Received: 7 November 2023 | Revised: 12 February 2024 | Accepted: 2 March 2024

DOI: 10.1111/sum.13045

RESEARCH ARTICLE

Soil Use and Management | BRITISH SOCIETY OF SOIL SCIENCE | WILEY

Differential responses of photosynthesis, yield and soil properties 4 years after a single application of zeolites and biochar in a rainfed olive orchard

Sandra Martins[1] | **Cátia Brito**[1,2] | **Alexandre Gonçalves**[3] | **José Moutinho-Pereira**[1,2] | **Ermelinda Pereira**[4] | **Margarida Arrobas**[4] | **Manuel Â. Rodrigues**[4] | **Fernando Nunes**[5] | **Carlos M. Correia**[1,2]

[1]CITAB – Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

[2]Inov4Agro – Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

[3]MORE – Collaborative Laboratory Mountains of Research, Brigantia Ecopark, Bragança, Portugal

[4]CIMO – Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal

[5]CQ-VR – Food and Wine Chemistry Laboratory, Chemistry Research Centre – Vila Real, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

Correspondence
Carlos M. Correia, CITAB – Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal.
Email: ccorreia@utad.pt

Funding information
European Regional Development Fund; Fundação para a Ciência e Tecnologia (FCT, Portugal); Fundação para a Ciência e Tecnologia (FCT)-Portuguese Foundation for Science and Technology, Grant/Award Number: PD/00122/2012; PT2020, Grant/Award Number: PD/BD/135327/2017; CQ-VR, Grant/Award Number: UIDB/00616/2020; CIMO, Grant/Award Number: UIDB/00690/2020; CITAB, Grant/Award Number: UIDB/04033/2020; AgriFood XXI Project, Grant/Award Number: NORTE-01-0145-FEDER-000041; North Regional Operational (NORTE)

Abstract

Olive orchards represent a key agricultural system in the Mediterranean Basin. Soil degradation processes associated with unsustainable agronomic practices and climate change could severely impact the sustainability of Mediterranean rainfed olive orchards. In this context, soil amendments are important tools that can be used to enhance soil fertility for sustained environmental quality and plant performance. In this study, a field trial was conducted for 4 years in olive tree (*Olea europaea* L.) to assess the effects of a mineral fertilizer compound and the combination with a single application of zeolites or biochar on the physiological and biochemical performance, tree nutritional status, crop yield and soil chemical and biological properties to gain knowledge towards more sustainable management. Our results showed that the addition of zeolites and biochar to mineral fertilizer ameliorated the physiological and biochemical performance, as evidenced by consistent increments of relative water content, stomatal conductance and net photosynthesis and by lower signs of oxidative stress during the periods of greater climate adversity. However, crop yield was not significantly different among soil treatments. On the other hand, soil chemical and biological traits at the surface layer (0–10 cm) have shown different and relevant responses after 4 years of soil amendment application. In fact, zeolite supply stood out as increased pH, extractable K, cation exchange capacity, microbial biomass carbon and microbial biomass quotient and reduced extractable Cu. Furthermore, zeolites induced positive changes in soil enzymatic activity, leading to increases in 10 enzymes involved in C, N and P cycles. In contrast, the effects of biochar on soil properties were much more reduced, given that it decreased the microbial biomass nitrogen and enhanced the activities of three P-cycle enzymes. In summary, our data demonstrated that both soil amendments can be an interesting complement to mineral fertilization, in order to increase trees' resilience under rainfed conditions and to promote soil health, although the use of zeolites appears to be a more promising strategy because of the induction of higher soil sustainability.

KEYWORDS

Olea europaea L., olive tree performance, rainfed orchards, soil amendments, soil fertility

1 | INTRODUCTION

Olive tree (*Olea europaea* L.) is an important crop in the Mediterranean Basin, which produces the great part of the olive oil world's supply and lends important nutritional and economic benefits (El & Karakaya, 2009; Michalopoulos et al., 2020). Agricultural soils from many areas of the Mediterranean region have been subjected to unsustainable agronomic practices, such as frequent and deep soil tillage, the excessive use of mineral fertilizers and the monoculture practice (Baldantoni et al., 2015; Giovanni et al., 2013). The intensive use of soils may create diverse problems of degradation, related to erosion, salinization, acidification, soil organic carbon (SOC) depletion or nutrient mining that seriously impair their fertility (Baldantoni et al., 2015). Moreover, the Mediterranean region is characterized by severe summer conditions, including low rainfall, excessive heat load and high daily irradiance (Brito et al., 2019). This region has been identified as one of the most climate-vulnerable regions and climate change "hotspot" (Ferreira et al., 2022). Thus, Mediterranean crops have to deal with severe environmental stress conditions, which are getting worse because of the advances of climate change (Brito et al., 2019). Drought stress contributes to marked reductions in soil moisture, which induces negative changes in the photosynthetic machinery, causing considerable perturbations in plant growth and yield losses (Fahad et al., 2017; Ferreira et al., 2022).

In order to maintain crop productivity, farmers mainly use chemical fertilizers, which are easy to apply and their nutrients are readily available to plants (Lopes et al., 2021, 2022). However, mineral fertilization is often associated with reduced nutrient use efficiency and a high risk of environmental contamination, because of water eutrophication and greenhouse gas (GHG) emissions to the atmosphere (Chandini et al., 2019). The overuse of chemical fertilizers can also lead to soil acidification and soil crust, thereby reducing soil organic matter (SOM) content and soil biological activity, stunting plant growth, changing soil pH and increasing pests (Chandini et al., 2019). Thus, it is increasingly necessary to reduce the dependence or improve the nutrient use efficiency of chemical fertilizers (Lopes et al., 2021). On the other hand, considering the relevance of olive cultivation in the Mediterranean region, it is necessary to implement the sustainable strategies that are able to restore and increase soil quality in order to improve crop growth and yield, while reducing the environmental hazards. Recently, soil amendments have been proposed to be a complement or an alternative to the use of fertilizers, to increase nutrient use efficiency and to minimize their negative impact on environment, once these materials are able to improve the physical, chemical and biological properties of soil (Babla et al., 2022; Yan et al., 2008). Soil amendment is recognized as a sustainable practice to support crop yields and, at the same time, maintain a good quality of agricultural soils (Giovanni et al., 2013). Among several soil amendments, biochar and zeolites are two of the most widely described. Biochar is a charcoal-like material that can be obtained from a range of biomass feedstocks mainly by pyrolysis (Wang et al., 2020). This material presents high porosity and low bulk density, and it is highly recalcitrant (Zaidun et al., 2019). Thus, the application of biochar can improve soil water-holding capacity, C sequestration, pH, cation exchange capacity (CEC) and soil nutrient availability (Ding et al., 2017; Zaidun et al., 2019). In recent years, several studies have described the potential of biochar to improve soil physico-chemical and biological properties, as well as crop growth and yield (Genesio et al., 2015; Lopes et al., 2022; Zaidun et al., 2019). Zeolites are crystalline aluminosilicates of alkali and alkaline-earth minerals, and their structure is characterized by a framework of $[SiO_4]^{-4}$ and $[AlO_4]^{-5}$ tetrahedron linked to each other's by sharing oxygen atoms (Zaidun et al., 2019). This structure provides important properties as a large internal porosity that results in water retention, a uniform particle-size distribution that allows them to be easily incorporated and high CEC that retains nutrients (Głąb et al., 2021). The most common zeolite for agricultural applications is clinoptilolite (Cataldo et al., 2021; Karaca, 2004). One of the main applications in agriculture is their use as an additive to fertilizer, promoting the nutrient-retention capacity of the soils by improving the slower release of these elements for uptake by crops (Cataldo et al., 2021; Karaca, 2004). The slow-release fertilizer effect mainly delays the release of the nutrients and extends the fertilizer effect period (Yan et al., 2008). The use of these materials as soil amendments has been reported as an efficient strategy to enhance soil health, crop performance and productivity, and environmental safety (Cataldo et al., 2021; Chen et al., 2017; Mondal et al., 2021). Therefore, this study was carried out to determine the effects of biochar and zeolites combined with the use of fertilizers on physiological performance, yield and chemical and biological soil properties of a rainfed olive orchard.

2 | MATERIALS AND METHODS

2.1 | Site description and experimental layout

The field experiment was carried out during 4 years (2018–2021) in São Pedro Vale do Conde (41°26′36.2″ N. 7°13′21.2″ W), in the Municipality of Mirandela, Trás-os-Montes region, northeast of Portugal. Meteorological data recorded during the experimental period in the weather station at Paradela, close to experimental plot, are presented in Figure 1.

The orchard characteristics, agronomic practices, and experimental design have been previously described by Martins et al. (2022). Briefly, the experiment was arranged in a randomized design with three replicates per treatment. The experimental units, or replicates, were composed of groups of three homogeneous trees. The distance between rows and plants within the rows was 7 × 7 m, with a planting density of 204 trees ha^{-1}. The experiment included the establishment of three soil treatments: conventional fertilization (FC) as control, FC plus zeolites (ZL), and FC plus biochar (BC). The conventional fertilization consisted of the annual application of 60 kg of N, P_2O_5 and K_2O ha^{-1}, and 2 kg ha^{-1} of B as borax (11% B). Biochar (Ibero Massa Florestal, Oliveira de Azeméis, Portugal) and clinoptilolite zeolites (Zeolita Natural AGRO®, 0.6–1.5 mm; ZeoCat, Barcelona, Spain) are commercial products, whose detailed composition (provided by the manufacturer) is given in Table 1. These products were only applied in the first year of the experiment, at a rate of 10 t ha^{-1} for biochar and 5 t ha^{-1} for zeolites. The amendments and fertilizers were homogeneously spread beneath the tree canopy in late March. No phytosanitary products were used during the experimental period. At the onset of the study, trees displayed a neglected appearance, as they had not received regular fertilizer application. According to the farmer, olive yield rarely exceeded 1 t ha^{-1}.

2.2 | Field and laboratory determinations

All the physiological and biochemical measurements at leaf level were performed in healthy, fully expanded and mature leaves. The leaf gas exchange measurements were taken periodically along the summer season, on 25 June 2018 (D1), 24 July 2018 (D2), 28 August 2018 (D3), 26 June 2019 (D4), 24 July 2019 (D5), 21 August 2019 (D6), 23 June 2020 (D7), 27 July 2020 (D8), 28 June 2021 (D9), 26 July 2021 (D10) and 23 August 2021 (D11).

The collection of leaf samples for biochemical analysis was performed last year, at D10. Fully expanded leaf samples were collected around the tree canopy and immediately frozen in liquid N_2. Posteriorly, leaf samples were stored at −80°C until proceed to biochemical analyses.

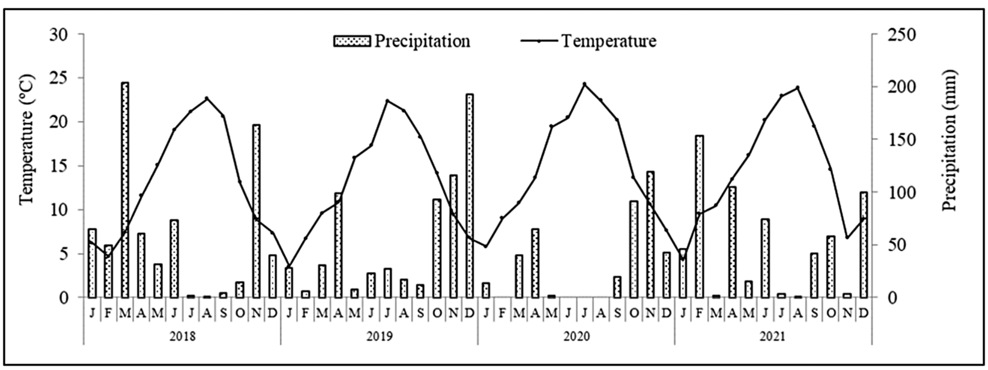

FIGURE 1 Average monthly temperature and precipitation conditions recorded during the experimental period in the weather station at Paradela close to experimental plot.

TABLE 1 Properties of zeolite and biochar used in this study as provided by the manufacturers.

Zeolite		Biochar	
Particle size	0.4–3.0 mm	Particle size	0.1–10
Bulk density	980	Bulk density	350–400 kg m^{-3}
Cation exchange capacity	1.5–1.9 meq g^{-1}	Moisture	≤30.0
Porosity	45.0%–50.0%	Conductivity	948 μs cm^{-1}
pH	7.0–8.0	pH	<9.0
Specific surface	70.0–80.0 m^2 g^{-1}	Total organic C	≥90.0%
SiO_2	65.0%–71.3%	Ash	≤5.0%
Al_2O_3	10.0%–12.0%	Volatile	≤5.0%
CaO	2.5%–3.5%	Total N	≤5.0 g kg^{-1}
K_2O	0.8%–1.9%	Cd	< 0.05 mg kg^{-1}
FeO^3	0.9%–1.2%	Pb	0.05 mg kg^{-1}
MgO	0.3%–0.7%	Fe	99.5 mg kg^{-1}
Na_2O	0%–0.1%	As	<0.10 mg kg^{-1}
TiO_2		Hg	< 0.10 mg kg^{-1}

Leaf samples for nutritional status analysis were collected during the winter resting period (December) and in summer (July) at endocarp sclerification.

Crop yields were evaluated on 6 November 2018, 4 November 2019, 29 October 2020 and 16 November 2021.

At the end of the experiment, soil samples (0–10 and 10–20 cm depths), prepared as a composite sample by collecting and mixing soil from nine points in each of the three replicates (three points per tree), were taken in order to assess the cumulative effect of the treatments on soil chemical properties and microbial activity. For chemical analyses, soil samples were air-dried after it had been collected and thereafter sieved to pass through a grid of 2 mm, whereas for microbiology and microbial activity assays the samples were frozen until laboratory determinations.

2.2.1 | Leaf gas exchange and water status

Leaf gas exchange measurements were performed using a portable IRGA (LCpro+, ADC, Hoddesdon, UK), operating in the open mode. Measurements were taken on two sun-exposed leaves per tree, on cloudless days under natural irradiance, during the morning period (10:00–11:15 local time). Net photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g_s, mmol m^{-2}s^{-1}) were determined following previously developed equations (von Caemmerer & Farquhar, 1981), while intrinsic water use efficiency and intercellular to atmospheric CO_2 concentration were calculated through the ratio of A/g_s and C_i/C_a, respectively.

For the evaluation of plant water status, the leaves used in leaf gas exchange were detached and immediately placed into air-tight tubes, and then, the following parameters were evaluated: fresh weight (FW, g); fresh mass at full turgor (TW, g), determined after immersion of leaf petioles in demineralized water for 48 h in the dark at 4°C; and dry weight (DW, g), measured after drying at 70°C to a constant weight. Then, the relative water content was calculated according to the following formula: RWC = (FW − DW)/(TW − DW) × 100.

2.2.2 | Photosynthetic pigments and antioxidant foliar metabolites

Chlorophylls and carotenoids were extracted with 80% (v/v) acetone and quantified according to Arnon (1949), Sesták et al. (1971) and Lichtenthaler (1987) methods. To extract the antioxidant compounds from olive leaves, 1.5 mL of MeOH:H_2O (70:30) was added to 30 mg of frozen olive leaf powder. After shaking for 1 h at room temperature, the samples were centrifuged at 12,000 rpm for 15 min, at 4°C. The methanolic extract resultant from centrifugation was reserved, and this step was repeated three times. The obtained extracts were used for the quantification of total phenols (TP), ortho-diphenols, flavonoids and total antioxidant activity (TAC), which were performed as described by Brito et al. (2018). TP and ortho-diphenols were expressed as mg of gallic acid equivalents (GAE), flavonoids were expressed as mg of catechin equivalents per g of olive leaf, and TAC was expressed as mmol of Trolox equivalent (TE) per g of olive leaf DW. All measurements were performed in triplicate.

2.2.3 | Tree nutritional status and olive yield

Samples of young fully expanded leaves were collected from shoots of the four quadrants around the tree canopy, in the middle of non-bearing current season, and oven-dried at 70°C to a constant weight, to proceed with elemental composition analyses. Tissue analyses were performed by Kjeldahl (N), colorimetry (B and P), flame emission spectrometry (K) and atomic absorption spectrophotometry (Ca, Mg, Cu, Fe, Zn and Mn) methods (Walinga et al., 1995).

Olive trees were harvested by a trunk shaker head, which detaches the olive fruits, and collected them by an associated inverted umbrella system. After that, sheets were spread on the ground to receive the fruits so that they could be weighed individually per group of three trees (the experimental unit).

2.2.4 | Soil chemical properties

The analyses performed on dried and sieved soil samples were as follows: pH (H_2O) (potentiometry); soil separation (by the Robinson pipette method); CEC (ammonium acetate, pH 7.0); extractable Fe, Mn, Zn and Cu (ammonium acetate and EDTA, determined by atomic absorption spectrometry) (van Reeuwijk, 2002); extractable P and K (ammonium lactate solution, pH 3.7) (Balbino, 1968); and extractable B (hot water and azomethine-H) (Jones, 2021).

2.2.5 | Soil microbiology and enzymatic activity

Soil microbial biomass C (Mic-C) and soil microbial biomass N (Mic-N) in the 0–10 cm layer were determined for fresh samples using the chloroform fumigation–extraction method (Vance et al., 1987), after 24 h of conditioning at 25°C and 60% water-holding capacity. Organic C (OC) and total soluble N were determined by near-infrared detection (NIRD) and by chemiluminescence detection after combustion at 850°C in an elemental analyser (Formacs; Skalar). The Mic-C and Mic-N were calculated using a KEC factor of 0.33 (Vance et al., 1987) and a KEN factor of 0.54 (Brookes et al., 1982), respectively. All results are expressed on an oven-dry (105°C) weight basis. Microbial biomass quotient (MBQ) was calculated as Mic-C/OC ratio.

The soluble enzyme activity in soil samples (0–10 cm) was determined according to Martínez et al. (2016), using a API ZYM strip system (BioMerieux) for semi-quantitative analysis of production of hydrolytic enzyme. At the end of the procedure, the colour reactions were read and a numerical value ranging from 0 to 5 was assigned according to the colour chart provided by the manufacturer. In an equivalence scale of readings according to Baldrian et al. (2011), 0 is 0 nanomoles substrate hydrolysed, 1 is 5 nanomoles substrate hydrolysed, 2 is 10 nanomoles substrate hydrolysed, 3 is 20 nanomoles substrate hydrolysed, 4 is 30 nanomoles substrate hydrolysed and 5 is higher or equal to 40 nanomoles substrate hydrolysed. The intensity of enzymatic reactions was measured by four independent people, and the final result was the average of their records.

2.3 | Statistical analysis

Data were tested for normality and homogeneity of variances using the Shapiro–Wilk test and Bartlett's test, respectively. A comparison of the effect of the fertilizer treatments in each field experiment was provided by one-way ANOVA, using IBM SPSS statistics 26 software. When significant differences were found ($p < .05$), the means were separated by the multiple-range Tukey HSD test.

Principal component analysis (PCA) was conducted using the SPSS software. PCA was used to avoid multicollinearity between the original variables and to reduce the dataset into new variables, the principal components (PCs), which explain most of the variation present in the original variables.

3 | RESULTS

3.1 | Leaf gas exchange and water status

Figure 2 shows the influence of soil treatments on leaf gas exchange variables. In general, ZL- and BC-treated trees presented higher A and g_s than control plants, namely in the sampling periods where those values reached the lowest levels, corresponding to periods of greatest accumulated stress. On the other hand, no significant differences on A/g_s and C_i/C_a were reported in the 8 first sampling dates, whereas in the last year of the experiment, FC showed the highest A/g_s and the lowest C_i/C_a at D9, and ZL revealed, generally, superior and inferior A/g_s and C_i/C_a at D10 and D11, respectively. Meanwhile, the influence of treatments on RWC (Figure 3) was a little less clear, given the predominance of non-significant differences. In any case, on 4 out of the 11 dates, higher values were recorded in ZL than in the FC treatment.

3.2 | Photosynthetic pigments and antioxidant foliar metabolites

The effects of soil management treatments on photosynthetic pigments and antioxidant leaf metabolites are given

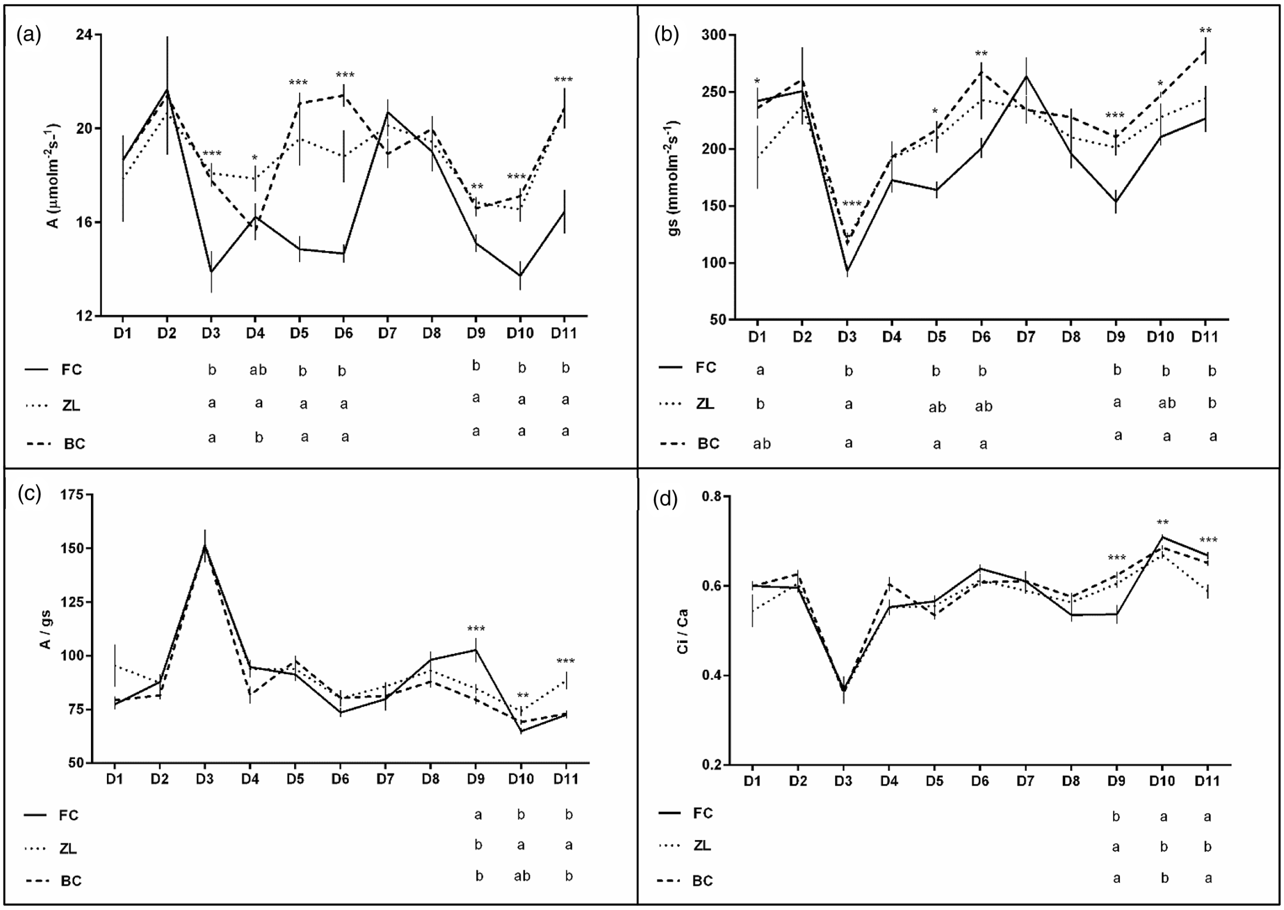

FIGURE 2 Evolution of leaf gas exchange variables in conventional fertilization as control (FC), zeolites (ZL) and biochar (BC) along the summer of 2018 (D1, D2 and D3), 2019 (D4, D5 and D6), 2020 (D7 and D8) and 2021 (D9, D10 and D11). Net photosynthetic rate (a), stomatal conductance (b), intrinsic water use efficiency (A/g_s) (c) and intercellular to atmospheric CO_2 concentration (C_i/C_a) (d). Each point with vertical bars represents the average and SE, respectively. Significance: *$p<.05$; **$p<.01$; ***$p<.001$.

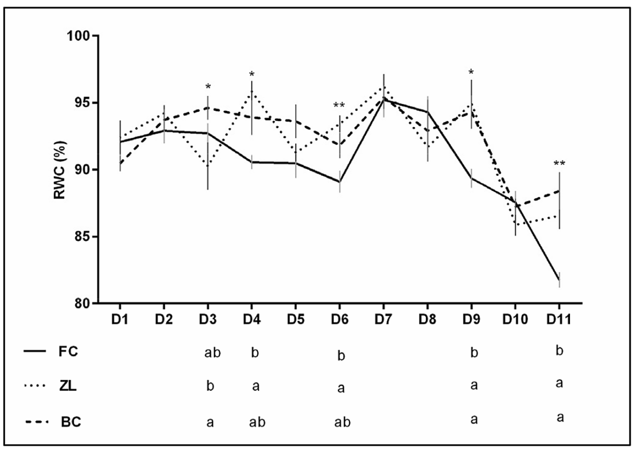

FIGURE 3 Influence of conventional fertilization as control (FC), zeolites (ZL), and biochar (BC) on relative water content (RWC) along the summer of 2018 (D1, D2 and D3), 2019 (D4, D5 and D6), 2020 (D7 and D8) and 2021 (D9, D10 and D11). Each point with vertical bars represents the average and SE, respectively. Significance: *$p<.05$; **$p<.01$.

in Table 2. In general, higher levels of total chlorophylls, total carotenoids, chlorophyll/carotenoid ratio, total phenols, ortho-diphenols, flavonoids and total antioxidant activity were registered in ZL and BC relatively to FC trees, although not always with significant differences. It is worth highlighting the higher values of carotenoids, ortho-diphenols, flavonoids and TAC with the application of biochar, and the superior chlorophyll/carotenoid ratio in ZL trees.

3.3 | Tree nutritional status and olive yield

The evolution of mineral macronutrient concentrations is represented in Figure 4. Overall, the influence of treatments was very low, although on certain dates there were some significant differences, and FC trees presented higher leaf N concentration than BC

TABLE 2 Photosynthetic pigments and metabolites from leaves of conventional fertilization as control (FC), zeolite (ZL) and biochar (BC) treatments collected at summer of 2021.

	FC	ZL	BC	*p*-Value
Chl $(a+b)$	2.18 ± 0.087^{b}	3.42 ± 0.068^{a}	3.20 ± 0.348^{a}	.001
Carotenoids	0.551 ± 0.016^{b}	0.581 ± 0.014^{ab}	0.703 ± 0.078^{a}	.040
Chl$(a+b)$/carotenoids	3.79 ± 0.086^{c}	5.62 ± 0.079^{a}	4.59 ± 0.046^{b}	<.001
Total phenols	24.7 ± 0.572^{b}	28.8 ± 0.389^{a}	27.9 ± 1.06^{a}	.001
Ortho-diphenols	29.4 ± 0.895^{b}	32.4 ± 1.72^{ab}	34.6 ± 0.919^{a}	.024
Flavonoids	19.1 ± 1.90^{b}	26.1 ± 1.37^{ab}	28.7 ± 3.51^{a}	.019
TAC	29.7 ± 0.648^{c}	31.7 ± 0.563^{b}	35.1 ± 0.365^{a}	<.001

Note: Total chlorophyll (Chl$(a+b)$) ($mg\,g^{-1}$ DW), total carotenoids ($mg\,g^{-1}$ DW), Chl$(a+b)$/carotenoids ratio, total phenols ($mg\,g^{-1}$ DW), ortho-diphenols ($mg\,g^{-1}$ DW), flavonoids ($mg\,g^{-1}$ DW) and total antioxidant activity (TAC, mmol TE g^{-1} DW). Values are means ± SE. Significance by Tukey's HSD test: $p < .05$. Means with different letters (a, b) represent significant differences between treatments. n.s. represents non-significant differences between treatments.

in December 2018. ZL showed superior leaf P concentration than BC in the last sampling date. Ca levels were higher in ZL and BC treatments in the first date, whereas in July 2019 BC trees stood out over FC treatment, and the leaf Mg concentration was superior in ZL than on FC trees in July 2018. Meanwhile, no significant effects on leaf K concentration were observed in the 7 sampling dates.

The most relevant results in this experiment related to nutritional status were the low levels of P, Ca and Mg, in general, kept below the minimum limits of the sufficiency range.

The evolution of mineral micronutrient concentrations (Figure 5) showed a behaviour similar to that of macronutrients, with the occasional recording of some significant differences, particularly on the first sampling date. The application of zeolites and biochar increased leaf B concentrations in 3 and 2 dates, respectively. When compared to FC, Zn levels were higher in BC than on FC trees in the last 2 sampling dates. Mn was superior in BC than on FC leaves in the first sampling, but an opposite trend was verified in December 2019, a period where the lowest levels of Mn were registered in ZL trees. The leaf Fe concentrations were higher in ZL and BC treatments in the first sampling date, whereas in December 2020 ZL trees presented higher values than the other treatments. Meanwhile, no significant effects on leaf Cu concentration were observed in the 7 sampling dates. Overall, the leaf micronutrient concentrations were maintained around or above the lower limit of the adequate range, being the exception, the Zn levels kept below this limit. It is also interesting to note the evolution of leaf Mn concentrations, with an increasing trend over time.

Olive yields are shown in Figure 6. Although no significant differences were found between treatments during the 4 years of the experiment, it was verified a trend to higher accumulated yield (63.3 ± 2.31) in BC relatively to ZL (56.3 ± 5.56) and to FC (52.5 ± 5.87) treatments.

3.4 | Soil chemical properties

The influence of treatments on soil chemical properties at two depths (0–10 and 10–20 cm), after 4 years, is presented in Table 3. No significant effects were found at the 10–20 cm layer, whereas in the superficial layer the application of zeolites enhanced pH relatively to control plants and extractable K and cation exchange capacity relatively to both treatments. In addition, the lowest extractable Cu at the 0–10 cm layer was observed in ZL-treated soil.

3.5 | Soil microbiology and enzymatic activity

The soil microbiological variables collected at the 0–10 cm layer presented in Table 4 revealed that in the absence of differences on organic carbon content, zeolites enhanced the soil microbial biomass carbon and the microbial biomass quotient, when compared with the control treatment, while biochar-treated soil had the lowest microbial biomass nitrogen.

From the 19 enzymatic reactions provided by the API ZYM assay, we reported for the soil fingerprint 17 enzyme activities that are involved in the main biogeochemical cycles (Table 5): carbon (β-glucosidase, α-glucosidase, α-galactosidase, β-galactosidase, α-mannosidase, α-fucosidase, esterase lipase and lipase activities), nitrogen (leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin and N-acetyl-β-glucosaminidase activities) and phosphorus (acid and alkaline phosphatase and naphthol-AS-BI-phosphohydrolase activities). Of those enzymes, esterase lipase leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase stood out as the most active soil enzymes, namely under the application of zeolites. Of the 17 enzymes studied, 7 enzyme activities were not

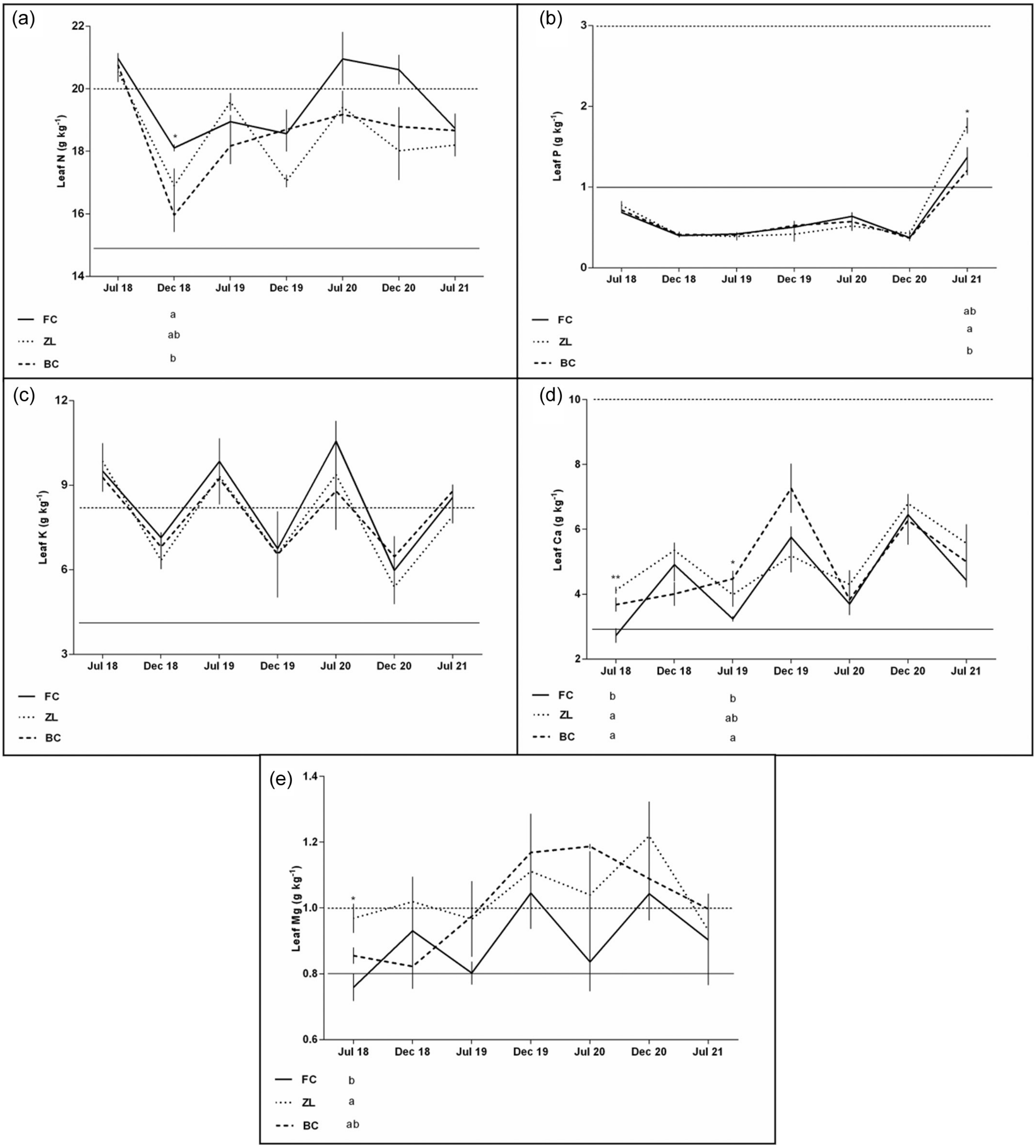

FIGURE 4 Leaf N (a), P (b), K (c), Ca (d) and Mg (e) concentrations ($g\,kg^{-1}$) in conventional fertilization as control (FC), zeolite (ZL) and biochar (BC) treatments from July 2018 to January 2021. Each point with vertical bars represents the average and SE, respectively. Dashed and solid lines are, respectively, the lower limit of the adequate range and the deficiency threshold for summer sampling, after Fernández-Escobar et al. (2017). Significance: *$p < .05$; **$p < .01$.

significantly different among treatments. Conversely, alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities were higher in ZL and BC than in control treatment; esterase lipase and α-chymotrypsin activities were superior in ZL than in FC soil; and leucine arylamidase, valine arylamidase, α-glucosidase, α-mannosidase and α-fucosidase activities were larger in ZL than in the other 2 treatments.

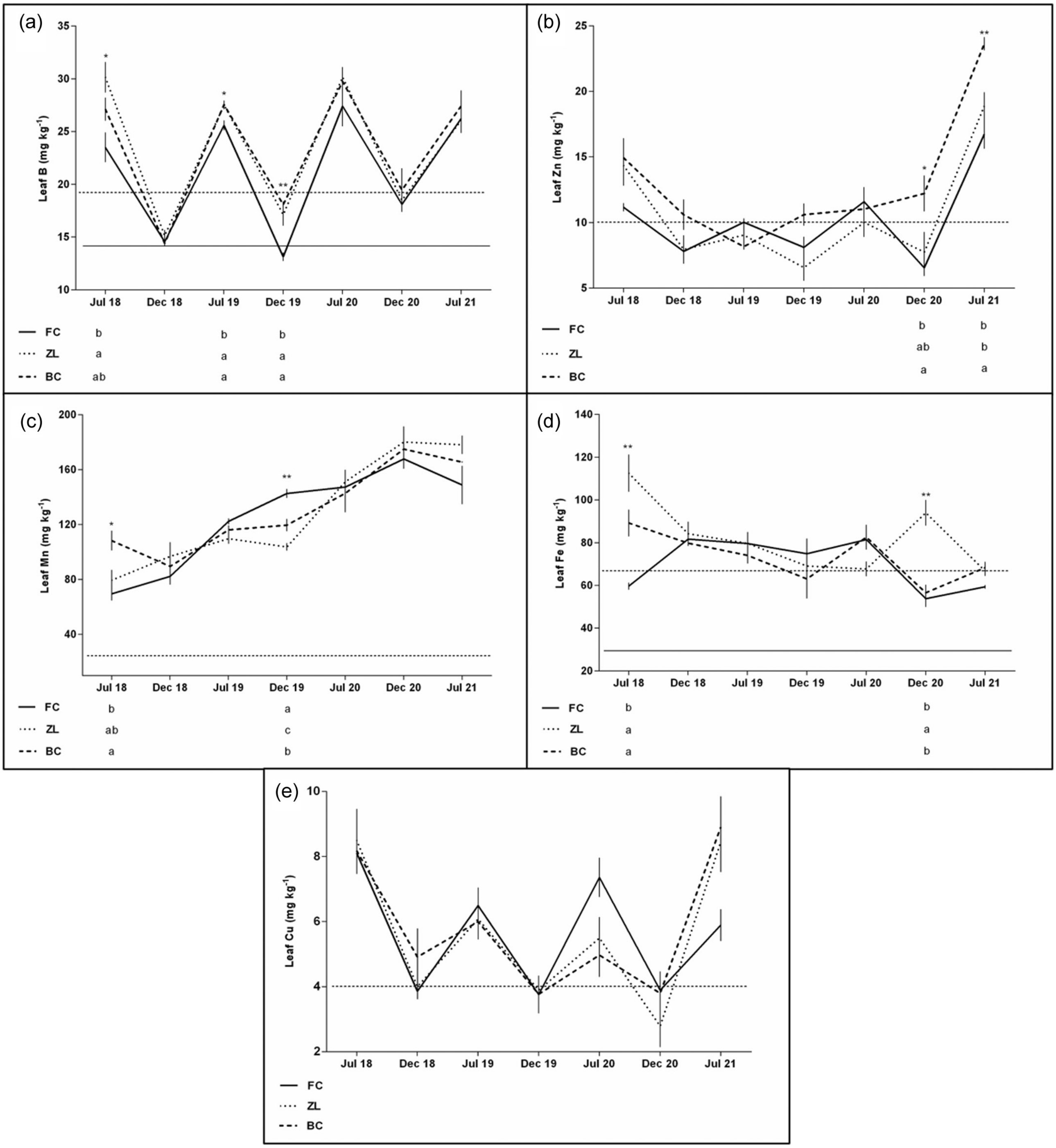

FIGURE 5 Leaf B (a), Zn (b), Mn (c), Fe (d) and Cu (e) concentrations (mg kg^{-1}) in conventional fertilization as control (FC), zeolite (ZL) and biochar (BC) treatments from July 2018 to January 2021. Each point with vertical bars represents the average and SE, respectively. Dashed and solid lines are, respectively, the lower limit of the adequate range and the deficiency threshold for summer sampling, after Fernández-Escobar et al. (2017). Significance: *$p < .05$; **$p < .01$.

3.6 | Principal component analysis

PCA was used to further identify relationships between soil properties, enzymatic activity and yield across treatments (Figure 7). This analysis considered variables such as cumulative yield, soil physico-chemical properties at a depth of 0–10 cm, microbiological properties and enzymatic activity. The results demonstrated a clear clustering of the three different soil management practices, and two principal components accounted for 81.7% of total variance (PC1 and PC2 explaining 57.8% and 23.9% of total variance, respectively). The parameters with high

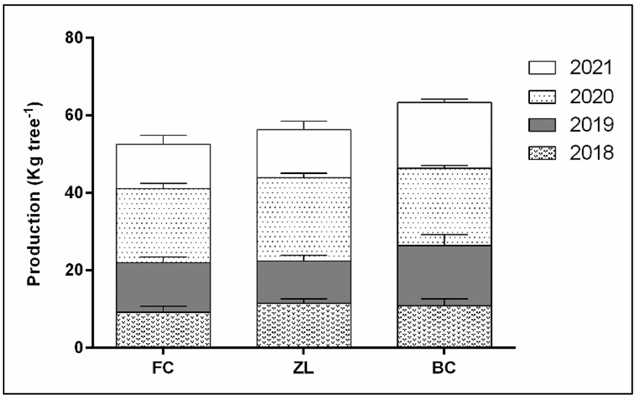

FIGURE 6 Variation in crop yield (kg tree^{-1}) from 2018 to 2021 in conventional fertilization as control (FC), zeolites (ZL) and biochar (BC).

impact on treatment separation were α-mannosidase, α-fucosidase, MBQ, leucine arylamidase, β-glucosidase, valine arylamidase, alkaline phosphatase, CEC, extractable Zn and acid phosphatase.

4 | DISCUSSION

The implementation of sustainable soil management practices in addition to restore degraded land and improve soil quality is crucial to promote several other ecosystem services, including regulation of water flows, changes in soil biodiversity, nutrient cycling and GHG reduction through increased C sequestration (Adhikari & Hartemink, 2016; Kihara et al., 2020).

In the present study, the application of soil amendments, biochar and zeolites did not change significantly the accumulated crop yield in the 4 years of the experiment in comparison with the control treatment, despite a trend towards an increase (up 20.6%) of productivity from FC to the BC treatment. Similarly, a lack of significant effect on crop yield by the use of zeolites and biochar was reported in other studies (Litaor et al., 2017; Lopes et al., 2022; Rodrigues et al., 2021; Vijay et al., 2021). Natural zeolites combined with chemical fertilizers may act as a slow-release fertilizer, allowing nutrients to be released gradually (Cataldo et al., 2021) and may not have an immediate effect on crop yield. In turn, Haider et al. (2017) reported that biochar requires a certain degree of ageing in the soil to positively affect crop yield.

On the other hand, as crop yield depends on the capacity of a plant canopy to intercept photosynthetically active radiation and on the conversion capacity into biomass (radiation use efficiency), at least one of these aspects was not significantly affected by the treatments in great part of the growing season. In fact, as the mean dry matter of leaves (about 21.0–21.5% of the pruning weight) removed in pruning in the 4 years did not vary between treatments, reaching 0.591 in FC, 0.599 in ZL and 0.609 kg/tree/year in BC, respectively, we can assume that there were no relevant differences in the canopy area and, thus, on the capacity to intercept solar radiation. On the other hand, radiation use efficiency, an index that is dependent on photosynthesis and respiration processes, seems also little altered by soil treatments, judging by the absence of differences in net photosynthetic rate in parts of the year where prevailed better conditions for the physiological activity of trees. The lack of biochar and zeolite effects on photosynthesis/respiration rate and/or canopy size was also previously observed (Hui et al., 2018; Lopes et al., 2020, 2022). Nevertheless, the integration of all leaf gas exchange variables allows to highlight the positive influence of both soil amendments on olive tree net photosynthesis during periods of greater climate adversity, coinciding with simultaneous low water availability, high temperature, and high photosynthetic photon flux density, because of the reductions in both stomatal and mesophyllic constraints to photosynthesis. Stimulations of net photosynthesis and stomatal conductance in response to the application of these soil conditioners were also recorded in other studies (Abideen et al., 2020; Bybordi, 2016; He et al., 2020; Jiang et al., 2022; Yeboah et al., 2017). In our experiment, the association of RWC with g_s data permits to infer that the application of zeolites and biochar improved the plant water status on some sampling dates, a fundamental reason for the enhancement of photosynthetic activity. In addition, some improvements in the nutritional status of trees subjected to the application of soil conditioners, particularly Ca, Mg, B, Zn, and Fe, were occasionally observed, although not simultaneously, an aspect that also contributed to the increase of photosynthetic performance. Improvements in the water and/or mineral nutritional status of plants in response to the addition of biochar and zeolites have been described by other authors (Abideen et al., 2020; Bybordi, 2016; Jiang et al., 2022). We believe that the results of the physiological and crop yield performance in the present study are strongly conditioned by severe limitations at the soil level, namely the high acidity, the low values of organic matter content and cation exchange capacity, as well as the deficiency of some minerals, which translate into plants with low leaf concentrations of P, Ca, Mg and Zn, generally kept below the minimum limits of the sufficiency range, as set by Fernández-Escobar et al. (2017). The application of biochar and zeolites, in the amounts here studied, was not enough to completely reverse those limitations, so in the future, their application should be studied in combination with soil correctives that enable substantial improvements in those parameters such as limestone and compost.

TABLE 3 Soil properties of conventional fertilization as control (FC), zeolite (ZL) and biochar (BC) treatments from samples taken at depths of 0–10 and 10–20 cm.

Soil properties	FC	ZL	BC	*p*-Value
Organic matter				
0–10 cm	16.8 ± 0.169	17.4 ± 0.297	17.4 ± 0.116	n.s.
10–20 cm	9.50 ± 0.212	8.66 ± 0.305	8.43 ± 0.602	n.s.
pH (H_2O)				
0–10 cm	4.64 ± 0.098[b]	5.01 ± 0.032[a]	4.76 ± 0.027[b]	.015
10–20 cm	4.45 ± 0.097	4.77 ± 0.095	4.42 ± 0.094	n.s.
Extract P				
0–10 cm	52.1 ± 10.05	48.6 ± 5.03	51.7 ± 3.91	n.s.
10–20 cm	25.2 ± 2.08	18.9 ± 3.87	15.4 ± 1.12	n.s.
Extract K				
0–10 cm	425.0 ± 26.5[b]	695.0 ± 16.1[a]	516.7 ± 33.7[b]	<.001
10–20 cm	295.1 ± 24.7	216.7 ± 30.9	234.3 ± 26.3	n.s.
Extract B				
0–10 cm	8.38 ± 1.093	7.88 ± 0.655	8.37 ± 0.863	n.s.
10–20 cm	6.93 ± 0.590	4.71 ± 0.702	5.54 ± 0.229	n.s.
Extract Fe				
0–10 cm	32.2 ± 2.14	24.9 ± 1.78	28.2 ± 3.36	n.s.
10–20 cm	22.2 ± 2.10	16.4 ± 1.18	17.8 ± 1.45	n.s.
Extract Mn				
0–10 cm	19.1 ± 4.04	30.2 ± 4.88	29.6 ± 1.63	n.s.
10–20 cm	14.1 ± 1.60	26.7 ± 5.09	20.9 ± 2.50	n.s.
Extract Zn				
0–10 cm	1.71 ± 0.113	1.77 ± 0.156	2.05 ± 0.110	n.s.
10–20 cm	1.04 ± 0.015	1.04 ± 0.075	1.00 ± 0.023	n.s.
Extract Cu				
0–10 cm	0.870 ± 0.112[a]	0.582 ± 0.045[b]	0.915 ± 0.031[a]	.035
10–20 cm	0.171 ± 0.010	0.172 ± 0.037	0.138 ± 0.001	n.s.
CEC				
0–10 cm	3.65 ± 0.278[b]	6.55 ± 0.261[a]	5.40 ± 0.339[b]	.001
10–20 cm	3.23 ± 0.520	5.19 ± 0.797	4.35 ± 0.303	n.s.

Note: Organic matter ($g\,kg^{-1}$), pH (H_2O), extractable P ($mg\ P_2O_5\ kg^{-1}$), extractable K ($mg\ K_2O\ kg^{-1}$), extractable B ($mg\,kg^{-1}$), extractable Fe ($mg\,kg^{-1}$), extractable Mn ($mg\,kg^{-1}$), extractable Zn ($mg\,kg^{-1}$), extractable Cu ($mg\,kg^{-1}$) and cation exchange capacity (CEC) ($cmol\ kg^{-1}$). Values are means ± SE. Significance by Tukey's HSD test: $p < .05$. Means with different letters (a, b) represent significant differences between treatments. n.s. represents non-significant differences between treatments.

TABLE 4 Microbiological properties of soils from conventional fertilization as control (FC), zeolites (ZL) and biochar (BC).

	FC	ZL	BC	*p*-Value
Organic C	9.7 ± 0.41	10.1 ± 0.017	10.1 ± 0.067	n.s.
Mic-C	80.8 ± 15.4[b]	226.6 ± 35.2[a]	166.2 ± 21.3[ab]	.019
Mic-N	1.18 ± 0.007[a]	1.22 ± 0.017[a]	1.04 ± 0.049[b]	.015
MBQ	0.83 ± 0.22[b]	2.24 ± 0.35[a]	1.64 ± 0.20[ab]	.019

Note: Organic C ($g\,kg^{-1}$), soil microbial biomass carbon (Mic-C, mg C microb kg^{-1} soil), microbial nitrogen (Mic-N, mg N kg^{-1} soil) and microbial biomass quotient (MBQ, %). Values are means ± SE. Significance by Tukey's HSD test: $p < .05$. Means with different letters (a, b) represent significant differences between treatments. n.s. represents non-significant differences between treatments.

TABLE 5 Enzymatic activity of soils from conventional fertilization as control (FC), zeolites (ZL) and biochar (BC) using API ZYM assay. All the results are presented as nmol of substrate hydrolysed.

	FC	ZL	BC	*p*-Value
Alkaline phosphatase	2.50 ± 1.44^b	12.5 ± 0.00^a	9.17 ± 1.67^a	.004
Esterase lipase	11.3 ± 0.722^b	37.9 ± 2.08^a	18.8 ± 7.53^ab	.015
Lipase	6.25 ± 2.17	10.8 ± 0.833	6.67 ± 0.833	n.s.
Leucine arylamidase	5.00 ± 0.00^b	31.9 ± 0.00^a	13.2 ± 6.02^b	.004
Valine arylamidase	21.7 ± 0.961^b	33.8 ± 0.00^a	18.9 ± 2.59^b	.001
Cystine arylamidase	2.50 ± 0.00	10.0 ± 5.00	4.58 ± 1.10	n.s.
Trypsin	2.50 ± 0.00	10.0 ± 5.00	4.58 ± 1.10	n.s.
α-Chymotrypsin	2.50 ± 0.00^b	5.00 ± 0.00^a	3.33 ± 0.833^ab	.027
Acid phosphatase	20.0 ± 0.00^b	40.0 ± 0.00^a	32.4 ± 4.86^a	.007
Naphthol-AS-BI-phosphohydrolase	23.3 ± 0.00^b	40.0 ± 0.00^a	36.7 ± 3.33^a	.002
α-Galactosidase	5.00 ± 1.44	8.75 ± 0.722	2.50 ± 1.50	n.s.
β-Galactosidase	5.00 ± 1.44	8.75 ± 0.722	2.50 ± 1.50	n.s.
α-Glucosidase	1.88 ± 1.08^b	12.5 ± 0.00^a	1.67 ± 0.833^b	<.0001
β-Glucosidase	20.7 ± 5.41	33.8 ± 0.00	14.2 ± 7.95	n.s.
N-acetyl-β-glucosaminidase	7.50 ± 1.44	3.75 ± 0.722	7.50 ± 2.50	n.s.
α-Mannosidase	1.25 ± 0.721^b	5.00 ± 0.00^a	0.833 ± 0.533^b	.007
α-Fucosidase	1.25 ± 0.721^b	5.00 ± 0.00^a	0.833 ± 0.533^b	.007

Note: Values are means ± SE. Significance by Tukey's HSD test: $p < .05$. Means with different letters (a, b) represent significant differences between treatments. n.s. represents non-significant differences between treatments.

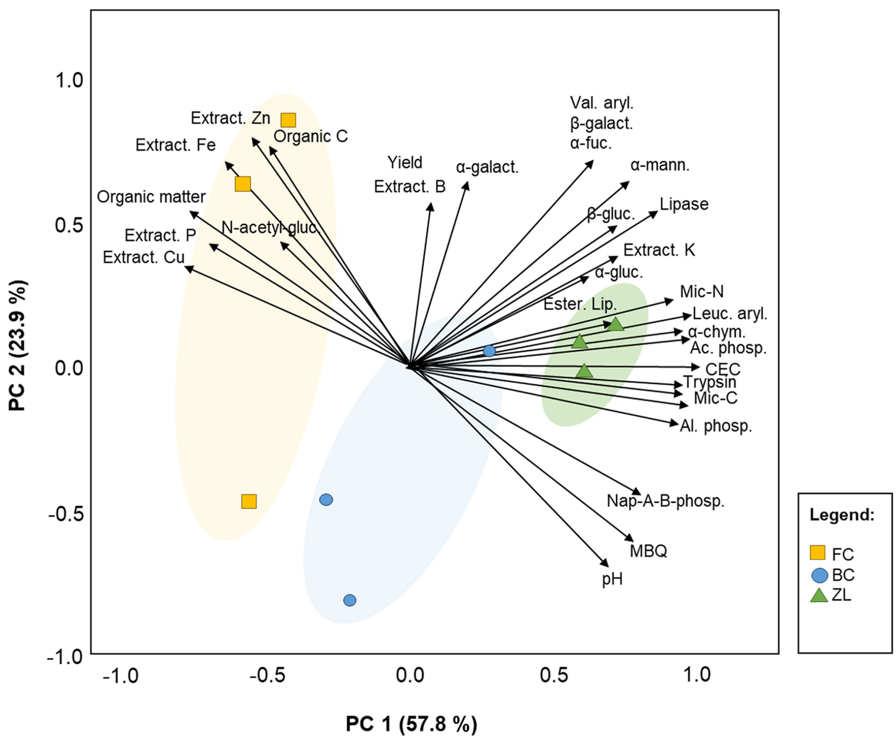

FIGURE 7 Principal component analysis (PCA) of cumulative yield, soil properties, microbiological and enzymatic activity and treatment clusters.

The effects of environmental stresses on plants can generate the over-reduction of photosynthetic electron chain, which is an important source of reactive oxygen species (ROS). ROS induce detrimental effects on plant metabolism, causing oxidative damage to a wide range of targets (Das & Roychoudhury, 2014; Mechri et al., 2020), including photosynthetic pigments. In our study, leaves from FC trees revealed high signs of oxidative stress, as confirmed by lower concentrations of chlorophylls than on ZL and BC treatments and lower concentrations of carotenoids than on BC trees, meaning that FC leaves presented higher disturbance of the physiological redox balance. Meanwhile, chlorophyll/carotenoid ratio was inferior in FC than in the other treatments, namely in comparison with ZL. Since carotenoids play a crucial function in photoprotection, scavenging ROS and releasing the excess of energy by thermal dissipation via xanthophyll cycle (Seyed et al., 2012), this lower ratio in FC leaves shows higher need for photoprotection of chlorophylls. Lower signs of oxidative stress in plants treated with biochar and zeolites were observed in previous studies (Abideen et al., 2020; Bybordi, 2016; Feng et al., 2022; He et al., 2020; Jiang et al., 2022).

Several stresses, and the concomitant oxidative stress, induce the phenylpropanoid metabolism in plants, leading to an accumulation of phenolics, compounds that hold an ideal chemistry to counteract ROS. Nonetheless, despite high signs of oxidative stress, FC leaves showed, in general, lower concentrations of TP, ortho-diphenols and flavonoids and inferior TAC in comparison with the other treatments, namely BC. The higher investment in secondary metabolism in ZL and, especially, in BC-treated plants could be related to the higher availability of carbohydrates as a consequence of the superior net photosynthesis during the stressful summer periods, as demonstrated previously (Pérez-López et al., 2013, 2015). The phenylpropanoid pathway is closely connected to the carbohydrate metabolism via the shikimate pathway (Crozier et al., 2006).

The analysis of the soil chemical properties after the 4 years of the study revealed a substantial improvement in its fertility, as demonstrated by the significant increases of extractable P and K values, as well as by the rise of more than 100% in the organic carbon content in all treatments. In contrast, there was a minor aggravation of soil acidity, with the exception of the ZL treatment. The main reasons that explain these variations were the profound changes in soil management and fertilization practices that occurred in the orchard. In fact, only one superficial soil tillage was carried out in the first year of the study in order to bury the soil amendments, jointly with annual applications of 60 kg of N, P_2O_5 and K_2O ha^{-1} as a compound NPK, plus 2 kg B ha^{-1}. These changes, together with the implementation of a moderate annual pruning regime justified the increase of olive yield to mean values slightly above 3 t ha^{-1} $year^{-1}$, when prior to the installation of the trial the productivity was quite low (about 1 t ha^{-1} $year^{-1}$). As this topic is not the main aim of the present work, we will now discuss, in greater detail, the influence of the studied treatments on soil chemical and biological properties, as well as on a wide range of soil enzyme activities.

Four years after the application of biochar and zeolites, no significant differences in the soil chemical properties at the 10–20 cm layer were observed, while at the superficial layer (0–10 cm) only significant effects of zeolites were recorded, as these plots presented higher pH, extractable K and CEC, and lower extractable Cu, averaging 8.0% (0.37 units), 63.5%, 79.5% and 33.1% relatively to FC plots, respectively, as also evidenced by the PCA. Similar trends were described earlier (Chatzistathis et al., 2021; Li et al., 2022; Lopes et al., 2022; Martins et al., 2023; Moeen et al., 2020; Quartacci et al., 2017). The increase of soil pH happened since zeolites are marginally alkaline and because of the likely influence on the reduction of nitrification, an acidifying process (Weil & Brady, 2017). On the other hand, as clinoptilolite zeolite has high cation exchange capacity, many times superior to soil CEC, is an *abundant K-rich silicate* and can act as a reservoir adsorbing K (Li et al., 2022; Yinghao et al., 2021), its application contributed to increase CEC and topsoil extractable K levels, resulting in less leaching losses, less luxury plant K uptake and greater soil K storage (Rosolem et al., 2010). Meanwhile, the lower concentration of extractable Cu in ZL-treated soil is also quite relevant, considering the problem of Cu accumulation in agricultural soils, as in olive orchards. The high affinity of natural zeolites for cationic pollutant sequestration, such as Cu, also reported previously by others (Kumar et al., 2007; Ponizovsky & Tsadilas, 2003; Tashauoei et al., 2010), contributes to decrease the morphological, physiological, biochemical and molecular phytotoxic effects induced by high Cu levels and also to drop the flow of metal to further links in the trophic chain. Although all these changes induced by zeolites are important from the soil fertility, environment and food safety point of view, during the 4 years of the experiment they did not have a relevant influence on crop yield. Nevertheless, this does not rule out the possibility of benefits that could be obtained in the following years. Soil quality is critical for plant growth and development because it directly affects nutrient availability, root development, water retention and microbial activity. Thus, it is expected to have a long-term beneficial effect on crop productivity (Ren et al., 2023).

In this study, the influence of biochar on soil chemical properties was residual, as has been reported in other studies (Nelissen et al., 2015; Tsolis & Barouchas, 2023), although in other experiments increases in pH, CEC, organic carbon and availability of essential minerals have been recorded (Ding et al., 2016; Laird et al., 2010; Tsolis & Barouchas, 2023; Wang et al., 2014; Xu et al., 2016). These discrepant results may be justified by a broad framework of factors such as the type of biochar (e.g. biochar feedstock and pyrolysis conditions), the soil type (e.g. pH and texture), the application rate, the degree of its incorporation into the soil, the depth of mixing with the soil, the availability of nutrients, the presence of environmental and biological stresses and the crop type (O'Connor et al., 2018; Tsolis & Barouchas, 2023). Furthermore, it should be noted that the effects of biochar are modified over time (the "ageing effect") through physico-chemical and biological processes (Sun et al., 2022). Thus, for all these causes it is crucial to consider the application period of biochar and to know the underlying influencing factors of biochar functions in order to choose the best one for each particular growing condition.

In our study, the use of zeolites and biochar changed important soil biological properties, as in other studies (Ding et al., 2016; Doni et al., 2021; Egamberdieva et al., 2022; Martins et al., 2023; Xu et al., 2016), but only in the 0–10 cm soil layer. Four years after the application, zeolite plots presented higher microbial biomass carbon and microbial biomass quotient than the FC treatment, whereas biochar-treated soil showed intermediate values. As Mic-C reflects the process of microbial growth, death and organic matter degradation, our results suggested that microbial growth could be accelerated (Zhang et al., 2014) by zeolite addition. This result is most likely associated with the increases of pH and K, which provided more energy source for microorganisms (Watzinger et al., 2014), and CEC, and the reduction of soil Cu. In addition, zeolites have high surface area and porous structure can create a more favourable habitat for microbial establishment. On the other hand, zeolite application enhanced MBQ by 169.9%, when compared to FC, allowing to reach 2.24%, a value considered within the normal range (1%–4% of organic carbon), as set by Jenkinson and Ladd (1981), while the MBQ of the control treatment was only 0.83%. High MBQ represents elevated availability of organic C for soil microbes and active organic matter (Sampaio et al., 2008). Meanwhile, biochar supply decreased the microbial biomass nitrogen relatively to the other treatments, as in the study of Durenkamp et al. (2010). The association between the lower Mic-N and the similar Mic-C indicates that biochar in soil acted as a carbon source rather than a nitrogen source for soil microbes, which could have consequences for N cycling as increased microbial N immobilization with the use of biochar (Zhang et al., 2014).

Enzymatic activities have been recognized as very delicate indicators of soil management, as they provide early detection of changes in soil health, answering to agronomical practices and environmental factors much faster than the other soil quality parameters (Ch et al., 2017). Soil enzymes are the key players in the biochemical processes of organic matter recycling in the soil system, being their activities closely related to changes on physical properties, pH, organic carbon, CEC, nutrient availability, and microbial population and activity (Ch et al., 2017; Martínez et al., 2016). The API ZYM system identified the enzymatic profile in our soil samples, revealing the activity of 17 out of 19 enzymes analyzed, belonging to the enzymatic families of phosphates, esterase lipases, peptidases, aminopeptidase, and glycosyl hydrolases. The high activity of acid phosphatase and naphthol-AS-BI-phosphohydrolase observed in this study has been related to the low availability of phosphorus, causing microorganisms to excrete enzymes to assimilate P into their structure, while the elevated esterase lipase activity was related to its role in the carbon cycle and in the degradation of water-soluble compounds, as ester bonds and organic acids (Boluda et al., 2014; Patel et al., 2019). Meanwhile, the soil of this orchard presented high activity of the aminopeptidase's leucine arylamidase and valine arylamidase, enzymes involved in the transformation of N protein into amino acids and its later degradation (Bedolla-Rivera et al., 2020). Furthermore, we noticed high β-glucosidase activity, a glycosyl hydrolase enzyme usually considered to be the most sensitive indicator of soil quality as it contributes to the degradation of cellulose and other β-1,4 glucans (Sinsabaugh et al., 2008) to produce sugars, which are the main source of energy to soil microorganisms (Acosta-Martínez et al., 2007).

The studied soil management treatments caused significant differences in 10 of the 17 enzymes detected, namely in three enzymes of the phosphorus cycle (alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase), in esterase lipase, in two aminopeptidases (leucine arylamidase and valine arylamidase), in one peptidase (α-chymotrypsin) and in three glycosyl hydrolases (α-glucosidase, α-mannosidase and α-fucosidase). In general, the addition of zeolites caused the most positive changes in soil enzymatic activity, leading to increases of all 10 enzymes involved in C, N and P cycles, when compared to control, and of the 2 aminopeptidases and 3 glycosyl hydrolases, when related to BC treatment. Meanwhile, biochar supply enhanced the activities of all 3 P-cycle-related enzymes relatively to control plots. From the above, we can admit that the application of the 2 soil conditioners improves soil health, especially the use of zeolites because they lead to increases in the activity of 10 enzymes involved in the C, N and P cycles, while biochar only increased the activity of three P-cycle enzymes. To avoid redundancy of information, below we will only briefly discuss the functions of the other soil enzymes where there were significant differences among treatments, but not yet discussed. Alkaline phosphatase, produced exclusively by microorganisms (Spohn & Kuzyakov, 2013), also plays important roles in P mineralization, even in acidic soils, as new evidence suggests that compared with acid phosphatases, primarily derived from plant roots and microorganisms, there is higher abundance of genes encoding alkaline phosphatases in acidic soils (Bergkemper et al., 2016), thereby underlining the importance of alkaline phosphatase (Li et al., 2021).

Apart from the greater activity of the 2 aminopeptidases, the supply of zeolites increased the activity of α-chymotrypsin, a general serine peptidase (Yamawaki et al., 2021), relatively to control soils, thus contributing to high rate of protein decomposition, commonly considered to be the rate-limiting step of N cycling in terrestrial (Schimel & Bennett, 2004). Meanwhile, zeolite-treated soil presented the highest activity of three glycosyl hydrolases that belong to a group of C-cycling enzymes. These responses are relevant to carbon cycle since α-glucosidase catalyses the hydrolysis of α-D-glucopyranosides to release α-D glucose (Uwituze et al., 2022), mannosidases are important in the biological processing of mannose-containing polysaccharides and complex glycoconjugates (Rovira et al., 2020) and α-fucosidase removes the terminal α-fucosyl residues from oligosaccharides and glycoconjugates (Wu et al., 2023). As high N availability is a main factor that increases microbial demand for C, inducing therefore the production of glucosidases (Asmar et al., 1994), our results suggest that zeolites may have increased soil N availability.

5 | CONCLUSIONS

Overall, our results showed that the addition of zeolites and biochar amendments to NPKB fertilizers ameliorated the physiological and biochemical performance of olive tree, as evidenced by consistent increments of RWC, g_s and net photosynthesis and by lower signs of oxidative stress during the periods of greater climate adversity. However, crop yield was not significantly different among soil treatments. On the other hand, soil chemical and biological traits at the surface layer (0–10 cm) have shown different and relevant responses after 4 years of soil amendment application. In fact, zeolite supply stood out as increased pH, extractable K, CEC, microbial biomass carbon and microbial biomass quotient and reduced extractable Cu. Furthermore, zeolites induced positive changes in soil enzymatic activity, leading to increases in 10 enzymes involved in C, N and P cycles. In contrast, the effects of biochar on soil properties were much more reduced, given that it decreased the microbial biomass nitrogen and enhanced the activities of three P-cycle enzymes.

To the best of our knowledge, this is the first study reporting the effects of zeolites and biochar combined with chemical fertilizer, covering a wide range of parameters related to soil quality and olive tree performance. Thus, this study increased the understanding of the influence of soil amendment with zeolites and biochar in rainfed olive orchards. In summary, our data demonstrated that both soil amendments can be an interesting complement to mineral fertilization, in order to increase trees' resilience under rainfed conditions and to promote soil health, although the use of zeolites appears to be a more promising strategy because of the induction of higher soil sustainability. Nevertheless, further studies should be done to find the optimal rates and application times, and the best physical and chemical characteristics, as well as to verify the effects of soil amendments on soil properties and crop yield in the long-term. Furthermore, future research should include an economic analysis to evaluate the cost–benefit ratios of the provided sustainable soil management strategies. Thus, we will obtain a more comprehensive representation of these management practices, which could be beneficial for further decision-making strategies.

ACKNOWLEDGMENTS

This research was funded by a doctoral fellowship under the Doctoral Program "Agricultural Production Chains—from fork to farm" (PD/00122/2012) provided by the FCT-Portuguese Foundation for Science and Technology to S. Martins (PD/BD/135327/2017) and by the Operational Group "Novas práticas em olivais de sequeiro: estratégias de mitigação e adaptação às alterações climáticas", funded by PT2020 and European Agricultural Fund for Rural Development (EAFRD). The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Program PT2020 for their financial support to CQ-VR (UIDB/00616/2020), CIMO (UIDB/00690/2020) and CITAB (UIDB/04033/2020) and also to the AgriFood XXI Project, No. NORTE-01-0145-FEDER-000041, co-financed by the European Regional Development Fund (ERDF) through the North Regional Operational (NORTE) Program 2014–2020.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Sandra Martins https://orcid.org/0000-0002-7519-4001
José Moutinho-Pereira https://orcid.org/0000-0001-5825-559X
Margarida Arrobas https://orcid.org/0000-0002-4652-485X
Manuel Â. Rodrigues https://orcid.org/0000-0002-5367-1129

Fernando Nunes https://orcid.org/0000-0003-2482-7873

REFERENCES

Abideen, Z., Koyro, H.-W., Huchzermeyer, B., Gul, B., & Khan, M. A. (2020). Impact of a biochar or a biochar-compost mixture on water relation, nutrient uptake and photosynthesis of Phragmites karka. *Pedosphere*, *30*(4), 466–477.

Acosta-Martínez, V., Cruz, L., Sotomayor-Ramírez, D., & Pérez-Alegría, L. (2007). Enzyme activities as affected by soil properties and land use in a tropical watershed. *Applied Soil Ecology*, *35*(1), 35–45.

Adhikari, K., & Hartemink, A. E. (2016). Linking soils to ecosystem services—A global review. *Geoderma*, *262*, 101–111.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, *24*(1), 1–15.

Asmar, F., Eiland, F., & Nielsen, N. E. (1994). Effect of extracellular-enzyme activities on solubilization rate of soil organic nitrogen. *Biology and Fertility of Soils*, *17*(1), 32–38.

Babla, M., Katwal, U., Yong, M.-T., Jahandari, S., Rahme, M., Chen, Z.-H., & Tao, Z. (2022). Value-added products as soil conditioners for sustainable agriculture. *Resources, Conservation and Recycling*, *178*, 1–13.

Balbino, L. R. (1968). *La Méthode Egner-Riehm et la Détermination du Phosfore et du Potassium «assimilável» des sols du Portugal*. (pp. 55–65). Medit Cont. Fert. Plantas Cultivadas, II Col.

Baldantoni, D., Bellino, A., Morra, L., & Alfani, A. (2015). Compost amendment enhances natural revegetation of a Mediterranean degraded agricultural soil. *Environmental Management*, *56*, 946–956.

Baldrian, P., Voříšková, J., Dobiášová, P., Merhautová, V., Lisá, L., & Valášková, V. (2011). Production of extracellular enzymes and degradation of biopolymers by saprotrophic microfungi from the upper layers of forest soil. *Plant and Soil*, *338*(1), 111–125.

Bedolla-Rivera, H. I., Xochilt Negrete-Rodríguez, M. D. L., Medina-Herrera, M. D. R., Gámez-Vázquez, F. P., Álvarez-Bernal, D., Samaniego-Hernández, M., Gámez-Vázquez, A. J., & Conde-Barajas, E. (2020). Development of a soil quality index for soils under different agricultural management conditions in the central lowlands of Mexico: Physicochemical, biological and ecophysiological indicators. *Sustainability*, *12*(22), 9754.

Bergkemper, F., Schöler, A., Engel, M., Lang, F., Krüger, J., Schloter, M., & Schulz, S. (2016). Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. *Environmental Microbiology*, *18*(6), 1988–2000.

Boluda, R., Roca-Pérez, L., Iranzo, M., Gil, C., & Mormeneo, S. (2014). Determination of enzymatic activities using a miniaturized system as a rapid method to assess soil quality. *European Journal of Soil Science*, *65*(2), 286–294.

Brito, C., Dinis, L.-T., Moutinho-Pereira, J., & Correia, C. M. (2019). Drought stress effects and olive tree acclimation under a changing climate. *Plants*, *8*(7), 1–20.

Brito, C., Dinis, L.-T., Silva, E., Gonçalves, A., Matos, C., Rodrigues, M., Moutinho Pereira, J., Barros, A., & Correia, C. (2018). Kaolin and salicylic acid foliar application modulate yield, quality and phytochemical composition of olive pulp and oil from rainfed trees. *Scientia Horticulturae*, *237*, 176–183.

Brookes, P. C., Powlson, D. S., & Jenkinson, D. S. (1982). Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry*, *14*(4), 319–329.

Bybordi, A. (2016). Influence of zeolite, selenium and silicon upon some agronomic and physiologic characteristics of canola grown under salinity. *Communications in Soil Science and Plant Analysis*, *47*(7), 832–850.

Cataldo, E., Salvi, L., Paoli, F., Fucile, M., Masciandaro, G., Manzi, D., Masini, C. M., & Mattii, G. B. (2021). Application of zeolites in agriculture and other potential uses: A review. *Agronomy*, *11*(8), 1–14.

Ch, S., Grover, M., Kundu, S., & Desai, S. (2017). In S. I. E. Science (Ed.), *Soil enzymes* (pp. 2100–2107). Springer.

Chandini, R. K., Kumar, R., & Prakash, O. (2019). The impact of chemical fertilizers on our environment and ecosystem. In *Research trends in environmental sciences* (Vol. 1, 2nd ed.). AkiNik Publications.

Chatzistathis, T., Papaioannou, E., Giannakoula, A., & Papadakis, I. E. (2021). Zeolite and vermiculite as inorganic soil amendments modify shoot-root allocation, mineral nutrition, photosystem II activity and gas exchange parameters of chestnut (Castanea sativa mill) plants. *Agronomy*, *11*(1), 109.

Chen, T., Xia, G., Wu, Q., Zheng, J., Jin, Y., Sun, D., Wang, S., & Chi, D. (2017). The influence of zeolite amendment on yield performance, quality characteristics, and nitrogen use efficiency of paddy rice. *Crop Science*, *57*(5), 2777–2787.

Crozier, A., Clifford, M., & Ashihara, H. (2006). *Plant secondary metabolites: Occurrence, structure and role in the human diet*. Blackwell Publishing Ltd.

Das, K., & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science*, *2*, 1–13.

Ding, Y., Liu, Y., Liu, S., Huang, X., Li, Z., Tan, X., Zeng, G., & Zhou, L. (2017). Potential benefits of biochar in agricultural soils: A review. *Pedosphere*, *27*(4), 645–661.

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., & Zheng, B. (2016). Biochar to improve soil fertility. A review. *Agronomy for Sustainable Development*, *36*(2), 36.

Doni, S., Gispert, M., Peruzzi, E., Macci, C., Mattii, G. B., Manzi, D., Masini, C. M., & Grazia, M. (2021). Impact of natural zeolite on chemical and biochemical properties of vineyard soils. *Soil Use and Management*, *37*(4), 832–842.

Durenkamp, M., Luo, Y., & Brookes, P. C. (2010). Impact of black carbon addition to soil on the determination of soil microbial biomass by fumigation extraction. *Soil Biology and Biochemistry*, *42*(11), 2026–2029.

Egamberdieva, D., Alaylar, B., Kistaubayeva, A., Wirth, S., & Bellingrath-Kimura, S. D. (2022). Biochar for improving soil biological properties and mitigating salt stress in plants on salt-affected soils. *Communications in Soil Science and Plant Analysis*, *53*(2), 140–152.

El, S. N., & Karakaya, S. (2009). Olive tree (*Olea europaea*) leaves: Potential beneficial effects on human health. *Nutrition Reviews*, *67*(11), 632–638.

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D., & Huang, J. (2017). Crop production under drought and heat stress: Plant responses and management options. *Frontiers in Plant Science*, *8*, 1–16.

Feng, S., Zhang, P., Hu, Y., Jin, F., Liu, Y., Cai, S., Song, Z., Zhang, X., Nadezhda, T., Guo, Z., Lynch, I., & Dang, X. (2022). Combined application of biochar and nano-zeolite enhanced cadmium immobilization and promote the growth of Pak Choi in cadmium contaminated soil. *NanoImpact*, *28*, 100421.

Fernández-Escobar, R., Barranco, D., & Rallo, L. (2017). *El Cultivo del Olivo* (7th ed.). Ediciones Mundi-Prensa.

Ferreira, C. S. S., Seifollahi-Aghmiuni, S., Destouni, G., Ghajarnia, N., & Kalantari, Z. (2022). Soil degradation in the European Mediterranean region: Processes, status and consequences. *Science of the Total Environment*, *805*, 1–17.

Genesio, L., Miglietta, F., Baronti, S., & Vaccari, F. P. (2015). Biochar increases vineyard productivity without affecting grape quality: Results from a four years field experiment in Tuscany. *Agriculture, Ecosystems and Environment*, *201*, 20–25.

Giovanni, S., Iovieno, P., Raffaella, M., Baldantoni, D., Morra, L., & Anna, A. (2013). Long-term compost amendment of a Mediterranean horticultural soil. Part I: Relationships among biological and chemical soil quality and the yield of two crops. In *Paper presented at the RAMIRAN 2013 15th International conference Versailles*, *Versailles*, *France*.

Głąb, T., Gondek, K., & Mierzwa–Hersztek, M. (2021). Biological effects of biochar and zeolite used for remediation of soil contaminated with toxic heavy metals. *Scientific Reports*, *11*(1), 2–11.

Haider, G., Steffens, D., Moser, G., Müller, C., & Kammann, C. I. (2017). Biochar reduced nitrate leaching and improved soil moisture content without yield improvements in a four-year field study. *Agriculture, Ecosystems and Environment*, *237*, 80–94.

He, Y., Yao, Y., Ji, Y., Deng, J., Zhou, G., Liu, R., Shao, J., Zhou, L., Li, N., Zhou, X., & Bai, S. H. (2020). Biochar amendment boosts photosynthesis and biomass in C3 but not C4 plants: A global synthesis. *GCB Bioenergy*, *12*(8), 605–617.

Hui, D., Yu, C.-L., Deng, Q., Saini, P., Collins, K., & Koff, J. D. (2018). Weak effects of biochar and nitrogen fertilization on switchgrass photosynthesis, biomass, and soil respiration. *Agriculture*, *8*(9), 143.

Jenkinson, D. S., & Ladd, J. N. (1981). Microbial biomass in soil: Measurement and turnover. In E. A. Paul & J. N. Ladd (Eds.), *Soil biochemistry* (Vol. 5). Marcel Dekker, Inc.

Jiang, W., Xu, L., Liu, Y., Su, W., Yan, J., & Xu, D. (2022). Effect of biochar on the growth, photosynthesis, antioxidant system and cadmium content of *Mentha piperita* 'chocolate' and Mentha spicata in cadmium-contaminated soil. *Agronomy*, *12*(11), 2737.

Jones, C. (2021). *Drought effects on soil enzyme activity* (Master of Science in Forestry), Lakehead University.

Karaca, M. (2004). Use of Natural Zeolite (Clinoptilolite) in agriculture. *Journal of Fruit and Ornamental Plant Research*, *12*, 183–189.

Kihara, J., Bolo, P., Kinyua, M., Nyawira, S. S., & Sommer, R. (2020). Soil health and ecosystem services: Lessons from sub-Sahara Africa (SSA). *Geoderma*, *370*, 114342.

Kumar, P., Jadhav, P. D., Rayalu, S. S., & Devotta, S. (2007). Surface-modified zeolite–A for sequestration of arsenic and chromium anions. *Current Science*, *92*(4), 512–517.

Laird, D. A., Fleming, P., Davis, D. D., Horton, R., Wang, B., & Karlen, D. L. (2010). Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. *Geoderma*, *158*(3), 443–449.

Li, J., Xie, T., Zhu, H., Zhou, J., Li, C., Xiong, W., Xu, L., Wu, Y., He, Z., & Li, X. (2021). Alkaline phosphatase activity mediates soil organic phosphorus mineralization in a subalpine forest ecosystem. *Geoderma*, *404*, 115376.

Li, Y., Xia, G., Wu, Q., Chen, W., Lin, W., Zhang, Z., Chen, Y., Chen, T., Siddique, K. H. M., & Chi, D. (2022). Zeolite increases grain yield and potassium balance in paddy fields. *Geoderma*, *405*, 115397.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, *148*, 350–382.

Litaor, M. I., Katz, L., & Shenker, M. (2017). The influence of compost and zeolite co-addition on the nutrients status and plant growth in intensively cultivated Mediterranean soils. *Soil Use and Management*, *33*(1), 72–80.

Lopes, J. I., Arrobas, M., Brito, C., Gonçalves, A., Silva, E., Martins, S., Raimundo, S., Rodrigues, M. Â., & Correia, C. M. (2020). Mycorrhizal fungi were more effective than zeolites in increasing the growth of non-irrigated young olive trees. *Sustainability*, *12*(24), 1–15.

Lopes, J. I., Arrobas, M., Raimundo, S., Gonçalves, A., Brito, C., Martins, S., Pinto, L., Moutinho-Pereira, J., Correia, C. M., & Rodrigues, M. Â. (2022). Photosynthesis, yield, nutrient availability and soil properties after biochar, zeolites or mycorrhizal inoculum application to a mature rainfed olive orchard. *Agriculture*, *12*(2), 1–15.

Lopes, J. I., Gonçalves, A., Brito, C., Martins, S., Pinto, L., Moutinho-Pereira, J., Raimundo, S., Arrobas, M., Rodrigues, M. Â., & Correia, C. M. (2021). Inorganic fertilization at high N rate increased olive yield of a rainfed orchard but reduced soil organic matter in comparison to three organic amendments. *Agronomy*, *11*(11), 1–15.

Martínez, D., Molina, M. J., Sánchez, J., Moscatelli, M. C., & Marinari, S. (2016). API ZYM assay to evaluate enzyme fingerprinting and microbial functional diversity in relation to soil processes. *Biology and Fertility of Soils*, *52*(1), 77–89.

Martins, S., Brito, C., Silva, E., Gonçalves, A., Arrobas, M., Pereira, E., Rodrigues, M. Â., Nunes, F. M., & Correia, C. M. (2023). Synergy between zeolites and leguminous cover crops improved olive tree performance and soil properties in a rainfed olive orchard. *Agronomy*, *13*(11), 2674.

Martins, S., Silva, E., Brito, C., Martins-Gomes, C., Gonçalves, A., Arrobas, M., Rodrigues, M., Correia, C. M., & Nunes, F. M. (2022). Zeolites and biochar modulate olive fruit and oil polyphenolic profile. *Antioxidants*, *11*(7), 1–24.

Mechri, B., Tekaya, M., Hammami, M., & Chehab, H. (2020). Effects of drought stress on phenolic accumulation in greenhouse-grown olive trees (*Olea europaea*). *Biochemical Systematics and Ecology*, *92*, 1–7.

Michalopoulos, G., Kasapi, K. A., Koubouris, G., Psarras, G., Arampatzis, G., Hatzigiannakis, E., Kavvadias, V., Xiloyannis, C., Montanaro, G., Malliaraki, S., Angelaki, A., Manolaraki, C., Giakoumaki, G., Reppas, S., Kourgialas, N., & Kokkinos, G. (2020). Adaptation of Mediterranean olive groves to climate change through sustainable cultivation practices. *Climate*, *8*(4), 1–11.

Moeen, M., Qi, T., Hussain, Z., Ge, Q., Maqbool, Z., Jianjie, X., & Kaiqing, F. (2020). Use of zeolite to reduce the bioavailability of heavy metals in a contaminated soil. *Ecological Engineering*, *21*(7), 186–196.

Mondal, M., Biswas, B., Garai, S., Sarkar, S., Banerjee, H., Brahmachari, K., Bandyopadhyay, P. K., Maitra, S., Brestic, M., Skalicky, M., Ondrisik, P., & Hossain, A. (2021). Zeolites enhance soil health, crop productivity and environmental safety. *Agronomy*, *11*(3), 1–29.

Nelissen, V., Ruysschaert, G., Manka'Abusi, D., D'Hose, T., De Beuf, K., Al-Barri, B., Cornelis, W., & Boeckx, P. (2015). Impact of a woody biochar on properties of a sandy loam soil and spring barley during a two-year field experiment. *European Journal of Agronomy*, *62*, 65–78.

O'Connor, D., Peng, T., Zhang, J., Tsang, D. C. W., Alessi, D. S., Shen, Z., Bolan, N. S., & Hou, D. (2018). Biochar application for the remediation of heavy metal polluted land: A review of in situ field trials. *Science of the Total Environment*, *619–620*, 815–826.

Patel, D., Gismondi, R., Alsaffar, A., & Tiquia-Arashiro, S. M. (2019). Applicability of API ZYM to capture seasonal and spatial variabilities in lake and river sediments. *Environmental Technology*, *40*(24), 3227–3239.

Pérez-López, U., Miranda-Apodaca, J., Muñoz-Rueda, A., & Mena-Petite, A. (2013). Lettuce production and antioxidant capacity are differentially modified by salt stress and light intensity under ambient and elevated CO2. *Journal of Plant Physiology*, *170*(17), 1517–1525.

Pérez-López, U., Miranda-Apodaca, J., Muñoz-Rueda, A., & Mena-Petite, A. (2015). Interacting effects of high light and elevated CO2 on the nutraceutical quality of two differently pigmented *Lactuca sativa* cultivars (Blonde of Paris Batavia and Oak Leaf). *Scientia Horticulturae*, *191*, 38–48.

Ponizovsky, A. A., & Tsadilas, C. D. (2003). Lead(II) retention by Alfisol and clinoptilolite: Cation balance and pH effect. *Geoderma*, *115*(3), 303–312.

Quartacci, M. F., Sgherri, C., & Frisenda, S. (2017). Biochar amendment affects phenolic composition and antioxidant capacity restoring the nutraceutical value of lettuce grown in a copper-contaminated soil. *Scientia Horticulturae*, *215*, 9–14.

Ren, X., Zou, W., Jiao, J., Stewart, R., & Jian, J. (2023). Soil properties affect crop yield changes under conservation agriculture: A systematic analysis. *European Journal of Soil Science*, *74*(5), e13413.

Rodrigues, M. Â., Torres, L. D. N. D., Damo, L., Raimundo, S., Sartor, L., Cassol, L. C., & Arrobas, M. (2021). Nitrogen use efficiency and crop yield in four successive crops following application of biochar and zeolites. *Journal of Soil Science and Plant Nutrition*, *21*(2), 1053–1065.

Rosolem, C. A., Sgariboldi, T., Garcia, R. A., & Calonego, J. C. (2010). Potassium leaching as affected by soil texture and residual fertilization in tropical soils. *Communications in Soil Science and Plant Analysis*, *41*(16), 1934–1943.

Rovira, C., Males, A., Davies, G. J., & Williams, S. J. (2020). Mannosidase mechanism: at the intersection of conformation and catalysis. *Current Opinion in Structural Biology*, *62*, 79–92.

Sampaio, D. B., Araújo, A. S. F., & Santos, V. B. (2008). Avaliação de indicadores biológicos de qualidade do solo sob sistemas de cultivo convencional e orgânico de frutas. *Ciência e Agrotecnologia*, *32*, 353–359.

Schimel, J. P., & Bennett, J. (2004). Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, *85*(3), 591–602.

Sesták, Z., Catský, J., Čatský, J., & Jarvis, P. G. (1971). *Plant photosynthetic production: Manual of methods*. Springer Netherlands.

Seyed, Y. S. L., Rouhollah, M., Mosharraf, M. H., & Ismail, M. M. R. (2012). Water stress in plants: Causes, effects and responses. In R. Ismail Md. Mofizur & H. Hiroshi (Eds.), *Water stress*. IntechOpen.

Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., Contosta, A. R., Cusack, D., Frey, S., Gallo, M. E., Gartner, T. B., Hobbie, S. E., Holland, K., Keeler, B. L., Powers, J. S., Stursova, M., Takacs-Vesbach, C., Waldrop, M. P., Wallenstein, M. D., … Zeglin, L. H. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, *11*(11), 1252–1264.

Spohn, M., & Kuzyakov, Y. (2013). Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation – Coupling soil zymography with 14C imaging. *Soil Biology and Biochemistry*, *67*, 106–113.

Sun, Z., Hu, Y., Shi, L., Li, G., Pang, Z., Liu, S., Chen, Y., & Jia, B. (2022). Effects of biochar on soil chemical properties: A global meta-analysis of agricultural soil. *Plant, Soil and Environment*, *68*(6), 272–289.

Tashauoei, H. R., Attar, H. M., Amin, M. M., Kamali, M., Nikaeen, M., & Dastjerdi, M. V. (2010). Removal of cadmium and humic acid from aqueous solutions using surface modified nanozeolite A. *International Journal of Environmental Science and Technology*, *7*(3), 497–508.

Tsolis, V., & Barouchas, P. (2023). Biochar as soil amendment: The effect of biochar on soil properties using VIS-NIR diffuse reflectance spectroscopy, biochar aging and soil microbiology—A review. *Land*, *12*(8), 1580.

Uwituze, Y., Nyiraneza, J., Fraser, T. D., Dessureault-Rompré, J., Ziadi, N., & Lafond, J. (2022). Carbon, nitrogen, phosphorus, and extracellular soil enzyme responses to different land use. *Frontiers in Soil Science*, *2*, 1–16.

van Reeuwijk, L. (2002). *Procedures for soil analysis* (6th ed.). FAO, ISRIC.

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *19*(6), 703–707.

Vijay, V., Shreedhar, S., Adlak, K., Payyanad, S., Sreedharan, V., Gopi, G., Sophia van der Voort, T., Malarvizhi, P., Yi, S., Gebert, J., & Aravind, P. (2021). Review of large-scale biochar field-trials for soil amendment and the observed influences on crop yield variations. *Frontiers in Energy Research*, *9*, 1–21.

von Caemmerer, S., & Farquhar, G. D. (1981). Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, *153*(4), 376–387.

Walinga, I., Lee, H., Vark, V. J. G., & Novozamsky, I. (1995). *Plant analysis manual*.

Wang, H., Ren, T., Feng, Y., Liu, K., Feng, H., Liu, G., & Shi, H. (2020). Effects of the application of biochar in four typical agricultural soils in China. *Agronomy*, *10*(11), 1–14.

Wang, Y., Yin, R., & Liu, R. (2014). Characterization of biochar from fast pyrolysis and its effect on chemical properties of the tea garden soil. *Journal of Analytical and Applied Pyrolysis*, *110*, 375–381.

Watzinger, A., Feichtmair, S., Kitzler, B., Zehetner, F., Kloss, S., Wimmer, B., Zechmeister-Boltenstern, S., & Soja, G. (2014). Soil microbial communities responded to biochar application in temperate soils and slowly metabolized 13C-labelled biochar as revealed by 13C PLFA analyses: Results from a short-term incubation and pot experiment. *European Journal of Soil Science*, *65*, 40–51.

Weil, R., & Brady, N. (2017). *The nature and properties of soils* (15th ed.). Pearson.

Wu, H., Owen, C. D., & Juge, N. (2023). Structure and function of microbial α-l-fucosidases: A mini review. *Essays in Biochemistry*, *67*(3), 399–414.

Xu, N., Tan, G., Wang, H., & Gai, X. (2016). Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *European Journal of Soil Science*, *74*, 1–8.

Yamawaki, Y., Yufu, T., & Kato, T. (2021). The effect of a peptide substrate containing an unnatural branched amino acid on chymotrypsin activity. *Processes*, *9*(2), 1–8.

Yan, X., Jin, J.-Y., He, P., & Liang, M.-Z. (2008). Recent advances on the technologies to increase fertilizer use efficiency. *Agricultural Sciences in China*, *7*(4), 469–479.

Yeboah, S., Zhang, R., Cai, L., Li, L., Xie, J., Luo, Z., Wu, J., & Antille, D. L. (2017). Soil water content and photosynthetic capacity of spring wheat as affected by soil application of nitrogen-enriched biochar in a semiarid environment. *Photosynthetica*, *55*(3), 532–542.

Yinghao, L., Junlin, Z., Qi, W., Wenhua, L., Xingmei, G., Yinglong, C., Taotao, C., Kadambot, H. M. S., & Daocai, C. (2021). Zeolite alleviates potassium deficiency and improves lodging-related stem morphological characteristics and grain yield in rice. *Crop & Pasture Science*, *72*(6), 407–415.

Zaidun, S., Jalloh, M. B., Awang, A., Sam, L., Awang Besar, N., Musta, B., Ahmed, O., & Omar, L. (2019). Biochar and clinoptilolite zeolite on selected chemical properties of soil cultivated with maize (*Zea mays* L.). *Eurasian Journal of Soil Science*, *8*, 1–10.

Zhang, Q. Z., Dijkstra, F. A., Liu, X. R., Wang, Y. D., Huang, J., & Lu, N. (2014). Effects of biochar on soil microbial biomass after four years of consecutive application in the north China Plain. *PLoS One*, *9*(7), e102062.

How to cite this article: Martins, S., Brito, C., Gonçalves, A., Moutinho-Pereira, J., Pereira, E., Arrobas, M., Rodrigues, M. Â., Nunes, F., & Correia, C. M. (2024). Differential responses of photosynthesis, yield and soil properties 4 years after a single application of zeolites and biochar in a rainfed olive orchard. *Soil Use and Management*, *40*, e13045. https://doi.org/10.1111/sum.13045