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Combined biochar and organic waste have little effect on chemical soil properties and plant growth

La combinación de biochar y residuos orgánicos tiene un efecto reducido en las propiedades químicas del suelo y el crecimiento de las plantas

Biochar combinado com resíduos orgânicos tem um reduzido efeito nas propriedades químicas do solo e no crescimento das plantas

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ABSTRACT

Biochar has received great attention as a soil conditioner since it can potentially sequester carbon (C) in soil, enhance soil physical, chemical and biological properties and improve crop productivity. This study reports the results of a pot experiment with olive (*Olea europaea* L.), carried out in an acidic and clay loam textured soil, and cultivated during two growing seasons under eight fertilization treatments. They included mineral fertilization equivalent to a rate of 100 kg ha^{-1} of N, P_2O_5 and K_2O (NPK), biochar applied at a rate of 10 (B10), and at 20 (B20) t biochar ha^{-1}, biochar-NPK mixture (B10+NPK), biochar-waste mixtures with mushroom waste compost (B10+MWC), olive mill waste (B10 + OMW), and municipal solid waste (B10+MSW), the organic materials applied at a rate of 20 t ha^{-1}, together with a treatment without fertilization (control). Biochar in the B20 treatment increased the soil C content in comparison to the control. Biochar in the B10+NPK treatment reduced soil nitrate levels compared to NPK treatment. No other benefits to soil properties, nutrient uptake or plant growth were observed with the use of biochar or any positive synergistic effect with the mixture of biochar with the other organic amendments. MSW, OMW and MWC tended to increase soil pH in comparison to the control. Most of the studies with biochar were carried out in soils with edaphic limitations or harsh environmental conditions limiting plant growth, which may have facilitated the detection of favorable effects. Under less limited soils or stressful conditions for plants, such as the ones established in this experiment, the benefits of using biochar were poor.

RESUMEN

*El biochar ha recibido gran atención como acondicionador de suelo ya que puede potencialmente secuestrar carbono (C) en el suelo, mejorar sus propiedades físicas, químicas y biológicas y promover la productividad de los cultivos. Este trabajo muestra los resultados de una experiencia en macetas con olivo (*Olea europaea *L.), realizada en un suelo ácido y con textura franco arcillosa, cultivado durante dos estaciones de crecimiento. Se utilizaron ocho tratamientos, incluyendo fertilización mineral equivalente a 100 kg ha*$^{-1}$ *de N,* P_2O_5 *y* K_2O *(NPK), biochar aplicado a las dosis de 10 (B10) y 20 (B20) t ha*$^{-1}$*, mezclas de biochar con fertilización mineral (B10+NPK) y con residuos de hongos*

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(B10+MWC), residuos de aceite de oliva de almazaras (B10+OMW) y residuos sólidos urbanos (B10+RSU), aplicados a una tasa de 20 t ha^{-1}, y un tratamiento sin fertilización (control). El biochar en el tratamiento B20 aumentó el contenido de C en el suelo en comparación con el control. El biochar en el tratamiento B10+NPK redujo los niveles de nitrato del suelo en comparación con el tratamiento NPK. No se registró ningún otro beneficio sobre las propiedades del suelo, la absorción de nutrientes o el crecimiento de las plantas por el uso de biochar o cualquier efecto sinérgico positivo con la mezcla de biochar con los otros correctivos orgánicos. MSW, OMW y MWC tendieron a aumentar el pH del suelo en comparación con el control. Muchos de los estudios anteriores con biochar se hicieron en suelos con limitaciones edáficas o condiciones ambientales adversas, lo que puede haber facilitado la aparición de efectos favorables. En condiciones menos estresantes para las plantas, como las establecidas en esta experiencia, los beneficios del uso del biochar parecen ser menos evidentes.

RESUMO

*O biochar tem recebido grande atenção como condicionador de solo, pois pode potencialmente sequestrar carbono (C) no solo, melhorar as propriedades físicas, químicas e biológicas do solo e promover a produtividade das culturas. Este trabalho relata os resultados de uma experiência em vasos com oliveira (*Olea europaea *L.), realizado em um solo ácido e de textura franco-argilosa, cultivada durante duas estações de crescimento. Oito tratamentos foram impostos, incluindo adubação mineral equivalente a 100 kg ha^{-1} de N, P_2O_5 e K_2O (NPK), biochar aplicado nas doses de 10 (B10) e 20 (B20) t ha^{-1}, misturas de biochar com adubação mineral (B10+NPK) e com composto de resíduos de cogumelos (B10+MWC), resíduos de lagar de azeite (B10+OMW) e resíduos sólidos urbanos (B10+RSU), aplicados a uma taxa de 20 t ha^{-1}, e um tratamento sem fertilização (testemunha). O biochar no tratamento B20 aumentou o teor de C no solo em comparação à testemunha. O biochar no tratamento B10+NPK reduziu os níveis de nitrato do solo em comparação com o tratamento NPK. Nenhum outro benefício sobre as propriedades do solo, a absorção de nutrientes ou o crescimento das plantas foi registado pelo uso de biochar ou qualquer efeito sinérgico positivo com a mistura de biochar com os outros corretivos orgânicos. MSW, OMW e MWC tenderam a aumentar o pH do solo em comparação à testemunha. Muitos dos estudos anteriores com biochar foram feitos em solos com limitações edáficas ou condições ambientais adversas, o que pode ter facilitado o surgimento de efeitos favoráveis. Em condições menos stressantes para as plantas, como as estabelecidas nesta experiência, os benefícios do uso do biochar parecem ser menos evidentes.*

KEY WORDS
Mineral NPK fertilizers, mushroom waste compost, olive mill waste, *Olea europaea*, pot experiment, nutrient uptake.

PALABRAS CLAVE
Fertilizantes minerales NPK, compost de residuos de hongos, residuos de almazaras, *Olea europaea*, cultivo en macetas, absorción de nutrientes.

PALAVRAS-CHAVE
Fertilizantes NPK, resíduos sólidos urbanos, composto de resíduos de cogumelos, resíduos de lagar de azeite, *Olea europaea*, cultivo em vasos, absorção de nutrientes.

1. Introduction

In many areas of the Mediterranean region, particularly in areas of steep terrain, water erosion and slow pedogenesis due to the climatic conditions have led to shallow soils with low organic matter that results in agroecosystems with low crop productivity (Rodrigues and Arrobas 2017). Herbaceous agriculture can be unproductive under these conditions and farmer incomes are dependent on species well adapted to these poorly developed soils and summer drought stress, such as olive, almond and grape. When this is coupled to a low production of farmyard manures due to limited feedlotting in areas with extensive grazing, sustainability of agriculture is impaired.

This can be faced by using alternative organic residues, such as municipal solid waste (MSW) and agro-industrial residues for the management of soil fertility in these agro-systems. A large number of previous studies have demonstrated the important role of organic amendments in the enhancement of physical, chemical and biological soil properties and crop productivity (Calleja-Cervantes et al. 2015; Obriot et al. 2016; Yazdanpanah et al.

2016; Bastida et al. 2017). This is why their use in agriculture is, admittedly, the best destination for these organic resources, allowing recycling of their nutrients for food production and restoring important soil properties instead of being burned or landfilled (Santos 2015), in agreement with the Circular Economy Principles of the EU in terms of self-sufficiency for raw material (Pardo and Schweitzer 2018). In NE Portugal the main organic resources available are municipal solid waste, olive mill waste and mushroom waste compost from local industries.

Biochar has also begun to be commercially used in the region. Biochar is a C-based material produced from the pyrolysis of biomass under partial or complete oxygen limitation and temperatures ranging from 300 to 1000 °C (Kavitha et al. 2018). Any type of biomass can be submitted to pyrolysis producing a material with a porous structure with high outer and inner surface area which is highly recalcitrant in soils because of the aromatic nature of the C in the resulting materials (Dispenza et al. 2016; Kavitha et al. 2018; Bian et al. 2019). Although it may contain high intrinsic amounts of nitrogen (N), phosphorus (P) and sulfur (S) (Sohi et al. 2010; Farrell et al. 2014), they are mostly in non-bioavailable forms, something that explains why biochar is mainly seen as a soil conditioner. On the other hand, it has been shown that biochar increases the C sequestration in the soil and improves its quality due to the highly recalcitrant nature of carbonated structures in them (Arif et al. 2017; Kavitha et al. 2018).

In addition, biochar can have a fundamental role in improving the physico-chemical and biological properties of soil, thereby potentially increasing crop yield (Liu et al. 2017; Jin et al. 2019; Langeroodi et al. 2019). The use of biochar can promote soil permeability and water holding capacity (Gul et al. 2015; Langeroodi et al. 2019), liming acid soils (Chan et al. 2007; Jin et al. 2019), and providing some soil available P (Farrell et al. 2014; Gao et al. 2019; Liu et al. 2017). Soil aeration provided by biochar may also increase soil N retention, by stimulating microbial N immobilization, and reducing N losses by leaching and denitrification (Esfandbod et al. 2017; Hawthorne et al. 2017; Li et al. 2019). Biochar has been also shown to enhance microbial communities (Masto et al. 2013; Liu et al. 2017; Wong et al. 2019) and to immobilize soil contaminants, and therefore reduce their bioavailability and prevent their toxicity, such as heavy metals or harmful organic compounds (Shaaban et al. 2018; Meng et al. 2019; Palansooriya et al. 2019).

However, biochar properties are highly variable dependent on the feedstock used, the pyrolysis conditions, and the soil type and the existing biotic interactions (Kavitha et al. 2018). A recent meta-analysis that included 124 biochar application response studies (Gao et al. 2019) and several review papers (Kavitha et al. 2018; Shaaban et al. 2018; Palansooriya et al. 2019) have claimed that biochar can have a positive effect on plant response. Nevertheless, they also have reported detrimental effects on crops in some scenarios. In addition, the same biochar may not necessarily play a positive role in all types of soils, and similarly, some biochars can significantly improve production in certain soil types, while the same biochar has no effect when placed in another soil or even causes significant decline in production (Shaaban et al. 2018).

In light of the above, it seems important to add more knowledge on biochar by doing research on a diversity of agro-pedo-ecological conditions. An example of this is the use of biochar in mixtures with other organic amendments, in an attempt to draw benefits from possible synergistic effects of the mixtures able to increase of the nutrient use efficiency of the organic amendment. The fertility of the soils of the Mediterranean basin is low and is expected to worsen in the context of global warming, so it is relevant to study a more efficient use of the scarce resources available. Thus, the working hypothesis is that biochar, particularly when mixed with organic amendments, could promote soil properties and olive plant growth thereby replacing mineral fertilization. Thus, in this work biochar was mixed with three organic amendments and a NPK mineral fertilizer and compared with biochar used alone, mineral fertilization, and a non-fertilized control for the promotion of olive cuttings grown in outdoor pots during two growing seasons and under Mediterranean conditions.

2. Material and Methods

The experiment was conducted in outdoor pots. Each pot received 10 kg of dry soil sieved in 6 mm mesh, to which the organic materials an d mineral fertilizers were added according to the experimental design. Some of the main soil properties at the beginning of the experiment are shown in **Table 1**.

Table 1. Selected physical and chemical properties (average ± standard deviation) of the soil used in the experiment

[1]Particle size distribution		Organic carbon (C)	
Clay (%)	37 ± 3.0	[5]Total C (g kg^{-1})	13.81 ± 1.47
Silt (%)	22 ± 2.6	[6]EOC (g kg^{-1})	4.59 ± 1.34
Sand (%)	41 ± 3.6	[7]Extractable P and K	
	(Clay loam)	P (mg P_2O_5 kg^{-1})	118.9 ± 59.9
[2]pH		K (mg K_2O kg^{-1})	97.3 ± 16.2
H_2O	5.56 ± 0.06	[8]Soil exchange complex	
KCl	4.14 ± 0.12	Ca^{++} ($cmol_c$ kg^{-1})	5.04 ± 0.81
Micronutrients		Mg^{++} ($cmol_c$ kg^{-1})	1.34 ± 0.27
[3]Boron (mg kg^{-1})	0.8 ± 0.07	K^{+} ($cmol_c$ kg^{-1})	0.27 ± 0.03
[4]Copper (mg kg^{-1})	6.5 ± 2.36	Na^{+} ($cmol_c$ kg^{-1})	0.19 ± 0.07
[4]Zinc (mg kg^{-1})	3.7 ± 1.86	Exchang. acidity ($cmol_c$ kg^{-1})	0.17 ± 0.06
[4]Iron (mg kg^{-1})	43.3 ± 9.57	Exchang. Al ($cmol_c$ kg^{-1})	0.17 ± 0.06
[4]Manganese (mg kg^{-1})	23.1 ± 6.82	CECe ($cmol_c$ kg^{-1})	7.00 ± 1.17

[1]Robinson pipette method; [2]Potenciometry; [3]Hot water azomethine-H; [4]EDTA+acetic acid+ammonium acetate; [5]Incineration; [6]Walkley-Black; [7]Ammonium lactate; [8]Ammonium acetate for bases and potassium chloride for exchangeable acidity (EA) from which effective cation-exchange capacity (CECe) was estimated (Houba et al. 1997).

The experimental setup was installed on 15 June 2016 at the University of Trás-os-Montes and Alto Douro, located at 41º 28' 82.59'' N and 7º 73' 68.52'' W, at an altitude of 450 m asl. The region benefits from a Mediterranean climate, Csb in the Köppen classification, with an average annual temperature and precipitation of 13.6 °C and 1018 mm, respectively.

The experiment included eight treatments arranged in a completely randomized design with four replicates (pots) each. The treatments corresponded to NPK (100 kg N ha^{-1} as ammonium nitrate, 34.5% N); 100 kg P_2O_5 ha^{-1} as superphosphate, 18% P_2O_5; and 100 K_2O ha^{-1} as potassium chloride, 60% K_2O), B10 (10 t ha^{-1} of biochar), B20 (20 t ha^{-1} of biochar), B10+NPK, B10+MWC (B10 + 20 t ha^{-1} of mushroom waste compost), B10+OMW (B10 + 20 t ha^{-1} of olive mill waste), B10+MSW (B10 + 20 t ha^{-1} of municipal solid waste) and control (non-fertilized treatment). Taking into account the concentration of N, P_2O_5 and K_2O in the mineral fertilizers used in the experiment, the moisture contents of biochar, MWC, OMW, MSW (**Table 2**) and that the mass of the arable layer (0.2 m depth) of a dried and sieved soil is 2000 (100 x 100 x 0.2 m) t, the rates of fertilizers, biochar and organic wastes (dry weight basis) applied per pot (10 kg dry soil) were 1.4 g ammonium nitrate, 2.8 g superphosphate, 0.8 g potassium chloride, 35.0 (B10) and 70.0 (B20) g biochar, 49.4 g MWC, 64.7 g OMW and 83.0 g MSW.

All the fertilizers and organic wastes were applied once at the time of experiment installation. Some of the main properties of organic wastes and the biochar are presented in **Table 2**.

Table 2. Selected properties of the bio-solids municipal solid waste (MSW), mushroom waste compost (MWC), olive mill waste (OMW) and biochar used in the pot experiment

	MSW	MWC	OMW		[7]Biochar
[1]Moisture (%)	17.0	50.6	35.3	Moisture (%)	≤ 30
[2]OC (g kg^{-1})	190.0	308.3	143.4	Bulk density (g cm^3)	0.35-0.40
[3]N(g kg^{-1})	16.3	23.8	12.2	Particle size (mm)	≤ 8
[4]P (g kg^{-1})	6.6	9.4	5.3	Ash (%)	≤ 5
[5]K (g kg^{-1})	15.8	23.1	8.3	OC (%)	≥ 90
[6]Ca (g kg^{-1})	63.4	69.1	48.2	Volatiles (%)	≤ 5
[6]Mg (g kg^{-1})	8.3	6.3	7.0	pH	8.0
[4]B (mg kg^{-1})	54.7	33.5	19.6	Total N (%)	< 0.5
[6]Cu (mg kg^{-1})	260.7	63.9	45.3	Fe (mg kg^{-1})	99.5
[6]Fe (mg kg^{-1})	10434.8	2656.4	12557.1	Pb (mg kg^{-1})	0.5
[6]Zn (mg kg^{-1})	484.1	335.4	148.3	Hg (mg kg^{-1})	< 0.1
[6]Mn (mg kg^{-1})	554.0	441.8	419.0	Cd (mg kg^{-1})	< 0.05
[6]Ni (mg kg^{-1})	875.3	182.6	337.2		
[6]Cd (mg kg^{-1})	7.5	7.4	7.3		
[6]Pb (mg kg^{-1})	170.8	42.6	50.4		
[6]Cr (mg kg^{-1})	84.9	12.4	21.6		
[6]EC (mS cm^{-1})	7.0	7.9	1.5		
[6]pH (H_2O)	8.5	7.2	7.8		
C:N ratio	12	13	12		

[1]Gravimetry, 105 ºC; [2]Organic carbon, incineration; [3]Kjeldahl; [4]Colorimetry; [5]Flame emission spectrophotometry; [6]Atomic absorption spectrophotometry; [7]Data provided by the manufacturer.

Young rooted olive cuttings of the 'Cobrançosa' variety were used in the trial. At planting, they had an average height of 30 cm and 28.8 g of fresh mass (19.8 g in the shoot and 8.9 g in the root).

During the experimental period the pots were kept free of weeds by manual weeding. In spring and summer the plants were watered as needed to ensure their regular growth. In the peak of summer, the plants were watered twice a week with 3 l of water per pot. Whenever fruits appeared they were removed at an early stage, since the fruiting of the young trees is very irregular, to reduce variability when comparing the plant performance between treatments.

During the growing season soil nitrate levels (the most stable microbial inorganic-N form) were monitored by using anion exchange membranes (AEM) in 1×2 cm strips which were inserted to a 4-8 cm depth and kept there for a week. The AEM strips were tied with a colored line allowing for easy identification and removal from the soil. After collection, the strips were rinsed with distilled water and nitrate ions were then eluted in flasks containing 30 ml of 0.5 N hydrochloric acid. After keeping them in an acid media for 1 h and 15 min, nitrate concentrations in the resulting acid extracts were determined by UV–Visible spectrophotometry. The strips were regenerated in 0.5 M $NaHCO_3$ before being reused.

After two growing seasons, the experiment was completed in November 2017. The soil of each pot was mixed and one homogenized representative soil sample was collected for analysis. The analyses performed were: 1) pH (H_2O, KCl); 2) easily oxidizable C (EOC) determined by the Walkley-Black method and total organic C by incineration; 3) cation exchange capacity (ammonium acetate, pH 7.0); 4) exchange acidity (KCl extraction); 5) extractable P and K (ammonium lactate); and 6) extractable B (azomethine-H). In the initial soil allotment soil particle distribution (clay, silt and sand fractions) were assessed by the Robinson pipette method. All these methods are fully described in Houba et al. (1997).

At soil pot removal, plants were gently removed from the bulk soil and separated into roots, stems and leaves. The roots were washed with water under low pressure. Soil samples were sent to the laboratory and oven-dried at 40 ºC and sieved in 2 mm mesh. The plant tissue samples were oven-dried at 65 °C, until weight stabilization, weighed for quantification of dry matter and subsequently ground. The removal of nutrients in plant tissues was estimated from the measured dry matter mass and the concentration of nutrients of each tissue.

The elemental analyses of plant tissues (roots, stems, leaves) and organic amendments were assessed by Kjeldahl (N), colorimetry (B and P), flame emission spectrometry (K) and atomic absorption spectrophotometry (Ca, Mg, Cu, Fe, Zn, Mn, Ni, Cd, Cr, Pb) (Walinga et al. 1989) after tissue samples were digested with nitric acid in a microwave.

Datasets were tested for normality and homogeneity of variances using the Shapiro-Wilk test and Bartlett's test, respectively, and then submitted to analysis of variance (ANOVA). When significant differences were found globally ($\alpha < 0.05$), the means of each treatment were compared for their significance by the multiple range Tukey HSD test ($\alpha = 0.05$).

3. Results

The organic amendments and mineral fertilization showed a trend of higher mean dry matter plant yield compared to controls and to the treatments receiving biochar, although these differences were not statistically significant for $P < 0.05$ (**Table 3**). However, N recovered was significantly higher in the NPK treatment than in the controls and in the treatments receiving only biochar, but not when compared to biochar-waste mixtures. For the remaining macronutrients (P, K, Ca and Mg) and B, Zn and Mn no significant differences among treatments where found, while for Fe and Cu significantly lower values were found between some fertilized treatments (B10+NPK, for instance) in comparison to the controls.

Figure 1 shows the dry matter distribution between stems, roots and leaves. Roots represented less than 20% of the total dry matter of the plants, leaves slightly over the 20%, and stems corresponded to around 60% of the total plant dry matter. However, no significant shifts in the plant allocation to these biomass compartments resulted from the effect of the treatments.

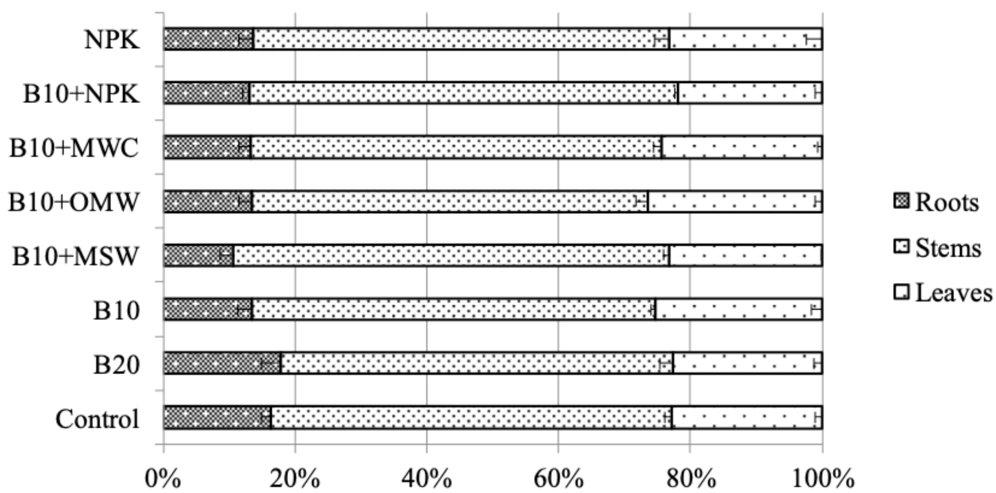

Figure 1. Dry matter portioning among plant parts. There were no significant differences among treatments (α < 0.05). Error bars are the mean standard deviations.

Table 3. Total (leaves+stems+roots) dry matter (TDM) yield and macro and micronutrient recovery as a function of fertilization treatment

	TDM	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Boron	Cooper	Iron	Zinc	Manganese
	(g pot^{-1})	(g pot^{-1})					(g pot^{-1})				
NPK	100.2 a	0.59 a	0.09 a	0.84 a	0.53 a	0.08 a	1.66 a	2.18 a	62.26 ab	2.78 a	2.70 a
B10+NPK	111.3 a	0.54 ab	0.09 a	0.90 a	0.53 a	0.07 a	1.72 a	0.87 b	49.20 b	2.66 a	2.22 a
B10+MWC	108.4 a	0.51 ab	0.10 a	0.92 a	0.61 a	0.07 a	1.91 a	2.11 a	54.40 b	2.45 a	2.10 a
B10+OMW	117.2 a	0.58 ab	0.11 a	0.95 a	0.67 a	0.08 a	1.99 a	1.83 ab	86.30 ab	2.89 a	2.60 a
B10+MSW	108.9 a	0.52 ab	0.09 a	0.86 a	0.51 a	0.07 a	1.78 a	1.87 ab	64.97 ab	2.51 a	2.91 a
B10	93.8 a	0.43 b	0.08 a	0.80 a	0.50 a	0.07 a	1.59 a	2.16 a	58.43 ab	2.14 a	2.20 a
B20	88.3 a	0.41 b	0.08 a	0.74 a	0.43 a	0.07 a	1.58 a	1.76 ab	82.45 ab	2.31 a	2.71 a
Control	93.2 a	0.43 b	0.09 a	0.72 a	0.50 a	0.07 a	1.67 a	2.06 a	100.84 a	2.38 a	3.09 a

The same letter in the values of each column indicate no significantly different means according to the by Tukey HSD test (α = 0.05).

Table 4. Shoot (leaves+stems) dry matter (SDM) and nutrient contents in shoot (leaves+stems) relatively to whole plant (leaves+stems+roots) as a function of fertilization treatment

	SDM	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Boron	Cooper	Iron	Zinc	Manganese
	(%)										
NPK	86.3 ab	82.3 ab	84.3 ab	86.0 a	84.9 a	70.4 a	86.2 a	79.7 ab	17.2 a	73.7 a	66.0 a
B10+NPK	86.9 ab	82.9 ab	85.5 ab	86.4 a	81.6 a	71.6 a	87.0 a	57.4 b	17.7 a	73.9 a	51.1 a
B10+MWC	86.8 ab	84.1 ab	87.1 a	86.6 a	83.3 a	69.7 a	87.8 a	81.0 a	12.0 a	71.2 a	51.6 a
B10+OMW	86.6 ab	83.9 ab	84.4 ab	85.5 a	84.4 a	68.1 a	85.7 a	73.5 ab	8.8 a	70.4 a	46.8 a
B10+MSW	89.3 a	86.4 a	88.2 a	88.3 a	85.6 a	71.5 a	88.7 a	82.2 a	17.7 a	74.4 a	66.1 a
B10	86.3 ab	83.3 ab	85.7 ab	86.3 a	84.0 a	68.8 a	88.1 a	83.4 a	13.7 a	60.8 a	52.3 a
B20	82.1 b	77.0 b	78.6 b	86.4 a	78.9 a	57.7 a	81.8 a	72.2 ab	14.0 a	60.8 a	52.2 a
Control	83.7 ab	79.7 ab	82.8 ab	83.7 a	82.9 a	63.6 a	85.4 a	74.8 ab	16.6 a	63.9 a	54.2 a

The same letter in the values of each column indicates no significantly different means according to the Tukey HSD test (α = 0.05).

When the whole aboveground dry matter (leaves + stems) was analyzed, significant differences among treatments showed up, but only the lower shoot (leaves + stems) dry matter (SDM), N and P values were recorded in B20 treatments compared to the other treatments (**Table 4**). A lower Cu content in shoots was found in the B10+NPK treatment.

The most relevant information in **Table 4** concerns the removal of nutrients in the aerial parts relative to the roots. All elements for which the percentage values are lower than that of the dry matter indicate that they are proportionally in greater amounts in the root than in the other tissues and vice versa. Thus, Fe is an element that primary accumulates in roots. Hence, the roots representing less than 20% of the total dry matter of the plant concentrate more than 80% of all the Fe in olive plants as average. Due to the high bioavailability of the Fe in this soil (**Table 1**), the plants seem to have a mechanism of exclusion, restricting its access to the aerial part. Mn and Zn also accumulate preferentially in the roots rather than in the aerial part, although these nutrients are generally found in the plant in much lower quantities than Fe. Regarding macronutrients, Mg accumulates preferentially in the root compared to the aerial part. N is similarly accumulated in roots and aerial parts. However, N appears very concentrated in the leaves and in very low concentrations in the stems, with the roots showing intermediate concentrations between leaves and stems.

The AEM results showed significantly higher nitrate values in the NPK treatment compared to the remaining treatments in the second (November 2016) and the third measurement (May 2017) (**Table 5**). In the first measurement (October 2016), the average values in the NPK treatment are also the highest but only significantly different to those of the B20 treatment. In the last measurement (June 2017), which corresponds to an active phase of plant growth, the values were very low and equivalent in all treatments.

Table 5. Nitrate concentration in anion exchange membranes (AEM) extracts from AEM inserted directly into the soil

Treatments	Oct 6th 2016	Nov 10th 2016	May 3rd 2017	Jun 13th 2017
		mg NO_3^- L^{-1}		
NPK	76.0 a	92.3 a	143.3 a	3.1 a
B10+NPK	70.0 ab	42.3 b	25.8 b	4.9 a
B10+MWC	65.0 ab	24.5 b	11.5 b	3.9 a
B10+OMW	21.7 ab	17.3 b	14.1 b	5.1 a
B10+MSW	41.0 ab	45.7 b	21.4 b	5.4 a
B10	26.6 ab	24.5 b	14.0 b	4.8 a
B20	20.6 b	21.5 b	16.2 b	5.1 a
Control	26.8 ab	21.9 b	16.6 b	3.8 a

The same letter in the values of each column indicates no significantly different means according to the Tukey HSD test ($\alpha = 0.05$).

The use of biochar at double the rate (B20), as expected, significantly increased the total organic C content in the soil when compared to the control treatment (**Table 6**), and was coupled with higher easily mineralizable organic C in the same treatment. Kjeldahl N did not vary among the fertilizer treatments and neither did P. However, the extractable K also varied among treatments, with the control and biochar (B20 and B10) treatments showing a tendency for lower values. In general, fertilization treatments did not influence the availability of micronutrients in the soil.

The organic amendments appear to have contributed to an increase in soil pH relative

Table 6. Total organic carbon (TOC), easily oxidizable carbon (EOC), Kjeldahl nitrogen and extractable nutrients in the soil at the end of experiment as a function of fertilization treatments

	TOC	EOC	Kjeldahl N	P (P_2O_5)	K (K_2O)	Boron	Cooper	Iron	Zinc	Manganese	Manganese
	------------ g kg^{-1} ------------			------------ mg kg^{-1} ------------							
NPK	18.9 b	16.0 b	1.64 a	95.7 a	65.0 abc	1.1 a	6.4 a	53.8 a	2.8 a	21.3 a	2.70 a
B10+NPK	24.7 ab	16.4 b	1.52 a	57.0 a	69.0 abc	1.0 a	5.8 a	44.8 a	2.7 a	19.3 a	2.22 a
B10+MWC	24.1 ab	18.9 ab	1.69 a	85.5 a	80.8 ab	0.9 a	6.6 a	51.9 a	2.9 a	20.3 a	2.10 a
B10+OMW	26.5 ab	17.9 b	1.67 a	95.5 a	60.8 bc	1.2 a	7.1 a	59.9 a	3.1 a	21.8 a	2.60 a
B10+MSW	21.0 ab	16.9 b	1.73 a	88.8 a	91.3 a	0.9 a	6.7 a	61.8 a	3.3 a	20.9 a	2.91 a
B10	21.7 ab	16.7 b	1.68 a	69.2 a	48.3 c	0.8 a	6.1 a	53.3 a	2.5 a	21.8 a	2.20 a
B20	28.9 a	28.2 a	1.75 a	75.4 a	61.7 bc	1.8 a	6.7 a	71.1 a	4.0 a	23.9 a	2.71 a
Control	19.3 b	16.3 b	1.58 a	66.7 a	52.3 c	1.0 a	5.9 a	58.1 a	2.7 a	21.9 a	3.09 a

The same letter in the values of each column indicates no significantly different means according to the Tukey HSD test (α = 0.05).

Table 7. pH, exchangeable bases, sum of exchangeable bases (SEB), exchangeable acidity and cation exchange capacity (CEC) as a function of fertilization treatments

	pH		Calcium	Magnesium	Potassium	Sodium	SEB	Exchangeable acidity	CEC	Zinc	Manganese
	(H_2O)	(KCl)	------------	------------	------------	------------	$cmol_{(+)}$ kg^{-1}	------------	------------	------------	------------
NPK	5.39 c	4.04 bc	5.17 a	1.31 a	0.17 cd	0.18 c	6.84 a	0.13 a	6.97 a	73.7 a	66.0 a
B10+NPK	5.63 bc	4.03 bc	4.24 ab	1.19 a	0.25 abc	0.29 bc	5.97 a	0.18 a	6.14 a	73.9 a	51.1 a
B10+MWC	5.77 ab	4.24 abc	4.47 ab	1.33 a	0.28 a	0.33 ab	6.41 a	0.10 a	6.51 a	71.2 a	51.6 a
B10+OMW	5.89 ab	4.31 ab	4.66 ab	1.36 a	0.20 bcd	0.27 bc	6.48 a	0.18 a	6.66 a	70.4 a	46.8 a
B10+MSW	5.96 a	4.40 a	4.85 ab	1.46 a	0.28 a	0.40 ab	7.00 a	0.18 a	7.17 a	74.4 a	66.1 a
B10	5.54 bc	4.00 bc	4.59 ab	1.39 a	0.18 cd	0.32 ab	6.47 a	0.13 a	6.60 a	60.8 a	52.3 a
B20	5.56 bc	4.06 bc	5.02 ab	1.29 a	0.25 abc	0.46 a	7.02 a	0.17 a	7.18 a	60.8 a	52.2 a
Control	5.55 bc	3.96 c	4.35 b	1.33 a	0.17 d	0.28 bc	6.13 a	0.23 a	6.36 a	63.9 a	54.2 a

The same letter in the values of each column indicates no significantly different means according to the Tukey HSD test (α = 0.05).

to the initial situation (Table 7). In the control treatment and in the biochar treated pots, pH did not change from the initial situation. However, in the NPK treatment pH (H_2O) appears to have decreased slightly, the lowest value being recorded. Exchangeable Mg, Ca, K and Na varied significantly with the fertilization treatment but in different directions, since the sum of exchangeable bases did not vary between treatments. The exchange acidity and the cation exchange capacity also did not vary between treatments.

4. Discussion

The organic amendments and the NPK treatment provided slightly higher average dry matter yields than the control and the treatments that received only biochar. However, there were no significant differences between treatments for dry biomass. When analyzing the amount of nutrients recovered in the whole plant, significant differences between treatments appeared in N, P and Cu concentrations due to the combined effect of the slight increase in dry matter production and nutrient concentration in the tissues. For N and P, the higher values were found in B10+MSW and the lower values in B20. For Cu, the higher and lower values were respectively found in B10 and B10+NPK. The difficulty in obtaining a significant response to the application of fertilizer and manures may be due to the reduced response of olive to fertilization that is usually found (Fernández-Escobar et al. 2009). Olive is a species of low N recovery (Rodrigues et al. 2012), especially when plants are young and annual biomass production is reduced (Ferreira 2018). A simple field-grown corn plant, for example, can recover 2 g of N in the aboveground biomass (Rodrigues et al. 2006) while in this study olive plants removed only 0.6 g in total biomass, including the roots, and in two growing seasons. Olive's response to the application of P and K is even more difficult to obtain (Ferreira et al. 2018 a, b). Several studies have shown increased bioavailability of P by the application of biochar in soils of restricted P availability (Arif et al. 2017; Liu et al. 2017; Jin et al. 2019). However, in the soils of this region it has been difficult to obtain a response in crop productivity to the application of P (Arrobas et al. 2018; Ferreira et al. 2018a; Rodrigues et al. 2018a), which helps to explain the lack of P effects in this study.

Fertilization has modified the growth pattern of the plants. The fertilized treatments displayed higher values of aboveground biomass than the treatments that received only biochar and the controls. The effect of N on the increase of the aboveground biomass of olive has previously been reported (Ferreira 2018). In turn, P, for instance, usually have an inverse effect (Ferreira et al. 2018a).

Significant differences were found among treatments in the amount of Fe and Cu removed. Some organic amendments contained high levels of Fe in their initial composition, such as OMW and MSW. However, the levels of these elements in the plant tissues were not related to their initial concentration in the manures, probably because soil also presented high levels of Fe. The presence of Fe in the soil solution is mainly related to pH and redox conditions (Broadley et al. 2012). These organic amendments led to an increase in soil pH which would have reduced the availability of these metals despite their higher initial concentration.

The monitoring of nitrates in the soil with anion exchange membranes showed nitrate levels initially higher in the fertilized treatments and lower in the treatments without application of mineral or organic N. The highest peaks were obtained in the middle of the experiment (November 2016 and May 2017) in the NPK treatment, probably coinciding with a period of high nitrification. The organic amendments might have released their N gradually as usually occurs with organic substrates (Rodrigues et al. 2006, 2018b), which may have avoided a high peak of nitrates in the soil. The biochar appears to have contributed to keeping low the levels of nitrates in the soil, since the values were lower in the B10+NKP in comparison to the NPK treatment. This effect of biochar in regulating the mineral N contents in the soil has been frequently recorded (Gao et al. 2019; Li et al. 2019) and may be due to NH_4^+ adsorption, making it less available for nitrification, or biological immobilization, if an increase of biological activity in the soil due to the application of biochar is assumed.

The organic C content in the soil had the highest value in B20 and the lowest in the control and NPK treatments. It seems that the biochar applied at the rate of 20 t ha^{-1} had a more significant effect in increasing soil C than the organic amendments MWC, OMW, MSW. One of the reasons why biochar has been promoted as a soil conditioner is because it can increase soil C sequestration due to its recalcitrant property in the soil (Arif et al. 2017; Shaaban et al. 2018; Gao et al. 2019; Palansooriya et al. 2019; Yu et al. 2019). It should be noted that biochar also increased the EOC content which may be related to an increase in microbial activity.

The pH in the pots receiving organic amendments rose significantly in comparison to the NPK treatment, which had recorded the lowest values. All organic amendments have alkaline pH, especially MSW, and this will have been reflected in the rise of soil pH. The lower NPK values may have been due to the effect of nitrification and NH_4^+ uptake by plants, both factors that tend to acidify the soil (Hawkesford et al. 2012; Havlin et al. 2014). Biochar appears to have no influence on soil pH, although this is an effect frequently reported in studies using this soil conditioner on acid soils (Jin et al. 2019).

Several studies with biochar are performed under poor soils or even harsh environmental conditions (Farrell et al. 2014; Arif et al. 2017; Liu et al. 2017; Jin et al. 2019; Palansooriya et al. 2019; Rashti et al. 2019). In a review of Yu et al. (2019), they found that a biochar amendment can improve plant growth in soils with physical constraints, whether they be acid, alkaline, nutrient-deficient, salt-affected or metal-contaminated. Biochar has also been used as a growing substrate in potted plants, where physical properties such as water retention and aeration are important for plant growth (Tian et al. 2012; Zhang et al. 2014; Dispenza et al. 2016). If plant growth conditions are not so stressful, the results may be less relevant, as probably occurred in our experiments.

5. Conclusions

Biochar appears to have had a significant effect on the regulation of the availability of inorganic N in the soil, since the levels of nitrates were reduced. The effect may have been due to adsorption of NH_4^+ and/or eventual biological immobilization. Biochar increased C sequestration in the soil due to its own properties, but possibly also by increasing microbial growth, since EOC also increased. The was no increase in soil P bioavailability or any effect on pH due to the application of biochar. Also no relevant synergistic effect was recorded from the use of biochar in mixtures with the other organic amendments. Under the conditions of this experiment, without significant constraints to plant growth, the beneficial effect of biochar addition was poor.

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