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Photosynthesis, Yield, Nutrient Availability and Soil Properties after Biochar, Zeolites or Mycorrhizal Inoculum Application to a Mature Rainfed Olive Orchard

João Ilídio Lopes [1], Margarida Arrobas [2], Soraia Raimundo [2], Alexandre Gonçalves [2,3,4], Cátia Brito [3], Sandra Martins [3], Luís Pinto [3,4], José Moutinho-Pereira [3], Carlos M. Correia [3] and Manuel Ângelo Rodrigues [2,*]

[1] Direção Regional de Agricultura e Pescas do Norte, 5370-347 Mirandela, Portugal; joao.lopes@drapnorte.gov.pt
[2] CIMO—Centro de Investigação de Montanha, Instituto Politécnico de Braganca, 5300-253 Braganca, Portugal; marrobas@ipb.pt (M.A.); soraia-raimundo@live.com.pt (S.R.); agoncalves@morecolab.pt (A.G.)
[3] CITAB—Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, Quinta dos Prados, 5000-801 Vila Real, Portugal; cvqbrito@utad.pt (C.B.); scpmartins@utad.pt (S.M.); lpinto@morecolab.pt (L.P.); moutinho@utad.pt (J.M.-P.); ccorreia@utad.pt (C.M.C.)
[4] MORE—Collaborative Laboratory Mountains of Research, Brigantia Ecopark, 5300-358 Braganca, Portugal
* Correspondence: angelor@ipb.pt

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Abstract: Soil conditioners and beneficial microorganisms are important tools that can be used to increase the sustainability of agro-systems. However, the high diversity of conditions where they can be applied may influence the results, which requires extensive field research. In this study, a field trial of four years was conducted in olive (*Olea europaea* L.) to assess the effect of biochar, zeolites and a commercial mycorrhizal inoculum in the photosynthetic performance, nutritional status of trees, olive yield and soil properties. The experimental design also included a fertilizer treatment with nitrogen (N), phosphorus (P), potassium (K) and boron (B), which nutrients were applied at 50 kg ha^{-1} of N, P_2O_5 and K_2O and 2 kg ha^{-1} of B, and an untreated control. The mineral fertilizer treatment increased significantly the dry mass of pruning wood and the average olive yield by 21% over the control treatment. The mineral treatment increased plant N nutritional status, the most likely reason why the trees of this treatment performed better. Overall, the soil treatments had net photosynthetic rates similar to each other and higher than the control treatment, from the second year onwards. Biochar increased soil organic matter, as a result of the carbon (C) contained in the amendment itself, and probably by stimulating soil biological activity. Biochar and zeolites did not improve the productive performances of the tress, but increased the soil cation exchange capacity (CEC), which can benefit the system in the long-term. Mycorrhizal fungi did not show any benefit for soil or plants, which could mean that mycorrhization was not established, or their effect was not better than that of native microorganisms. In the conditions of this study, the interest of using commercial mycorrhizal fungi in a mature olive orchard seems to be low.

Keywords: *Olea europaea*; soil conditioners; soil improvers; biofertilizers; plant biostimulants

1. Introduction

The intensification of agriculture has been a response to the global demand for food. However, the intensive use of soils may create diverse problems of degradation, related to erosion, salinization, acidification or nutrient mining that reduce their productive potential [1]. There is currently a widespread awareness that soil is a non-renewable resource that must be preserved for future generations [2].

In the Northeast Portugal, soils can also be a breaking point in the increasing intensification of cropping, due to their low natural fertility. They are shallow, due to the sloping relief and continuous erosion, and have low levels of organic matter [3,4]. There is also no water available or irrigation infrastructures, most crops being rainfed managed. Plants have to deal with several types of environmental stress, including the high temperatures of the Mediterranean summer, which are getting worse due to climate change [5]. Currently, farmers tend to maintain crop productivity mainly by using chemical fertilizers. However, these products are often associated with environmental contamination, neamely water eutrophication and greenhouse gas emissions into the atmosphere [6] and shoul be reduced as much as possible. In recent years, several materials have been proposed to be a complement or an alternative to the use of fertilizers, as they can improve physical, chemical and/or biological soil properties and reduce the harmful effects of fertilization on the environment. Some materials, such as biochar and zeolites, usually known as soil conditioners, may have the potential to regulate nutrient bioavailability and to improve some physical and biological processes in soils [7,8]. The use of beneficial microorganisms, currently included in the group of plant biostimulants [9], is also expanding fast since they can facilitate plants to access nutrients and water, and provide protection against abiotic and biotic stresses. One of the groups that has been received increased attention are arbuscular mycorrhizal (AM) fungi [10].

Biochars are carbon-rich products obtained from thermochemical conversion of organic materials under limited oxygen conditions [11]. Biochars have been recommended as soil amendments for their positive effects on soil properties [12,13], environmental protection, by reducing greenhouse gas emissions [14], nitrate leaching [15] or phytostabilization of heavy metals [16], and/or on crop productivity [12–14]. However, although a large number of studies have shown some positive effects from the use of biochars, studies already exist were diverse inconsistencies were found [11,17,18]. A meta-analysis performed by Gao et al. [7] showed a fairly consistent increase in available P and an overall negative effect on the accumulation of inorganic N when biochars were applied to agricultural soils. The slowing of the N cycle is a major risk of the use of biochars as this can cause N deprivation during the growing season [15,19].

Zeolites represent a broad range of crystalline aluminosilicates of natural occurrence. There are over 40 species of natural zeolites, of which clinoptilolite is apparently the most abundant, both in soils and in sediments [20]. Synthetic zeolites can also be made from coal fly ash or biomass fly ash by hydrothermal treatments [21,22]. Generally, the structure of zeolites can be considered as an inorganic polymer built from $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedral linked by the sharing of all oxygen atom. When some of the Si^{4+} in the silica framework is replaced by Al^{3+}, this makes the framework negatively charged, which is compensated by the presence of extra-framework cations, usually alkali or alkali-earth metals [20]. Zeolites are characterized by the ease of retaining and releasing water and exchanging cations without structural changes [23]. Some experimental work has shown that the use of zeolites can improve soil properties [23,24] and enhance nutrient use efficiency and crop productivity [25–27]. In any case, studies with zeolites are much less abundant than with biochars, which increase the importance of obtaining more data about these materials, especially from field trials.

Most land plants including cultivated species can establish mutualistic relationships with endophytic fungi. Some fungi of the subphylum Glomeromycotina form arbuscules in the cortical cells of roots that facilitate exchanges of nutrients between the fungi and the host plants [28]. AM fungi may establish underground hyphal networks that increase the ability to explore the soil as an extension of the plant's root system [10]. As AM fungi are obligate biotrophs, the plant provides photosynthates for their proliferation [28,29]. In exchange, fungi provide nutrients and water and help plants to cope with abiotic and biotic stresses, which usually enhance crop productivity [30–32]. The importance of microorganisms for plants and the need to promote the sustainability of the agro-systems have increased the industry of commercial plant biostimulants, which include fertilizing materials containing microorganisms [9,33]. However, plant mycorrhization is ubiquitous in nature and the use of commercial mycorrhizal fungi can be redundant in face of the usual presence of indigenous inocula in the soil, which justifies further studies in field conditions.

Olive is the main crop in the Northeast of Portugal, one of the few that can generate some income even though it is grown in such poor ecological conditions. Due to the weaknesses of these agro-systems, it would be important to know whether soil conditioners and biofertilizers can enhance soil properties and improve tree crop performance and productivity. Thus, in this study the effect of two soil conditioners (biochar and zeolites) and a biofertilizer or plant biostimulant (commercial mycorrhizal inoculum) was compared to mineral fertilization and an untreated control, by measuring the photosynthetic performance of the trees, their nutritional status, the olive yield and soil properties in a rainfed managed orchard. Known from other studies the theoretical potential of these products to regulate nutrient cycling and nutrient use efficiency and, directly or indirectly, several soil properties, the working hypothesis for this study is that these materials will bring measurable benefits to soil and/or trees compared to mineral fertilization and control treatment.

2. Materials and Methods

2.1. Study Site

The field trial was carried out during four years in a non-irrigated olive orchard of mature trees (~30-year-old) of cv. Cobrançosa, located in Mirandela (41.513946; −7.187348), Northeast Portugal. The planting density is ~204 trees ha^{-1} (trees spaced at 7 m × 7 m). The climate of the region is typically Mediterranean, with an average annual air temperature of 14.3 °C and a cumulative annual rainfall of 509 mm. Average monthly temperature and precipitation recorded during the experimental period is shown in Figure 1. The parent material of the experimental plot is schist and the soil is loamy-sand textured (78.4% sand, 15.4% silt and 6.2% clay). Other selected soil properties determined in the beginning of the field trial are presented in Table 1.

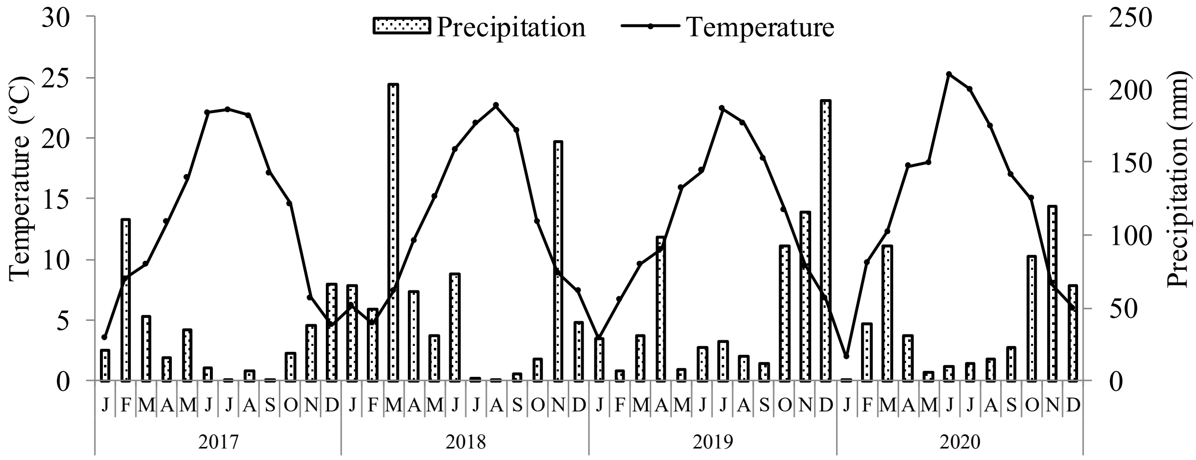

Figure 1. Monthly precipitation and average monthly temperature during the experimental period in Mirandela, Northeast Portugal.

Table 1. Selected properties (average ± standard deviation) of the 0–20 cm soil layer.

Soil Properties		Soil Properties (cont.)	
[1] Organic C (g kg^{-1})	7.82 ± 1.51	[5] Extract. Cu (mg kg^{-1})	2.8 ± 0.37
[2] pH (H_2O)	5.88 ± 0.10	[6] Exchang. Ca (cmol$_c$ kg^{-1})	3.93 ± 0.84
[2] pH (KCl)	4.66 ± 0.06	[6] Exchang. Mg (cmol$_c$ kg^{-1})	0.73 ± 0.20
[3] Extract. P (mg P_2O_5 kg^{-1})	77.8 ± 23.8	[6] Exchang. K (cmol$_c$ kg^{-1})	0.22 ± 0.08
[4] Extract. B (mg kg^{-1})	1.4 ± 0.23	[6] Exchang. Na (cmol$_c$ kg^{-1})	0.59 ± 0.52
[5] Extract. Fe (mg kg^{-1})	38.1 ± 5.62	[7] Exchang. acidity (cmol$_c$ kg^{-1})	0.08 ± 0.02
[5] Extract. Mn (mg kg^{-1})	61.5 ± 13.40	[8] CEC (cmol$_c$ kg^{-1})	5.55 ± 1.50
[5] Extract. Zn (mg kg^{-1})	2.0 ± 0.21		

[1] Walkley-Black; [2] Potentiometry; [3] Ammonium lactate; [4] Hot water, azomethine-H; [5] ammonium acetate and EDTA; [6] Ammonium acetate; [7] Potassium chloride; [8] Cation exchange capacity.

2.2. Experimental Design, Fertilizing Materials and Orchard Management

The experiment was arranged as a completely randomized design with five treatments: (i) biochar; (ii) zeolites; (iii) arbuscular mycorrhizal fungi; (iv) inorganic fertilization; and (v) non-fertilized control. Each treatment was applied to six trees (six replicates) of similar canopy volume in a row separated to a row of untreated trees.

Biochar was obtained in a pyrolytic reactor from woody biomass of silver wattle (*Acacia dealbata*) and was applied at a rate of 5 t ha^{-1}. The main physical and chemical properties of biochar are presented in Table 2. The zeolite was applied at a rate of 1 t ha^{-1}. The zeolite is a natural aluminosilicate of alkaline metals and alkaline earth metals. The main properties of the zeolite are also presented in Table 2. The mycorrhizal inoculum (Offyougrow Standard®, manufacturer Symbiom s.r.o., Sázava, Czech Republic) contained the propagules (spores, mycelium and colonized root fragments) of five different species of AM fungi (*Rhizophagus irregularis*, *Funneliformis mosseae*, *F. geosporum*, *F. coronatum* and *Claroideoglomus claroideum*) and a natural zeolite acting as a carrier. The rate of the commercial product applied was 680 kg ha^{-1} yr^{-1}. Biochar, zeolite and mycorrhizal fungi were applied only in the first two years (2017 and 2018) in this four-year study, in rates within the ranges recommended by the vendor, and considered sufficient to assess their effect on soil and plants but also for safety reasons, because the first two contain heavy metals although in low levels. Inorganic fertilization consisted of the program usually followed by local farmers, 50 kg ha^{-1} of N, P_2O_5 and K_2O, applied as a compound NPK (10: 10: 10) fertilizer every year. B was also applied in the inorganic treatment as borax (11% B) at a rate of 2 kg B ha^{-1} yr^{-1}. The amendments and fertilizers were applied late in march and were homogeneously spread beneath the tree canopy.

Table 2. Selected properties of biochar and zeolites used in the experiment (data provided by the manufacturer).

Biochar		Zeolites			
Moisture (%)	≤30	General formula: $(Ca, K_2, Na_2, Mg)_4Al_8Si_{40}O_{96}\cdot 24H_2O$			
Conduct (µS cm^{-1})	948	Mineral composition (%)		Chemical composition (%)	
Bulk dens. (g cm^3)	0.35–0.40	Clinoptilolite	84	SiO2	65.0–71.3
Particle size (mm)	≤8	Cristobalite	8	Al2O3	11.5–13.1
Ash (%)	≤5	Clay mica	4	CaO	2.7–5.2
Organic C (%)	≥90	Plagioclase	3–4	K2O	2.2–3.4
Volatiles (%)	≤5	Rutile	0.1–0.3	FeO3	0.7–1.9
pH	8	Quartz	Traces	MgO	0.6–1.2
Total N (%)	<0.5	Ion exchange (mol kg^{-1})		Na2O	0.2–0.3
Fe (mg kg^{-1})	99.5	Ca++	0.64–0.08	Physical and mechanical properties	
Pb (mg kg^{-1})	0.5	K+	0.22–0.45	Vol. density	1600–1800 kg m^{-3}
Hg (mg kg^{-1})	<0.1	Mg++	0.06–0.19	Porosity	24–32%
Cd (mg kg^{-1})	<0.05	Na+	0.01–0.19	Diameter of pores	0.4 nm
		CEC	1.2–1.59	Specific surface	30–60 m^2 g^{-1}

In this orchard the weeds have been usually controlled by conventional tillage with two passes of cultivator during spring. This soil management system was maintained during the experimental period. The first tillage was done shortly after the application of the amendments and fertilizers allowing their incorporation into the soil along with the control of weeds. The second tillage was performed late in spring, the date depending on whether the spring was more or less rainy. Tillage depth was between 10 to 15 cm.

During the experimental period the tagged trees received a light pruning every year as a mean of maintaining a similar foliar area over the years. The pruning wood was used as an indicator of the response of the trees to the fertilizer and amendment treatments. It was not necessary to apply pesticides during the experimental period since no relevant phytosanitary problems were observed. The harvest was done by a branch shaking machine by which the fruit were pull down. Sheets spread on the floor allowed to recover the fruits, which were weighed separately per tree.

2.3. Field Determinations

The trees were pruned every year as mentioned above. Total pruning wood was weighed in fresh per individual tree. Thereafter, a random subsample was taken and separated into stems and leaves and weighed also in fresh. The subsamples were then sent to the laboratory, oven-dried at 70 °C and weighed dry. The procedure allowed to use the pruning wood as a result of this experiment. Olive yield per tree was also determined in the field as mentioned above.

Leaf gas exchange measurements were performed during the 4 years of the experiment in healthy and full expanded mature leaves on cloudless mornings (photosynthetic photon flux density above 1500 µmol m^{-2} s^{-1}) using a portable IRGA (LCpro+, ADC, Hoddesdon, UK), operating in the open mode. Net photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1}) and the ratio of intercellular to atmospheric CO_2 concentration (C_i/C_a) were estimated using the equations developed by von Caemmerer and Farquhar [34]. Intrinsic water use efficiency was calculated as the ratio of A/g_s (µmol mol^{-1}).

2.4. Field Sampling and Laboratory Analysis

Twice a year, in the summer (late July) and in the winter (December) leaf samples were taken by the standard procedure for olive to monitor the nutritional status of the trees. Young fully developed leaves were detached from the middle of the shoots developing in the current growing season from all quadrants. In June 2020, the soil was also sampled at three depths (0.0–0.1 m, 0.1–0.2 m and 0.2–0.3 m), beneath the canopy in the zone were the fertilizers and amendments were applied. Each soil sample was prepared as a composite sample by collecting and mixing soil from 10 points. From each treatment, three composite soil samples were prepared, sent to the laboratory, oven-dried at 40 °C and sieved (2 mm mesh).

The dried and sieved soil samples were submitted to the following analytical determinations: (1) pH (H_2O and KCl) (by potentiometry); (2) organic C (Walkley-Black method); (3) cation exchange capacity (ammonium acetate, pH 7.0); (4) extractable P and K (ammonium lactate solution at pH 3.7); (5) extractable B (hot water, and azomethine-H method); (6) extractable Fe, Mn, Zn and Cu (ammonium acetate and EDTA, determined by atomic absorption spectrometry). In the initial samples there were also determined (7) clay, silt and sand fractions (Robinson pipette method). Methods 1–3 and 5–7 are fully described by Van Reeuwijk [35] and method 4 by Balbino [36].

Elemental analyses of tissue samples were performed by Kjeldahl (N), colorimetry (B and P), and atomic absorption spectrophotometry (K, Ca, Mg, Fe, Mn, Cu, Zn) methods [37] after tissue samples had been digested with nitric acid in a microwave.

2.5. Data Analysis

Data was firstly tested for normality and homogeneity of variances using the Shapiro-Wilk test and Bartlett's test, respectively. The comparison of the effect of the fertilizer treatments was provided by one-way ANOVA. When significant differences were found ($\alpha < 0.05$), the means were separated by the multiple range Tukey HSD test ($\alpha = 0.05$). In the analysis of data related to soil properties, the randomized block model was considered, with soil depths entering the model as blocks.

3. Results

3.1. Olive Yield and Pruning Wood

Average total olive yield varied between 31.8 kg $tree^{-1}$ in the biochar treatment and 44.4 kg $tree^{-1}$ in the mineral fertilizer treatment (Figure 2). However, these values were not significantly different at $p < 0.05$ level. The olive yields of 2017 and 2019 varied significantly between treatments. The higher average values in 2017 were found in the mycorrhizal (12.7 kg $tree^{-1}$) and in 2019 in the mineral fertilizer (18.7 kg $tree^{-1}$) treatments.

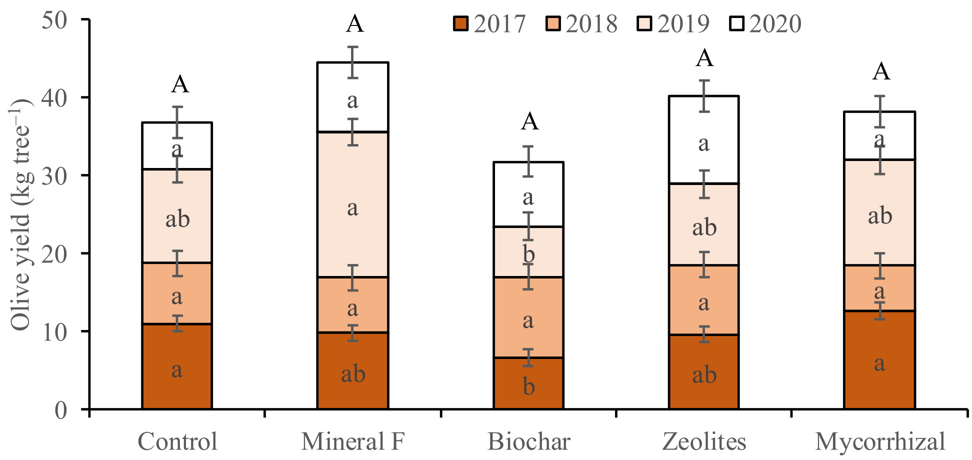

Figure 2. Annual olive yield and accumulated total. Separated by year (lowercase letters) and total (uppercase letters), means followed by the same letter were not significantly different by Tukey HSD test (α = 0.05). Vertical bars are the standard errors.

Pruning wood followed a similar hierarchy between treatments to that observed with olive yield (Figure 3). However, in this case, total pruning wood varied significantly between treatments. The highest average value was found in the mineral fertilizer treatment (21.5 kg tree^{-1}), whereas the lowest one was in the control treatment (17.5 kg tree^{-1}). Separated by years, significant differences between treatments were found in 2017 and 2020, with the highest average values to be found respectively in the zeolites (5.0 kg tree^{-1}) and in mineral fertilizer (6.8 kg tree^{-1}) treatments, respectively.

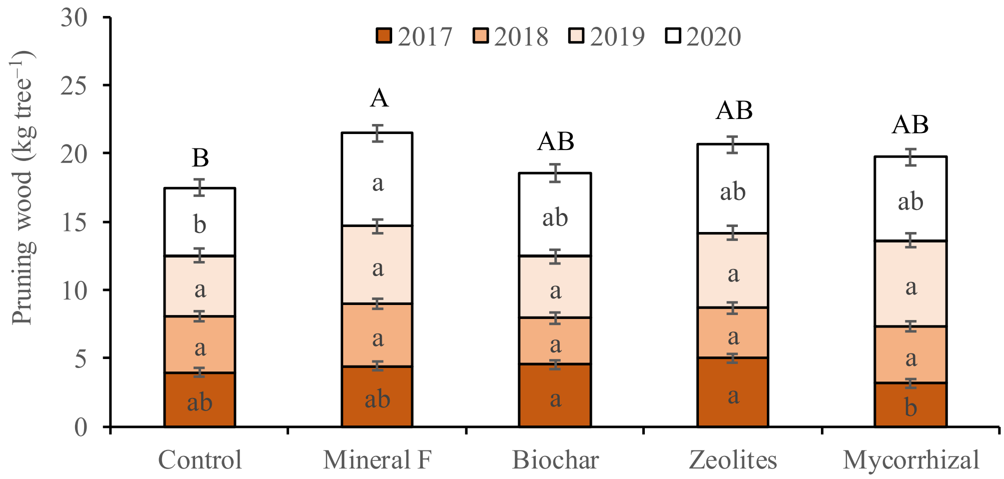

Figure 3. Annual pruning wood and accumulated total. Separated by year (lowercase letters) and total (uppercase letters), means followed by the same letter were not significantly different by Tukey HSD test (α = 0.05). Vertical bars are the standard errors.

3.2. Leaf Gas Exchange

The response of leaf gas exchange variables to the applied treatments varied with the monitored dates (Figures 4 and 5). Regarding net photosynthesis and stomatal conductance, a significant influence of treatments was only observed from the second year of the study (Figure 4). Although several fluctuations among the sampling dates were recorded, in general trees from control treatment had more frequently lower CO_2 assimilation rates in a closely association with g_s values. Nevertheless, non-stomatal limitations were also evident as supported by the trend towards lower A/g_s and greater C_i/C_a in control treatment (Figure 5). Meanwhile, there was no relevant trend for the existence of significant differences among soil treatments that received mineral fertilization, soil conditioners or the plant biostimulant.

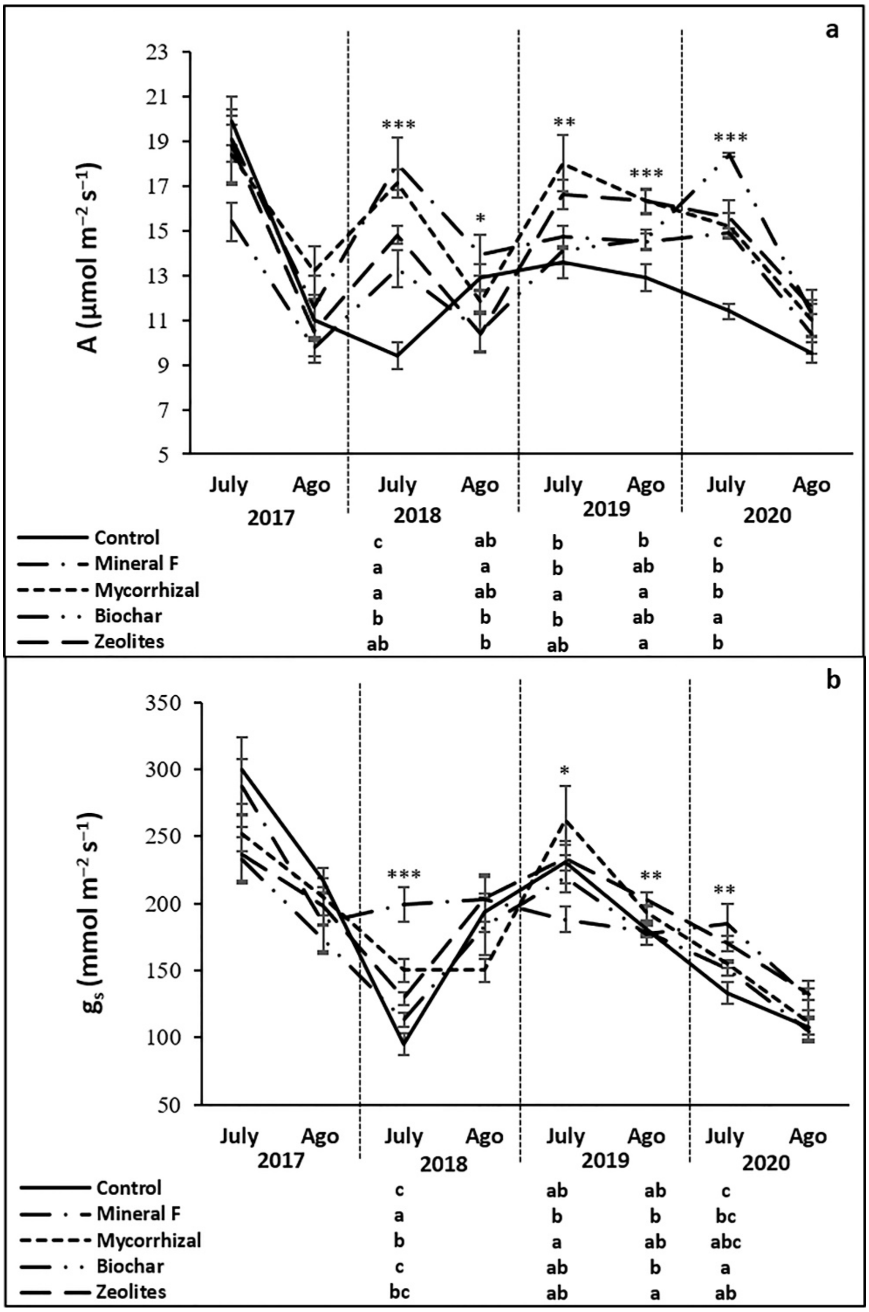

Figure 4. Evolution of net photosynthetic rate (**a**) and stomatal conductance (**b**) throughout the experiment. Different letters demonstrate significant differences by Tukey HSD test ($p < 0.05$) between treatments in each date. Vertical bars are the standard errors; * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) are the results of analysis of variance.

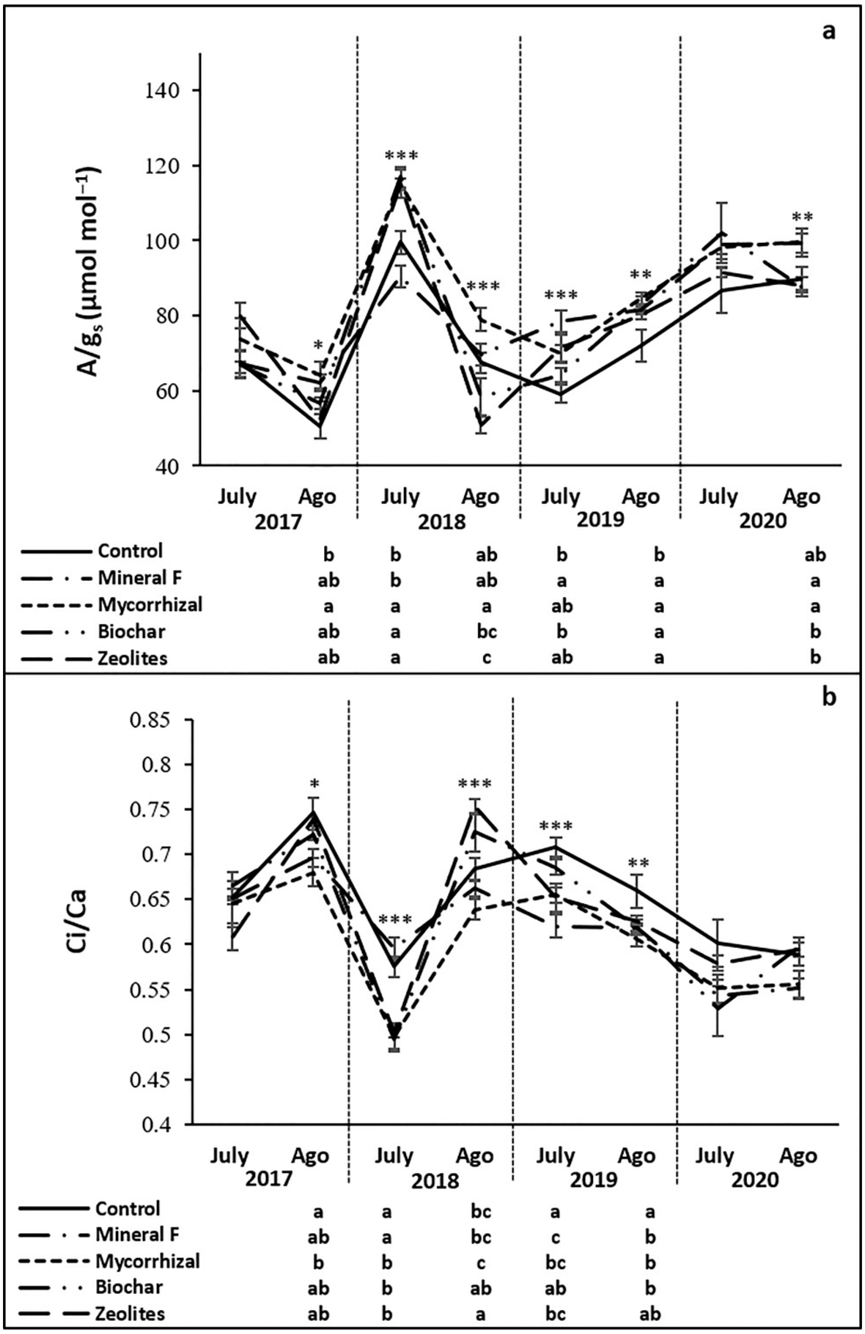

Figure 5. Evolution of intrinsic water use efficiency (**a**) and the ratio of intercellular to atmospheric CO_2 concentration (**b**) throughout the experiment. Different letters demonstrate significant differences by Tukey HSD test ($p < 0.05$) between treatments in each date. Vertical bars are the standard errors; * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) are the results of analysis of variance.

3.3. Plant Nutritional Status

Leaf N concentration varied significantly between treatments in four of the seven sampling dates (Figure 6). The line corresponding to the mineral fertilizer treatment usually appeared at the top of the figure. The lines of biochar, mycorrhizal and control are those that most frequently appeared below the set of lines. In general, leaf N concentrations appeared close to or below the lower limit of the sufficiency range. Leaf P concentration varied significantly between soil treatments in six of the seven samplings. The line of control and mycorrhizal treatments were those observed more frequently in the lower part of the set of lines, even if the last sampling date was an exception for mycorrhizal treatment. Anyway, leaf P levels were usually found within the sufficiency range of the element for olive. Leaf K concentration varied significantly between treatments in two of the seven samplings. Overall, the values fluctuated greatly between sampling dates but without a coherent trend between treatments. Sometimes, K levels were found below the lower limit of the sufficiency range. Regarding the other nutrients analysed, no significant differences between treatments were usually found, and when they occasionally occurred, no consistent trend was found with the other sampling dates (data not shown).

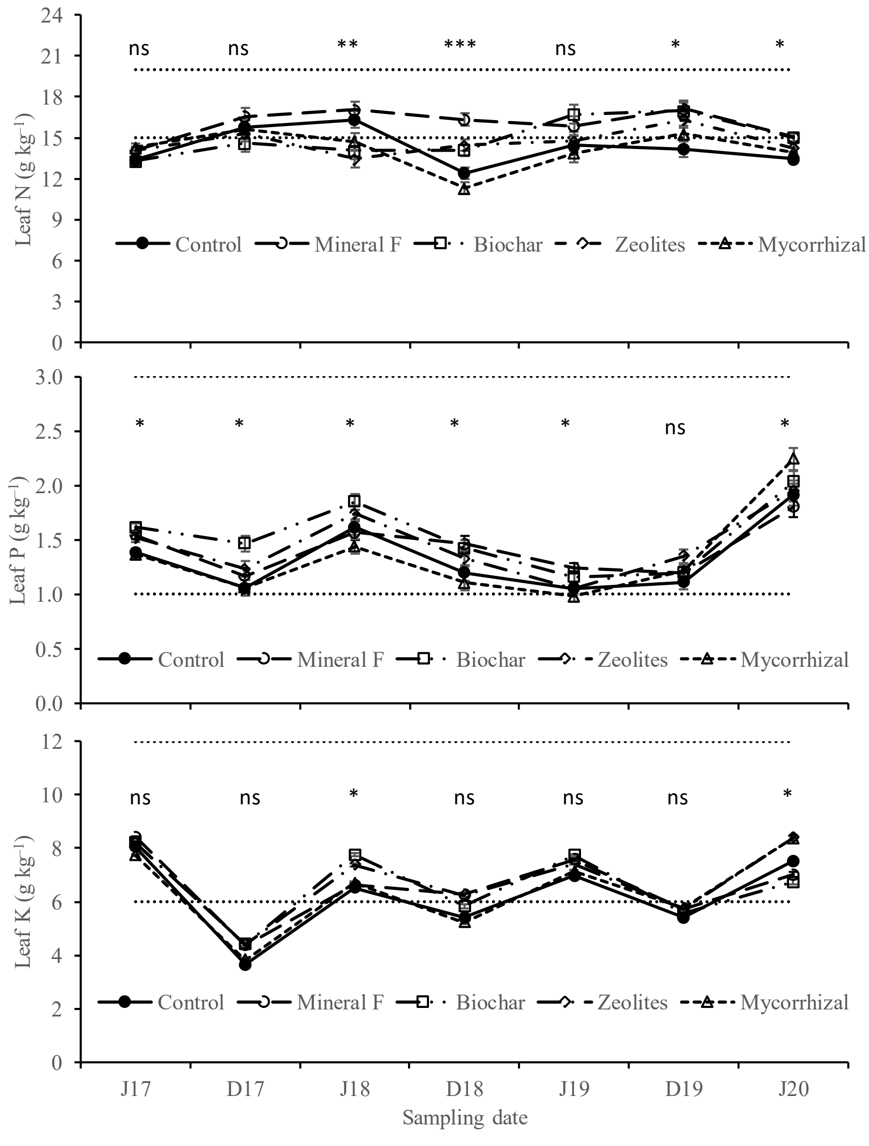

Figure 6. Leaf concentrations of nitrogen (N), phosphorus (P) and potassium (K) in seven consecutive samplings in July (J) and December (D) from July 2017 (J17) to July 2020 (J20). Horizontal dashed lines are the lower and higher limits of the sufficiency ranges; vertical bars the standard errors; ns (not significant), * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) are the results of analysis of variance.

3.4. Soil Properties

Soil treatments influenced significantly some relevant soil properties, such as organic matter, extractable P and CEC (Figure 7). Soil organic C varied significantly between soil layers, with the average values decreasing from 10.0 to 3.4 g kg^{-1} between the layers 0.0–0.1 m and 0.2–0.3 m. The mineral fertilizer and biochar treatments resulted in significantly higher levels of organic C than in the control, zeolites and mycorrhizal treatments. Extractable P decreased significantly from the surface layer to the layers below, with values ranging from 76.6 mg P_2O_5 kg^{-1} to 24.7 mg P_2O_5 kg^{-1}, respectively in the layers 0.0–0.1 m and 0.2–0.3 m. The levels of P in the soil also varied significantly between treatments. The mineral fertilizer treatment displayed the higher value (71.5 mg P_2O_5 kg^{-1}), the lower one being found in the mycorrhizal treatment (30.1 mg P_2O_5 kg^{-1}). CEC varied significantly from the depth 0.0–0.1 m (7.4 $cmol_c$ kg^{-1}) to 0.1–0.2 m (6.4 $cmol_c$ kg^{-1}), but not from the latter to 0.2–0.3 m (5.7 $cmol_c$ kg^{-1}). CEC also varied significantly with soil treatments. The biochar and zeolites treatments gave values significantly higher than the control, mineral fertilizer and mycorrhizal treatments. In the surface layer, the increase in exchange sites was occupied mainly by Ca and to a lesser extent by K. In the case of the biochar and zeolites treatments, the increase of exchange sites was occupied mainly by Na, Ca and to a lesser extent by K. Many other soil properties were determined, usually without significant differences between treatments, and were considered of little relevance for the interpretation of the results of this experiment (data not shown).

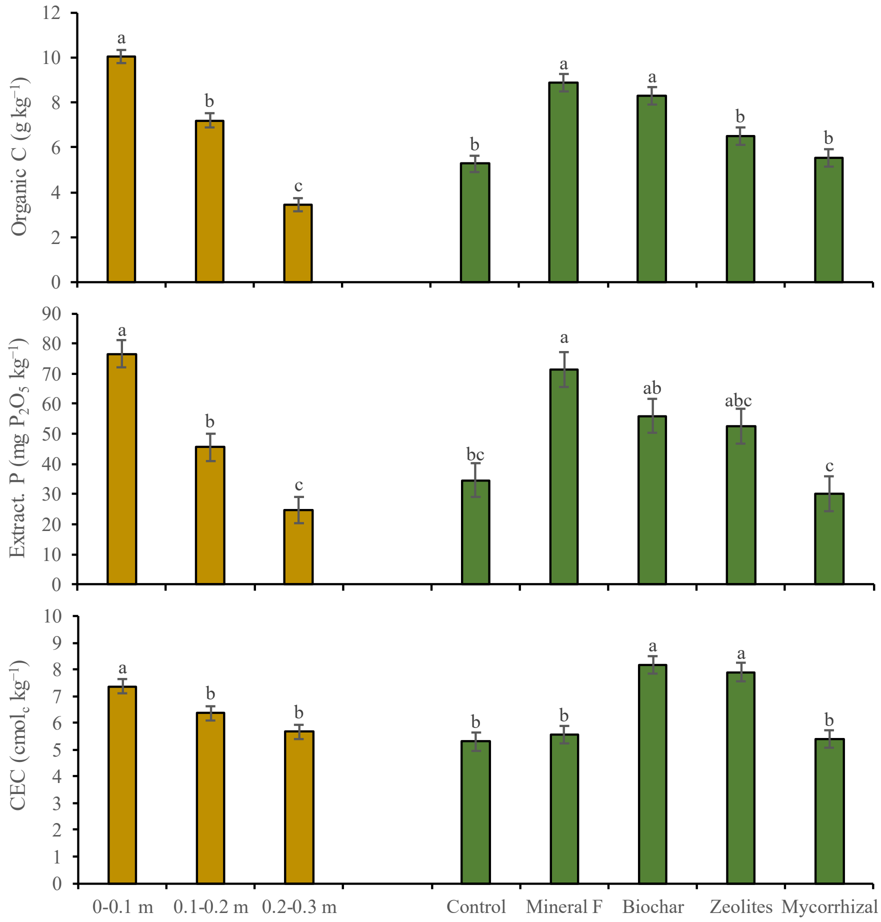

Figure 7. Organic carbon (C), extractable phosphorus (P) and cation exchange capacity (CEC) as a function of soil depth and treatment. Within soil depth and treatment, means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05). Error bars are the standard errors.

4. Discussion

Although no significant differences were found in the accumulated olive yield in the four years of study, the average value of the mineral fertilizer treatment was higher than that of the other treatments. Furthermore, the sum of the pruning wood from the four pruning events was significantly higher in the mineral fertilizer in comparison to the other treatments, namely the control, allowing to agree that mineral fertilization had a very positive effect on the performance of the trees. The mineral fertilizer treatment also increased the N nutritional status of the trees. Leaf N concentration in mineral fertilizer treatment persisted within the sufficiency range, as set by Fernández-Escobar [38], while in the other treatments leaf N concentration was found close to the lower limit of the sufficiency range or even often below. Considering the concentration of other minerals in the leaves, usually within the sufficiency ranges, we may infer that the effect of treatments on trees' performance can be mainly attributed to N. This soil has a low content of organic matter and clay minerals, which are the soil constituents responsible for the largest soil N reserves, organic matter holding the organic N fraction and clay minerals interlamellar NH_4^+, with a role in balancing the negative charges resulting from the isomorphic substitutions of Si^{4+} by Al^{3+} [1]. Thus, this type of soil provides little N to the plants. In previous studies it was found that olive [39,40] and many other tree species [3,41] grown in similar soils respond clearly to the application of N, in contrast to that sometimes occurs in regions of clayey soils with a greater capacity to supply nutrients to plants [42,43].

Generally, all soil treatments contributed to improve olive tree gas exchange responses relatively to control plants, at least during certain periods of the experiment. The positive influence was more evident on A than on g_s and, as a consequence, A/g_s was generally higher with the application of soil amendments than in control, while the ratio Ci/Ca tend to be lower. This combination of results demonstrates that the positive effects of soil treatments were mainly due to lower non-stomatal limitations to photosynthesis. It should also be noted that, despite the non-application of mineral fertilizers in biochar, zeolites and mycorrhizal treatments, their photosynthetic performance was identical to mineral fertilizer treatment, which is of great interest. Biochar has been reported to benefit soil properties and, consequently, crop productivity [12–14], although inconsistencies in the observation of positive effects have also been frequent [11,44]. It has also been found that the effect of biochar on soils and crops may vary depending on feeding material and pyrolysis conditions, particle size and application rate and methodology, as well on soil texture and pH [45–47], which makes it difficult to clarify the value of these materials as soil conditioners. Moreover, most of the positive impacts of biochar application has been observed when biochar was applied with other organic amendments and fertilizers [48]. Normally, there is greater consensus to consider that biochar is a win-win solution to C sequestration and ecosystem function. Zeolites are characterized by the ease of retaining and releasing water and exchanging cations without structural changes [21]. In this study, however, the use of zeolites was also not reflected in the improvement of the tree's performances. Arbuscular mycorrhizal fungi are known for their potential to favour water and nutrient acquisition by plants [10], and in a very particular way the increased supply of P [49,50] due to their role in solubilizing P from P-sparse sources usually not available to host plants [28]. AM fungi usually also increase Ca and Mg acquisition by plants in acidic soils [51]. However, although this soil is very acidic, which could facilitate the observation of positive effects on P, Ca and Mg acquisition from the application of AM fungi, perhaps the native microorganisms, well adapted to these conditions, have obscured the role of the commercial mycorrhizal fungi. Thus, in this study, no beneficial effect of using commercial AM fungi was observed either due to a deficient establishment of mycorrhiza or due to their inability to provide better conditions to plants than native soil microbiology. Mycorrhizal fungi can also favour the supply of N to plants. Koller et al. [29] hypothesized that AM fungi and protozoa interactively facilitate plant N acquisition from organic matter. However, these soils are very poor in organic matter which minimizes a possible benefit for the plant through this route.

Soil organic matter was significantly higher in the mineral fertilizer and biochar in comparison to the other treatments. However, the higher levels of organic matter in the soil in the mineral fertilizer and biochar treatments may be due to different reasons. Mineral fertilization, in particular soil N availability, tends to increase herbaceous vegetation [4,40] and, just as there was recorded an expansion of the aerial part of the trees measured as pruning wood, probably the root system also increased due to the higher availability of photosynthates. Thus, it is likely that the increase in organic matter in the soil in the mineral fertilizer treatment was due to the increase in the debris of the herbaceous vegetation and the enhanced activity of the root system of the trees. In the case of biochar, it is likely that it is only C added by the application of the product due to its recalcitrant property in the soil [12,17]. However, it is known that biochar can protect some soil microbiology, due to its high porosity, and increase biological activity due to better soil aeration [17,52], which may mean that part of the organic C found in this treatment may correspond to microbial C.

Soil P levels were higher in the mineral fertilizer than in the other treatments, probably due to the direct application of P in the compound NPK fertilizer. It should be noted that mycorrhizal fungi, although can benefit the plant from several ways, the most universally reported effect is their contribution to P acquisition, as previously mentioned. The low levels of P in the soil, are a convincing proof that mycorrhization has not occurred at a relevant level. In addition to the fact that higher levels of P in the leaves were not detected by the application of mycorrhizal fungi, which could be justified by the fact that P accumulates preferentially in the roots [53], soil samples should have shown higher levels of P. Soil sampling and sieving (2 mm) procedures did not remove the hyphae of the fungi and thinner roots, which means that if the mycorrhiza had been established, an increase of P in the soil should be observed due to the increased content of P in these tissues, as reported by Rodrigues et al. [54]. The application of commercial mycorrhizal fungi on mature trees has not been common. The positive effects of plant inoculation with mycorrhizal fungi have been obtained mainly in young nursery plants or when they are being installed in the field [30,51,54].

The application of biochar and zeolites increased soil CEC. Biochar possesses high surface area and porosity and a variety of functional groups with high ion adsorption potential [11,17], these being the reasons of the increase of CEC through the application of biochar. Some of the properties which make zeolites and interesting soil conditioner for agricultural purposes are high cation exchange capacity, high water holding capacity and high adsorption capacity [23]. Under certain conditions, these properties can benefit plant growth, which was not the case of this experiment. Although no short-term benefits for plants were recorded, CEC is an important soil property, as it helps to regulate the retention of cations such as NH_4^+, Ca^{2+}, Mg^{2+} and K^+ in the soil [1] and should be considered a positive aspect of the application of these soil conditioners.

5. Conclusions

The mineral fertilization improved the performance of trees, mainly due to the supply of N, in a soil of reduced natural N availability, due to low level of organic matter and 2: 1 clay minerals. Biochar and zeolites did not show beneficial short-term effects to plants. However, they contributed to increase CEC, a relevant soil property that helps to regulate the availability of cation nutrients (such as NH_4^+, Ca^{2+}, Mg^{2+} and K^+) in the soil reducing the risk of being leached out. No benefits were found from the use of commercial mycorrhizal fungi. No improvement was noted in the trees' performance, nutritional status or soil properties. The reason for the failure was attributed to its application to mature trees probably already mycorrhized with native soil microorganisms better adapted to local ecological conditions.

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