1) maintenance of a low soil water content throughout the experiment or (2) a
612 finite pool of applied water in soil. This could allow to obtain a more complete understanding
613 of the effects of organic amendments on plant and soil functioning.

614

615 **Conclusion**

616 Drought event frequency is forecasted to increase in the near future, with potential negative
617 impacts on plant growth, soil microorganisms, and soil microbial activity. In this study, we
618 investigated whether water shortage effects might be alleviated by soil amendment with fresh
619 and aged biochar-compost mixtures. We conclude that application of mixtures of biochar and
620 compost have few significant effects on plant physiological parameters, while they seem to alter
621 the response pattern of soil microbial enzyme activities and also plant response to water
622 shortage. Amendment presence could reduce the stress imposed to plants and microorganisms
623 by the reduction of water supply, especially after their ageing in soil. These effects might be
624 explained by a combination of their influence on water, nutrient and carbon cycling. Our results
625 suggest that the use of a biochar-compost mixture reduces water stress in plants especially after
626 their aging in soil. However, organic amendments may not be suitable to increase plant water
627 use efficiency.

628

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- 635 Abdelmagid, H.M., Tabatabai, M.A., 1987. Nitrate reductase activity of soils. *Soil Biol. Biochem.* 19,
636 421–427. [https://doi.org/10.1016/0038-0717\(87\)90033-2](https://doi.org/10.1016/0038-0717(87)90033-2)
- 637 Abdelrasheed, K.G., Mazrou, Y., Omara, A.E.-D., Osman, H.S., Nehela, Y., Hafez, E.M., Rady, A.M.S., El-
638 Moneim, D.A., Alowaiesh, B.F., Gowayed, S.M., 2021. Soil Amendment Using Biochar and
639 Application of K-Humate Enhance the Growth, Productivity, and Nutritional Value of Onion
640 (*Allium cepa* L.) under Deficit Irrigation Conditions. *Plants* 10, 2598.
641 <https://doi.org/10.3390/plants10122598>
- 642 Abujabhah, I.S., Doyle, R., Bound, S.A., Bowman, J.P., 2016. The effect of biochar loading rates on soil
643 fertility, soil biomass, potential nitrification, and soil community metabolic profiles in three
644 different soils. *J. Soils Sediments* 16, 2211–2222. <https://doi.org/10.1007/s11368-016-1411-8>
- 645 Acosta-Martínez, V., Tabatabai, M.A., 2001. Tillage and residue management effects on arylamidase
646 activity in soils. *Biol. Fertil. Soils* 34, 21–24. <https://doi.org/10.1007/s003740100349>
- 647 Acosta-Martínez, V., Tabatabai, M.A., 2000. Enzyme activities in a limed agricultural soil. *Biol. Fertil.*
648 *Soils* 31, 85–91. <https://doi.org/10.1007/s003740050628>
- 649 Agegnehu, G., Bass, A.M., Nelson, P.N., Bird, M.I., 2016a. Benefits of biochar, compost and biochar-
650 compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural
651 soil. *Sci. Total Environ.* 543, 295–306. <https://doi.org/10.1016/j.scitotenv.2015.11.054>
- 652 Agegnehu, G., Bass, A.M., Nelson, P.N., Bird, M.I., 2016b. Benefits of biochar, compost and biochar-
653 compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural
654 soil. *Sci. Total Environ.* 543, 295–306. <https://doi.org/10.1016/j.scitotenv.2015.11.054>
- 655 Agegnehu, G., Bass, A.M., Nelson, P.N., Muirhead, B., Wright, G., Bird, M.I., 2015. Biochar and
656 biochar-compost as soil amendments: Effects on peanut yield, soil properties and
657 greenhouse gas emissions in tropical North Queensland, Australia. *Agric. Ecosyst. Environ.*
658 213, 72–85. <https://doi.org/10.1016/j.agee.2015.07.027>
- 659 Agegnehu, G., Srivastava, A.K., Bird, M.I., 2017. The role of biochar and biochar-compost in improving
660 soil quality and crop performance: A review. *Appl. Soil Ecol.* 119, 156–170.
661 <https://doi.org/10.1016/j.apsoil.2017.06.008>
- 662 Ahmed, F., Arthur, E., Plauborg, F., Razzaghi, F., Kørup, K., Andersen, M.N., 2018. Biochar amendment
663 of fluvio-glacial temperate sandy subsoil: Effects on maize water uptake, growth and
664 physiology. *J. Agron. Crop Sci.* 204, 123–136. <https://doi.org/10.1111/jac.12252>
- 665 Ajayi, A.E., Holthusen, D., Horn, R., 2016. Changes in microstructural behaviour and hydraulic
666 functions of biochar amended soils. *Soil Tillage Res.* 155, 166–175.
667 <https://doi.org/10.1016/j.still.2015.08.007>
- 668 Aranjuelo, I., Arrese-Igor, C., Molero, G., 2014. Nodule performance within a changing environmental
669 context. *J. Plant Physiol.* 171, 1076–1090. <https://doi.org/10.1016/j.jplph.2014.04.002>
- 670 Aubertin, M.-L., Girardin, C., Houot, S., Nobile, C., Houben, D., Bena, S., Brech, Y.L., Rumpel, C.,
671 2021a. Biochar-Compost Interactions as Affected by Weathering: Effects on Biological
672 Stability and Plant Growth. *Agronomy* 11, 336. <https://doi.org/10.3390/agronomy11020336>
- 673 Aubertin, M.-L., Girardin, C., Houot, S., Nobile, C., Houben, D., Bena, S., Brech, Y.L., Rumpel, C.,
674 2021b. Biochar-Compost Interactions as Affected by Weathering: Effects on Biological
675 Stability and Plant Growth. *Agronomy* 11, 336. <https://doi.org/10.3390/agronomy11020336>
- 676 Azeem, M., Hale, L., Montgomery, J., Crowley, D., McGiffen, M.E., 2020. Biochar and compost effects
677 on soil microbial communities and nitrogen induced respiration in turfgrass soils. *PLOS ONE*
678 15, e0242209. <https://doi.org/10.1371/journal.pone.0242209>
- 679 Baccari, S., Elloumi, O., Chaari-Rkhis, A., Fenollosa, E., Morales, M., Drira, N., Ben Abdallah, F., Fki, L.,
680 Munné-Bosch, S., 2020. Linking Leaf Water Potential, Photosynthesis and Chlorophyll Loss
681 With Mechanisms of Photo- and Antioxidant Protection in Juvenile Olive Trees Subjected to
682 Severe Drought. *Front. Plant Sci.* 11, 614144. <https://doi.org/10.3389/fpls.2020.614144>

683 Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress
684 studies. *Plant Soil* 39, 205–207. <https://doi.org/10.1007/BF00018060>

685 Bell, L.W., Williams, A.H., Ryan, M.H., Ewing, M.A., 2007. Water relations and adaptations to
686 increasing water deficit in three perennial legumes, *Medicago sativa*, *Dorycnium hirsutum*
687 and *Dorycnium rectum*. *Plant Soil* 290, 231–243. <https://doi.org/10.1007/s11104-006-9155-7>

688 Ben Rejeb, K., Abdelly, C., Saviouré, A., 2014. How reactive oxygen species and proline face stress
689 together. *Plant Physiol. Biochem.* 80, 278–284. <https://doi.org/10.1016/j.plaphy.2014.04.007>

690 Blouin, M., 2018. Chemical communication: An evidence for co-evolution between plants and soil
691 organisms. *Appl. Soil Ecol.* 123, 409–415. <https://doi.org/10.1016/j.apsoil.2017.10.028>

692 Browman, M.G., Tabatabai, M.A., 1978. Phosphodiesterase Activity of Soils. *Soil Sci. Soc. Am. J.* 42,
693 284–290. <https://doi.org/10.2136/sssaj1978.03615995004200020016x>

694 Cha, J.S., Park, S.H., Jung, S.-C., Ryu, C., Jeon, J.-K., Shin, M.-C., Park, Y.-K., 2016. Production and
695 utilization of biochar: A review. *J. Ind. Eng. Chem.* 40, 1–15.
696 <https://doi.org/10.1016/j.jiec.2016.06.002>

697 Chang, R., Sohi, S.P., Jing, F., Liu, Y., Chen, J., 2019. A comparative study on biochar properties and Cd
698 adsorption behavior under effects of ageing processes of leaching, acidification and
699 oxidation. *Environ. Pollut.* 254, 113123. <https://doi.org/10.1016/j.envpol.2019.113123>

700 del Pozo, A., Ovalle, C., Espinoza, S., Barahona, V., Gerding, M., Humphries, A., 2017. Water relations
701 and use-efficiency, plant survival and productivity of nine alfalfa (*Medicago sativa* L.)
702 cultivars in dryland Mediterranean conditions. *Eur. J. Agron.* 84, 16–22.
703 <https://doi.org/10.1016/j.eja.2016.12.002>

704 Deshpande, M.V., Eriksson, K.-E., Pettersson, L.G., 1984. An Assay for Selective Determination of Exo-
705 1,4,-beta-glucanases in a Mixture of Cellulolytic Enzymes 138, 481–487.

706 Diacono, M., Montemurro, F., 2010. Long-term effects of organic amendments on soil fertility. A
707 review. *Agron. Sustain. Dev.* 30, 401–422. <https://doi.org/10.1051/agro/2009040>

708 Din, S.N.M., Sakimin, S.Z., Sijam, K., Ramlan, F., Zakaria, A.T., Manickam, T., Tan, J., 2018. EFFECT OF
709 BIORICHAR AMENDMENT ON GROWTH, NUTRITIONAL PROPERTIES AND BIOCHEMICAL
710 CHANGES OF BANANA (*Musa acuminata*) cv. BERANGAN ESTABLISHED IN AN ULTISOL SOIL
711 AT VEGETATIVE STAGE 10, 23–35.

712 Doan, T.T., Henry-des-Tureaux, T., Rumpel, C., Janeau, J.-L., Jouquet, P., 2015. Impact of compost,
713 vermicompost and biochar on soil fertility, maize yield and soil erosion in Northern Vietnam:
714 A three year mesocosm experiment. *Sci. Total Environ.* 514, 147–154.
715 <https://doi.org/10.1016/j.scitotenv.2015.02.005>

716 Eden, M., Gerke, H.H., Houot, S., 2017. Organic waste recycling in agriculture and related effects on
717 soil water retention and plant available water: a review. *Agron. Sustain. Dev.* 37, 11.
718 <https://doi.org/10.1007/s13593-017-0419-9>

719 FAO, F. and A.O., 2013. *The State of the World's Land and Water Resources for Food and Agriculture*,
720 0 ed. Routledge. <https://doi.org/10.4324/9780203142837>

721 Farooqi, Z.U.R., Ayub, M.A., Zia ur Rehman, M., Sohail, M.I., Usman, M., Khalid, H., Naz, K., 2020.
722 Regulation of drought stress in plants, in: *Plant Life Under Changing Environment*. Elsevier,
723 pp. 77–104. <https://doi.org/10.1016/B978-0-12-818204-8.00004-7>

724 Fischer, D., Glaser, B., 2012. Synergisms between Compost and Biochar for Sustainable Soil
725 Amelioration, in: Kumar, S. (Ed.), *Management of Organic Waste*. InTech.
726 <https://doi.org/10.5772/31200>

727 Głąb, T., Żabiński, A., Sadowska, U., Gondek, K., Kopeć, M., Mierzwa-Hersztek, M., Tabor, S., Stanek-
728 Tarkowska, J., 2020. Fertilization effects of compost produced from maize, sewage sludge
729 and biochar on soil water retention and chemical properties. *Soil Tillage Res.* 197, 104493.
730 <https://doi.org/10.1016/j.still.2019.104493>

731 Gorovtsov, A.V., Minkina, T.M., Mandzhieva, S.S., Perelomov, L.V., Soja, G., Zamulina, I.V., Rajput,
732 V.D., Sushkova, S.N., Mohan, D., Yao, J., 2020. The mechanisms of biochar interactions with
733 microorganisms in soil. *Environ. Geochem. Health* 42, 2495–2518.
734 <https://doi.org/10.1007/s10653-019-00412-5>

735 Guo, L., Bornø, M.L., Niu, W., Liu, F., 2021. Biochar amendment improves shoot biomass of tomato
736 seedlings and sustains water relations and leaf gas exchange rates under different irrigation
737 and nitrogen regimes. *Agric. Water Manag.* 245, 106580.
738 <https://doi.org/10.1016/j.agwat.2020.106580>

739 Hafez, E.M., Omara, A.E.D., Alhumaydhi, F.A., El-Esawi, M.A., 2021. Minimizing hazard impacts of soil
740 salinity and water stress on wheat plants by soil application of vermicompost and biochar.
741 *Physiol. Plant.* 172, 587–602. <https://doi.org/10.1111/ppl.13261>

742 Hari, V., Rakovec, O., Markonis, Y., Hanel, M., Kumar, R., 2020. Increased future occurrences of the
743 exceptional 2018–2019 Central European drought under global warming. *Sci. Rep.* 10, 12207.
744 <https://doi.org/10.1038/s41598-020-68872-9>

745 Hashem, A., Kumar, A., Al-Dbass, A.M., Alqarawi, A.A., Al-Arjani, A.-B.F., Singh, G., Farooq, M.,
746 Abd_Allah, E.F., 2019. Arbuscular mycorrhizal fungi and biochar improves drought tolerance
747 in chickpea. *Saudi J. Biol. Sci.* 26, 614–624. <https://doi.org/10.1016/j.sjbs.2018.11.005>

748 Iizumi, T., Wagai, R., 2019. Leveraging drought risk reduction for sustainable food, soil and climate via
749 soil organic carbon sequestration. *Sci. Rep.* 9, 19744. <https://doi.org/10.1038/s41598-019-55835-y>

750

751 IPCC, 2021. *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the*
752 *Sixth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge
753 University Press, Cambridge University Press.

754 Irigoyen, J.J., Emerich, D.W., Sanchez-Diaz, M., 1992. Phosphoenolpyruvate carboxylase, malate and
755 alcohol dehydrogenase activities in alfalfa (*Medicago sativa*) nodules under water stress.
756 *Physiol. Plant.* 84, 61–66. <https://doi.org/10.1111/j.1399-3054.1992.tb08765.x>

757 Jačka, L., Trakal, L., Ouředníček, P., Pohořelý, M., Šípek, V., 2018. Biochar presence in soil significantly
758 decreased saturated hydraulic conductivity due to swelling. *Soil Tillage Res.* 184, 181–185.
759 <https://doi.org/10.1016/j.still.2018.07.018>

760 Jien, S.-H., 2019. Physical Characteristics of Biochars and Their Effects on Soil Physical Properties, in:
761 *Biochar from Biomass and Waste.* Elsevier, pp. 21–35. <https://doi.org/10.1016/B978-0-12-811729-3.00002-9>

762

763 Kammann, C.I., Linsel, S., Gößling, J.W., Koyro, H.-W., 2011. Influence of biochar on drought
764 tolerance of *Chenopodium quinoa* Willd and on soil–plant relations. *Plant Soil* 345, 195–210.
765 <https://doi.org/10.1007/s11104-011-0771-5>

766 Khan, Z., Khan, M.N., Zhang, K., Luo, T., Zhu, K., Hu, L., 2021. The application of biochar alleviated the
767 adverse effects of drought on the growth, physiology, yield and quality of rapeseed through
768 regulation of soil status and nutrients availability. *Ind. Crops Prod.* 171, 113878.
769 <https://doi.org/10.1016/j.indcrop.2021.113878>

770 Kiani, R., Nazeri, V., Shokrpour, M., Hano, C., 2020. Morphological, Physiological, and Biochemical
771 Impacts of Different Levels of Long-Term Water Deficit Stress on *Linum album* Ky. ex Boiss.
772 Accessions. *Agronomy* 10, 1966. <https://doi.org/10.3390/agronomy10121966>

773 Kopittke, P.M., Berhe, A.A., Carrillo, Y., Cavagnaro, T.R., Chen, D., Chen, Q.-L., Román Dobarco, M.,
774 Dijkstra, F.A., Field, D.J., Grundy, M.J., He, J.-Z., Hoyle, F.C., Kögel-Knabner, I., Lam, S.K.,
775 Marschner, P., Martinez, C., McBratney, A.B., McDonald-Madden, E., Menzies, N.W., Mosley,
776 L.M., Mueller, C.W., Murphy, D.V., Nielsen, U.N., O'Donnell, A.G., Pendall, E., Pett-Ridge, J.,
777 Rumpel, C., Young, I.M., Minasny, B., 2022. Ensuring planetary survival: the centrality of
778 organic carbon in balancing the multifunctional nature of soils. *Crit. Rev. Environ. Sci.*
779 *Technol.* 1–17. <https://doi.org/10.1080/10643389.2021.2024484>

780 Kramer, P.J., Boyer, J.S., 1995. Roots and root systems, in: *Water Relations of Plants and Soils.* San
781 Diego, CA.

782 Kranz, C.N., McLaughlin, R.A., Johnson, A., Miller, G., Heitman, J.L., 2020. The effects of compost
783 incorporation on soil physical properties in urban soils – A concise review. *J. Environ.*
784 *Manage.* 261, 110209. <https://doi.org/10.1016/j.jenvman.2020.110209>

785 Król, A., Amarowicz, R., Weidner, S., 2014. Changes in the composition of phenolic compounds and
786 antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of

787 long-term drought stress. *Acta Physiol. Plant.* 36, 1491–1499.
788 <https://doi.org/10.1007/s11738-014-1526-8>

789 Li, W., Zhang, S., Shan, L., Eneji, A.E., 2011. Changes in root characteristics, gas exchange and water
790 use efficiency following water stress and rehydration of Alfalfa and Sorghum. *Aust. J. Crop*
791 *Sci.* 5, 1521–1532.

792 Liu, D., She, D., Mu, X., 2019. Water flow and salt transport in bare saline-sodic soils subjected to
793 evaporation and intermittent irrigation with saline/distilled water. *Land Degrad. Dev.* 30,
794 1204–1218. <https://doi.org/10.1002/ldr.3306>

795 Liu, H., Sultan, M.A.R.F., Liu, X. li, Zhang, J., Yu, F., Zhao, H. xian, 2015. Physiological and Comparative
796 Proteomic Analysis Reveals Different Drought Responses in Roots and Leaves of Drought-
797 Tolerant Wild Wheat (*Triticum boeoticum*). *PLOS ONE* 10, e0121852.
798 <https://doi.org/10.1371/journal.pone.0121852>

799 Liu, W., Sun, F., Lim, W.H., Zhang, J., Wang, H., Shiogama, H., Zhang, Y., 2018. Global drought and
800 severe drought-affected populations in 1.5 and 2 °C warmer worlds. *Earth Syst. Dyn.* 9, 267–
801 283. <https://doi.org/10.5194/esd-9-267-2018>

802 Liu, Y., Chen, J., 2022. Effect of ageing on biochar properties and pollutant management.
803 *Chemosphere* 292, 133427. <https://doi.org/10.1016/j.chemosphere.2021.133427>

804 Luo, Y.-Z., Liu, H., Yan, G., Li, G., Turner, N., 2019. Roots of Lucerne Seedlings are More Resilient to a
805 Water Deficit than Leaves or Stems. *Agronomy* 9, 123.
806 <https://doi.org/10.3390/agronomy9030123>

807 Maljanen, M., Sigurdsson, B.D., Guðmundsson, J., Óskarsson, H., Huttunen, J.T., Martikainen, P.J.,
808 2010. Greenhouse gas balances of managed peatlands in the Nordic countries – present
809 knowledge and gaps. *Biogeosciences* 7, 2711–2738. <https://doi.org/10.5194/bg-7-2711-2010>

810 Mia, S., Dijkstra, F.A., Singh, B., 2017. Long-Term Aging of Biochar, in: *Advances in Agronomy*.
811 Elsevier, pp. 1–51. <https://doi.org/10.1016/bs.agron.2016.10.001>

812 Mierzwa-Hersztek, M., 2016. Effect of poultry litter biochar on soil enzymatic activity, ecotoxicity and
813 plant growth. *Appl. Soil Ecol.* 8.

814 Mouradi, M., Bouizgaren, A., Farissi, M., Ghoulam, C., 2018. Assessment of Deficit Irrigation
815 Responses of Moroccan Alfalfa (*Medicago Sativa* L.) Landraces Grown Under Field
816 Conditions: Drought responses of Moroccan alfalfa genotypes under field conditions. *Irrig.*
817 *Drain.* 67, 179–190. <https://doi.org/10.1002/ird.2190>

818 Mouradi, M., Farissi, M., Bouizgaren, A., Makoudi, B., Kabbadj, A., Very, A.-A., Sentenac, H.,
819 Qaddourya, A., Ghoulam, C., 2016. Effects of water deficit on growth, nodulation and
820 physiological and biochemical processes in *Medicago sativa* -rhizobia symbiotic association.
821 *Arid Land Res. Manag.* 30, 193–208. <https://doi.org/10.1080/15324982.2015.1073194>

822 Mukherjee, A., Zimmerman, A.R., Hamdan, R., Cooper, W.T., 2014. Physicochemical changes in
823 pyrogenic organic matter (biochar) after 15 months of field aging. *Solid Earth* 5, 693–704.
824 <https://doi.org/10.5194/se-5-693-2014>

825 Nada, W., Blumenstein, O., Claassens, S., Rensburg, L. van, 2012. Effect of Wood Compost on
826 Extreme Soil Characteristics in the Lusatian Lignite Region. *Open J. Soil Sci.* 02, 347–352.
827 <https://doi.org/10.4236/ojss.2012.24041>

828 Nadeem, S.M., Imran, M., Naveed, M., Khan, M.Y., Ahmad, M., Zahir, Z.A., Crowley, D.E., 2017.
829 Synergistic use of biochar, compost and plant growth-promoting rhizobacteria for enhancing
830 cucumber growth under water deficit conditions: Water deficit stress mitigation in cucumber
831 by PGPR/organic amendments. *J. Sci. Food Agric.* 97, 5139–5145.
832 <https://doi.org/10.1002/jsfa.8393>

833 Naeem, M.A., Khalid, M., Aon, M., Abbas, G., Amjad, M., Murtaza, B., Khan, W.-D., Ahmad, N., 2018.
834 Combined application of biochar with compost and fertilizer improves soil properties and
835 grain yield of maize. *J. Plant Nutr.* 41, 112–122.
836 <https://doi.org/10.1080/01904167.2017.1381734>

837 Naidoo, G., Naidoo, K.K., 2018. Drought stress effects on gas exchange and water relations of the
838 invasive weed *Chromolaena odorata*. *Flora* 248, 1–9.
839 <https://doi.org/10.1016/j.flora.2018.08.008>

840 Obadi, A., Alharbi, A., Abdel-Razzak, H., Al-Omran, A., 2020. Biochar and compost as soil
841 amendments: effect on sweet pepper (*Capsicum annuum* L.) growth under partial root zone
842 drying irrigation. *Arab J Geosci* 12.

843 Ortíz-Castro, R., Contreras-Cornejo, H.A., Macías-Rodríguez, L., López-Bucio, J., 2009. The role of
844 microbial signals in plant growth and development. *Plant Signal. Behav.* 4, 701–712.
845 <https://doi.org/10.4161/psb.4.8.9047>

846 Paetsch, L., Mueller, C.W., Kögel-Knabner, I., von Lützow, M., Girardin, C., Rumpel, C., 2018. Effect of
847 in-situ aged and fresh biochar on soil hydraulic conditions and microbial C use under drought
848 conditions. *Sci. Rep.* 8, 6852. <https://doi.org/10.1038/s41598-018-25039-x>

849 Paz-Ferreiro, J., Lu, H., Fu, S., Méndez, A., Gascó, G., 2014. Use of phytoremediation and biochar to
850 remediate heavy metal polluted soils: a review. *Solid Earth* 5, 65–75.
851 <https://doi.org/10.5194/se-5-65-2014>

852 Phares, C.A., Atiah, K., Frimpong, K.A., Danquah, A., Asare, A.T., Aggor-Woananu, S., 2020.
853 Application of biochar and inorganic phosphorus fertilizer influenced rhizosphere soil
854 characteristics, nodule formation and phytoconstituents of cowpea grown on tropical soil.
855 *Heliyon* 6, e05255. <https://doi.org/10.1016/j.heliyon.2020.e05255>

856 Rahman, M.T., Guo, Z.C., Zhang, Z.B., Zhou, H., Peng, X.H., 2018. Wetting and drying cycles improving
857 aggregation and associated C stabilization differently after straw or biochar incorporated into
858 a Vertisol. *Soil Tillage Res.* 175, 28–36.

859 Ritchie, G.A., Hinckley, T.M., 1975. The Pressure Chamber as an Instrument for Ecological Research,
860 in: *Advances in Ecological Research*. Elsevier, pp. 165–254. [https://doi.org/10.1016/S0065-2504\(08\)60290-1](https://doi.org/10.1016/S0065-2504(08)60290-1)

861

862 Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2011. Drought effects on
863 microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant
864 community composition. *Appl. Soil Ecol.* 48, 38–44.
865 <https://doi.org/10.1016/j.apsoil.2011.02.004>

866 Schulze, J., Tesfaye, M., Litjens, R.H.M.G., Bucciarelli, B., Trepp, G., Miller, S., Samac, D., Allan, D.,
867 Vance, C.P., 2002. Malate plays a central role in plant nutrition. *Prog. Plant Nutr. Plenary*
868 *Lect. XIV Int. Plant Nutr. Colloq.* 133–139. https://doi.org/10.1007/978-94-017-2789-1_10

869 Seehausen, M., Gale, N., Dranga, S., Hudson, V., Liu, N., Michener, J., Thurston, E., Williams, C., Smith,
870 S., Thomas, S., 2017. Is There a Positive Synergistic Effect of Biochar and Compost Soil
871 Amendments on Plant Growth and Physiological Performance? *Agronomy* 7, 13.
872 <https://doi.org/10.3390/agronomy7010013>

873 Sicher, R.C., Timlin, D., Bailey, B., 2012. Responses of growth and primary metabolism of water-
874 stressed barley roots to rehydration. *J. Plant Physiol.* 169, 686–695.
875 <https://doi.org/10.1016/j.jplph.2012.01.002>

876 Sinsabaugh, R.L., Reynolds, H., Long, T.M., 2000. Rapid assay for amidohydrolase (urease) activity in
877 environmental samples. *Soil Biol. Biochem.* 32, 2095–2097. [https://doi.org/10.1016/S0038-0717\(00\)00102-4](https://doi.org/10.1016/S0038-0717(00)00102-4)

878

879 Sisouvanh, P., Trelo-ges, V., Isarangkool Na Ayutthaya, S., Pierret, A., Nunan, N., Silvera, N.,
880 Xayyathip, K., Hartmann, C., 2021. Can Organic Amendments Improve Soil Physical
881 Characteristics and Increase Maize Performances in Contrasting Soil Water Regimes?
882 *Agriculture* 11, 132. <https://doi.org/10.3390/agriculture11020132>

883 Sun, D., Li, K., Bi, Q., Zhu, J., Zhang, Q., Jin, C., Lu, L., Lin, X., 2017. Effects of organic amendment on
884 soil aggregation and microbial community composition during drying-rewetting alternation.
885 *Sci. Total Environ.* 574, 735–743. <https://doi.org/10.1016/j.scitotenv.2016.09.112>

886 Swann, A.L.S., n.d. *Plants and Drought in a Changing Climate* 18.

887 Szabados, L., Saviouré, A., 2010. Proline: a multifunctional amino acid. *Trends Plant Sci.* 15, 89–97.
888 <https://doi.org/10.1016/j.tplants.2009.11.009>

889 Taguchi, H., Hamasaki, T., Akamatsu, T., Okada, H., 1996. A Simple Assay for Xylanase Using *o*-
890 Nitrophenyl- β - D -xylobioside. *Biosci. Biotechnol. Biochem.* 60, 983–985.
891 <https://doi.org/10.1271/bbb.60.983>

892 Tani, E., Chronopoulou, E., Labrou, N., Sarri, E., Goufa, M., Vaharidi, X., Tornesaki, A., Psychogiou, M.,
893 Bebeli, P., Abraham, E., 2019a. Growth, Physiological, Biochemical, and Transcriptional
894 Responses to Drought Stress in Seedlings of *Medicago sativa* L., *Medicago arborea* L. and
895 Their Hybrid (Alborea). *Agronomy* 9, 38. <https://doi.org/10.3390/agronomy9010038>

896 Tani, E., Chronopoulou, E., Labrou, N., Sarri, E., Goufa, M., Vaharidi, X., Tornesaki, A., Psychogiou, M.,
897 Bebeli, P., Abraham, E., 2019b. Growth, Physiological, Biochemical, and Transcriptional
898 Responses to Drought Stress in Seedlings of *Medicago sativa* L., *Medicago arborea* L. and
899 Their Hybrid (Alborea). *Agronomy* 9, 38. <https://doi.org/10.3390/agronomy9010038>

900 Tanure, M.M.C., da Costa, L.M., Huiz, H.A., Fernandes, R.B.A., Cecon, P.R., Pereira Junior, J.D., da Luz,
901 J.M.R., 2019. Soil water retention, physiological characteristics, and growth of maize plants in
902 response to biochar application to soil. *Soil Tillage Res.* 192, 164–173.
903 <https://doi.org/10.1016/j.still.2019.05.007>

904 Thies, J.E., Rillig, M.C., 2009. Characteristics of Biochar: Biological Properties, in: *Biochar for*
905 *Environmental Management* (1st Edition). London, p. 22.

906 Ullah, N., Ditta, A., Imtiaz, M., Li, X., Jan, A.U., Mehmood, S., Rizwan, M.S., Rizwan, M., 2021.
907 Appraisal for organic amendments and plant growth-promoting rhizobacteria to enhance
908 crop productivity under drought stress: A review. *J. Agron. Crop Sci.* jac.12502.
909 <https://doi.org/10.1111/jac.12502>

910 Wang, D., Li, C., Parikh, S.J., Scow, K.M., 2019. Impact of biochar on water retention of two
911 agricultural soils – A multi-scale analysis. *Geoderma* 340, 185–191.
912 <https://doi.org/10.1016/j.geoderma.2019.01.012>

913 Wang, F., Liu, Y., Li, X., Liu, F., 2018. Effects of biochar amendment, CO₂ elevation and drought on
914 leaf gas exchange, biomass production and water use efficiency in maize. *Pak J Bot* 4, 1347–
915 1353.

916 Wang, J., Xiong, Z., Kuzyakov, Y., 2016. Biochar stability in soil: meta-analysis of decomposition and
917 priming effects. *GCB Bioenergy* 8, 512–523. <https://doi.org/10.1111/gcbb.12266>

918 Wang, L., O’Connor, D., Rinklebe, J., Ok, Y.S., Tsang, D.C.W., Shen, Z., Hou, D., 2020. Biochar Aging:
919 Mechanisms, Physicochemical Changes, Assessment, And Implications for Field Applications.
920 *Environ. Sci. Technol.* 54, 14797–14814. <https://doi.org/10.1021/acs.est.0c04033>

921 Xu, C., He, C.G., Wang, Y.J., Bi, Y.F., Jiang, H., 2020. Effect of drought and heat stresses on
922 photosynthesis, pigments, and xanthophyll cycle in alfalfa (*Medicago sativa* L.).
923 *Photosynthetica* 58, 1226–1236. <https://doi.org/10.32615/ps.2020.073>

924 Xue, R., Shen, Y., Marschner, P., 2017. Soil water content during and after plant growth influence
925 nutrient availability and microbial biomass. *J. Soil Sci. Plant Nutr.* 17, 702–715.
926 <https://doi.org/10.4067/S0718-95162017000300012>

927 Yacoumas, A., Honvault, N., Houben, D., Fontaine, J., Meglouli, H., Laruelle, F., Tisserant, B., Faucon,
928 M.-P., Sahraoui, A.L.-H., Firmin, S., 2020. Contrasting Response of Nutrient Acquisition Traits
929 in Wheat Grown on Bisphenol A-Contaminated Soils. *Water. Air. Soil Pollut.* 231, 23.
930 <https://doi.org/10.1007/s11270-019-4383-7>

931 Yang, A., Akhtar, S.S., Li, L., Fu, Q., Li, Q., Naeem, M.A., He, X., Zhang, Z., Jacobsen, S.-E., 2020.
932 Biochar Mitigates Combined Effects of Drought and Salinity Stress in Quinoa. *Agronomy* 10,
933 912. <https://doi.org/10.3390/agronomy10060912>

934 Zaheer, M.S., Ali, H.H., Soufan, W., Iqbal, R., Habib-ur-Rahman, M., Iqbal, J., Israr, M., El Sabagh, A.,
935 2021. Potential Effects of Biochar Application for Improving Wheat (*Triticum aestivum* L.)
936 Growth and Soil Biochemical Properties under Drought Stress Conditions. *Land* 10, 1125.
937 <https://doi.org/10.3390/land10111125>

938 Zhang, J., Jiang, H., Song, X., Jin, J., Zhang, X., 2018. The Responses of Plant Leaf CO₂/H₂O Exchange
939 and Water Use Efficiency to Drought: A Meta-Analysis 13.

940 Zhao, B., Chen, J., Zhang, J., Qin, S., 2010. Soil microbial biomass and activity response to repeated
941 drying–rewetting cycles along a soil fertility gradient modified by long-term fertilization
942 management practices. *Geoderma* 160, 218–224.
943 Zia, R., Nawaz, M.S., Siddique, M.J., Hakim, S., Imran, A., 2021. Plant survival under drought stress:
944 Implications, adaptive responses, and integrated rhizosphere management strategy for stress
945 mitigation. *Microbiol. Res.* 242, 126626. <https://doi.org/10.1016/j.micres.2020.126626>
946

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948 Table 1. Soil, biochar and compost chemical characteristics.

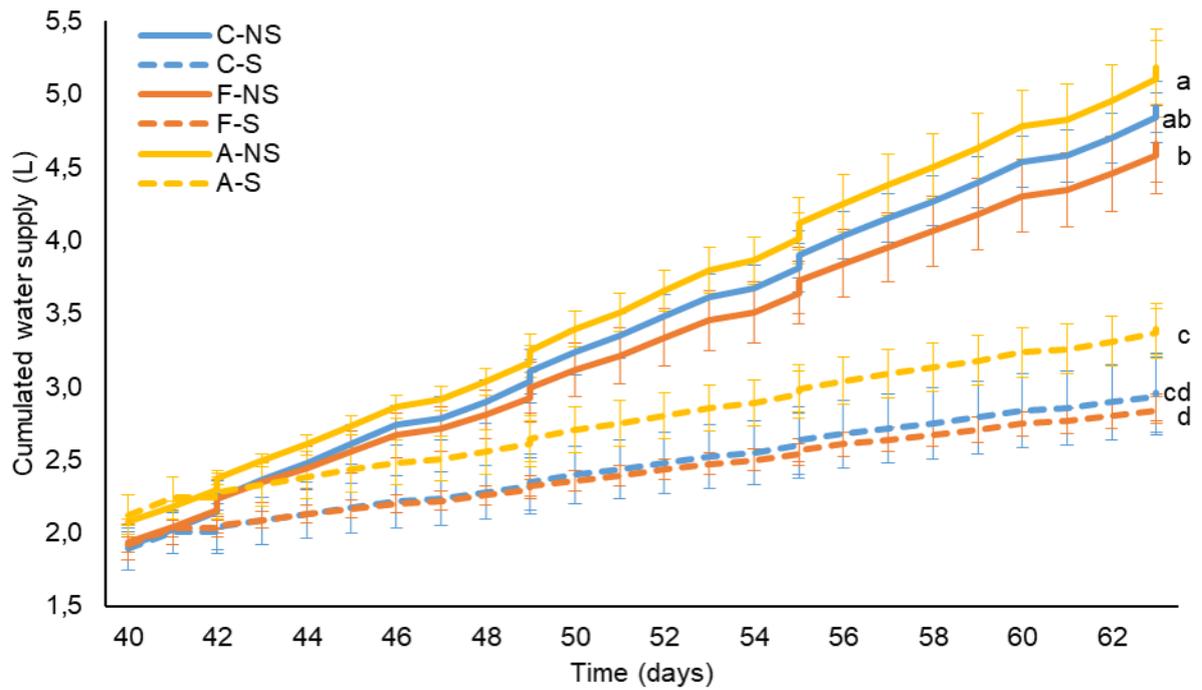
		Soil	Biochar	Compost
pH ^a		7.93	11.4	8
Total carbonate (%) ^b		0.8		
Organic matter (g.kg ⁻¹) ^c		17.67		
Organic carbon (g.kg ⁻¹) ^c		10.22	555	292
Total nitrogen (g.kg ⁻¹) ^d		1.13	8.4	27
Cation exchange capacity (cmol.kg ⁻¹) ^e		10.9	0.85	3.49
Exchangeable element (g.kg ⁻¹) ^f	P	0.07		1.89
	K	0.12		
	Mg	0.07		
	Ca	3.94		
	Na	0.01		
Total elements (g.kg ⁻¹) ^g	P	0.57	3.71	2.05
	K	14.32	17.85	0.86
	Mg	3.2	5.07	0.18
	Ca	7.77	34.02	1.37
	Na	5.85		

^a1 :5 ratio NF ISO 10390 ; ^bNF ISO 10693; ^cdry combustion NF ISO 14235; ^dDumas method; ^eMetson NFX 31-130; ^fNFX 31-108; ^g*aqua regia* NF EN 13650 followed by ICP MS NF ISO 17294.

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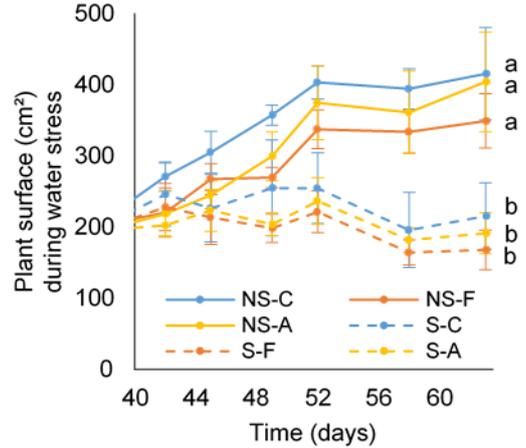
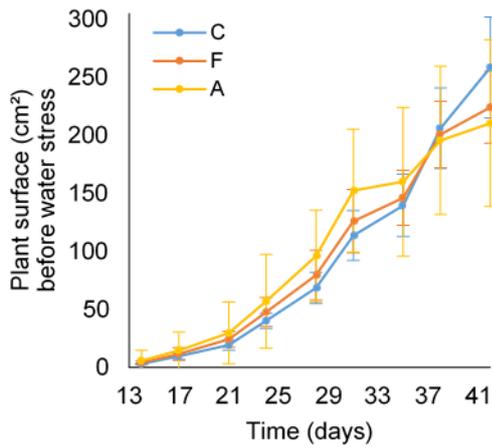
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953 Figure 1. Cumulated water supply from the beginning of the water stress until the end of the
 954 experiment for pots with control soil (C), fresh (F) and aged (A) mixtures of amendments in
 955 well-watered (NS) and under water stress (S) conditions. Statistics were performed at day 63
 956 of the experiment.

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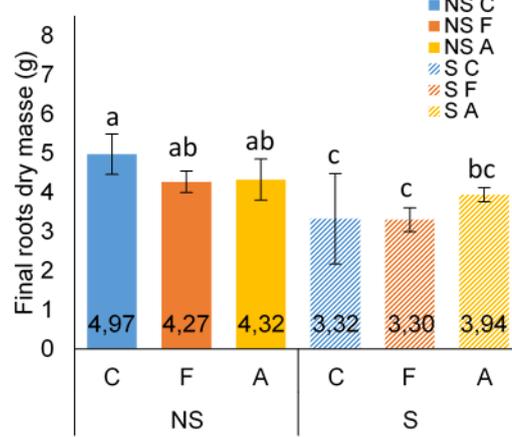
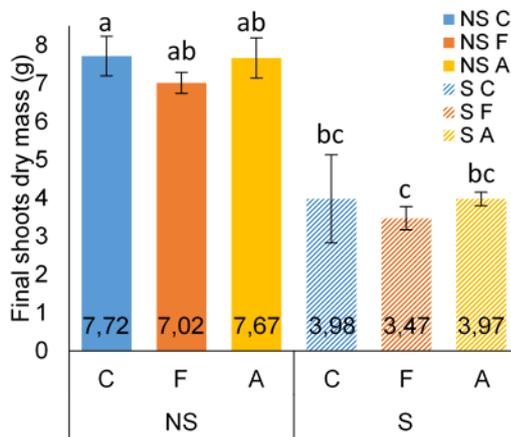
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(a)

(b)



(c)

(d)

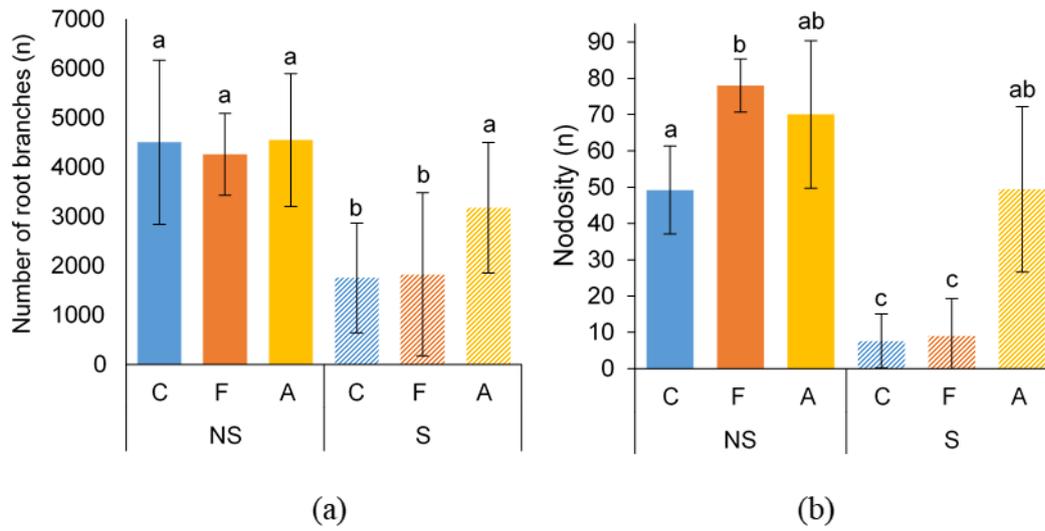
960

961 Figure 2. Surface of plant biomass before water stress (10 replicates per treatments) (a), after
 962 the application of the water stress (5 replicates per treatments) (b) and shoot (c) and root (d)
 963 biomasses after the harvest for control soil (C), fresh (F) and aged (A) mixtures of amendments
 964 in well-watered (NS) and under water stress (S) conditions. Statistics were performed at 41 days
 965 (a) and at the end of the experiment (b, c and d).

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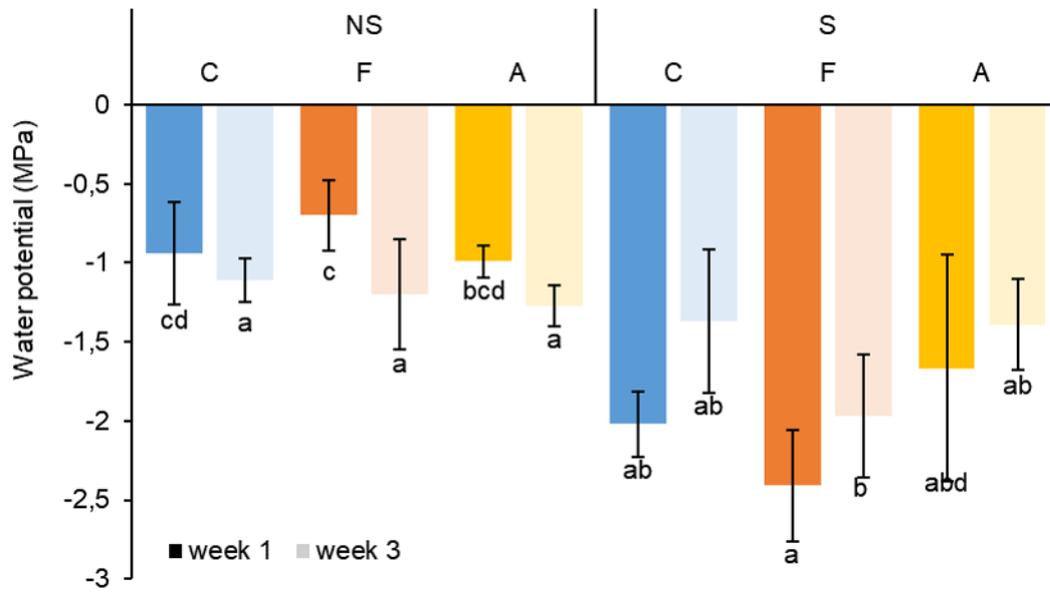
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970 Figure 3. Number of branches (a) and nodulation observed on roots after the harvest.

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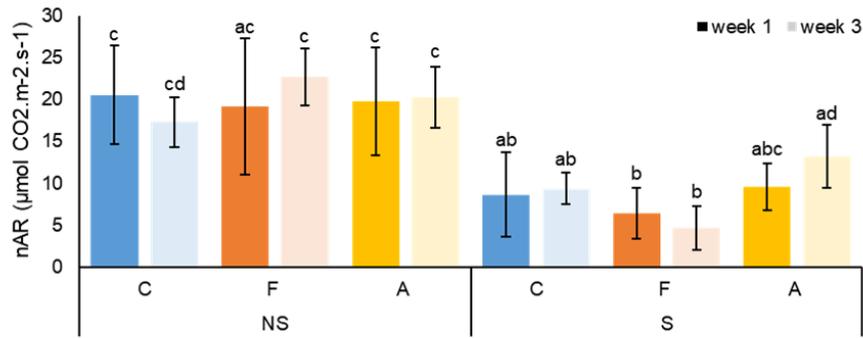
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975 Figure 4. Water potential during the first (lighter colours) and third weeks (darker colours) of
 976 the water stress for control soil (C), fresh (F) and aged (A) mixtures of amendments in well-
 977 watered (NS) and under water stress (S) conditions. Statistics were performed each week
 978 separately.

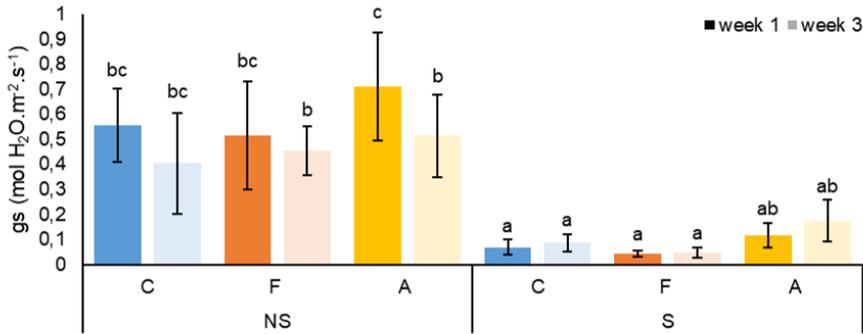
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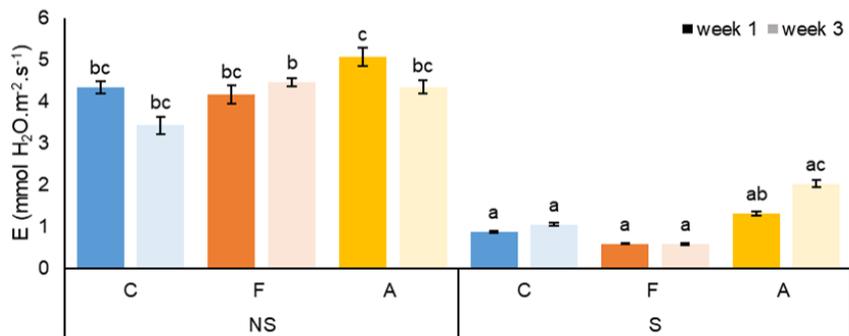
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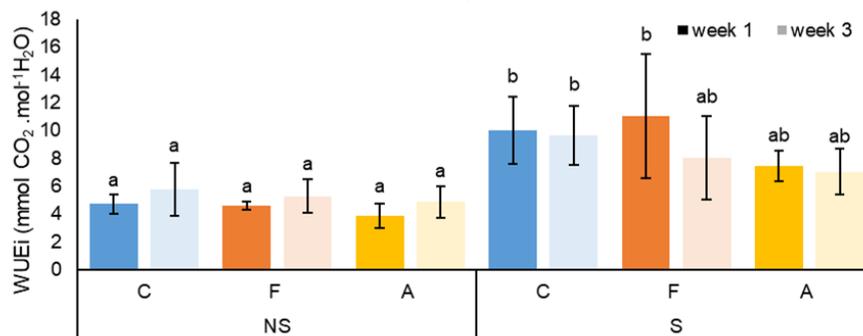
(a)



(b)



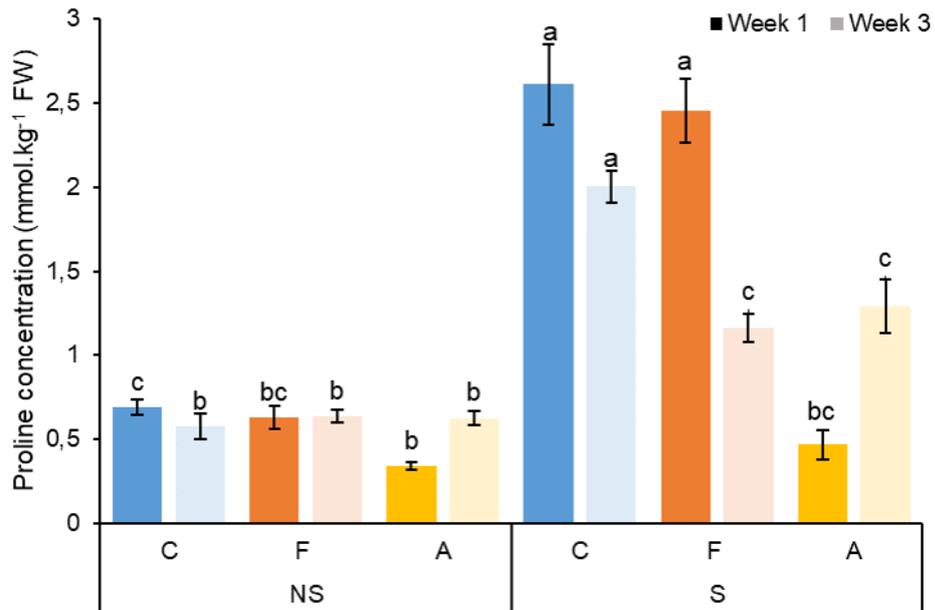
(c)



(d)

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983 Figure 5. Net assimilation rate, nAR (a), stomatal conductance, gs (b), transpiration rate, E (c)
 984 and instantaneous water use efficiency (d) during the first (darker colour) and third weeks
 985 (lighter colour) of the water stress for control soil (C), fresh (F) and aged (A) mixtures of
 986 amendments in well-watered (NS) and under water stress (S) conditions. Statistics were
 987 performed each week separately.



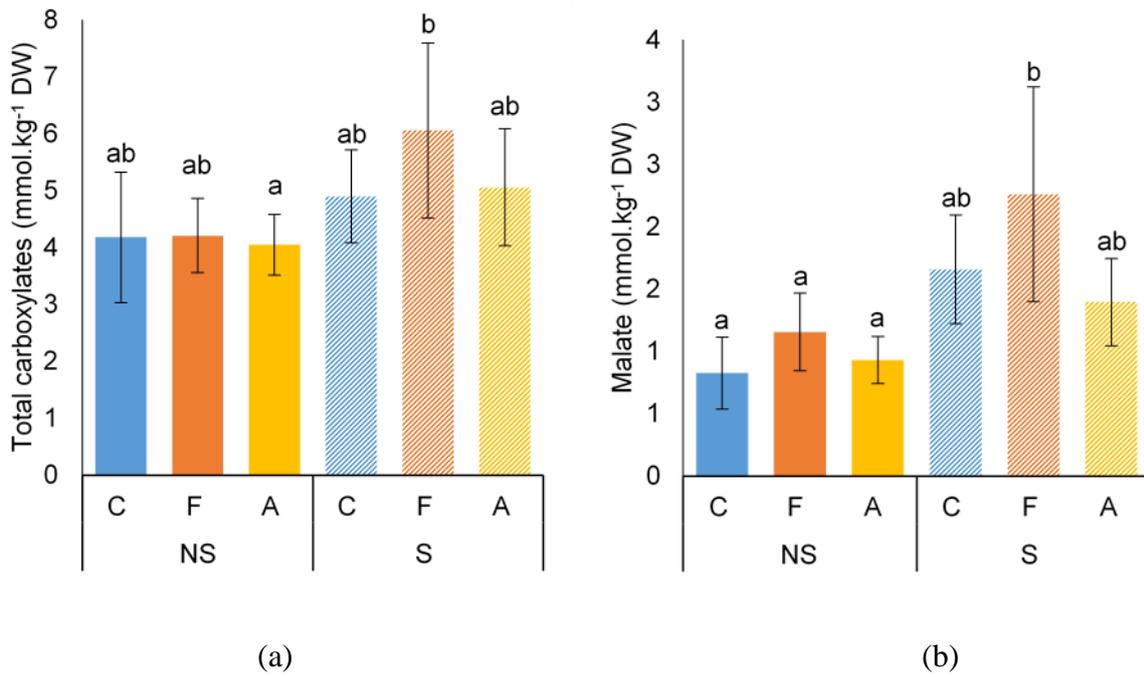
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989 Figure 6. Proline concentration during the first (darker colour) and third (lighter colour)
 990 weeks of the water stress for control soil (C), fresh (F) and aged (A) mixtures of amendments
 991 in well-watered (NS) and under water stress (S) conditions. Statistics were performed each
 992 week separately.

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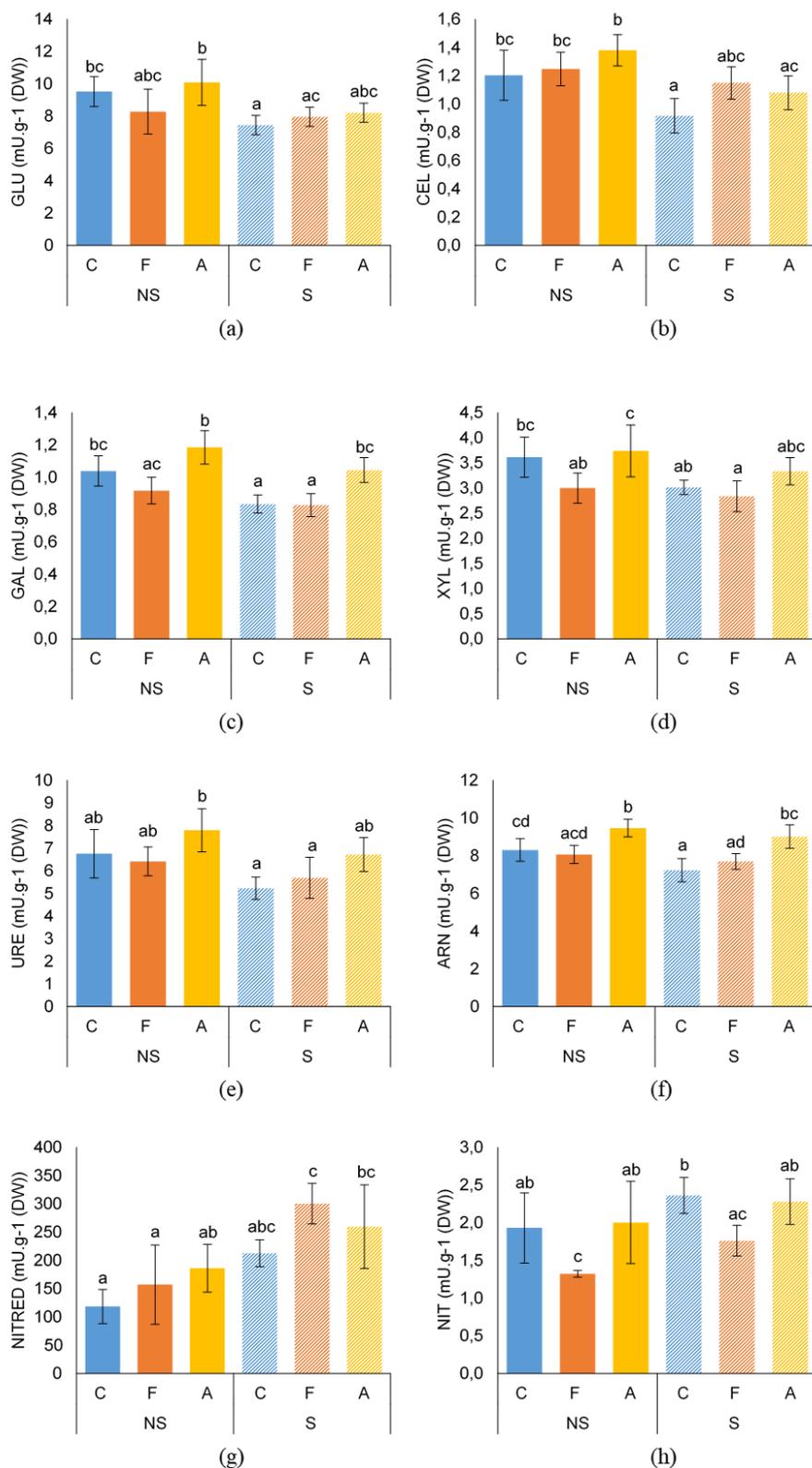
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999 Figure 7. Total carboxylates (a) and malate (b) release at the end of the experiment for control
 1000 soil (C), fresh (F) and aged (A) mixtures of amendments in well-watered (NS) and under
 1001 water stress (S) conditions.

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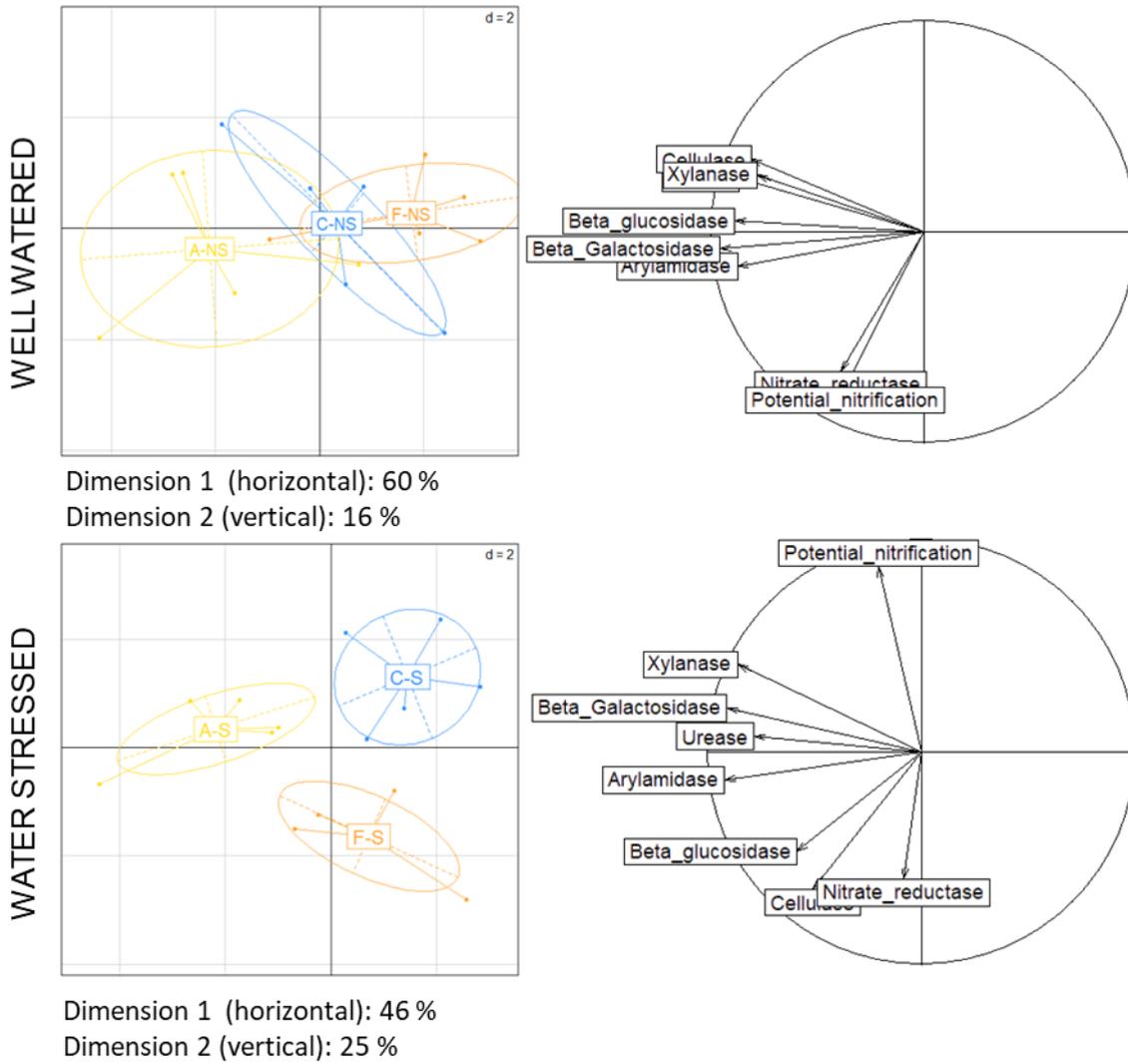
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1006 Figure 8. Enzymes activities, i.e. β -glucosidase (GLU) (a), cellulase (CEL) (b), β -galactosidase
 1007 (GAL) (c), xylanase (XYL) (d), urease (URE) (e), arylamidase (ARN) (f), nitrate reductase
 1008 (NITRED) (g) and potential nitrification (h), in soil according to soil without amendment (C)
 1009 or amended with fresh (F) or altered (A) mixture of biochar + compost and in well-watered
 1010 (NS) or water stress (S) conditions.

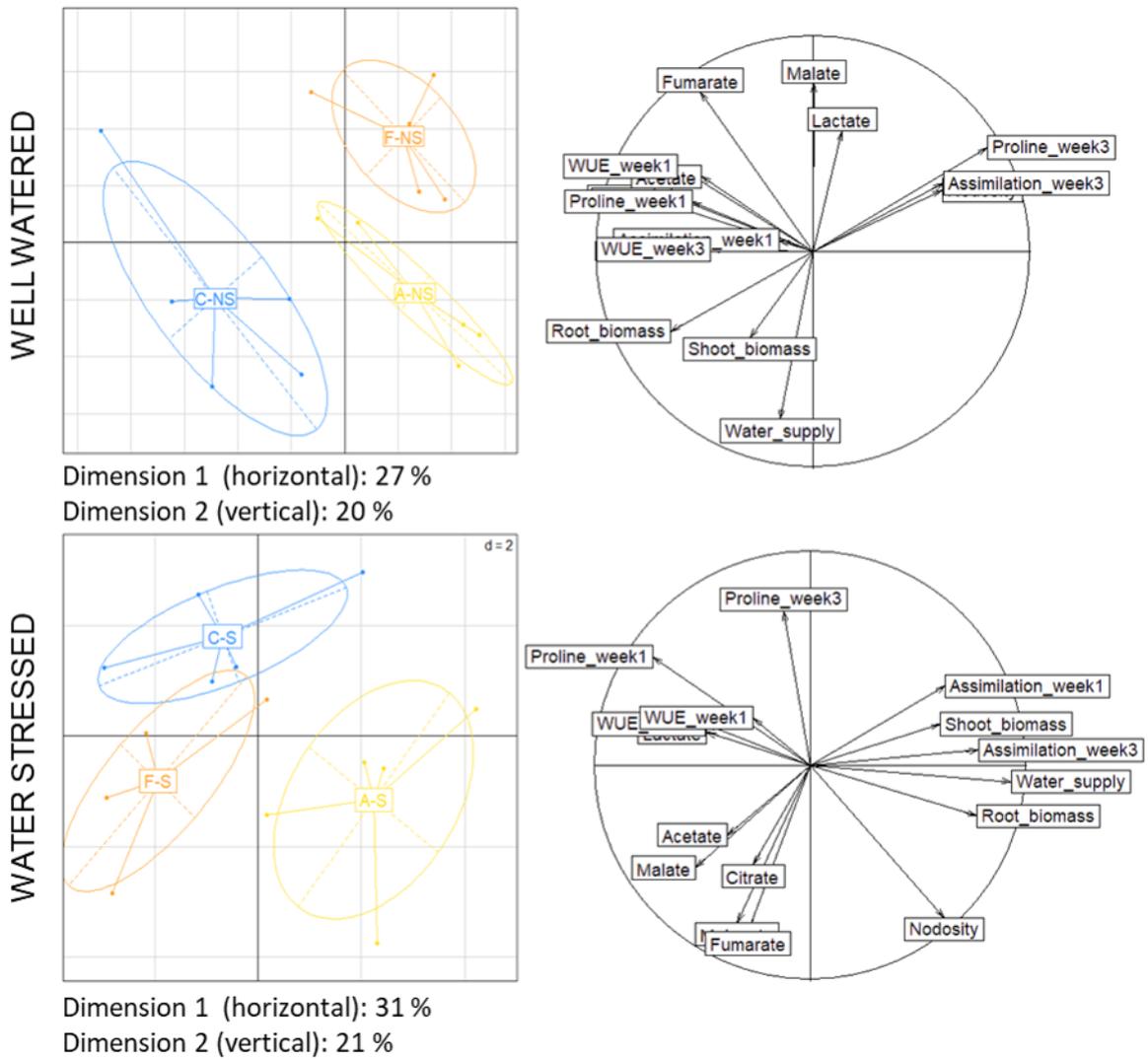


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1012 Figure 9. PCA of soil enzyme activities for control soil (C), fresh (F) and aged (A) mixtures
1013 of amendments in well-watered (top) and under water stress (bottom).

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1017 Figure 10. PCA of plant parameters for control soil (C), fresh (F) and aged (A) mixtures of
1018 amendments in well-watered (top) and under water stress (bottom).

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