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

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## **Abstract**

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

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

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

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

## 1. Introduction

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

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

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

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

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

composts contain a large amount of labile organic matter, which stimulates soil biota, influences microbial community composition, promotes plant growth and, through its effect on microbial activity, improves soil physical and chemical properties (Fischer and Glaser, 2012; Eden et al., 2017). Some studies unraveled the potential mechanisms of compost and biochar to improve the capacity of plants to sustain drought: (i) improvement of soil moisture (Wang et al., 2019) through water binding on the surface (Jačka et al., 2018) and amelioration of soil structure (Wang et al., 2019) allowing a better development and penetration of plant roots in soil (Nadeem et al., 2017), and (ii) improvement of soil nutrient availability (Tanure et al., 2019), especially nitrogen, which is essential for CO<sub>2</sub> assimilation and thus plant growth.

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

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

effect of aged biochar-compost mixtures on plant physiology and soil microbial functioning, although both compartments may be crucial to understand the long-term benefits of this option.

In this study, we carried out a greenhouse experiment with alfalfa plants and a silt-loamy temperate agricultural soil and investigated the effect of biochar-compost mixtures and their natural ageing on the plant-soil system. We aimed to investigate (1) if organic amendment application attenuates negative effects of water stress on plant development and microbial soil functioning and (2) if this effect is changing upon their ageing during field exposure.

To this end, we monitored plant parameters, such as growth, aboveground and belowground biomass, photosynthetic activities, plant stress indicators and their stem water potential during water stress. To examine the contribution of soil processes and plant physiology to water stress responses, we compared plant indicators and soil enzyme activities.

We hypothesised that biochar-compost mixtures affect plant physiological responses to water stress because of their effect on soil water conditions and microbial functioning. We also hypothesised that ageing of biochar-compost mixtures will modify plant responses under water stress due to enhancement of their soil effects.

## **2. Materials and Methods**

### *2.1. Amendments and soil*

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

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

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

## *2.2. Experimental design*

As the purpose of this study was to evaluate the effect of amendment ageing, we had three treatments: (i) the non-amended soil sampled from our field experiment (control -C), (ii) the same soil as the control amended with fresh biochar-compost mixture (F), composed of biochar applied at 0.39 kg.m<sup>-2</sup> dry weight (DW) and compost applied at 0.78 kg.m<sup>-2</sup> (DW) respectively (equivalent to 3.9 t.ha<sup>-1</sup> and 8.8 t.ha<sup>-1</sup>, respectively), and (iii) the soil amended with the same biochar-compost amendment dose (incorporated at 5 cm depth with a rotative harrow), which had been subjected to field ageing for two years (A) and collected on the site at the same time as the non-amended soil. The rate of application of biochar-compost mixtures was based on a 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  $1.3 \text{ g.cm}^{-3}$ , with ten replicates per  
177 treatment. After two weeks of acclimatisation at 70 % field capacity (FC), *i.e.* 25 g H<sub>2</sub>O 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<sup>-2</sup>, 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 42<sup>nd</sup> 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 H<sub>2</sub>O 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.

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

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

### *2.3. Plant development*

In order to monitor plant growth, we took a photo twice a week at a fixed time during the whole experiment, between 10 am – 11 am. Photos were taken at the same place on a table each time in front of a white wall on which was attached a black disk of a known surface. The camera was set on a first fixed distance and height from the pot until they grew too much to fit in the frame. We then replaced it by a new distance until the end of monitoring without changing the height. Three photos were taken per plant with a lateral rotation of 90° each in order to limit the effect of the spatial variability of each plant. After image processing, the average plant surface on these three photos was saved for each plant and expressed in cm<sup>2</sup>. Image processing was performed with the software ImageJ 1.52a (Wayne Rasband, National institutes of Health, USA) by determining the plant surface comparing the number of plant pixels to the number of pixels identified on the disk (Supplementary Materials, S2, Figure 4).

### *2.4. Stem water potential*

A Schölander pressure chamber (PMS instrument, model 600) was used to measure the stem water potential in the first and third week of water stress (Ritchie and Hinckley, 1975). In order to allow the leaves to equilibrate with the stem, each leaf was sealed with aluminium foil to be in the dark during 2h prior to measurement (del Pozo et al., 2017). Data were recorded at solar noon by cutting the leaf from the plant, removing the leaf from the aluminium and placing it in the chamber in which an increasing pressure was applied. From the moment bubbles were 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  $E$  ( $\text{mmol}$   
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  $\text{H}_2\text{O}$ ) 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 5<sup>th</sup> 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,  $\text{CO}_2$  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 <sup>1</sup>. The  $\text{CO}_2$  concentration value set up to simulate the average  $\text{CO}_2$  concentration in our  
234 atmosphere. The PAR value was decided after realisation of light curves, before induction of  
235 the stress, which showed maximum  $\text{CO}_2$  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

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

For carboxylates measurements, after whole plant sampling (see section 2.7), its roots were immersed in a  $\text{CaCl}_2$  solution at  $0.2 \text{ mol.m}^{-3}$  for an hour in order to collect plant root exudates. Thereafter, the solution was frozen until it could be filtered and analysed using a HPLC system (Thermo Fisher, San Jose, CA, USA) reverse phase Luna  $5\mu\text{m}$  C18 column  $250\text{mm} \times 4 \text{ mm}$  (Phenomenex, 147 Le Pecq, France) as described by Yacoumas et al., (2020). We quantified seven carboxylates, i.e. malate, malonate, lactate, acetate, citrate and fumarate (supplementary materials, Figure 6) and calculated their sum as an indicator of total carboxylic acid exudation.

## 2.7. Soil analyses.

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

(Génius 2 Lector, Safas). Enzyme activities are expressed in mU (nmol of release per minutes).g<sup>-1</sup> of dry soil (DW).

## 2.10. Statistics

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

## 3. Results

### 3.1. Cumulative water supply

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

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

### 3.2. Plant development

Five days after seeding, plants grown on soil amended with aged compost-biochar mixtures (A) showed a significantly higher germination rate than the two other treatments (supplementary materials, Figure 8). Before water stress, plant surface linearly increased to reach 258.5 ( $\pm$  43.3) cm<sup>2</sup> for controls (C), 224.1 ( $\pm$  31.0) cm<sup>2</sup> for fresh compost-biochar mixtures (F) and 210.5 ( $\pm$  71.8) cm<sup>2</sup> for aged compost-biochar mixtures (A) (Fig. 2a). Plants of A and F treatments showed a higher plant surface area compared to the control (C) until day 38 (non-significant differences). At 42 days of growth, *i.e.* the day before water stress application, the control plants show a significant higher surface than F and A (Fig. 2a). In treatments without water stress (NS), plant surface continued to increase until 52 days after the start of the experiment, thereafter they stabilised around 404.3 ( $\pm$  74.3) cm<sup>2</sup> for NS-C, 380.0 ( $\pm$  104.6) cm<sup>2</sup> for NS-A and 340.2 ( $\pm$  56.0) cm<sup>2</sup> for NS-F (Fig. 2b). NS-C treatments continued to show a higher surface than those amended with mixtures until the end of the experiment (63 days) and plants grown in NS-A treatments showed higher development than

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

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

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

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

### 3.3. Stem water potential measurements

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

### 3.4. Leaf gas exchange

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

During the first week of stress, the leaf stomatal conductance (gs) of plants grown on the control 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 supply was reduced (Fig. 5b). Similarly, gs was reduced in the stressed compared to non-stressed plant in the amended treatments F ( $0.04 \text{ mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \pm 0.01$  compared to  $0.52 \text{ mol}$



367  $\text{H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$   $\pm$  0.21) and A ( $0.12 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$   $\pm$  0.05 compared to  $0.71 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$   
 368  $\pm$  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  $0.56 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  ( $\pm$  0.20) in the well-  
 372 watered C treatment, and reduced by 78 % under stress ( $0.09 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$   $\pm$  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  $4.34 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  ( $\pm$  1.03) in non-stressed  
 380 control and reduced to  $0.87 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  ( $\pm$  0.41) under stress. In F treatment, the E value  
 381 was  $4.16 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  ( $\pm$  1.80) in non-stressed plants and dropped to  $0.58 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  ( $\pm$  0.19) under stress, while in A treatment, E was  $5.07 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  ( $\pm$  1.05) and  
 382  $1.31 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  ( $\pm$  0.46) in non-stressed and stressed plants, respectively. The highest  
 383 reduction of E under stress was measured in plants of the F treatment (85 %) followed by those  
 384 of C (80 %) and A (74 %) treatments. After three weeks of water stress, E was significantly  
 385 reduced in the C and F treatments by 69 % and 87 %, whereas E of plants of A treatments was  
 386 unaffected.

388 As a result, after a week of water shortage, instantaneous water use efficiency (WUEi) 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

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

Finally, internal CO<sub>2</sub> concentrations (Ci) were not affected by amendment application in the first and third weeks of stress (supplementary material, Figure 9). However, Ci was reduced under water shortage. Specifically, in the first week of water stress, Ci values were 308 (+/- 21)  $\mu\text{mol CO}_2\cdot\text{mol}^{-1}$ , 313 (+/- 10)  $\mu\text{mol CO}_2\cdot\text{mol}^{-1}$  and 327 (+/- 10)  $\mu\text{mol CO}_2\cdot\text{mol}^{-1}$  in plants grown of C, F and A treatments, respectively, which was reduced by 40%, 52% and 24% when water stress was applied. In the third week under water stress, Ci was 294 (+/- 27)  $\mu\text{mol CO}_2\cdot\text{mol}^{-1}$  for non-stressed control and 199 (+/- 46)  $\mu\text{mol CO}_2\cdot\text{mol}^{-1}$  for the stressed control, corresponding to a 32% reduction in Ci, whereas for F and A treatments, Ci was 291 (+/- 16)  $\mu\text{mol CO}_2\cdot\text{mol}^{-1}$  and 306 (+/- 14)  $\mu\text{mol CO}_2\cdot\text{mol}^{-1}$  in non-stressed condition, and was unaffected by water limitation.

### 3.5. Stress indicators

#### 3.5.1 Proline

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

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

### 3.5.2. Root exudation

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

### 3.6. Enzymes activities

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

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

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

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

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

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

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

## **4. Discussion**

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

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

Amendment addition resulted in a rise in carbon and nitrogen contents in the amended soil, as observed in previous studies (Agegnehu et al., 2016; Mierzwa-Hersztek, 2016; Azeem et al., 2020). This could lead to changes of the arylamidase synthesis, which catalyses the release of amino acids from SOM (Acosta-Martínez and Tabatabai, 2000, 2001). Potential nitrification, considered as the maximum capacity of soil microorganisms to transform  $\text{NH}_4^+$  into  $\text{NO}_3^-$ , was sometimes reduced by the addition of biochar-compost mixture, which could be due to the  $\text{NH}_4^+$  sorption capacity of biochar (Abujabhah et al., 2016). However, most enzyme activities were positively affected by amendment application.

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

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

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

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

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

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

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

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

Few significant amendment effects were noted for plant physiological parameters. However, amendment addition affected the overall plant response to (water) stress as shown by PCA analysis (Fig. 10). Indeed, plant stress seems to be reduced by amendment addition as indicated by the significantly lower proline contents in plant leaves of amended treatments. In addition, even if the differences between well-watered and stressed plants were not significant, organic acid exudation increased more between well-watered and stressed plants under fresh mixture application mainly because of malate production which is involved, among others, in plant nutrition and N<sub>2</sub> fixation through nodule activity (Irigoyen et al., 1992; Schulze et al., 2002; Aranjuelo et al., 2014). This could have participated to the improvement of plant resistance to water stress and is in accordance to the lower level of proline found in treatments with fresh mixture addition.

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

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



nitrate reduction during denitrification.

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

Compared to fresh mixtures, the aged biochar-compost mixture increased root branch numbers and nodule formation under water stress. In addition, the internal CO<sub>2</sub> concentration and transpiration rates were maintained with aged amendments despite the negative impacts of water shortage on these parameters. Leaf proline content also increased less during water stress with aged mixtures. In agreement with the maintenance of the assimilation rate in treatment with aged amendments, this might indicate an improvement of the plants' water stress resistance.

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

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

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

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

## **Conclusion**

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

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

		Soil	Biochar	Compost
pH <sup>a</sup>		7.93	11.4	8
Total carbonate (%) <sup>b</sup>		0.8		
Organic matter (g.kg <sup>-1</sup> ) <sup>c</sup>		17.67		
Organic carbon (g.kg <sup>-1</sup> ) <sup>c</sup>		10.22	555	292
Total nitrogen (g.kg <sup>-1</sup> ) <sup>d</sup>		1.13	8.4	27
Cation exchange capacity (cmol.kg <sup>-1</sup> ) <sup>e</sup>		10.9	0.85	3.49
Exchangeable element (g.kg <sup>-1</sup> ) <sup>f</sup>	P	0.07		1.89
	K	0.12		
	Mg	0.07		
	Ca	3.94		
	Na	0.01		
Total elements (g.kg <sup>-1</sup> ) <sup>g</sup>	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		

<sup>a</sup>1 :5 ratio NF ISO 10390 ; <sup>b</sup>NF ISO 10693; <sup>c</sup>dry combustion NF ISO 14235; <sup>d</sup>Dumas method; <sup>e</sup>Metson NFX 31-130; <sup>f</sup>NFX 31-108; <sup>g</sup>*aqua regia* NF EN 13650 followed by ICP MS NF ISO 17294.

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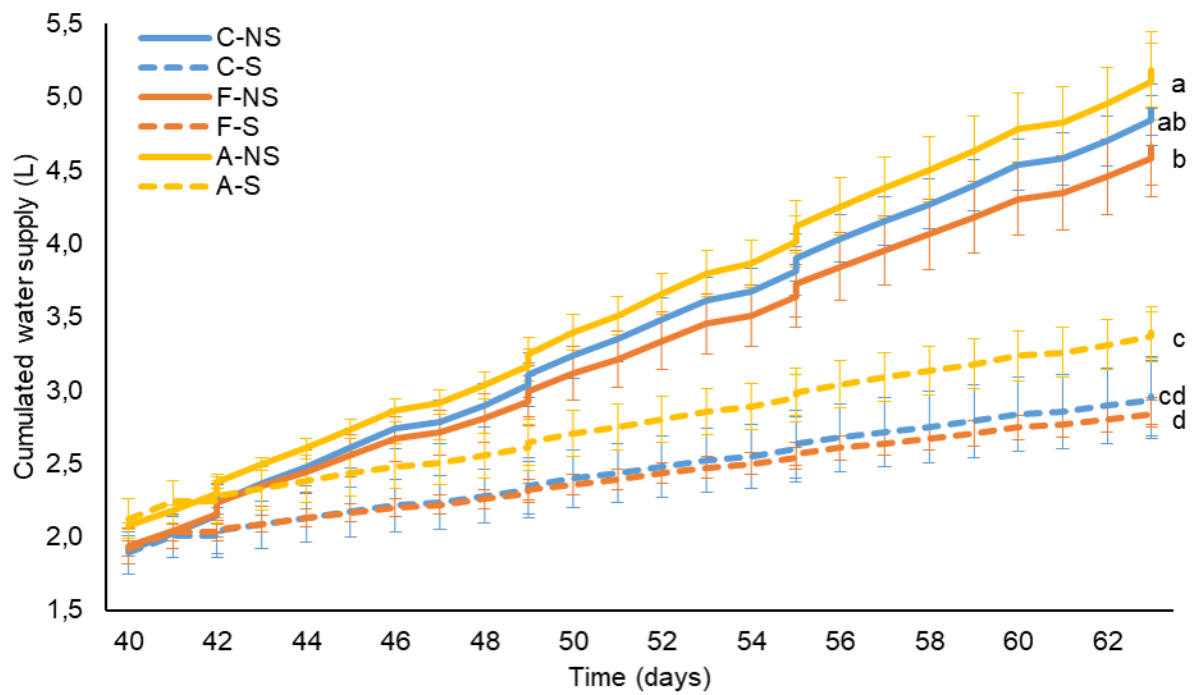


Figure 1. Cumulated water supply from the beginning of the water stress until the end of the experiment for pots with control soil (C), fresh (F) and aged (A) mixtures of amendments in well-watered (NS) and under water stress (S) conditions. Statistics were performed at day 63 of the experiment.

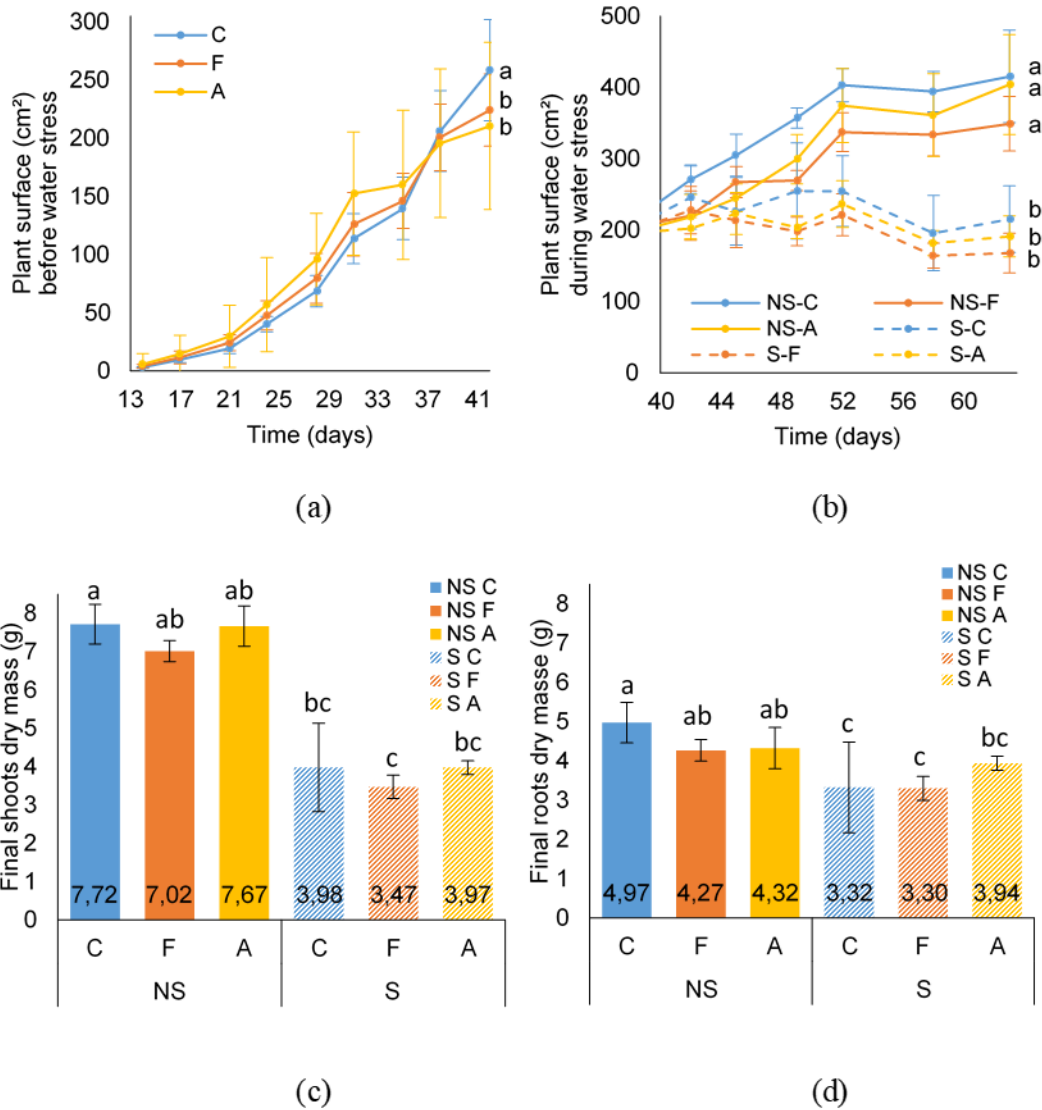


Figure 2. Surface of plant biomass before water stress (10 replicates per treatments) (a), after the application of the water stress (5 replicates per treatments) (b) and shoot (c) and root (d) biomasses after the harvest for control soil (C), fresh (F) and aged (A) mixtures of amendments in well-watered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

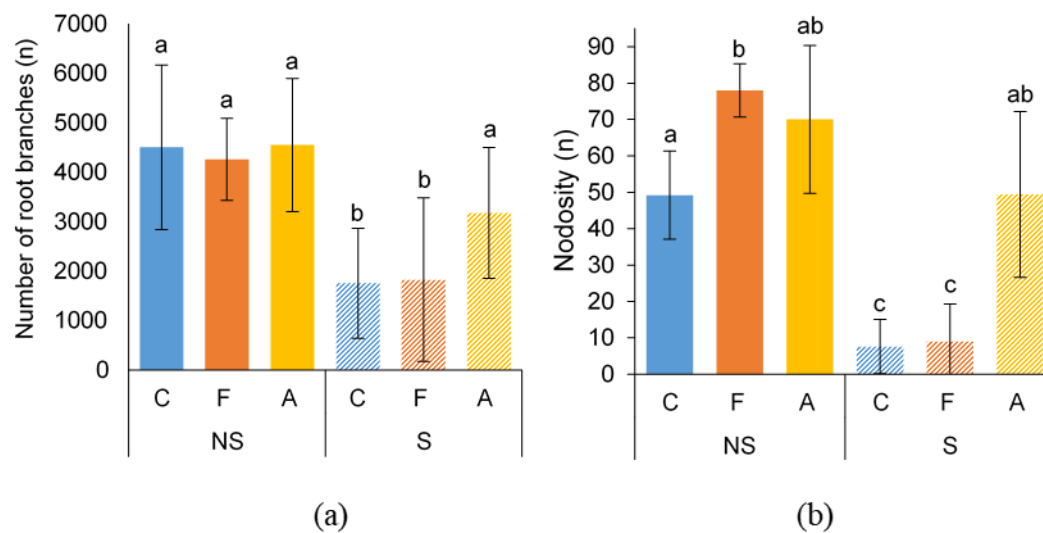


Figure 3. Number of branches (a) and nodulation observed on roots after the harvest.

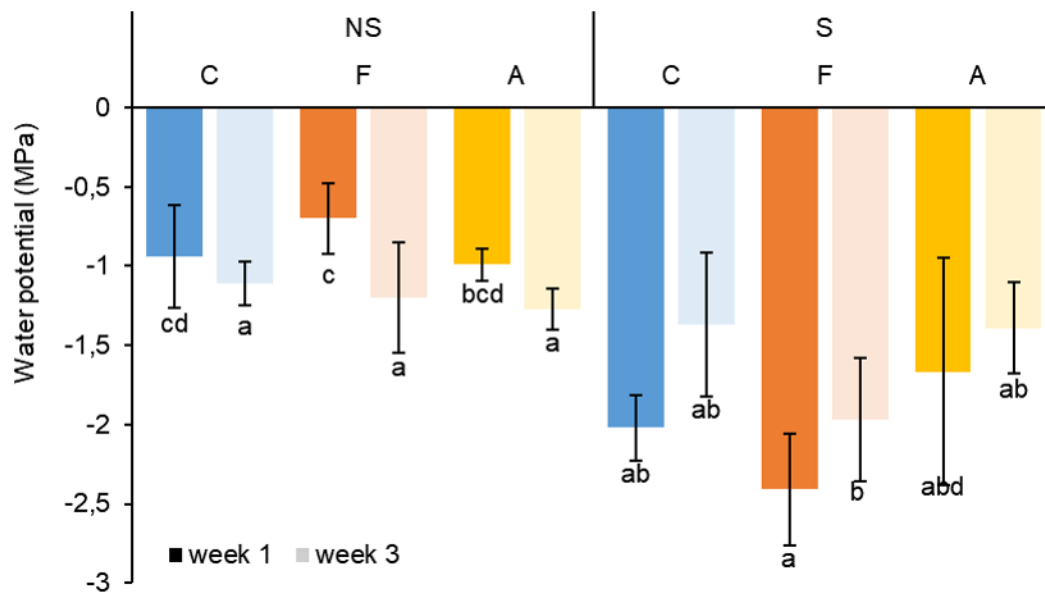
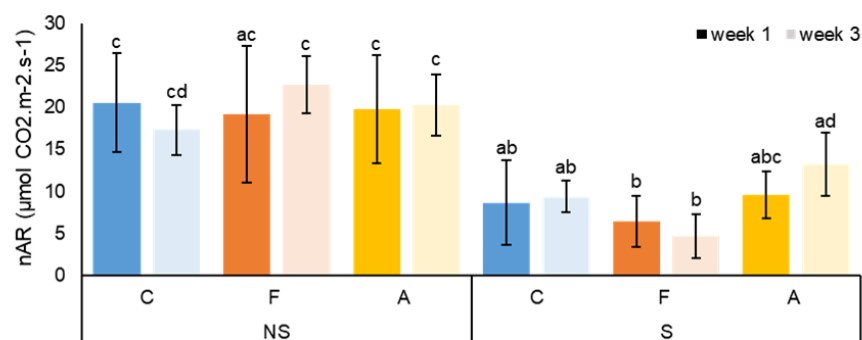
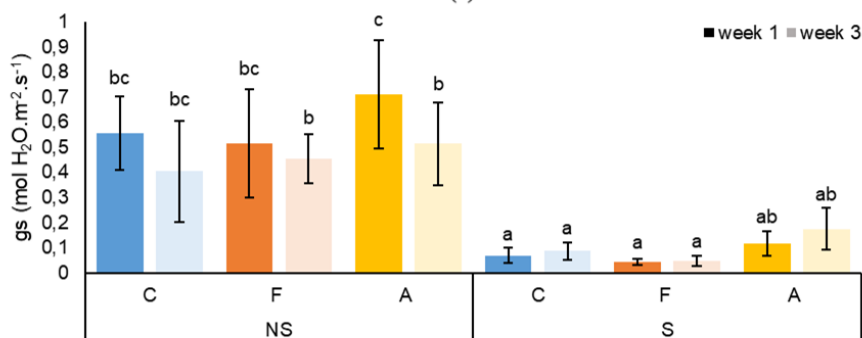


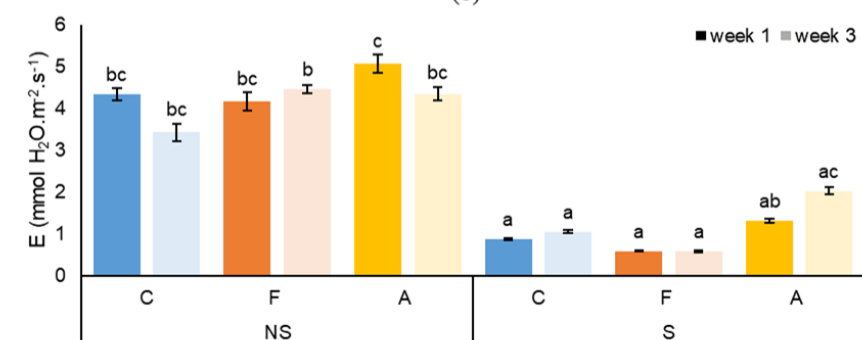
Figure 4. Water potential during the first (lighter colours) and third weeks (darker colours) of the water stress for control soil (C), fresh (F) and aged (A) mixtures of amendments in well-watered (NS) and under water stress (S) conditions. Statistics were performed each week separately.



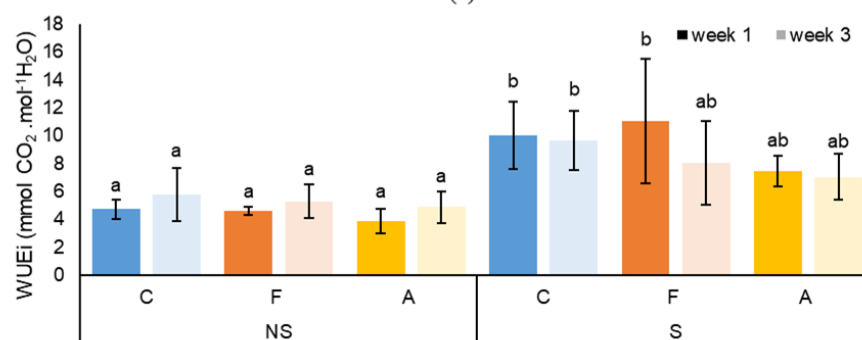
(a)



(b)



(c)



(d)

982  
 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.



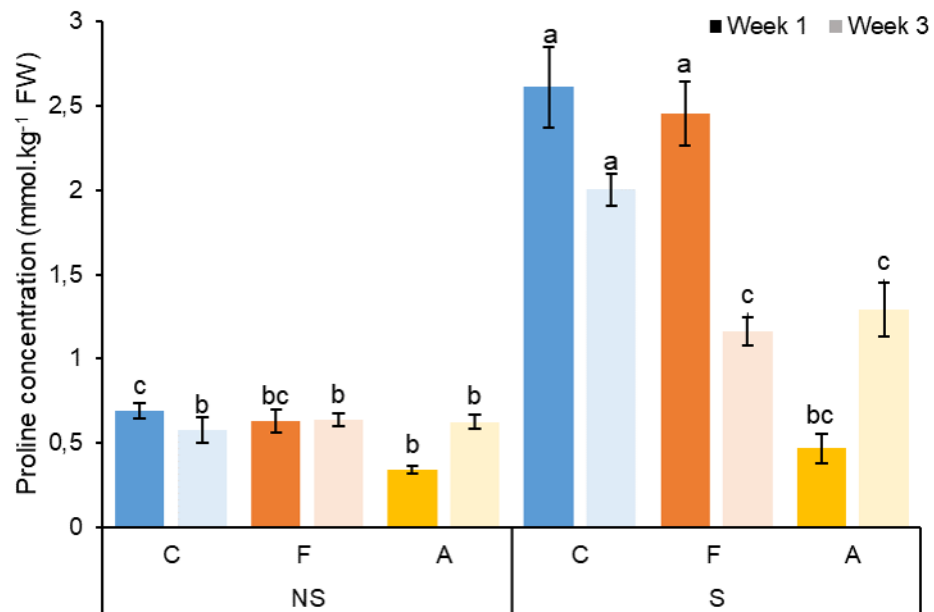


Figure 6. Proline concentration during the first (darker colour) and third (lighter colour) weeks of the water stress for control soil (C), fresh (F) and aged (A) mixtures of amendments in well-watered (NS) and under water stress (S) conditions. Statistics were performed each week separately.

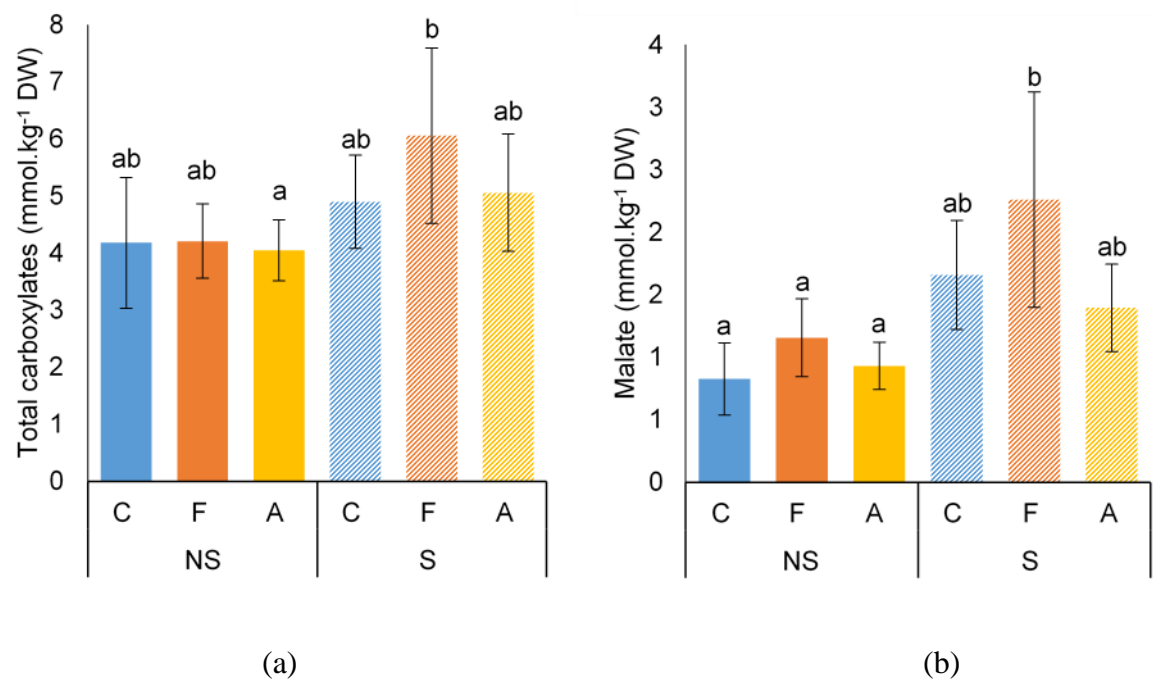


Figure 7. Total carboxylates (a) and malate (b) release at the end of the experiment for control soil (C), fresh (F) and aged (A) mixtures of amendments in well-watered (NS) and under water stress (S) conditions.

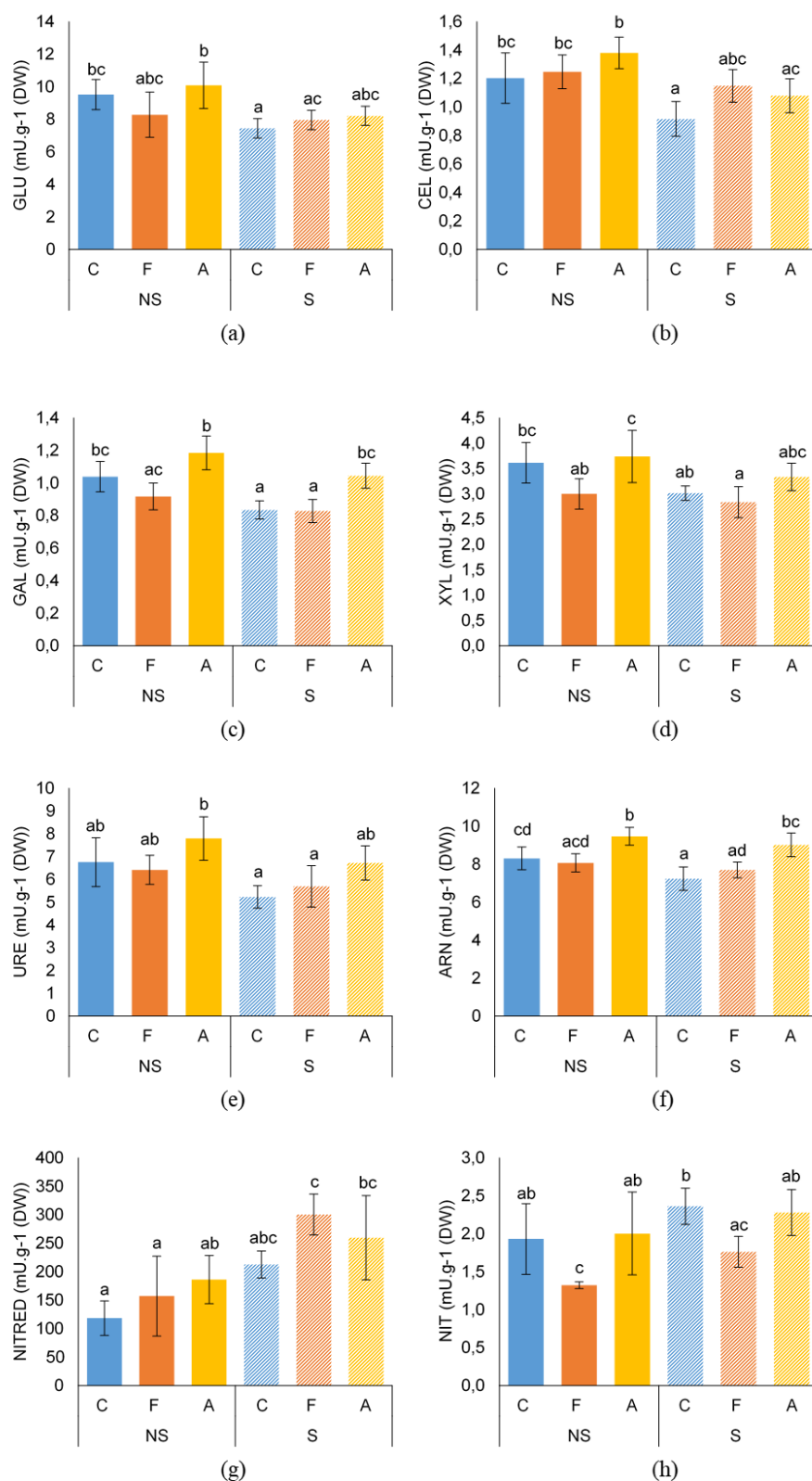


Figure 8. Enzymes activities, i.e.  $\beta$ -glucosidase (GLU) (a), cellulase (CEL) (b),  $\beta$ -galactosidase (GAL) (c), xylanase (XYL) (d), urease (URE) (e), arylamidase (ARN) (f), nitrate reductase (NITRED) (g) and potential nitrification (h), in soil according to soil without amendment (C) or amended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

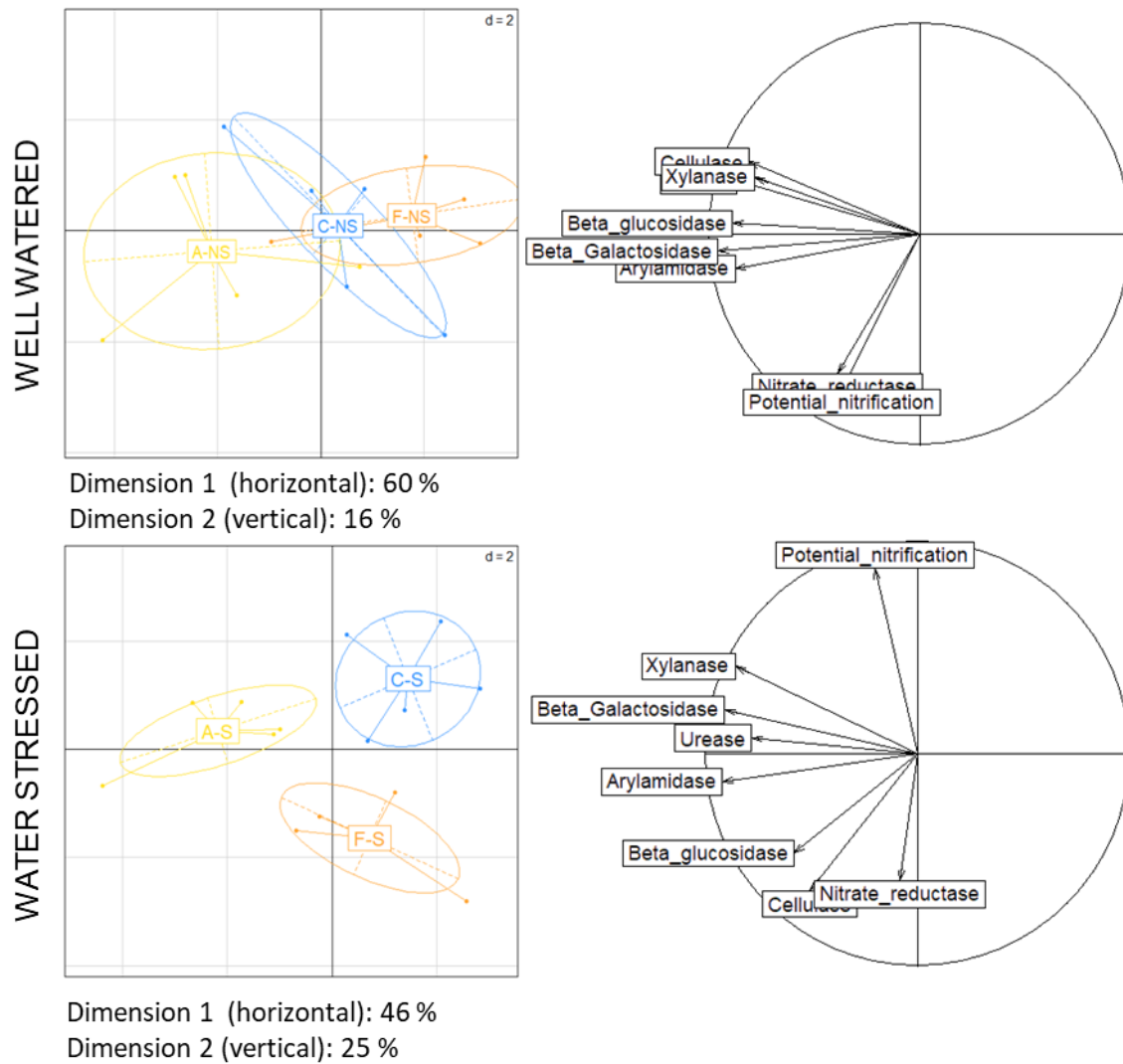


Figure 9. PCA of soil enzyme activities for control soil (C), fresh (F) and aged (A) mixtures of amendments in well-watered (top) and under water stress (bottom).

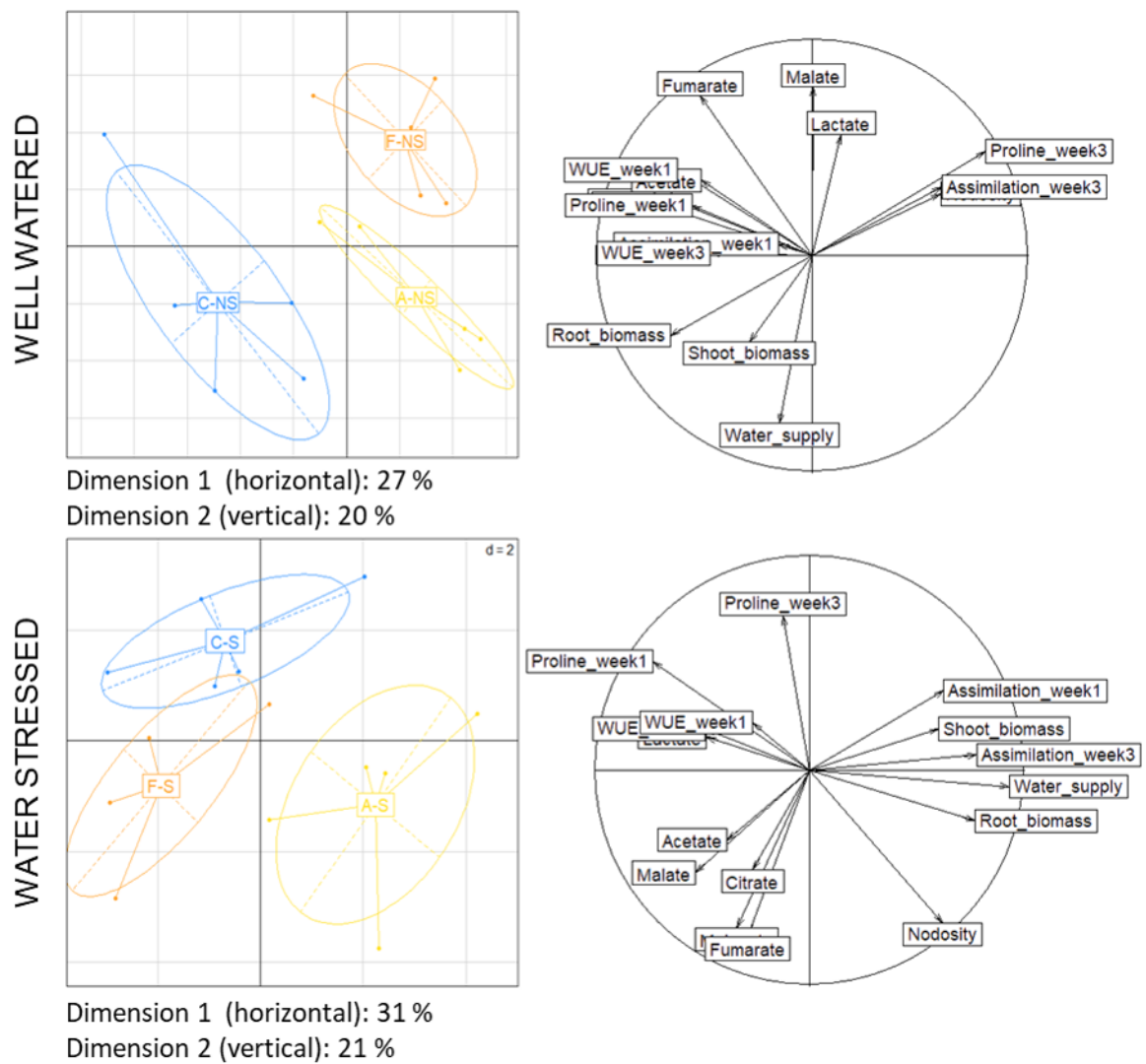


Figure 10. PCA of plant parameters for control soil (C), fresh (F) and aged (A) mixtures of amendments in well-watered (top) and under water stress (bottom).