agronomy

MDPI

*Article*

# 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

**Theocharis Chatzistathis [1,*], Evgenia Papaioannou [2], Anastasia Giannakoula [3] and Ioannis E. Papadakis [4]**

[1] Hellenic Agricultural Organization (H.A.O.) 'Demeter', Institute of Soil and Water Resources, 57001 Thermi, Thessaloniki, Greece
[2] Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; eapapaioa@agro.auth.gr
[3] Department of Agriculture, International Hellenic University, 54700 Sindos, Thessaloniki, Greece; agianna@cp.teithe.gr
[4] Laboratory of Pomology, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece; papadakis@aua.gr
* Correspondence: chchatzista@gmail.com

**Abstract:** One of the most challenging topics for the sustainable agriculture is how to decrease high fertilization rates. A pot experiment, exploring the effects of zeolite (ZEO) and/or vermiculite (VER) as soil amendments, comparing to the soil application of a controlled release fertilizer (CRF), was realized in chestnut plants. Various parameters related to soil fertility, and plant growth, nutrition, and physiology were investigated to gain knowledge towards more sustainable management. After ZEO application and in comparison to CRF, an impressive boost in soil K was achieved. Moreover, soil P and Zn levels were higher in the VER-treated soil, compared to CRF. Leaf K and Ca concentrations were significantly higher in ZEO, compared to the VER treatment; the highest foliar N and Zn concentrations were measured in CRF and VER, respectively. However, significantly lower foliar Mn and Cu were found in VER. The highest root biomass produced in the ZEO treated plants. For most nutrients, their total uptake per plant was higher in CRF and ZEO. Finally, photosynthetic rates were higher in VER (mainly due to non-stomatal factors) and CRF (mainly due to stomatal factors). Our data open a discussion towards the application of ZEO and/or VER as soil amendments in chestnut nurseries and orchards, aiming at partially decreasing fertilization rates and boosting sustainable nutrient management.

**Keywords:** chestnut nurseries; chestnut orchards; controlled release fertilizer (CRF); nutrient uptake; plant nutrition; chlorophyll fluorescence; photosynthesis; soil fertility; root growth



**Citation:** Chatzistathis, T.; Papaioannou, E.; Giannakoula, A.; Papadakis, I.E. 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* **2021**, *11*, 109. https://doi.org/10.3390/agronomy11010109

Received: 30 November 2020
Accepted: 5 January 2021
Published: 8 January 2021



## 1. Introduction

Chestnut (*Castanea sativa* Mill) has been used for centuries, for both wood and fruit (nut) production. Although wood production is declining due to the fall in price, the production of nuts is expanding in mountainous areas [1]. In addition, during the last years, chestnut management cultivation has been shifted from abandoned, huge, isolated trees of low density to more intensified cropping systems of higher plant density; this practice also includes chemical fertilization, pruning and mechanical harvesting. This shift in chestnut management cultivation may be explained by the higher profit that recently nut prices may provide to the growers [1]. With regard to chestnut management and especially its fertilization, very few things are known on the nutritional demands of chestnut trees [2]. Only Zhang et al. [3] investigated the understory vegetation management and fertilization on greenhouse gas emissions and labile organic C pools in intensively managed Chinese

chestnut plantations. Furthermore, most of the times fertilization recommendations for chestnut trees are highly empirical, mainly based on personal experience of the growers and researchers, without taking into account scientific data (soil fertility and determination of leaf nutrient concentrations) (Chatzistathis, unpublished data). Most of the existing published papers on *C. sativa* Mill are mainly focused on the improvement of its germination and micro-propagation [4–6], as well as on the non-destructive leaf area estimation [7]. In addition, Silvanini et al. [8] investigated the altitude effects on fruit morphology of two chestnut cultivars. According to our knowledge, only Guzman et al. [9] and Zysset et al. [10] studied nutritional topics in *C. sativa* Mill (Fe nutrition and the response of European chestnut to varying Ca/Al ratios, respectively).

Modern agriculture focuses on the necessity to boost crop productivity, without increasing (or even by decreasing) mineral fertilization rates in soils [11]. Thus, the intensification in cultivation of chestnut orchards will soon provoke the interest of many researchers on how to decrease the high fertilization rates, in order to: (i) protect the environment, (ii) enhance sustainability of chestnut orchards, (iii) decrease cost production and increase profitability for the growers. There are mainly two categories of soil amendments to achieve decrease in fertilization rates, by also enhancing soil fertility: (a) organic amendments, such as the different kind of manures [12], composts [13] and other kind of vegetative materials [14], as well as olive mill wastewaters-OMWs [15,16], provided as organic crop fertilizers, (b) inorganic amendments, such as the different kind of zeolites, vermiculites etc. [17–21]. Thus, it is expected that with the suitable mix/combination of soil with organic/inorganic amendments chemical fertilization inputs will be substantially reduced.

Natural or synthetic inorganic amendments have been used in agriculture as a tool to: (i) improve soil properties, (ii) enhance crop productivity, (iii) restore/remediate polluted, saline and acid soils and decrease metal uptake, (iv) reduce the potential environmental and climate impacts arising from excessive nutrient availability (mainly N and P) in soil [17,19,21–23]. According to our knowledge, no published data exist with regard to the application of inorganic amendments on soil fertility and chestnut trees' nutrition. Thus, our study is innovative, since it is the first one investigating the role of zeolite and vermiculite (as potential fertilizers) on boosting soil fertility and enhancing nutrient uptake for chestnut rootstocks. This was realized via a comparative study with inorganic fertilization (CRF), in order to investigate if zeolite and vermiculite were capable of satisfying the nutritional needs of chestnut rootstocks. The hypothesis of our study was based on the premise that zeolite and vermiculite could act as potential fertilizers (in the frame of sustainable agriculture) to satisfy the nutritional needs of chestnut plants. Since it was found that inorganic amendments (especially zeolite) can improve soil chemical properties (based on published data), together with the high content of zeolite in K and Ca (Table 1), it was quite reasonable to assume that nutrient uptake by chestnut plants could be enhanced, meeting their nutritional needs. In addition, since zeolite is rich in Ca and both amendments have high pH (Table 1), their application is more interesting in acid soils, as that existed in our study.

**Table 1.** Chemical properties of inorganic materials, i.e., zeolite ('Zeoterra', powder size 0.8–2.5 mm) and vermiculite ('Vermiterra medium'), used as soil amendments.

| Amendment | pH | Organic Matter (%) | $NO_3^-$-N | P | K | Ca | Mg | Fe | Mn | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mg kg$^{-1}$ | | | | | | | | |
| Zeolite | 7.28 | 0.00 | 9.36 | 1.87 | 14,984 | 16050 | 922 | 0.57 | 1.12 | 0.24 | 0.05 |
| Vermiculite | 8.83 | 0.18 | 6.58 | 2.53 | 445 | 1727 | 146 | 14.44 | 1.92 | 0.33 | 0.47 |

The purposes of our study were to investigate the effects of zeolite and vermiculite application (in comparison to conventional/inorganic fertilization) on: (i) soil fertility, (ii) plant growth, (iii) plant mineral nutrition, and (iv) plant physiological performance (photosystem II activity, photosynthetic and transpiration rates, stomatal conductance,

intercellular $CO_2$ concentration and intrinsic water use efficiency-WUEi) of chestnut plants. These purposes were posed in order to gain knowledge towards more sustainable management in chestnut nurseries and orchards, aiming to decrease use of chemical fertilizers.

## 2. Materials and Methods

### *2.1. Plant Material, Soil Sampling and Treatments*

Chestnut (*C. sativa* Mill) plants, coming from seeds (collected from natural forest vegetation), were grown outdoors, in 3L pots, for 153 days (from early May to early October), on soil substrate from parent material Gneiss. The soil samples with which were filled the pots were collected from the region of Gomati (40°23′46″ N 23°47′45″ E), Chalkidiki, Northern Greece. A significant part of the natural vegetation of this area is occupied by chestnut trees. Samples were collected from the upper 60 cm of soil profile, since most part of the active (responsible for nutrient and water uptake) root system of trees grows in this layer. After soil samples were taken (before filling the experimental pots), they were carefully mixed, in order to achieve homogenization.

After seeds' germination, the plants were randomized and divided into four similar groups (corresponding to each one of the four soil substrates-treatments), based on their height. The four treatments were the following: (i) plants grown on soil substrate, mixed with VER ('Vermiterra medium' Exfoliated vermiculite for horticultural uses, provided by the company NORDIA A.E., Greece) (soil:vermiculite = 80:20, *v*/*v*), (ii) plants grown on soil substrate, mixed with ZEO ('Zeoterra', powder size 0.8–2.5 mm, provided by the company NORDIA A.E., Greece)) (soil:zeolite = 80:20, *v*/*v*), (iii) plants grown on soil substrate, mixed with zeolite and vermiculite (ZEO + VER; soil:ZEO:VER = 80:10:10, *v*/*v*/*v*), and (iv) plants grown on control soil (no mixture of soil with either ZEO or VER), but they were fertilized during their development (experimental period) with a controlled release fertilizer (CRF, Nova Tec-Suprem, 21-5-10 + 3MgO + trace elements). The fertilizer was solid and it was slightly incorporated into soil. The choice of the ratios 80:20 or 80:10:10 between the soil substrate and the inorganic amendments was based on previously published data [24]. In the 4th treatment (control soil, with CRF application), a total quantity of 10 g fertilizer per pot (during the whole experimental period) was applied, in two doses of 5 g each: the first one was applied in June and the second one in July. In each of the 4 treatments, 10 plants-replicates (one plant per pot) were included; thus, the total number of the experimental plants was 40. During the experiment, all the plants were daily irrigated with distilled water, in order to achieve soil moisture in approximately 70% of water holding capacity.

### *2.2. Chemical Analyses of Soil Samples and Mixtures*

The particle size of the ZEO used was 0.8–2.5 mm (powder size), while the exfoliated type of VER was chosen as second soil amendment. The chemical properties of pure ZEO and VER are presented in the Table 1. The fertility of the mixtures of soil with ZEO and VER were determined according to the international protocol methods described below. After representative soil samples were received from forest sampling areas, they were initially dried for at least 48 h (inside an experimental greenhouse), they were mixed with the two amendments (as described above, in the experimental treatments), and a quantity of approximately 1–1.5 kg from each mixture-treatment (used as plant substrate) was transferred to laboratory for chemical analyses, in order to determine the fertility of the soil mixtures. Before analyses, soil mixtures were air-dried for 48 h at room temperature, their stones were removed, and afterwards they were sieved to pass a 10-mesh screen. Chemical analyses included: pH, organic matter, total N, available P, exchangeable cations (Ca, Mg and K) and extractable micronutrients (Fe, Mn, Zn and Cu). The above-mentioned parameters were determined as follows: pH in a soil-distilled water paste (1:1) [25], organic matter with potassium dichromate ($K_2Cr_2O_7$) [26] and the available P was carried out according to the Olsen method [27]. Nitrogen was determined with the Kjeldahl method [28]. The exchangeable cations (K, Ca and Mg) were determined according to the

method of ammonium acetate ($CH_3COONH_4$) [29], while the extractable micronutrients (Fe, Mn, Zn and Cu) were determined after extraction of 10 g soil with DTPA solution, pH 7.3 [30]. Finally, the concentrations of K, Ca, Mg, Fe, Mn, Zn and Cu were measured by the ICP (OPTIMA 2100 DV Optical Emission Spectrometer, Perkin Elmer, Waltham, MA, USA) method [31].

### *2.3. Plant Growth Data*

At the end of the experiment, the main shoot length for all the experimental plants was measured. Then, leaves, stem and root of each plant were separated from each other. Before being washed, the fresh weights (F.W.) of root, stem and leaves were measured. By adding the F.W. of all the plant parts, the total plant F.W. was calculated. After all the plant tissues being carefully washed (once with tap and twice with distilled water), they were dried at 75 °C, for 48 h. Then, the dry weights (D.W.) of root, stem and leaves were determined. By adding the D.W. of all the plant parts, the total plant D.W. was also calculated. In addition, the ratios between shoot (i.e., leaves + stem) and root, as well as between root and total plant biomass were calculated, on both dry (D.W.) and fresh weight (F.W.) basis.

### *2.4. Leaf Nutrient Analyses and Total Plant Nutrient Content*

At the end of the 153-day experimental period, after the chestnut plants being harvested and the tissues being separated, washed and dried, they were ground to a fine powder, in order to pass a 30-mesh screen. A portion of 0.5 g of the fine powder of each sample was dry-ashed in a muffle furnace, at 515 °C for 5 h. Then, the ash was dissolved with 3 mL of 6 N HCl and diluted with double distilled water up to 50 mL. The concentrations of P, K, Ca, Mg, Fe, Mn, Zn and Cu were determined by ICP (OPTIMA 2100 DV Optical Emission Spectrometer, Perkin Elmer, USA) [31].

Nitrogen was determined by the Kjeldahl method [32]. Macronutrient (N, P, K, Ca and Mg) concentrations were expressed in % D.W., while those of micronutrients (Fe, Mn, Zn and Cu) were expressed in mg $kg^{-1}$ D.W. Multiplying the concentration of each nutrient (mg or μg $g^{-1}$ dry weight for macro- or micro-nutrients, respectively) found in each plant part by the corresponding D.W., the content (absolute quantity) was calculated. By addition of the nutrient contents of different plant parts, the total nutrient content (μg for micronutrients and mg for macronutrients) per plant, thus the total nutrient uptake per plant, was computed.

### *2.5. Chlorophyll Fluorescence and Gas Exchange Measurements*

At the end of the experimental period, the following chlorophyll fluorescence parameters were determined: $F_v/F_m$: maximum quantum yield of primary photochemistry, $F_0$: minimum fluorescence, $F_m$: maximum fluorescence, $F_v = F_m - F_0$: variable fluorescence, and performance index (PI): reflects the functionality of both photosystems I and II and provides quantitative information on the current state of plant performance under stress conditions [33]. All these parameters were determined in mature leaves (from the upper part of the main shoot) of chestnut plants, by the PAM-2000 fluorometer (Heinz Walz GmbH, Effeltrich, Germany), after preconditioning of leaves in the dark for 20 min [34].

For the gas exchange measurements (i.e., net photosynthetic rate, transpiration rate, stomatal conductance, intercellular $CO_2$ concentration), the LC PRO portable gas exchange measuring system (ADC Bioscientific Ltd., Hoddesdon, UK) was used. The measurements were carried out in mature, fully expanded leaves, from the middle of the main shoot. Measurements were performed the time period from 10:00 to 12:00 a.m., at natural full light intensity. Finally, intrinsic water use efficiency (WUEi), which is the ratio between the net photosynthetic rate and stomatal conductance, was calculated since it provides information on the water use efficiency at the plant level.

### 2.6. Statistical Analysis

The experimental design consisted of a 4 × 1 completely randomized factorial, with 4 soil treatments and one plant species. In each of the 4 treatments, 10 plants-replicates (one plant per experimental pot) were included (thus, the total number of experimental plants was 4 × 10 = 40). The data were statistically analyzed by the SPSS statistical program (ONE-WAY ANOVA) and for the comparison of the mean values among the treatments, the Duncan's multiple range test, for $p \leq 0.05$, was used.

## 3. Results

### 3.1. Soil Fertility in the Four Soil Treatments

The chemical properties of the two pure soil amendments used in the current study, i.e., zeolite and vermiculite, are presented in Table 1. As it is clear from that Table, K concentration was more than 34 times higher in zeolite, than in vermiculite; similarly, Ca and Mg concentrations were more than nine times and approximately 6.5 times higher, respectively, in zeolite, than in vermiculite (Table 1). In contrast, Fe, Mn, Zn and Cu concentrations were 25, 1.7, 1.4 and 9.5 times higher in vermiculite than in zeolite, respectively. This means that zeolite is a better source of K and Ca, while vermiculite is a better amendment to enhance soil micronutrient availability (especially Fe). Finally, pH was also higher in pure vermiculite (8.83), than in pure zeolite (7.28) (Table 1).

After zeolite and/or vermiculite application in soil, its pH was approximately increased by 0.5 or 1 unit (Table 2). Organic matter was not substantially affected by the application of amendment(s), while the ratio C/N was slightly decreased in the VER and ZEO + VER treatments, compared to the control soil. An impressive boosting of more than 28 times in exchangeable K was determined after ZEO application (Table 2), while extractable Olsen P in the VER treatment was approximately two times higher, compared to the control soil. Exchangeable Ca was increased after ZEO supply, from 9.23 to 13.95 cmol $kg^{-1}$ (Table 2). Finally, DTPA extractable Mn and Zn were increased by approximately 40% after the combinational application of ZEO and VER (in the case of Mn) and VER supply (in the case of Zn) (Table 3).

**Table 2.** Properties of the soil mixtures (with VER, ZEO, ZEO + VER) and control soil among the four treatments, before the experiment.

| Treatment (Soil Amendment) | pH | Organic C (%) | Organic Matter (%) | N (%) | C/N | P (mg/100 g) | K (cmol $kg^{-1}$) | Ca (cmol $kg^{-1}$) | Mg (cmol $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| VER | 5.59 | 7.91 | 13.64 | 0.48 | 16.48 | 1.95 | 0.80 | 10.28 | 3.88 |
| ZEO | 5.16 | 7.77 | 13.39 | 0.40 | 19.43 | 1.51 | 14.62 | 13.95 | 3.99 |
| ZEO + VER | 4.98 | 7.85 | 13.53 | 0.45 | 17.44 | 1.34 | 8.27 | 12.89 | 3.57 |
| Control | 4.61 | 7.79 | 13.42 | 0.34 | 22.85 | 1.06 | 0.48 | 9.23 | 3.63 |

**Table 3.** Concentrations of micronutrients in the soil mixtures (with VER, ZEO, ZEO + VER) and control soil among the four treatments, before the experiment.

| Treatment (Soil Amendment) | Fe | Mn | Zn | Cu |
|---|---|---|---|---|
| | mg $kg^{-1}$ | | | |
| VER | 28.18 | 6.90 | 0.89 | 0.36 |
| ZEO | 26.06 | 6.64 | 0.71 | 0.25 |
| ZEO + VER | 22.10 | 12.05 | 0.68 | 0.30 |
| Control | 24.84 | 7.00 | 0.63 | 0.33 |

### 3.2. Plant Growth

Significantly higher leaf and stem weight was found in the CRF, compared to the VER and ZEO + VER treatments. However, insignificant differences in leaf and stem

F.W. and D.W. were found between the ZEO and CRF (Table 4). The highest total plant biomass was recorded in ZEO; this was ascribed to the highest root weight determined in this treatment, compared to the other ones (Table 4). Similarly, significantly lower values of the ratio shoot/root were found in the ZEO treatments (ZEO, ZEO + VER), compared to the CRF; in contrast, higher values of the ratio root/total plant biomass were found in these two treatments (ZEO, ZEO + VER), compared to CRF (Table 4). At the end of the experiment, we found better root growth (higher development of thin absorptive roots, which are responsible for nutrient and water uptake) of chestnut plants in the ZEO treatment, compared to VER and CRF (data not shown).

### *3.3. Leaf Nutrient Concentrations and Total Plant Nutrient Content*

The highest foliar N concentration (2.47% D.W.) was found in CRF; in contrast, the highest leaf K was determined in ZEO (0.65% D.W.) (Table 5). Significant difference in foliar K and Ca was recorded only between ZEO and VER, and not between ZEO and the other two treatments (ZEO + VER, CRF). Similarly, significantly lower leaf Mn was found in the VER treatment (83 mg $kg^{-1}$ D.W.), compared to the very high Mn levels determined in the other three treatments (337–405 mg $kg^{-1}$ D.W.) (Table 5). In contrast to Mn, significantly higher foliar Zn concentration was found in VER, compared to the other 3 treatments (ZEO, ZEO + VER, CRF).

Total plant N content was significantly higher in the CRF and ZEO treatments, compared to the other two ones (Figure 1a); similarly for P and Mg, their highest contents were observed in CRF (Figure 1a,e). In contrast, the highest total plant K and Ca contents were determined in ZEO; however, the differences between: (i) ZEO and (ii) CRF or ZEO + VER were insignificant. Significant differences in K and Ca contents were only found between ZEO and VER (Figure 1c,d). Clearer was the image for Fe uptake: the highest Fe content was recorded in the ZEO treatment, which was significantly higher to the contents determined in the VER and ZEO + VER treatments (Figure 2a). With regard to Zn uptake, significantly higher Zn content was recorded in the ZEO treatment, compared to ZEO + VER; between: (i) ZEO and (ii) VER or CRF treatments insignificant differences were found (Figure 2c). In contrast, significant differences among all the treatments were determined for Mn: its highest content was recorded in CRF, followed by those determined in ZEO, ZEO + VER and VER (Figure 2b). Finally, regarding Cu uptake, significantly lower total plant Cu content was found in the VER treatment, compared to the other three ones; among ZEO, ZEO + VER and CRF insignificant differences were determined (Figure 2d).

### *3.4. Photosystem II Activity and Gas Exchange Measurements*

Significantly higher values of the ratios $F_v/F_m$ and $F_v/F_0$ were found in the VER treatment, compared to those determined in the other three treatments (ZEO, ZEO + VER, CRF) (Figure 3a,b). However, Performance Index (PI) did not significantly differ among VER, ZEO and CRF; significantly lower PI was found in ZEO + VER (Figure 3c). With regard to gas exchange measurements, significantly higher photosynthetic rates were determined in VER and CRF, compared to ZEO and ZEO + VER (Figure 4a). However, stomata opening was significantly lower in VER and ZEO, compared to CRF, as well as to the ZEO + VER (Figure 4b). Moreover, transpiration rate was significantly higher in CRF than in VER, ZEO and/or ZEO + VER (Figure 4c). Finally, intercellular $CO_2$ concentration was significantly higher in CRF and ZEO + VER than in VER and ZEO (Figure 4d). In contrast, intrinsic water use efficiency (WUEi) was higher in ZEO and VER, compared to CRF and ZEO + VER (Figure 4e).

**Table 4.** Plant growth parameters of *C. sativa* Mill plants among the four soil treatments (VER, ZEO, ZEO + VER, CRF).

| Treatment | LEAF (g) | | STEM (g) | | ROOT (g) | | Total Plant Biomass (g) | | Shoot/Root | | Root/Total Plant Biomass | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F.W. | D.W. | F.W. | D.W. | F.W. | D.W. | F.W. | D.W. | F.W. | D.W. | F.W. | D.W. |
| VER | (25.68 ± 2.44) $^{b}$ | (10.59 ± 1.46) $^{b}$ | (23.63 ± 3.81) $^{b}$ | (10.84 ± 1.95) $^{b}$ | (75.59 ± 12.01) $^{b}$ | (18.43 ± 3.65) $^{b}$ | (127.77 ± 19.06) $^{b}$ | (40.95 ± 4.27) $^{b}$ | (0.63 ± 0.05) $^{ab}$ | (1.15 ± 0.19) $^{ab}$ | (0.62 ± 0.05) $^{ab}$ | (0,47 ± 0.06) $^{ab}$ |
| ZEO | (27.53 ± 5.58) $^{ab}$ | (10.60 ± 1.12) $^{b}$ | (30.77 ± 5.54) $^{ab}$ | (14.00 ± 2.77) $^{ab}$ | (109.37 ± 12.44) $^{a}$ | (31.23 ± 6.02) $^{a}$ | (180.61 ± 30.05) $^{a}$ | (57,44 ± 6.67) $^{a}$ | (0.53 ± 0.06) $^{bc}$ | (0.96 ± 0.14) $^{b}$ | (0.65 ± 0.05) $^{a}$ | (0.52 ± 0.05) $^{a}$ |
| ZEO + VER | (21.71 ± 3.90) $^{b}$ | (9.05 ± 1.27) $^{b}$ | (20.87 ± 4.45) $^{b}$ | (9.69 ± 1.63) $^{b}$ | (93.63 ± 9.97) $^{ab}$ | (22.30 ± 4.84) $^{ab}$ | (136.21 ± 25.17) $^{ab}$ | (41.74 ± 6.04) $^{b}$ | (0.48 ± 0.06) $^{c}$ | (0.81 ± 0.16) $^{b}$ | (0.68 ± 0.07) $^{a}$ | (0.53 ± 0.07) $^{a}$ |
| CRF | (35.32 ± 5.89) $^{a}$ | (15.08 ± 2.20) $^{a}$ | (38.33 ± 7.92) $^{a}$ | (17.71 ± 3.97) $^{a}$ | (85.69 ± 10.11) $^{b}$ | (21.00 ± 4.50) $^{ab}$ | (151.19 ± 22.38) $^{ab}$ | (50.21 ± 7.42) $^{ab}$ | (0.82 ± 0.16) $^{a}$ | (1.53 ± 0.24) $^{a}$ | (0.55 ± 0.04) $^{b}$ | (0.40 ± 0.04) $^{b}$ |

F.W. = Fresh Weight; D.W. = Dry Weight; Values are means of 10 replicates (N = 10). The different letters, in the same column, symbolize statistically significant differences among the 4 treatments, according to the Duncan's multiple range test, for $p \leq 0.05$.

**Table 5.** Leaf nutrient concentrations for *C. sativa* Mill plants among the four soil treatments (VER, ZEO, ZEO + VER, CRF).

| Nutrients | | Treatment | | | |
|---|---|---|---|---|---|
| | | VER | ZEO | ZEO + VER | CRF |
| N | % D.W. | (2.12 ± 0.28) $^{ab}$ | (2.10 ± 0.39) $^{ab}$ | (1.95 ± 0.26) $^{b}$ | (2.47 ± 0.24) $^{a}$ |
| P | | (0.38 ± 0.04) $^{a}$ | (0.33 ± 0.06) $^{a}$ | (0.35 ± 0.05) $^{a}$ | (0.33 ± 0.07) $^{a}$ |
| K | | (0.49 ± 0.05) $^{b}$ | (0.65 ± 0.08) $^{a}$ | (0.61 ± 0.06) $^{a}$ | (0.56 ± 0.06) $^{ab}$ |
| Ca | | (1.12 ± 0.14) $^{b}$ | (1.54 ± 0.18) $^{a}$ | (1.47 ± 0.16) $^{a}$ | (1.17 ± 0.21) $^{ab}$ |
| Mg | | (0.49 ± 0.06) $^{a}$ | (0.45 ± 0.06) $^{a}$ | (0.53 ± 0.09) $^{a}$ | (0.57 ± 0.08) $^{a}$ |
| Fe | mg $kg^{-1}$ | (207 ± 29.08) $^{a}$ | (202 ± 30.46) $^{a}$ | (243 ± 34.27) $^{a}$ | (190 ± 25.17) $^{a}$ |
| Mn | | (83 ± 9.96) $^{b}$ | (380 ± 50.11) $^{a}$ | (337 ± 47.17) $^{a}$ | (405 ± 60.44) $^{a}$ |
| Zn | | (35 ± 3.27) $^{a}$ | (24 ± 2.80) $^{b}$ | (24 ± 4.55) $^{b}$ | (21 ± 3.12) $^{b}$ |
| Cu | | (18 ± 2.98) $^{c}$ | (27 ± 4.06) $^{b}$ | (38 ± 5.88) $^{a}$ | (27 ± 3.70) $^{b}$ |

D.W. = Dry Weight; Values are means of 10 replicates (N = 10). The different letters, in the same row, symbolize statistically significant differences among the 4 treatments, according to the Duncan's multiple range test, for $p \leq 0.05$.

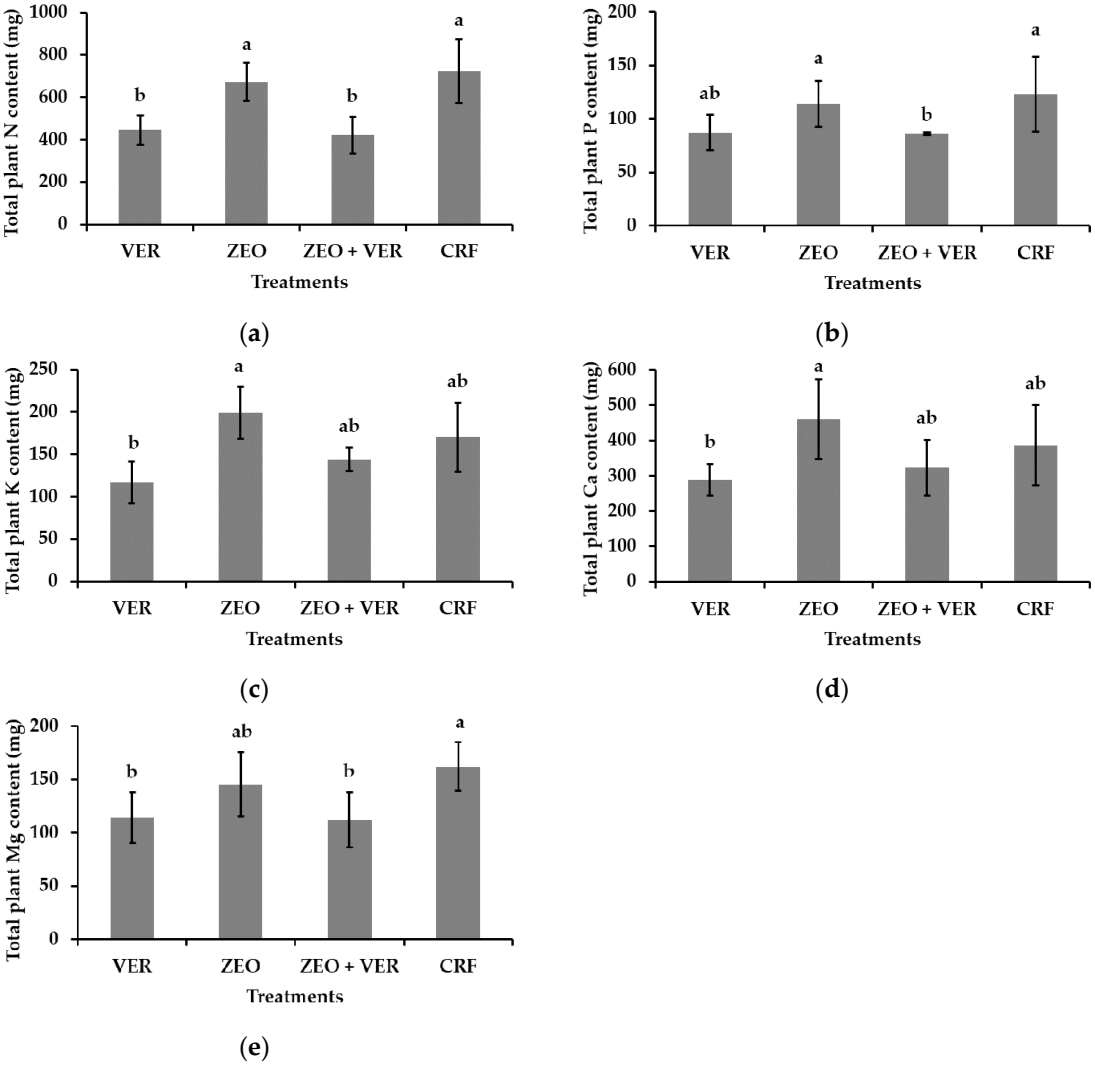



**Figure 1.** Total plant N (**a**), P (**b**), K (**c**), Ca (**d**) and Mg (**e**) content (mg) among the 4 soil treatments (VER, ZEO, ZEO + VER, CRF). The bars symbolize standard deviation values. The different letters on the bars symbolize statistically significant differences among the treatments.

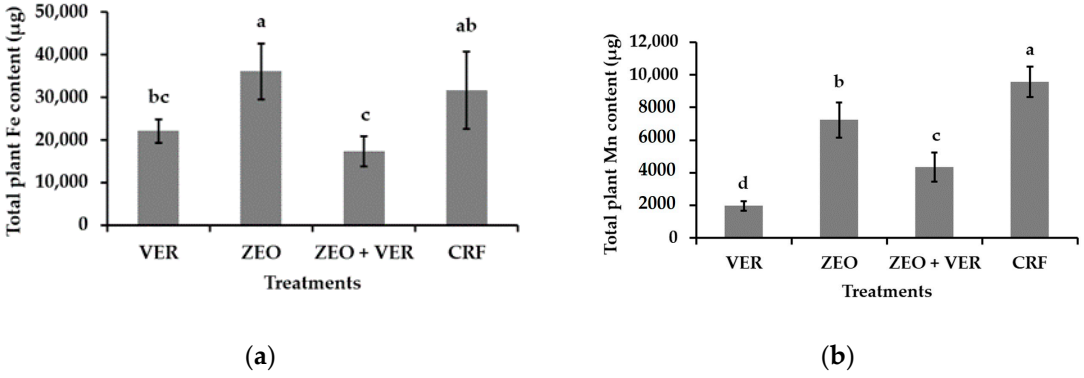
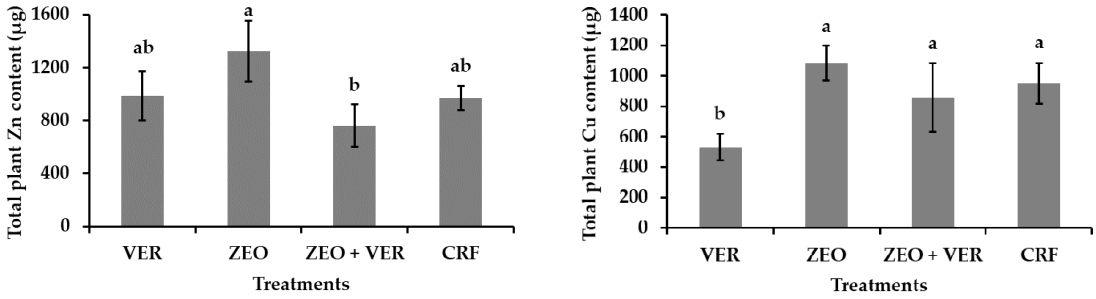
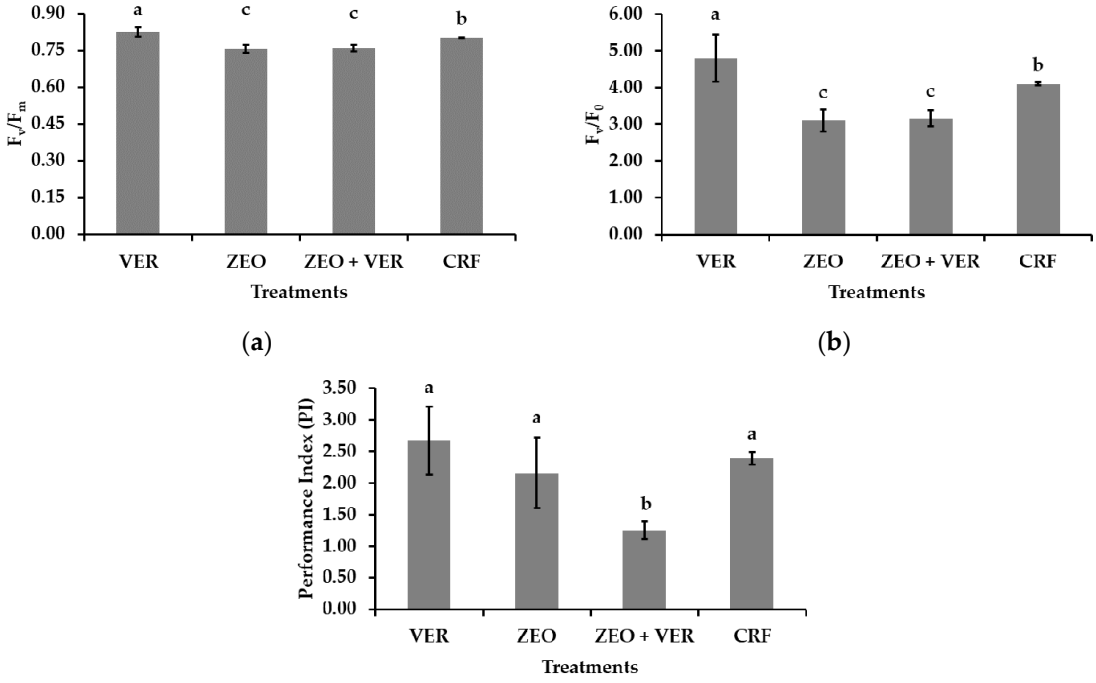



**Figure 2.** *Cont.*




(**c**) (**d**)

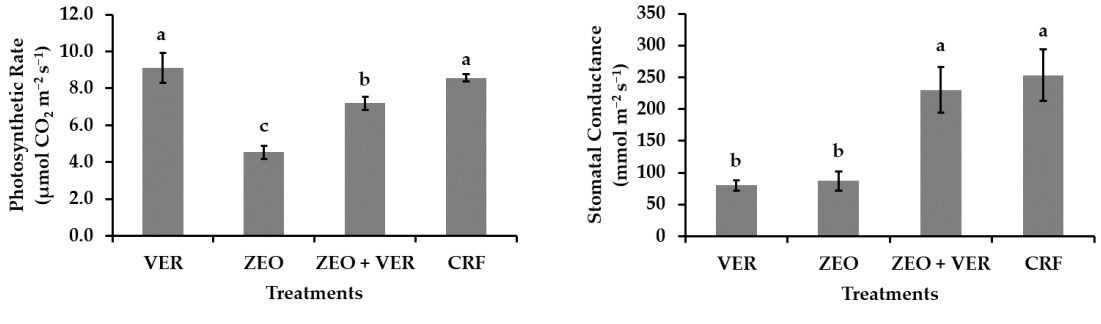
**Figure 2.** Total plant Fe (**a**), Mn (**b**), Zn (**c**) and Cu (**d**) content (μg) among the 4 soil treatments (VER, ZEO, ZEO + VER, CRF). The bars symbolize standard deviation values. The different letters on the bars symbolize statistically significant differences among the treatments.




(**a**) (**b**)

(**c**)

**Figure 3.** Photosystem II activity, as expressed by the chlorophyll fluorescence parameters $F_v/F_m$ (**a**), $F_v/F_0$ (**b**), and Performance Index-PI (**c**) among the 4 soil treatments (VER, ZEO, ZEO + VER, CRF). The bars symbolize standard deviation values. The different letters on the bars symbolize statistically significant differences among the treatments.




(**a**) (**b**)

**Figure 4.** *Cont.*

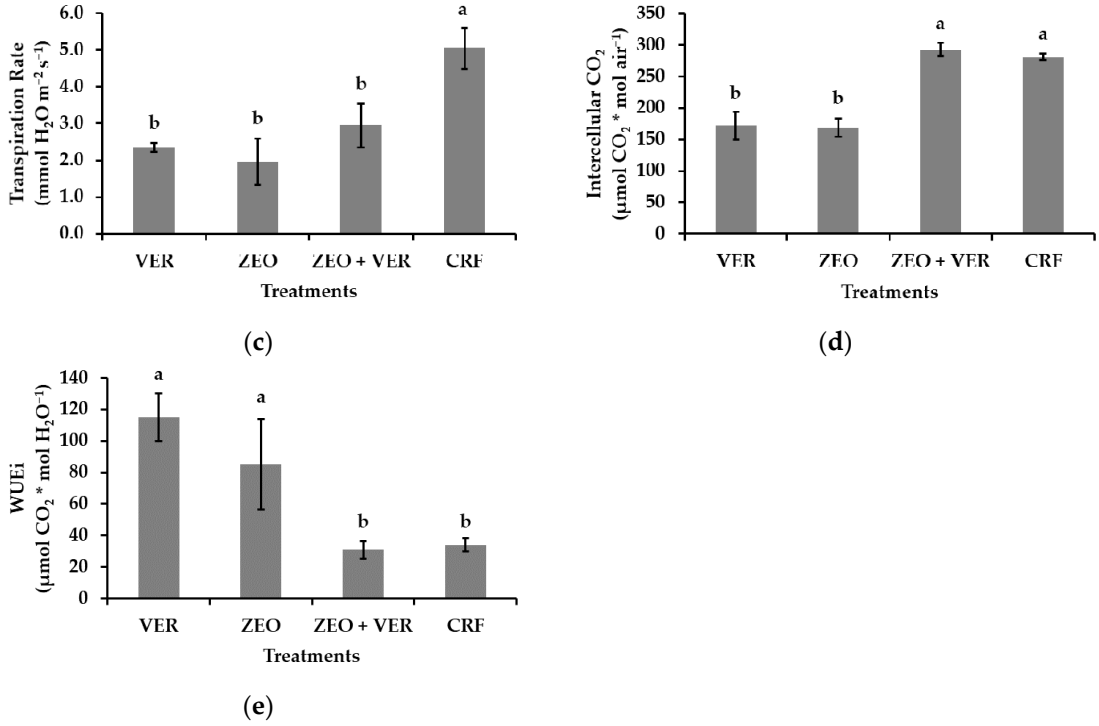



**Figure 4.** Photosynthetic rate (**a**), stomatal conductance (**b**), transpiration rate (**c**), intercellular $CO_2$ concentration (**d**) and intrinsic water use efficiency (WUEi) (**e**) among the 4 soil treatments (VER, ZEO, ZEO + VER, CRF). The bars symbolize standard deviation values. The different letters on the bars symbolize statistically significant differences among the treatments.

## 4. Discussion

Potassium, Ca and Mg contents were significantly higher in pure zeolite, than in pure vermiculite (Table 1); the significantly higher K content in zeolite led to the significantly higher soil exchangeable K, more than 18 times higher in ZEO compared to VER, and to an impressive boosting in soil K (more than 30 times higher), compared to the control soil (CRF) (Table 2). The beneficial effects of zeolite application as soil amendment (ZEO treatment), especially on the increase of soil exchangeable K and to a lesser extent on the increase of Ca, have been also referred by other researchers [35,36]. The increase of exchangeable K and Ca should be also ascribed to the potential boosting of Cation Exchange Capacity (C.E.C.) after ZEO application, which should be also incorporated into similar future studies. The higher exchangeable K in ZEO, compared to the VER treatment, was also followed by significantly higher foliar K in ZEO (0.65% D.W.), compared to VER (0.49% D.W.) (Table 5). From the data of Table 5 it is also concluded that leaf K concentrations varied from 0.49 to 0.65% D.W., while Ca concentrations fluctuated from 1.12 to 1.54% D.W. among the 4 treatments. It is generally accepted that there is a great gap in the international literature with regard to the nutrition of *C. sativa* Mill, thus, no published data exist on the optimum range of foliar nutrients. Nanos and Tsintirakou [37] stated that the optimum foliar K concentrations in mature leaves of European chestnut trees (during the summer period) should be around 2%, or slightly lower to that value. Based on this remark, leaf K levels of our experiment were probably low; however, our experimental conditions (pot experiment with non-fruiting plants) were completely different compared to field (chestnut orchards) data, thus no direct comparisons for K sufficiency, or insufficiency, can be made.

Soil extractable P was approximately 1.5 times and 2 times higher in ZEO and VER treatments, compared to the control soil (Table 2). However, insignificant differences among the 4 treatments were recorded in foliar P; in addition, leaf P concentrations were >0.30% D.W. in all the four treatments (Table 5). This means that P was probably within the over-sufficiency levels, since for many fruit tree species, such as *Juglans regia* and *Prunus*

*amygdalus*, foliar P concentrations > 0.30% D.W. (from the middle of annual shoots, during the summer period) are considered as over-sufficient (Chatzistathis, unpublished data). In addition, it is concluded that ZEO and VER (as soil amendments) were equally effective to the controlled release fertilizer (CRF) in satisfying the P nutritional needs of *C. sativa* plants. Similarly, insignificant differences among CRF, ZEO and VER treatments were found regarding the foliar N; Nitrogen levels were approximately 2–2.5% D.W. (Table 5), which may be considered as sufficient. According to Nanos and Tsintirakou [37], optimum leaf N concentrations from annual shoots of fully productive *C. sativa* Mill trees should be around 2% D.W. With regard to total plant N and P content, for both nutrients higher contents were found in the CRF and ZEO treatments, compared to the other two ones (VER, ZEO + VER) (Figure 1a,b). Based on all the above data for macronutrients, it is concluded that ZEO was a promising soil amendment to sufficiently satisfy the macronutrient needs (especially those of K and Ca and afterwards those of N and P) of *C. sativa* plants. However, further research is needed under field conditions (in *Castanea sativa* orchards) to verify the suitability of zeolite as fertilizer for chestnut trees. In addition, in the future studies, N leaching and volatilization losses should be included, in order to further quantify the response of the soil-plant system to CRF and VER applications.

ZEO and VER soil application slightly increased extractable Zn, while the combined application (ZEO + VER) increased by approximately 40% soil Mn (Table 3). In plant level, the highest leaf Zn concentration was determined in the VER treatment (35 mg $kg^{-1}$ D.W.), while the lowest Mn was also recorded in the same treatment (83 mg $kg^{-1}$ D.W.). In the other three treatments (ZEO, ZEO + VER, CRF), very high foliar Mn levels (varying from 337 to 405 mg $kg^{-1}$ D.W.) were recorded, while significantly lower Zn concentrations were determined, compared to VER (Table 5). This phenomenon may be ascribed to an antagonism between $Zn^{2+}$ and $Mn^{2+}$ for common uptake [38,39]. Previous studies have highlighted the potential role of zeolite to enhance micronutrient uptake and supply crops with micronutrients, such as Mn, Cu and Zn [40,41], sufficiently confirming our data (Figure 2a–d; Table 5). Maybe the beneficial role of zeolite in boosting the total plant uptake of Fe, Zn and Cu (and to a lesser extent of Mn) by chestnut plants (Figure 2a–d) was probably due to the enhanced retention capacity of zeolite [42], which probably prevented these micronutrients from leaching, rather than to directly supply plants with them, since both zeolite and vermiculite content in Mn, Zn and Cu was poor (Table 1). In the study of Najafi-Ghiri and Rahimi [43], it was found that application of zeolite and vermicompost significantly increased Zn uptake by spinach plants. In contrast to micronutrients, in the cases of K and Ca, the highest total plant K and Ca content in the ZEO treatment (Figure 1c–d) should be possibly ascribed to a combined effect of increased content of K and Ca in zeolite, together with its increased retention capacity.

Higher leaf dry weight (D.W.) was recorded in CRF, compared to the other treatments; this may be ascribed to the higher foliar N concentration determined in CRF (2.47% D.W.) (Table 5). Nitrogen availability influences plant growth and vegetation flush [44]. However, the highest root D.W. was found in ZEO (Table 4); this influenced total plant biomass production and the ratios: (i) shoot/root, and (ii) root/total plant biomass (Table 4). Thus, zeolite application (as soil amendment) favorably influenced the root growth of *C. sativa* plants, a fact that was probably also contributed to the higher nutrient uptake recorded in the ZEO treatment, compared to those of VER and ZEO + VER (Table 5; Figure 1a–e; Figure 2a–d). However, in some cases (Figure 1b,e; Figure 2a,c,d), the higher plant nutrient content/accumulation found in ZEO was ascribed rather to higher biomass production (Table 4) than to higher nutrient concentrations existed in various plant organs (Table 5). In the study of Chatzistathis et al. [13] it was also found that the highest nutrient accumulation in olive plants (occurred when they received inorganic fertilization, compared to the organic treatments) was owed to higher biomass production, rather than to higher concentrations of mineral nutrients.

The highest photosystem II activity (performance of PSII, as described by the parameters $F_v/F_m$, $F_v/F_0$ and Performance Index-PI) (Figure 3a–c) was found in VER, compared to

the other treatments. This result could be attributed to the optimum foliar Mn concentration determined in VER (83 mg $kg^{-1}$ D.W.), compared to the other three treatments, where very high leaf Mn levels were found (337–405 mg $kg^{-1}$ D.W.) (Table 5), something which could probably play a harmful effect on PSII activity in these cases. Manganese plays a key-role in photosystem II of photosynthesis, and particularly in the reactions liberating $O_2$ [44]. Similar photosynthetic rates were found between the VER and CRF treatments; these two rates were significantly higher to those determined in ZEO and ZEO + VER (Figure 4a). However, stomata opening were quite different between CRF and VER (Figure 4b). Thus, the high photosynthetic rate in CRF was ascribed to the higher stomatal conductance, while the high photosynthetic rate in VER was probably owed to the better PSII performance (Figure 3a–c) and other non-stomatal factors (e.g., increased activity of photosynthetic enzymes). The fact that stomata opening affects the photosynthetic rate of plants (as happened in the CRF treatment of our study) was also supported by other researchers, for other plant species [45–48]. The intercellular $CO_2$ concentration was significantly higher in the ZEO + VER and CRF treatments (Figure 4d), following the trend of stomata opening (Figure 4b). Finally, intrinsic water use efficiency (WUEi), which provides information on how efficiently water (low losses by transpiration and high internal utilization, in terms of $CO_2$ assimilation) is used by plants, was significantly higher in VER and ZEO, compared to the other two treatments (Figure 4e). It was found that WUEi was influenced by: (i) K application [45,49], (ii) N fertilization and arbuscular mycorrhiza fungi (AMF) inoculation under conditions of water scarcity [20], as well as by: (iii) the kind of organic fertilization [12]. Our study proves that WUEi was influenced not only by organic soil amendments (manures), but also by the application of inorganic soil amendments, such as ZEO and VER.

## 5. Conclusions

In conclusion, ZEO and VER application influenced some soil fertility parameters, such as pH, extractable P, and exchangeable K. More specifically, ZEO addition leaded to an impressive boosting in exchangeable K concentration of more than 30 times, compared to the control soil (CRF treatment). The highest root biomass was recorded in ZEO. In many cases, foliar nutrition was affected by ZEO and VER (e.g., foliar K and Ca), while the highest leaf N was achieved in CRF. The optimum foliar Mn concentration (83 mg $kg^{-1}$ D.W.) was recorded in the VER treatment, while in the other three ones very high leaf Mn levels (>335 mg $kg^{-1}$ D.W.) were found. The highest photosynthetic rates were found in the VER and CRF treatments; however, only in CRF the higher rate was ascribed to higher stomata opening. It is believed that our data is a first step towards a more sustainable nutrient management in chestnut nurseries, limiting the high conventional fertilization rates in the young plantlets. However, further research is needed (under field conditions) to investigate whether zeolite and/or vermiculite could act as potential, complementary fertilizers, aiming at partially decreasing the high fertilization rates and enhancing the sustainability of the intensified chestnut orchards.

**Author Contributions:** Conceptualization, T.C.; methodology, T.C. and A.G.; software, T.C. and I.E.P.; validation, T.C., A.G. and I.E.P.; formal analysis, T.C.; investigation, T.C., E.P. and A.G.; resources, T.C.; data curation, T.C., E.P., A.G. and I.E.P.; writing—original T.C. and I.E.P.; writing—review and editing, T.C. and I.E.P.; visualization, T.C. and I.E.P.; supervision, T.C.; project administration, T.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author (T.C.). The data are not publicly available due to privacy reasons.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References


1. Arrobas, M.; Afonso, S.; Ferreira, I.Q.; Moutinho-Pereira, J.; Correia, C.M.; Rodrigues, M.A. Liming and application of nitrogen, phosphorus, potassium and boron on a young plantation of chestnut. *Turk. J. Agric. For.* **2017**, *41*, 441–451. [CrossRef]
2. Portela, E.; Martins, A.; Pires, A.L.; Raimundo, F.; Marques, G. Cap 6—Práticas culturais no souto: O manejo do solo. In *Vila Real, Portugal: Programa AGRO 499; Castanheiros*; Gomes-Laranjo, J., Ferreira-Cardoso, J., Portela, E., Abreu, C.G., Eds.; Universidade de Trás-os-Montes e Alto Douro: Vila Real, Portugal, 2007; pp. 207–264. (In Portuguese)
3. Zhang, J.; Li, Y.; Chang, S.X.; Jiang, P.; Zhou, G.; Liu, J.; Wu, J.; Shen, Z. Understory vegetation management affected greenhouse gas emissions and labile organic C pools in an intensively managed Chinese chestnut plantation. *Plant Soil* **2014**, *376*, 363–375. [CrossRef]
4. Corredoira, E.; Valladares, S.; Vieitez, A.M.; Ballester, A. Improved germination of somatic embryos and plant recovery of European chestnut. *In Vitro Cell. Dev. Biol. Plant* **2008**, *44*, 307–315. [CrossRef]
5. Cuenca, B.; Sanchez, C.; Aldrey, A.; Bogo, B.; Blanco, B.; Correa, B.; Vidal, N. Micropropagation of axillary shoots of hybrid chestnut (*Castanea sativa* x *C. crenata*) in liquid medium in a continuous immersion system. *Plant Cell Tiss Organ Cult.* **2017**, *131*, 307–320. [CrossRef]
6. Larson, C.G.; Hasbun, R.; Paz Jofre, M.; Sanchez-Olate, M.; Darcy Rios, L. Effect of genotype and cytokines source at the initiation stage in vitro culture of adult tissue of *Castanea sativa* Mill. *Gayana Bot.* **2017**, *74*, 30–40. [CrossRef]
7. Serdar, U.; Dermirsoy, H. Non-destructive leaf area estimation in chestnut. *Sci. Hortic.* **2006**, *108*, 227–230. [CrossRef]
8. Silvanini, A.; Dall'Asta, C.; Morrone, L.; Cirlini, M.; Beghe, D.; Fabbri, A.; Ganino, T. Altitude effects on fruit morphology and flour composition of two chestnut cultivars. *Sci. Hortic.* **2014**, *176*, 311–318. [CrossRef]
9. Guzman, M.; Urrestarazu, M.; Romero, L. Active and total Fe in *Castanea sativa* and their relation to other nutrients. *J. Plant Nutr.* **1986**, *9*, 909–921. [CrossRef]
10. Zysset, M.; Brunner, I.; Frey, B.; Blaser, P. Response of European chestnut to varying Calcium/Aluminum ratios. *J. Environ. Qual.* **1996**, *25*, 702–708. [CrossRef]
11. Eprikashvili, L.; Zautashvili, M.; Kordzakhia, T.; Pirtskhalava, N.; Dzagania, M.; Rubashvili, I.; Tsitsishvili, V. Intensification of bio-productivity of agricultural cultures by adding natural zeolites and brown coals into soils. *Ann. Agrar. Sci.* **2016**, *14*, 67–71. [CrossRef]
12. Chatzistathis, T.; Papadakis, I.E.; Papaioannou, A.; Chatzissavvidis, C.; Giannakoula, A. Comparative study effects between manure application and a controlled release fertilizer on the growth, nutrient uptake, photosystem II activity and photosynthetic rate of *Olea europaea* L. (cv. 'Koroneiki'). *Sci. Hortic.* **2020**, *264*, 109176. [CrossRef]
13. Benabderrahim, M.A.; Elfalleh, W.; Belayadi, H.; Haddad, M. Effect of date palm waste compost on forage alfalfa growth, yield, seed yield and minerals uptake. *Int. J. Recycl. Org. Waste Agric.* **2018**, *7*, 1–9. [CrossRef]
14. Li, Z.; Schneider, R.L.; Morreale, S.J.; Xie, Y.; Li, C.; Li, J. Woody organic amendments for retaining soil water, improving soil properties and enhancing plant growth in desertified soils of Ningxia, China. *Geoderma* **2018**, *310*, 143–152. [CrossRef]
15. Chartzoulakis, K.; Psarras, G.; Moutsopoulou, M.; Stefanoudaki, E. Application of olive mill wastewater to a Cretan olive orchard: Effects on soil properties, plant perfromance and the environment. *Agric. Ecosyst. Environ.* **2010**, *138*, 293–298. [CrossRef]
16. Chatzistathis, T.; Koutsos, T. Olive mill wastewater as a source of organic matter, water and nutrients for restoration of degraded soils and for crops managed with sustainable systems. *Agric. Water Manag.* **2017**, *190*, 55–64. [CrossRef]
17. Paskovic, I.; Bronic, J.; Subotic, B.; Pecina, M.; Perica, S.; Palcic, I.; Herak Custic, M. Impact of zeolite fertilization on radicchio mineral composition and nutritive value. *J. Food Agric. Environ.* **2013**, *11*, 498–502.
18. Doostikhah, N.; Panahpour, E.; Nadian, H.; Gholami, A. Tomato (*Lycopersicon esculentum* L.) nutrient and lead uptake affected by zeolite and DTPA in a lead-polluted soil. *Plant Biol.* **2020**, *22*, 317–322. [CrossRef]
19. Mazloomi, F.; Jalali, M. Effects of vermiculite, nanoclay and zeolite on ammonium transport through saturated sandy loam soil: Column experiments and modeling approaches. *Catena* **2019**, *176*, 170–180. [CrossRef]
20. Zhang, J.; Jiang, X.; Miao, Q.; Yu, B.; Xu, L.; Cui, Z. Combining mineral amendments improves wheat yield and soil properties in a coastal saline area. *Agronomy* **2019**, *9*, 48. [CrossRef]
21. Assimakopoulou, A.; Dimitroulia, D.; Kosmidis, S.; Doula, M.K. Growth, yield and nutrient status of pepper plants grown on a soil substrate with olive mill waste sludge and natural zeolite addition. *J. Plant Nutr.* **2020**, *43*, 629–640. [CrossRef]
22. Malandrino, M.; Abollino, O.; Buoso, S.; Giacomino, A.; La Gioia, C.; Mentasti, E. Accumulation of heavy metals from contaminated soil to plants and evaluation of soil remediation by vermiculite. *Chemosphere* **2011**, *82*, 169–178. [CrossRef] [PubMed]
23. Hamidpour, M.; Akbari, L.; Shirani, H. Effects of co-application of zeolites and vermicompost on speciation and phytoavailability of cadmium, lead and zinc in a contaminated soil. *Commun. Soil Sci. Plant Anal.* **2017**, *48*, 262–273. [CrossRef]
24. Chatzistathis, T.; Tzanakakis, V.; Giannakoula, A.; Psoma, P. Inorganic and organic amendments affect soil fertility, nutrition, photosystem II activity and fruit weight, and may enhance the sustainability of *Solanum lycopersicon* L. (cv. 'Mountain Fresh') crop. *Sustainability* **2020**, *12*, 9028. [CrossRef]
25. McLean, E. Soil pH and lime requirement. In *Agronomy Monograph, Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; ASA, SSSA: Madison, WI, USA, 1982; pp. 199–224. [CrossRef]
26. Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon and organic matter. In *Agronomy Monograph, Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; ASA, SSSA: Madison, WI, USA, 1982; pp. 539–547. [CrossRef]

27. Olsen, S.; Sommers, L. Phosphorus. In *Agronomy Monograph, Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; ASA, SSSA: Madison, WI, USA, 1982; pp. 403–430.
28. Bremner, J.M.; Mulvaney, C.S. Total nitrogen. In *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; ASA, SSSA: Madison, WI, USA, 1982; pp. 595–615.
29. Thomas, G.W. Exchangeable cations. Methods of soil analysis. In *Agronomy Monograph, Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; ASA, SSSA: Madison, WI, USA, 1982; pp. 159–166.
30. Lindsay, W.L.; Norvell, W.A. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* **1978**, *42*, 421–428. [CrossRef]
31. Chyla, M.A.; Żyrnicki, W. Determination of metal concentrations in animal hair by the ICP method Biological. *Biol. Trace Elem. Res.* **2000**, *75*, 187–194. [CrossRef]
32. Chapman, H.D.; Pratt, P.F. *Methods of Analysis for Soils, Plants and Waters*; Division of Agricultural Sciences, University of California: Riverside, CA, USA, 1961; p. 309.
33. Strasser, R.J.; Tsimilli-Michael, M.; Srivastava, A. Analysis of the chlorophyll a fluorescence transient. In *Chlorophyll a Fluorescence a Signature of Photosynthesis*; Papageorgiou, G., Govindjee, C., Eds.; Springer: Dordrecht, The Netherlands, 2004; Volume 19, pp. 321–362. [CrossRef]
34. Schreiber, U.; Schliwa, U.; Bilger, W. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* **1986**, *10*, 51–62. [CrossRef] [PubMed]
35. Basri, M.H.; Abdu, A.; Jusop, S.; Ahmed, O.H.; Abdul-Hamid, H.; Kusno, M.A.; Zainal, B.; Senin, A.L.; Junejo, N. Effects of mixed organic and inorganic fertilizers application on soil properties and the growth of Kenaf (*Hibiscus cannabinus* L.) cultivated on Bris soils. *Am. J. Appl. Sci.* **2013**, *10*, 1586–1597. [CrossRef]
36. Litaor, M.I.; Katz, L.; Shenker, M. The influence of compost and zeolite co-addition on the nutrients' status and plant growth in intensively cultivated Mediterranean soils. *Soil Use Manag.* **2017**, *33*, 72–80. [CrossRef]
37. Nanos, G.; Tsintirakou, I. Fertilization of *Castanea sativa* Mill. *Agric. Livest. Farming Agrotypos Publ.* **2013**, *74*. (In Greek)
38. El-Jaoual, T.; Cox, D.A. Manganese toxicity in plants. *J. Plant Nutr.* **1998**, *21*, 353–386. [CrossRef]
39. Li, G.; Li, C.; Rengel, Z.; Liu, H.; Zhao, P. Excess Zn-induced changes in physiological parameters and expression levels of TaZips in two wheat genotypes. *Environ. Exp. Bot.* **2020**, *177*, 104133. [CrossRef]
40. Mahmoodabadi, M.R.; Ronaghi, A.M.; Khayyat, M.; Hadarbadi, G. Effects of zeolite and cadmium on growth and chemical composition of soybean (*Glycine max* L.). *Trop. Subtrop. Agroecosyst.* **2009**, *10*, 515–521.
41. Ozbahce, A.; Tari, A.F.; Gönülal, E.; Simsekli, N.; Padem, H. The effect of zeolite applications on yield components and nutrient uptake of common bean under water stress. *Arch. Agron. Soil Sci.* **2015**, *61*, 615–626. [CrossRef]
42. Iskander, A.L.; Khald, E.M.; Sheta, A.S. Zinc and manganese sorption behavior by natural zeolite and bentonite. *Ann. Agric. Sci.* **2011**, *56*, 43–48. [CrossRef]
43. Najafi-Ghiri, M.; Rahimi, T. Zinc uptake by spinach (*Spinacia oleracea* L.) as affected by Zn application rate, zeolite and vermicompost. *Compost Sci. Util.* **2016**, *24*, 203–207. [CrossRef]
44. Chatzistathis, T.; Therios, I. How soil nutrient availability influences plant biomass and how biomass stimulation alleviates heavy metal toxicity in soils: The cases of nutrient use efficient genotypes and phytoremediators, respectively. In *Biomass Now-Cultivation and Utilization*; Matovic, D.M., Ed.; IntechOpen: Rijeka, Croatia, 2013; pp. 427–448. [CrossRef]
45. Zhu, L.D.; Shao, X.H.; Zhang, Y.C.; Zhang, H.; Hou, M.M. Effects of K fertilizer application on photosynthesis and seedling growth of sweet potato under drought stress. *J. Food Agric. Environ.* **2012**, *10*, 487–491.
46. Chen, Y.; Liu, L.; Guo, Q.; Zhu, Z.; Zhang, L. Effects of water management options and fertilizer supply on photosynthesis, fluorescence parameters and water use efficiency of *Prunella vulgaris* seedlings. *Biol. Res.* **2016**, *49*, 12. [CrossRef]
47. Gao, Y.; Wu, W.H.; Wang, Y. The $K^+$ channel KZM2 is involved in stomatal movement by modulating inward $K^+$ currents in maize guard cells. *Plant J.* **2017**, *92*, 662–675. [CrossRef]
48. Urban, J.; Ingwers, M.; McGuire, M.A.; Teskey, R.O. Stomatal conductance increases with rising temperature. *Plant Signal. Behav.* **2017**, *12*, e1356534. [CrossRef]
49. Saykhul, A.; Chatzistathis, T.; Chatzissavvidis, C.; Koundouras, S.; Therios, I.; Dimassi, K. Potassium utilization efficiency of three olive cultivars grown in a hydroponic system. *Sci. Hortic.* **2013**, *162*, 55–62. [CrossRef]