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Large Chestnut Trees Did Not Respond to Annual Fertiliser Applications, Requiring a Long-Term Approach to Establishing Effective Fertilisation Plans

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Abstract: Due to the high value of the fruit, the European chestnut (*Castanea sativa* Mill.), usually grown in agroforestry systems, has been planted as a single species in orchards managed with increasingly intensive cropping practices, such as the regular use of fertilisers. This justifies research into establishing fertilisation programmes oriented towards ecological intensification. In this study, the results of fruit production, plant nutritional status and soil properties are reported from a field trial in which three NPK fertilisers (20:7:10, 13:11:21 and 7:14:14) and a control treatment were used. Chestnut yields did not vary significantly between treatments, although the mean values of the control showed a clear downward trend. N supplied by the fertilisers seems to have been the most important factor in the difference between the fertilised and control treatments, since leaf N concentrations were lower in the control and often below the lower limit of the sufficiency range. Soil inorganic N levels in the autumn, and tissue N concentrations of the herbaceous vegetation developing beneath the trees, indicated risks of N loss to the environment and highlighted the importance of this vegetation remaining during the winter. The chestnuts' poor response to fertiliser applications was attributed to the buffering effect of the large perennial structure of the trees on the distribution of nutrients to the growing plant parts. In large trees, it seems appropriate to base the annual fertilisation plan on leaf nutrient concentration. Thus, farmers probably should avoid spending money on fertilizer applications as long as leaf nutrient concentrations do not approach the lower limits of sufficiency ranges.

Keywords: chestnut tree; *Castanea sativa*; chestnut yield; plant nutritional status; soil inorganic nitrogen

1. Introduction

Chestnuts are the main source of income for farmers in the upland areas of the north of Portugal. However, farmers are facing a quite complex situation due to a set of pests and diseases that weaken the trees, thereby reducing their productivity and, in some cases, causing their death. Currently, ink disease (*Phytophthora* sp.pl.), chestnut blight

(*Cryphonectria parasitica* (Murrill) Barr.) and the Asian gall wasp (*Dryocosmus kuriphilus* Yasumatsu) are the main health problems affecting chestnut trees [1–3]. Notwithstanding this, chestnuts have maintained very good market prices [4], which has led farmers to devote great attention and care to their crops, either replacing dead trees or establishing new orchards [5].

Chestnut is grown all over the world as part of agroforestry systems with little phytotechnical intensification [6]. In the mountainous areas of the north of Portugal, the lack of other crop options has raised chestnut to the status of the main crop, having been grown in monoculture and integrated into increasingly intensive farming systems, in a similar way to orchards of other important fruit trees [7–9]. One of the practices that has received greater attention from producers is fertilisation, with trees currently being fertilised regularly [5,10,11].

Crop fertilisation, being essential for obtaining high productivity in any species [12–14], can also be associated with high risks of environmental contamination, especially the use of N fertilisers that can lead to the eutrophication of ground water [15,16] and the emission of greenhouse gases into the atmosphere, in particular, N oxides [17,18]. Thus, crop fertilisation must be managed judiciously, in order to apply the appropriate nutrient rates, thereby reducing the risk of environmental damage [19–22].

The prospects of a growing global population and the need to feed it, associated with the risks of environmental contamination, have led to the need to develop farming practices based on the concept of ecological intensification [23,24], which, in practice, means maintaining high productivity, but by using production factors in a more rational way. Thus, as with the main world crops, but also with chestnut, it is necessary to manage resources properly, using them in the smallest amounts necessary to maintain productivity.

Previous work carried out in NE Portugal has shown that in chestnut groves, nutrients are often below the lower limit of the sufficiency range [25] and that trees generally tend to respond to fertiliser applications [11,26], although in some studies, they did not [5]. However, there are still only a few studies on chestnut fertilisation, and the use of fertilisers is far from being optimized, with more data being required to establish adequate fertilisation programmes. It is therefore necessary to establish better guidelines for the fertilisation of these trees, to try to keep them healthy and productive, so that these magnificent ecosystems may persist, allowing man to continue to occupy these mountain territories which are showing concerning signs of depopulation [27]. This study reports the results from a field experiment of chestnut fertiliser application using NPK fertilisers with different combinations of macronutrients, trying as best as possible to replicate the diversity of fertilisers found on the market, which farmers have access to. The objectives of the study are to understand better how these huge trees respond to fertiliser application so as to help farmers make better decisions when they need to acquire them.

2. Materials and Methods

2.1. Experimental Conditions

The field experiment took place in Vinhais (41°50′15.8″ N; 7°03′40.4″ W, 800 m above sea level), northeastern Portugal, in a 50-year-old chestnut orchard of the cultivar Judia with trees spaced at 10 m × 10 m. The region benefits from a warm-summer Mediterranean climate (Csb), according to the Köppen–Geiger classification [28]. The annual mean temperature and the accumulated annual precipitation are 11.9 °C and 880 mm, respectively. Average monthly temperatures and precipitation of the climatological normal (1981–2010), together with those recorded during the experimental period (2018–2021), are presented in Figure 1.

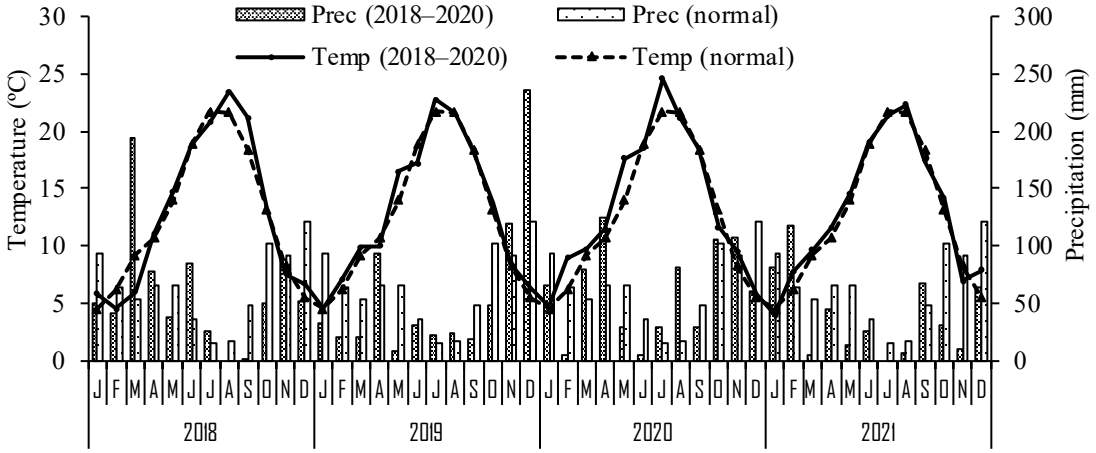

Figure 1. Average monthly temperature and accumulated monthly precipitation during the experimental period, and climatological normal values for the region.

The soil where the chestnut orchard is planted is a Leptosol, sandy-loam textured. It is a very shallow soil (~ 0.20 m deep), which separates, determined from composite soil samples (n = 3) taken from the 0.0–0.2 m soil layer at the beginning of the study, were 11.8% clay, 17.3% silt and 70.9% sand. Soil organic C was low, pH acidic and extractable P and K were medium and very high, respectively. Some other soil properties determined at the beginning of the field trial are presented in Table 1.

Table 1. Selected soil properties (average ± standard deviation, n = 3) from composite samples (10 cores per composite sample) taken at 0–0.20 m depth at the beginning of the study.

Soil Properties		Soil Properties (Cont.)	
[1] Organic carbon (g kg^{-1})	13.4 ± 0.50	[4] Exchang. sodium ($cmol_c$ kg^{-1})	0.1 ± 0.02
[2] pH (H_2O)	5.3 ± 0.19	[5] Exchang. acidity ($cmol_c$ kg^{-1})	0.7 ± 0.08
[2] pH (KCl)	4.2 ± 0.15	[6] CEC ($cmol_c$ kg^{-1})	6.0 ± 0.26
[3] Extract. phosphorus (mg kg^{-1}, P_2O_5)	93.1 ± 15.75	[7] Extract. boron (mg kg^{-1})	0.4 ± 0.06
[3] Extract. potassium (mg kg^{-1}, K_2O)	344.7 ± 20.63	[8] Extract. iron (mg kg^{-1})	62.2 ± 4.78
[4] Exchang. calcium ($cmol_c$ kg^{-1})	3.1 ± 0.20	[8] Extract. zinc (mg kg^{-1})	2.5 ± 0.31
[4] Exchang. magnesium ($cmol_c$ kg^{-1})	1.1 ± 0.11	[8] Extract. copper (mg kg^{-1})	1.2 ± 0.25
[4] Exchang. potassium ($cmol_c$ kg^{-1})	1.0 ± 0.13	[8] Extract. manganese (mg kg^{-1})	132.6 ± 18.59

[1] Wet digestion (Walkley–Black); [2] Potentiometry; [3] Ammonium lactate; [4] Ammonium acetate; [5] Potassium chloride; [6] Cation Exchange Capacity; [7] Hot water, azomethine-H; [8] Ammonium acetate and EDTA (ethylenediaminetetraacetic acid).

2.2. Experimental Design and Management of the Field Trial

Sixteen large-sized trees with similar, spherical canopies (~270 m^3) were selected for the study. They were randomly distributed into four groups, corresponding to four fertilisation treatments, with four trees (replicates) in each treatment, in a completely randomized design. The treatments consisted of three compound NPK fertilisers with different levels of N, P and K, and an unfertilised control.

One of the treatments, named 7:14:14, consisted of the application of a 7:14:14 NPK compound fertiliser that doses 7% N (5% ammoniacal-N and 2% ureic-N), 14% P_2O_5 (11% water soluble) and 14% K_2O. This fertiliser also contains 4% CaO, 2% MgO, 15% SO_3, and 0.02% B. Another treatment named YA20:7:10, corresponds to the application of the commercial fertiliser Yara MilaTM Actyva 20:7:10, with 20% N (9.4% nitric-N, 10.6% ammoniacal-N), 7% P_2O_5 (25 to 30% as polyphosphates) and 10% K_2O. The fertiliser also contains other important nutrients, namely S (10% SO_3) and Mg (3% MgO). The third treatment, named YS13:11:21, consisted of the application of the NPK compound fertiliser Yara MilaTM Solán 13:11:21, which doses 13% N (5.5% nitric-N, 7.7% ammoniacal-N), 11% P_2O_5

(20 to 30% as polyphosphates) and 21% K_2O. This fertiliser also contains relevant amounts of Mg (2% MgO) and B (0.2%).

All fertilisers were applied at a rate of 4 kg per tree (~400 kg ha^{-1}). Thus, the YA20:7:10 treatment corresponded to an application of 80, 28 and 40 kg ha^{-1} of N, P_2O_5 and K_2O, respectively; the 7:14:14 treatment to an application of 28, 56 and 56 kg ha^{-1} of N, P_2O_5 and K_2O, respectively, and the YS13:11:21 to an application of 52, 44, 84 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. Similar fertilisations are commonly used by local farmers, and these particular fertilisers were chosen for the trial because they present a good variation in the levels of macronutrients.

The fertilisers were evenly applied beneath the canopy of the trees in the first week of April over the four years of the study and incorporated with a cultivator. The orchard was tilled a second time every year at the end of May to control the weeds. No further cropping practices were carried out in the orchard during the four years of the study.

2.3. Measurements in the Field

The effect of the treatments was assessed in the field by measuring the greenness of the leaves and by chlorophyll a fluorescence analysis.

The SPAD (Soil and Plant Analysis Development)-502 Plus chlorophyll meter (Spectrum Technologies, Inc., Aurora, IL, USA) was used to measure leaf greenness. SPAD-502 provides *dimensionless* readings, proportional to the chlorophyll content of the leaves, by measuring the transmittance of light through the leaves at 650 nm (red light, absorbed by chlorophyll) and 940 nm (infrared light, non-absorbed by chlorophyll). Each mean value was obtained after 30 individual readings taken around the crown on fully expanded young leaves.

Chlorophyll a fluorescence was assessed using the dark adaptation protocols with the OS-30p+ fluorometer (Opti-sciences, Inc., Hudson, NH, USA). F_M, F_0 and F_V are, respectively, maximum, minimum and variable fluorescence from dark-adapted leaves. F_V/F_M is estimated as $(F_M - F_0)/F_M$.

To harvest the chestnuts, it is necessary to wait for them to fall to the ground and then pick them up manually or mechanically. In this experiment, the fruits were harvested manually, in three passes during the autumn, to allow individual weighing per tree. In 2021, the COVID-19 pandemic restrictions did not allow the completion of harvest records, and therefore, only the values for 2018–2020 are available.

2.4. Soil and Plant Tissue Sampling and Analytical Determinations

Three composite samples were taken at the beginning of the experiment to characterize the experimental plot. The soil was sampled again in October 2021 to evaluate the effect of the treatments on soil properties. All soil samples taken to the laboratory were composite samples, taken at six different sampling points. Sampling was carried out in the 0.0–0.20 m soil layer, beneath the canopy of the trees, where the fertilisers had been applied.

Soil samples were oven-dried at 40 °C and sieved (2 mm mesh). Thereafter, the samples were analysed for pH (H_2O and KCl) (soil: solution, 1:2.5), cation-exchange capacity (ammonium acetate, pH 7.0), organic C (wet digestion, Walkley-Black method) and extractable P and K (Egner–Riehm method, ammonium lactate extract). Soil B was extracted by hot water and determined by the method of azomethine-H. For more details on these analytical procedures, the reader is referred to van Reeuwijk [29]. The availability of other micronutrients (Cu, Fe, Zn, and Mn) in the soil was determined by atomic absorption spectrometry after extraction with ammonium acetate and EDTA, according to the method described by Lakanen and Erviö [30]. Soil inorganic-N was determined in soil extracts prepared from 20 g of soil and 40 mL 2 M KCl. The suspension was shaken for 1 h and filtered through Watmann No. 42 filter paper. Nitrate and ammonium concentrations in the extracts were analysed in an UV–Vis spectrophotometer [31].

By the end of July, in each of the four years, samples of young, fully developed leaves were taken for elemental analysis. Following each harvest, samples of 50 nuts per tree were randomly taken to evaluate their size and also for elemental analysis. After counting and weighing, the kernel was separated from shell and pellicle, and the two parts analysed separately. In April 2022, the spontaneous vegetation which had developed beneath the canopy of the trees was mowed to serve as a biological index of soil-available nutrients. The samples were collected by randomly placing a grid of 0.5 m × 0.5 m on the vegetation.

The samples of leaves, fruit kernels, shells and pellicles and spontaneous vegetation, were oven-dried at 70 °C until they reached a constant weight and ground (1 mm mesh). Elemental analyses of tissue samples were performed by Kjeldahl (N), colorimetry (B and P) and atomic absorption spectrophotometry (K, Ca, Mg, Fe, Mn, Cu, Zn) methods [32] after tissue samples had been previously digested with nitric acid in a microwave.

2.5. Data Analysis

The data was analysed for normality and homogeneity of variance using the Shapiro–Wilk and Bartlett's test, respectively. The analysis of variance was performed as a one-way ANOVA, using the Statistical Package for the Social Sciences (SPSS) version 25 (IBM Corporation, New York, NY, USA). When significant differences were found, the means were separated by the Tukey HSD post hoc test ($\alpha = 0.05$).

3. Results

3.1. Chestnut Yield

Chestnut yield did not vary significantly in the three years in which it was possible to collect the fruits (Figure 2). However, the accumulated yield of the three years showed a clear tendency towards reduction in the control in comparison to the fertilised treatments. In addition, the probability values decreased over time, nearing significant differences between treatments in the last year (2020) for which it was possible to obtain records. In the YS13:11:21 treatment, an accumulated average nut yield of 94.9 kg tree^{-1} was recorded, while in the control treatment, the value was 80 kg tree^{-1}.

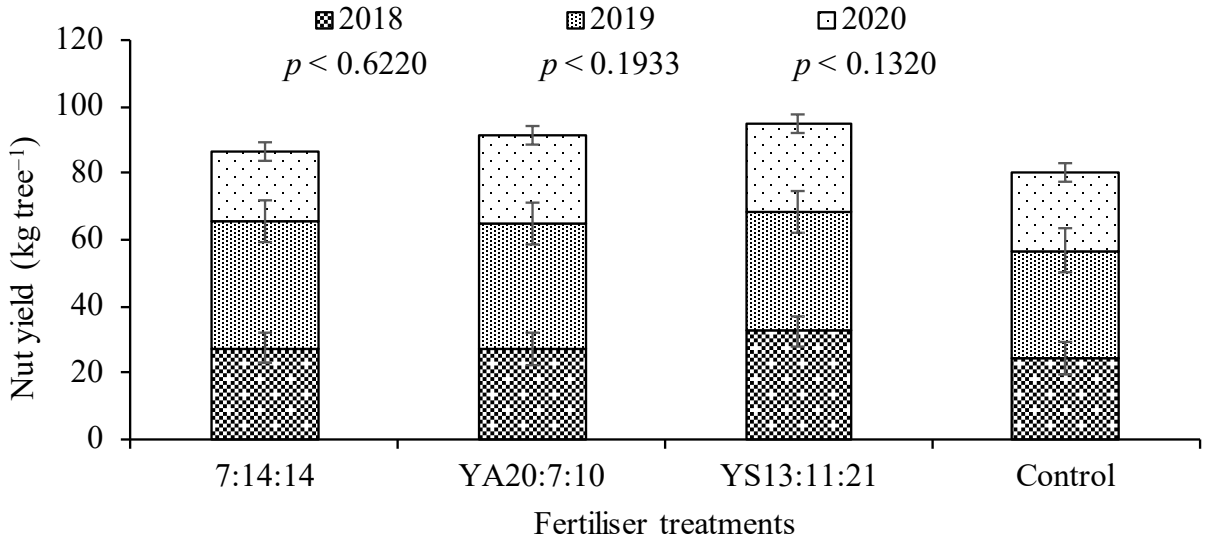

Figure 2. Average annual nut yield as a response to fertilisation treatments. Error bars are the standard errors.

3.2. Nutrients in Plant Tissues and Chlorophyll a Fluorescence

Leaf nitrogen concentrations varied significantly between treatments only in the 2021 sampling (Figure 3). In the last year, the control treatment displayed an average concentration of N in the leaves (18.9 g kg^{-1}) much lower than the values recorded in the fertilised treatments (22.4 to 22.9 g kg^{-1}) and below the lower limit of the sufficiency range. Fertiliser YA20:7:10, being more concentrated in N, showed average concentrations of leaf N tending to be higher than the other treatments. Leaf P concentrations did not vary significantly between treatments and remained above the lower limit of the sufficiency range. No sign of any coherence was observed between the application of P and the concentration of the nutrient in the leaves. The concentrations of K in leaves fluctuated inconsistently with treatments over the years, although there were found to be significant differences between treatments in the last sampling. As observed for P, there seem to have been more important variables other than the application of these nutrients determining their concentration in the leaves. The values of K tended to remain above the lower limit of the sufficiency range. Leaf concentrations of Ca and Mg did not vary significantly between treatments. In the case of Ca, the values were close to the lower limit of the sufficiency range and those of Mg were clearly within the interval of adequate concentrations.

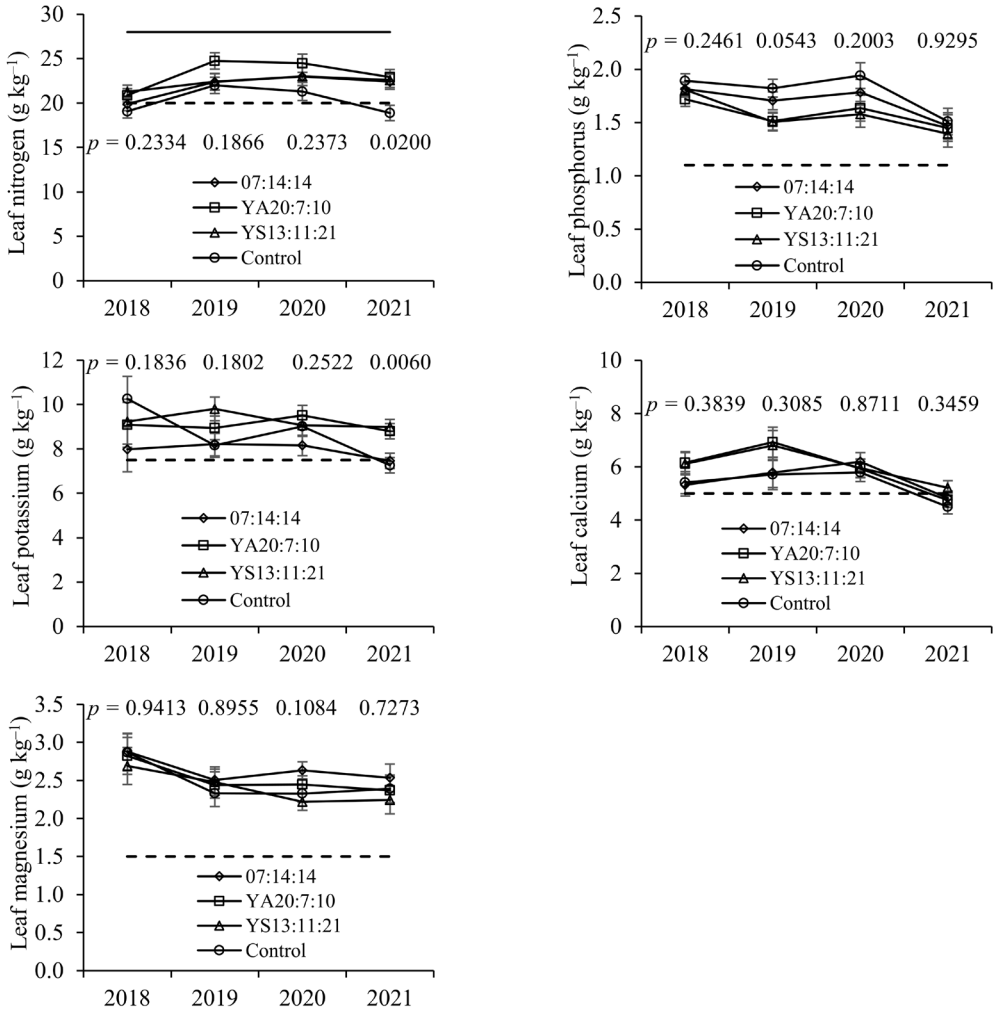

Figure 3. Leaf concentrations of nitrogen, phosphorus, potassium, calcium and magnesium as a response to fertilisation treatments. Dashed and solid lines are, respectively, the lower and upper limits of the sufficiency ranges. Error bars are the standard errors.

Leaf B concentrations remained low in three treatments (07:14:14, YA20:7:10 and control) for all sampling dates, but without going down to the deficiency zone (Figure 4). The

fertiliser YS13:11:21 which, in addition to the macronutrients N, P and K, also contains B (0.2%), maintained a nutrient concentration in the leaves higher than the other treatments, especially in the last two samplings. Fe concentrations in the leaves fluctuated within the sufficiency range, but without a clear coherence between treatments. The average levels of Mn in the leaves appeared in the upper part of the sufficiency range, although they never reached the toxicity zone. Control treatment values remained consistently lower than those for the fertilised treatments. The concentrations of Zn and Cu in the leaves showed no relationship with the fertilisation treatments and were, in general, within their sufficiency ranges.

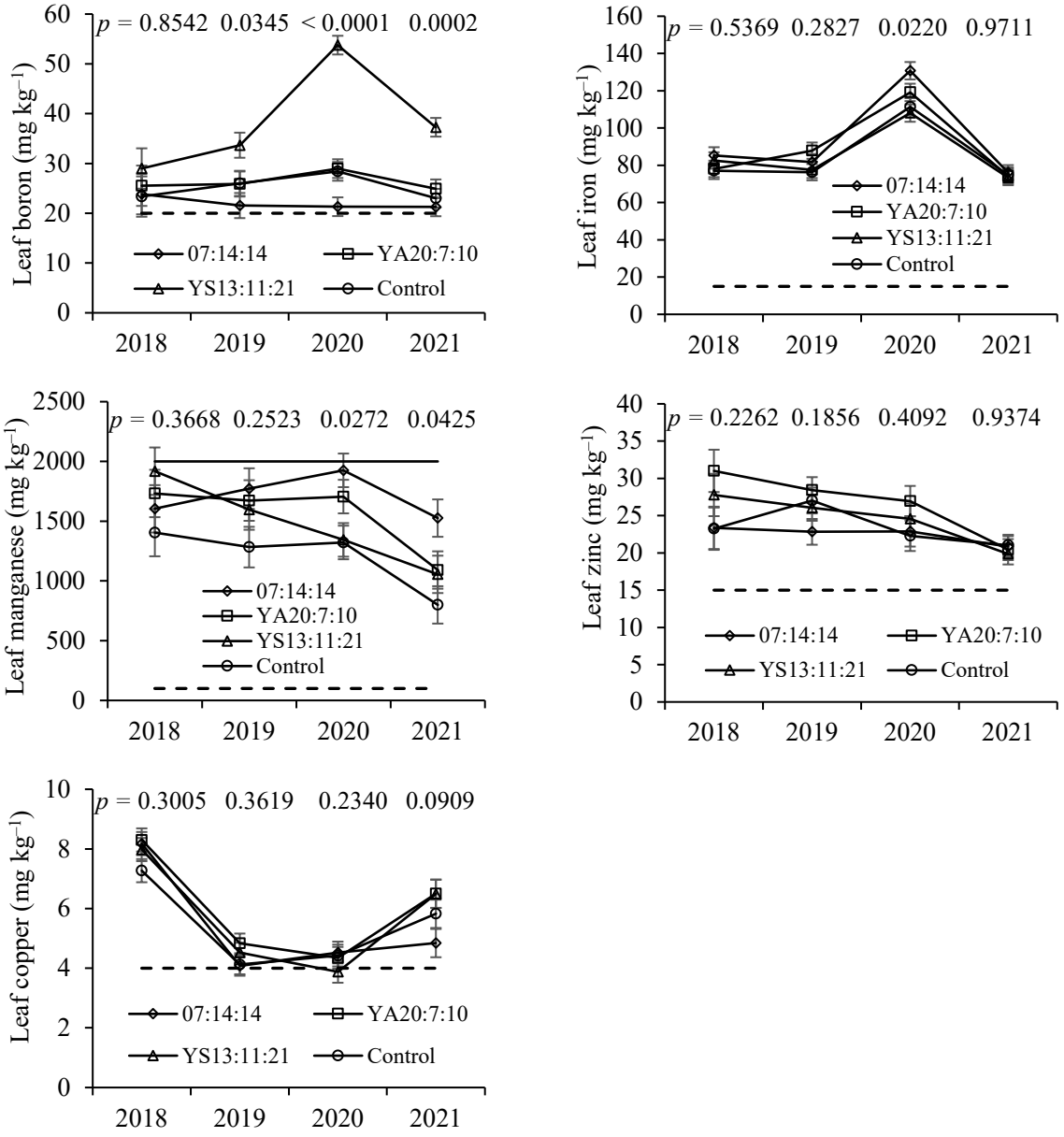

Figure 4. Leaf concentration of boron, iron, manganese, zinc and copper as a response to fertilisation treatments. Dashed and solid lines are, respectively, the lower and upper limits of the sufficiency ranges. Error bars are the standard errors.

The concentration of the majority of nutrients in the kernel and shell did not vary significantly with fertilisation treatments (data not shown). Only the concentration of B in these tissues showed a pattern that is worth reporting. In the kernel, significant differences between treatments were found in the last sampling (2020), with the highest values recorded in treatment YS13:11:21 (Figure 5). In the shell, the same pattern of the kernel was maintained, but with more accentuated average differences between the YS13:11:21 and other treatments. In the shell, the average B concentrations were also higher than in the kernel for the same treatment and sampling date.

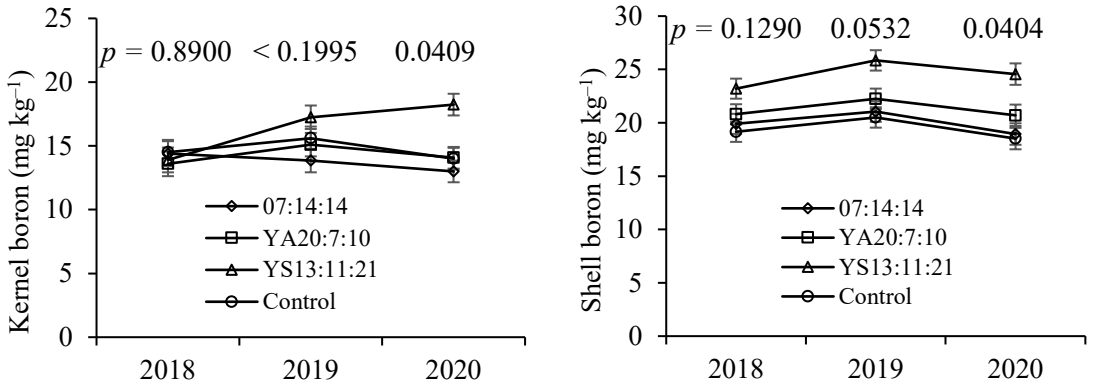

Figure 5. Boron concentrations in kernel and shell as a response to fertilisation treatments. Error bars are the standard errors.

Mean SPAD values showed a tendency to be lower in the control treatment compared to the fertilised treatments (Table 2). In the 2021 reading, significant differences were found between the values of the YA20:710 fertiliser (47.5), the most concentrated in N, and the control (44.2). A somewhat similar trend showed F_V/F_M, although for this variable, the differences were only significant in the 2019 readings, with the mean value of the YA20:7:10 treatment (0.834) being higher than that of the control (0.807).

Table 2. SPAD-readings and F_V/F_M (ratio of variable fluorescence/maximum fluorescence) as a function of fertilisation treatments.

Fertilisation	**SPAD**			**F_V/F_M**		
treatment	2019	2020	2021	2019	2020	2021
7:14:14	44.4 a *	43.6 a	44.8 ab	0.814 ab	0.833 a	0.821 a
YA20:7:10	45.4 a	45.6 a	47.5 a	0.834 a	0.845 a	0.828 a
YS13:11:21	46.3 a	45.2 a	45.9 ab	0.824 ab	0.829 a	0.829 a
Control	43.9 a	43.4 a	44.2 b	0.807 b	0.827 a	0.817 a
Prob > F	0.0762	0.1837	0.0432	0.0331	0.1797	0.3712
St. error	0.63	0.82	0.77	0.006	0.005	0.005

* In columns, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

3.3. Chemical Soil Properties

Fertilisation treatments carried out during the four consecutive years did not influence soil organic C content (Table 3). However, fertilisation acidified the soil (pH_{H2O} and pHKCl) compared to the control treatment. Extractable P also varied significantly with the fertilisation treatment, with the highest mean values appearing in the treatments corresponding to the fertilisers more concentrated in P. Soil K levels also varied significantly with treatments and, as for P, there was also a good consistency between the application of the nutrient and its resulting level in the soil.

Table 3. Soil organic carbon (C), pH and extractable phosphorus (P) and potassium (K) (Egner-Riehm) as a function of fertilisation treatments.

Fertilisation	Organic C			Extractable P	Extractable K
Treatment	g kg^{-1}	pH(H_2O)	pH(KCl)	mg kg^{-1}, P_2O_5	mg kg^{-1}, K_2O
7:14:14	13.8 a *	5.08 bc	4.11 b	142.6 a	414.0 ab
YA20:7:10	13.4 a	4.89 c	4.02 b	95.0 b	327.8 b
YS13:11:21	13.5 a	5.11 bc	4.13 b	121.1 ab	519.0 a
Control	12.5 a	5.41 a	4.33 a	81.3 b	303.8 b
Prob > F	0.6947	<0.0001	0.0017	0.0064	0.0006
St. error	0.79	0.048	0.043	10.5	28.1

* In columns, means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05).

Soil exchangeable Ca^{2+} and Mg^{2+} did not vary significantly with fertilisation treatments (Table 4). The exchangeable K^+ (extracted by ammonium acetate) varied significantly with the treatments and the mean values were related to the amount of nutrient provided by the fertilisers, as had been verified with the K extracted by the Egner-Riehm method (ammonium lactate). Soil Na+ levels also varied with treatments, with mean values being significantly higher in the 07:14:14 and YS13:11:21 fertiliser plots compared to the values in the YA20:7:10 and control plots. Exchangeable acidity also varied significantly between treatments, with the lowest mean value being recorded in the control treatment. The cation-exchange capacity did not vary significantly with the treatments, maybe because no significant differences were found between two important bases, Ca^{2+} and Mg^{2+}.

Table 4. Soil exchangeable bases, exchangeable acidity (EA) and cation-exchange capacity (CEC) as a function of fertilisation treatments.

	Exchangeable Complex					
Fertilisation	Ca^{2+}	Mg^{2+}	K^+	Na^+	EA	CEC
Treatment	cmol$_c$ kg^{-1}					
7:14:14	3.43 a *	1.00 a	1.08 ab	0.44 a	0.85 ab	6.73 a
YA20:7:10	2.88 a	1.02 a	0.81 b	0.07 b	1.15 a	5.95 a
YS13:11:21	2.36 a	0.78 a	1.37 a	0.44 a	1.17 a	6.00 a
Control	3.25 a	1.16 a	0.80 b	0.16 b	0.70 b	6.34 a
Prob > F	0.3233	0.2371	0.0017	0.0002	0.0073	0.7907
St. error	0.42	0.12	0.09	0.05	0.09	0.61

* In columns, means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05).

Soil B levels were significantly higher in the YS13:11:21 treatment than in the other fertilisation treatments and in the control (Table 5). In contrast, soil Fe, Zn and Cu levels did not vary significantly with treatments. In the case of Mn, significant differences between treatments were observed, with the lowest mean values being recorded in the control treatment.

Table 5. Soil boron, iron, zinc, copper and manganese as a function of fertilisation treatments.

Fertilisation	Boron	Iron	Zinc	Copper	Manganese
Treatment	mg kg^{-1}				
7:14:14	0.35 b *	65.2 a	2.7 a	2.0 a	152.5 a
YA20:7:10	0.43 b	72.6 a	2.3 a	1.9 a	136.2 ab
YS13:11:21	1.28 a	59.2 a	2.8 a	1.8 a	149.1 ab
Control	0.32 b	60.5 a	2.2 a	2.6 a	127.8 b

Prob > F	<0.0001	0.2244	0.0852	0.0654	0.0240
St. error	0.102	4.67	0.17	0.17	5.40

* In columns, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

Soil ammonium levels extracted by hot or cold KCl did not vary significantly with the fertilisation treatments, although the average values were higher in treatment YA20:7:10, the fertiliser being more concentrated in N (Table 6). The hydrolysable NH_4^+ showed significant differences between treatments, with the mean value of YA20:7:10 being higher than that of the other treatments. Soil nitrate levels also varied significantly between treatments, with YA20:7:10 and the control recording the highest and lowest mean values, respectively.

Table 6. Soil ammonium (NH_4^+) extracted by hot and cold potassium chloride, NH_4^+ hydrolysable (Hyd) (NH_4^+ hot − NH_4^+ cold) and nitrate extracted by cold KCl.

Fertilisation	NH_4^+ Hot	NH_4^+ Cold	NH_4^+ Hyd	NO_3^- Cold
Treatment		**mg kg^{-1}**		
7:14:14	82.1 a *	69.0 a	13.1 b	59.0 b
YA20:7:10	108.1 a	92.20 a	15.9 a	100.5 a
YS13:11:21	72.3 a	58.1 a	14.2 b	78.0 ab
Control	65.4 a	51.7 a	13.6 b	44.9 b
Prob > F	0.4803	0.5649	0.9213	0.0038
St. error	19.99	21.12	2.99	8.65

* In columns, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

The development of spontaneous vegetation beneath the canopy of trees, where fertilisers were applied, showed significant differences between treatments (Table 7). Dry matter yield appeared in three response groups, in which the values were higher in the YA20:7:10 treatment, followed by the YS13:11:21 and 7:14:14 treatments and finally, the control treatment. N concentrations in the dry matter followed exactly the same trend, while the concentrations of the macronutrients P and K did not vary significantly between treatments. Tissue B concentration was significantly higher in YS13:11:21 than in the other treatments. Tissue Mn levels did not differ significantly between treatments, although the control treatment had the lowest mean value, following the trend observed in chestnut leaves and the soil. For the other nutrients analysed (Ca, Mg, Fe, Cu and Zn), there were no significant differences or any trend that deserve to be reported.

Table 7. Dry matter yield and nutrient concentrations in the herbaceous vegetation developing under the canopy of chestnut trees where the fertilisation treatments were applied.

		Tissue Nutrient Concentration				
Fertilisation	**DM Yield**	**N**	**P**	**K**	**B**	**Mn**
Treatment	**Mg ha^{-1}**		**g kg^{-1}**		**mg kg^{-1}**	
7:14:14	2.3 b *	24.5 b	2.6 a	32.2 a	20.5 b	636.3 a
YA20:7:10	3.5 a	29.6 a	2.4 a	33.8 a	20.4 b	722.9 a
YS13:11:21	2.6 b	26.2 b	2.5 a	35.3 a	43.0 a	681.9 a
Control	1.8 c	19.5 c	2.4 a	27.0 a	18.4 b	534.8 a
Prob > F	0.0001	< 0.0001	0.0898	0.3458	0.0003	0.1152
St. error	0.16	0.45	0.07	3.19	2.51	49.14

* In columns, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

4. Discussion

The annual and accumulated (2018–2020) chestnut yields did not vary significantly with fertilisation treatments. However, the average accumulated yield showed a clear tendency of reduction in the control in relation to the fertilised treatments. Chestnut trees are particularly large, with a huge perennial structure and canopy. In previous studies, it has already been observed that chestnut tends to respond poorly to fertilisers applied to the soil, probably due to the buffering effect that the perennial structure exerts in regulating the supply of nutrients for the growth of the aerial plant parts [5].

Leaf N concentrations tended to be higher in the fertilised treatments, which were more concentrated in N compared to the control, although significant differences only occurred on the last sampling date. In the control treatment, the values were close to the lower limit of the sufficiency range, having even fallen into the deficiency range on some sampling dates. The relevant structural role of N in plant tissues is undeniable [33**],** and it is still recognized as the main nutrient that limits plant productivity in both natural ecosystems and cultivated fields [19]. This result points to N as the most likely cause for the apparent drop in productivity in the control treatment. The SPAD values, which have been used mainly as an index of the N nutritional status of crops [5,29,34], agreed with tissue N concentrations, and in 2021, significant differences were found between the YA20:7:10 treatment and the control. The F_V/F_M ratio, a widely used indicator of photoinhibition or other injuries at the PSII complexes [35], followed the same trend as the N nutritional status indices, with values in the control being lower than in the treatment YA20:7:10 in the 2019 reading. However, the values never dropped below 0.78, which is the threshold limit below which most plants are considered to be under clear environmental stress [26,36–38]. Thus, the values of the maximum quantum efficiency of PSII also highlight the poor response of the photochemical reactions of photosynthesis of these huge trees to nutrient supply.

Leaf P concentrations did not vary significantly with treatments and always remained within the sufficiency range established for this species (1.1 to 3.0%) [25,39]. Initial soil P levels were at a level classified as medium (Table 1), and in the region, it has been difficult to obtain a response of different crop species to P applications [5,40,41]. In chestnut, the lack of response may be due to those reasons but also to the buffering effect of the perennial parts, already mentioned for N, and to a possible role of mycorrhizal fungi. Chestnut is recognized as a plant that establishes symbiotic relationships with several mycorrhizal fungi [42]. One of the main benefits for mycorrhizal plants is the access to sparingly soluble P sources that non-mycorrhizal plants do not have [43–47]. Thus, whatever the reason, the results seem to indicate a reduced importance of P in chestnut tree fertilisation programmes.

Although soil K levels increased with the application of fertilisers, no significant differences were observed in the K concentrations of the chestnut leaves. Leaf K concentrations varied greatly over the years and between treatments, although they generally remained within the sufficiency range. This pattern of K is common in shrub and tree species [13,21,38,48] and may be due to source/sink relationships and/or environmental constraints. Growing fruits are a primary sink for available K, the nutrient being remobilized from leaves [33]. In chestnut, fruit growth coincides with the end of summer, a period in which there is often little soil moisture, which limits the movement of nutrients in the soil by mass flow and diffusion, making nutrient uptake difficult [19]. In addition, the original levels of K in the soil were relatively high, which would have reduced the impact of applying K as a fertiliser. Finally, the buffering effect of the perennial tree structure may have moderated the effects of the fertiliser applications, as mentioned for N and P.

Tissue B levels differed between treatments on three dates, with YS13:11:21 fertiliser (B-rich) values being significantly higher than in the other treatments. Additionally in the fruits, B concentrations were the highest in the YA13:11:21 treatment, in particular, in the shell. Boron is very important in dicots, where it plays an important role in cell wall and membrane integrity, with these plants requiring greater amounts of B than monocots [48–

50]. In the region, dicots often respond to the application of B [12,41,51]. In this field trial, however, tissue B levels were never below the sufficiency range even though they were close to the limit. Perhaps for this reason, B was not determinant in crop productivity, in contrast to what has been shown in other studies with chestnut [11,52–54].

In the control treatment, mean leaf Mn levels were lower than in the fertilised treatments, and the differences were statistically significant on the last sampling date. A tendency for lower Mn levels in soils was also observed in the control treatment. Soil pH in the control was higher than in the fertilised treatments, particularly in those that had a greater amount of applied N. Nitrification can decrease soil pH [19], and the increase of available Mn levels in the fertilised treatments may have been a reflection of pH reduction [19,49]. Even so, leaf Mn levels never exceeded the upper limit of the sufficiency range, which is set at 2000 mg kg^{-1} [25,39], so its effect on crop productivity must not have been relevant.

In the autumn, the availability of inorganic N in the soil as measured by hydrolysable NH_4^+ and cold-extracted NO_3^- was higher in the treatments with more N-concentrated fertilisers. This may indicate a greater risk of N loss through leaching and/or denitrification, since the rainy season follows, a precondition for the occurrence of these phenomena [19]. However, in April, dry matter and tissue N concentrations in the spontaneous vegetation were also higher in the treatments that received more N as a fertiliser. These plants, which appear after the first autumn rains and develop during the winter, can play important roles by controlling soil erosion [55,56], increasing soil organic matter [57,58] and developing ecosystem biodiversity [59,60]. They also act as an N catch crop [21,61], justifying the promotion of their presence in orchard soils [62]. This result also shows that the effect of N applications is easier to obtain in herbaceous vegetation than in a tree, probably due to the latter's large perennial structure. It seems clear that in large trees, it is more difficult to get a response to fertilisation and therefore, more difficult to optimize a fertilisation programme. In trees, a dynamic optimization method should always be used [63], by which, based on a given annual fertilisation plan, nutrient concentrations in leaves are monitored and fertiliser rates adjusted according to increasing (reduce fertilisation) or decreasing (increase fertilisation) concentrations of a particular nutrient being observed in leaves. This is a programme optimized for long-term monitoring and not just based on annual observations, which is the procedure currently used in fruit crops.

In Mediterranean climates, with rainfall concentrated in the winter, and the summer being particularly dry, in rainfed orchards, where there are no fertigation practices, there is only one window of opportunity to apply fertilizers, which is in early spring, just before the regrowth of vegetation. If applying earlier, there is a risk of loss of mobile nutrients, such as N, by leaching and denitrification, whereas if applying later, there is a risk of loss of effectiveness due to reduced soil moisture [21]. In addition, slow and controlled release fertilizers tend to be less effective, as they delay nutrient availability for the summer, when the opportunity for root uptake is low [64].

5. Conclusions

The results of this study indicate that these large trees had a poor response to the annual application of fertilisers. Even so, the nutrient that had the greatest effect on the plant was N, since on some dates, significant differences were observed between treatments in leaf N concentrations, and the nutrient in the control treatment was close to, or even below the lower limit of the sufficiency range. The poor response of trees to fertiliser applications was probably due to the buffering effect that the huge perennial structure has on the redistribution of nutrients by the growing plant parts. Thus, in large trees, the fertilisation plan must be based on monitoring leaf nutrient concentration over time and on evaluating the trend of the nutrient concentrations in the leaves. As long as there is no decrease of leaf nutrient concentration that approaches the lower limit of the sufficiency ranges, the farmer should probably avoid spending money on fertiliser applications. In

contrast, the herbaceous vegetation developing beneath the canopy responded to the application of fertilisers, in particular to N, with increased dry matter yield and tissue nutrient concentrations. This vegetation, which begins to develop with the first autumn rains, seems to play an important role in protecting the soil and acts as a catch crop, reducing the risk of N loss during the winter.

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References

1. Gouveia, E. Doenças [Crop diseases]. In *Manual de boas Práticas do Castanheiro [Handbook of Good Management Practices on Chestnut Orchards]*; Bento, A., Ribeiro, A.C., Eds.; Terras de Trás-os-Montes: Bragança, Portugal, 2020; pp. 191–203. (In Portuguese).
2. Santos, A.; Marrão, R.; Bento, A. Pragas [Pests]. In *Manual de Boas Práticas do Cas-Tanheiro [Handbook of Good Management practices on Chestnut Orchards]*; Bento, A., Ribeiro, A.C., Eds.; Terras de Trás-os-Montes: Bragança, Portugal, 2020; pp. 205–227. (In Portuguese)
3. Rosário, J.N.; Coelho, V.; Rodrigues, M.; Raimundo, S.; Afonso, S.; Arrobas, M.; Gouveia, M.E. Metalaxyl-M, phosphorous acid and potassium silicate applied as soil drenches show different chestnut seedling performance and protection against Phytophthora root rot. *Eur. J. Plant Pathol.* **2021**, *161*, 147–159. https://doi.org/10.1007/s10658-021-02309-5.
4. INE (Instituto Nacional de Estatística); [National Institute of Statistics]. *Estatísticas Agrícolas–2020 [Agricultural Statistics–2020]*; INE: Lisboa, Portugal, 2021. (In Portuguese).
5. Rodrigues, M.; Raimundo, S.; Pereira, A.; Arrobas, M. Large Chestnut Trees (Castanea sativa) Respond Poorly to Liming and Fertilizer Application. *J. Soil Sci. Plant Nutr.* **2020**, *20*, 1261–1270. https://doi.org/10.1007/s42729-020-00210-4.
6. Aguiar, C.F. Sistemática, morfologia, fenologia e biologia da reprodução [Systematics, morphology, phenology and biology of reproduction]. In *Manual de boas Práticas do Castanheiro [Handbook of Good Management Practices on Chestnut Orchards]*; Bento, A., Ribeiro, A.C., Eds.; Terras de Trás-os-Montes: Bragança, Portugal, 2020; pp. 31–72. (In Portuguese)
7. Almeida, A.C.F. Instalação da cultura [Crop planting]. In *Manual de boas Práticas do Castanheiro [Handbook of Good Management Practices on Chestnut Orchards]*; Bento, A., Ribeiro, A.C., Eds.; Terras de Trás-os-Montes: Bragança, Portugal, 2020; pp. 85–92. (In Portuguese)
8. Rodrigues, M.A.; Arrobas, M. Gestão do solo [Soil management]. In *Manual de boas Práticas do Castanheiro [Handbook of Good Management Practices on Chestnut Orchards]*; Bento, A., Ribeiro, A.C., Eds.; Terras de Trás-os-Montes: Bragança, Portugal, 2020; pp. 119–129. (In Portuguese)
9. Patrício, M.A. Sistemas de condução e poda [Training and pruning systems]. In *Manual de boas Práticas do Castanheiro [Handbook of Good Management Practices on Chestnut Orchards]*; Bento, A., Ribeiro, A.C., Eds.; Terras de Trás-os-Montes: Bragança, Portugal, 2020; pp. 149–170. (In Portuguese)
10. Arrobas, M.; Rodrigues, M.A. Fertilização [Crop fertilization]. In *Manual de boas Práticas do Castanheiro [Handbook of Good Management Practices on Chestnut Orchards]*; Bento, A., Ribeiro, A.C., Eds.; Terras de Trás-os-Montes: Bragança, Portugal, 2020; pp. 131–148. (In Portuguese)
11. Rodrigues, M.; Grade, V.; Barroso, V.; Pereira, A.; Cassol, L.C.; Arrobas, M. Chestnut Response to Organo-mineral and Controlled-Release Fertilizers in Rainfed Growing Conditions. *J. Soil Sci. Plant Nutr.* **2019**, *20*, 380–391. https://doi.org/10.1007/s42729-019-00119-7.
12. Arrobas, M.; Ribeiro, A.; Barreales, D.; Pereira, E.L.; Rodrigues, M. Soil and foliar nitrogen and boron fertilization of almond trees grown under rainfed conditions. *Eur. J. Agron.* **2019**, *106*, 39–48. https://doi.org/10.1016/j.eja.2019.02.014.

13. Lopes, J.I.; Gonçalves, A.; Brito, C.; Martins, S.; Pinto, L.; Moutinho-Pereira, J.; Raimundo, S.; Arrobas, M.; Rodrigues, M.; Correia, C.M. 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* **2021**, *11*, 2172. https://doi.org/10.3390/agronomy11112172.
14. Ferreira, I.Q.; Arrobas, M.; Moutinho-Pereira, J.M.; Correia, C.M.; Rodrigues, M. The effect of nitrogen applications on the growth of young olive trees and nitrogen use efficiency. *Turk. J. Agric. For.* **2020**, *44*, 278–289. https://doi.org/10.3906/tar-1905-26.
15. Yang, X.; Zhang, P.; Li, W.; Hu, C.; Zhang, X.; He, P. Evaluation of four seagrass species as early warning indicators for nitrogen overloading: Implications for eutrophic evaluation and ecosystem management. *Sci. Total Environ.* **2018**, *635*, 1132–1143. https://doi.org/10.1016/j.scitotenv.2018.04.227.
16. Poikane, S.; Phillips, G.; Birk, S.; Free, G.; Kelly, M.G.; Willby, N.J. Deriving nutrient criteria to support 'good' ecological status in European lakes: An empirically based approach to linking ecology and management. *Sci. Total Environ.* **2019**, *650*, 2074–2084. https://doi.org/10.1016/j.scitotenv.2018.09.350.
17. Coyne, M.S. Biological denitrification. In *Nitrogen in Agricultural Systems, Agrono-My Monograph n.◦ 49*; Schepers, J.S., Raun, W.R., Eds.; ASA, CSSA, SSSA: Madison, WI, USA, 2008; pp. 201–253.
18. Pelster, D.E.; Larouche, F.; Rochette, P.; Chantigny, M.H.; Allaire, S.; Angers, D.A. Nitrogen fertilization but not soil tillage affects nitrous oxide emissions from a clay loam soil under a maize–soybean rotation. *Soil Tillage Res.* **2011**, *115–116*, 16–26. https://doi.org/10.1016/j.still.2011.06.001.
19. Weil, R.R.; Brady, N.C. *The Nature and Properties of Soils*, 15th ed.; Global Edition: London, UK, 2017.
20. Bryson, G.; Mills, H.A.; Sasseville, D.N.; Jones, J.B., Jr.; Barker, A.V. *Plant Analysis Handobook III. A Guide to Sampling, Preparation, Analysis and Interpretation for Agronomic and Horticultural Crops*; Micro-Macro Publishing, Inc.: Athens, GA, USA, 2014.
21. Silva, E.; Arrobas, M.; Gonçalves, A.; Martins, S.; Raimundo, S.; Pinto, L.; Brito, C.; Moutinho-Pereira, J.; Correia, C.M.; Rodrigues, M. A controlled-release fertilizer improved soil fertility but not olive tree performance. *Nutr. Cycl. Agroecosyst.* **2021**, *120*, 1–15. https://doi.org/10.1007/s10705-021-10134-9.
22. Rodrigues, M.; Torres, L.D.N.D.; Damo, L.; Raimundo, S.; Sartor, L.; Cassol, L.C.; Arrobas, M. Nitrogen Use Efficiency and Crop Yield in Four Successive Crops Following Application of Biochar and Zeolites. *J. Soil Sci. Plant Nutr.* **2021**, *21*, 1053–1065. https://doi.org/10.1007/s42729-021-00421-3.
23. Tilman, D.; Balzer, C.; Hill, J.; Befort, B.L. Global food demand and the sustainable intensification of agriculture. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 20260–20264. https://doi.org/10.1073/pnas.1116437108.
24. Tittonell, P. Ecological intensification of agriculture—Sustainable by nature. *Curr. Opin. Environ. Sustain.* **2014**, *8*, 53–61. https://doi.org/10.1016/j.cosust.2014.08.006.
25. Arrobas, M.; Afonso, S.; Rodrigues, M. Diagnosing the nutritional condition of chestnut groves by soil and leaf analyses. *Sci. Hortic.* **2018**, *228*, 113–121. https://doi.org/10.1016/j.scienta.2017.10.027.
26. Arrobas, M.; Afonso, S.; Ferreira, I.Q.; Moutinho-Pereira, J.; Correia, C.M.; Rodrigues, M. Liming and application of nitrogen, phosphorus, potassium,and boron on a young plantation of chestnut. *Turk. J. Agric. For.* **2017**, *41*, 441–451. https://doi.org/10.3906/tar-1705-79.
27. INE (Instituto Nacional de Estatística); [National Institute of Statistics]. *Recenseamento Agrícola–2019 [Agricultur-al Census–2019]*; Lisboa, Portugal: INE. Portuguese, 2019.
28. IPMA (Instituto Português do Mar e da Atmosfera); [Portuguese Institute of the Sea and the Atmosphere]. Normais Climatológicas [Climate Normals]. 2022. Available online: https://www.ipma.pt/pt/oclima/normais.clima/ (accessed on 15 April 2022). (In Portuguese).
29. Van Reeuwijk, L. *Procedures for Soil Analysis*; Technical Paper 9; Int Soil Ref Inform Center: Wageningen, MI, USA, 2002.
30. Lakanen, E.; Erviö, R. A Comparison of eight extractants for the determination of plant available micronutrients in soils. *Acta Agral. Fenn*. **1971**, *123*, 223–232.
31. Baird, R.B.; Eaton, A.D.; Rice, E.W. Nitrate by ultraviolet spectrophotometric method. In *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association, American Water Works Association, Water Environment Federation: Washington, DC, USA, 2017.
32. Temminghoff, E.E.J.M.; Houba, V.G. *Plant Analysis Procedures*, 2nd ed. Kluwer Acad Publ.: Dordrecht, The Netherlands, 2004. https://doi.org/10.1007/978-1-4020-2976-9.
33. Hawkesford, M.; Horst, W.; Kichey, T.; Lambers, H.; Schjoerring, J.; Skrumsager Møller, I.; White, P. Functions of macronutrients. In *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Marschner, P., Ed.; Elsevier Ltd.: Amsterdam, The Netherlands, 2012; pp. 135–189. https://doi.org/10.1016/B978-0-12-384905-2.00006-6.
34. Afonso, S.; Arrobas, M.; Ferreira, I.Q.; Rodrigues, M. Assessing the potential use of two portable chlorophyll meters in diagnosing the nutritional status of plants. *J. Plant Nutr.* **2017**, *41*, 261–271. https://doi.org/10.1080/01904167.2017.1385798.
35. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot*. **2000**, *51*, 659–668.
36. Opti-Sciences. *Desktop Plant Stress Guide*; Edition 3.0; Opti-Sciences: Hudson, WI, USA, 2014. Available online: www.optisci.com (accessed on 15 April 2022).
37. Rodrigues, M.; Afonso, S.; Ferreira, I.Q.; Arrobas, M. Response of stevia to nitrogen fertilization and harvesting regime in north-eastern Portugal. *Arch. Agron. Soil Sci.* **2017**, *63*, 626–637. https://doi.org/10.1080/03650340.2016.1230272.
38. Ferreira, I.Q.; Arrobas, M.; Moutinho-Pereira, J.; Correia, C.; Rodrigues, M. Olive response to potassium applications under different water regimes and cultivars. *Nutr. Cycl. Agroecosyst.* **2018**, *112*, 387–401. https://doi.org/10.1007/s10705-018-9954-2.

39. Portela, E.; Martins, A.; Pires, A.L.; Raimundo, F.; Marques, G. Práticas culturais no souto: O manejo do solo [Cropping practices in chestnut orchards: Soil management]. In *Castanheiros [Chestnut Groves]*; 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).
40. Ferreira, I.Q.; Rodrigues, M.; Moutinho-Pereira, J.M.; Correia, C.M.; Arrobas, M. Olive tree response to applied phosphorus in field and pot experiments. *Sci. Hortic.* **2018**, *234*, 236–244. https://doi.org/10.1016/j.scienta.2018.02.050.
41. Rodrigues, M.; Ferreira, I.Q.; Afonso, S.; Arrobas, M. Sufficiency ranges for lemon balm and nutrient removals in aboveground phytomass. *J. Plant Nutr.* **2018**, *41*, 996–1008. https://doi.org/10.1080/01904167.2018.1431671.
42. Pereira, E.; Coelho, V.; Tavares, R.M.; Lino-Neto, T.; Baptista, P. Effect of competitive interactions between ectomycorrhizal and saprotrophic fungi on Castanea sativa performance. *Mycorrhiza* **2012**, *22*, 41–49. https://doi.org/10.1007/s00572-011-0379-x.
43. Mechri, B.; Attia, F.; Tekaya, M.; Cheheb, H.; Hammami, M. Colonization of olive trees (*Olea europaea* L.) with the arbuscular mycorrhizal fungus *Glomus* sp. modified the glycolipids biosynthesis and resulted in accumulation of unsaturated fatty acids. *J. Plant Physiol.* **2014**, *171*, 1217–1220. https://doi.org/10.1016/j.jplph.2014.04.005.
44. Lanfranco, L.; Bonfante, P.; Genre, A. The mutualistic interaction between plants and arbuscular mycorrhizal fungi. *Microbiol. Spectr.* **2016**, *4*, 1–20. https://doi.org/ 10.1128/microbiolspec.FUNK-0012-201.
45. Tekaya, M.; Mechri, B.; Mbarki, N.; Cheheb, H.; Hammami, M.; Attia, F. Arbuscular mycorrhizal fungus Rhizophagus irregularis influences key physiological parameters of olive trees (*Olea europaea* L.) and mineral nutrient profile. *Photosynthetica* **2017**, *55*, 308–316. https://doi.org/10.1007/s11099-016-0243-5.
46. Ortaş, I.; Bykova, A. The Effect of Mycorrhiza Inoculation and Phosphorus Application on Phosphorus Efficiency of Wheat Plants. *Commun. Soil Sci. Plant Anal.* **2018**, *49*, 1199–1207. https://doi.org/10.1080/00103624.2018.1455849.
47. Lopes, J.; Arrobas, M.; Brito, C.; Gonçalves, A.; Silva, E.; Martins, S.; Raimundo, S.; Rodrigues, M.; Correia, C. Mycorrhizal Fungi were More Effective than Zeolites in Increasing the Growth of Non-Irrigated Young Olive Trees. *Sustainability* **2020**, *12*, 10630. https://doi.org/10.3390/su122410630.
48. Afonso, S.; Arrobas, M.; Morais, J.S.; Rodrigues, M. Hop dry matter yield and cone quality responses to amino acid and potassium-rich foliar spray applications. *J. Plant Nutr.* **2021**, *44*, 2042–2056. https://doi.org/10.1080/01904167.2021.1889597.
49. Broadley, M.; Brown, P.; Cakmak, I.; Rengel, Z.; Zhao, F. Function of nutrients: Micronutrients. In *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Marschner, P., Ed.; Elsevier Ltd.: Amsterdam, The Netherlands, 2012; pp. 191–248. https://doi.org/10.1016/b978-0-12-384905-2.00007-8.
50. Wimmer, M.A.; Eichert, T. Review: Mechanisms for boron deficiency-mediated changes in plant water relations. *Plant Sci.* **2013**, *203–204*, 25–32. https://doi.org/10.1016/j. plantsci.2012.12.012.
51. Ferreira, I.Q.; Rodrigues, M.; Arrobas, M. Soil and foliar applied boron in olive: Tree crop growth and yield, and boron remobilization within plant tissues. *Span. J. Agric. Res.* **2019**, *17*, e0901. https://doi.org/10.5424/sjar/2019171-13796.
52. Portela, E.A.C.; Ferreira-Cardoso, J.V.; Louzada, J.L. Boron application on a chestnut orchard: Effect on yield and quality of nuts. *J. Plant Nutr.* **2011**, *34*, 1245–1253. https://doi.org/10.1080/01904167.2011.580812.
53. Portela, E.; Ferreira-Cardoso, J.; Louzada, J.; Gomes-Laranjo, J. Assessment of Boron Application in Chestnuts: Nut Yield and Quality. *J. Plant Nutr.* **2015**, *38*, 973–987. https://doi.org/10.1080/01904167.2014.963116.
54. Portela, E.M.A.C.; Louzada, J.L.P. Early diagnosis of boron deficiency in chestnut. *J. Plant Nutr.* **2012**, *35*, 304–310. https://doi.org/10.1080/01904167.2012.636132.
55. Keesstra, S.; Pereira, P.; Novara, A.; Brevik, E.C.; Azorin-Molina, C.; Parras-Alcántara, L.; Jordán, A.; Cerdà, A. Effects of soil management techniques on soil water erosion in apricot orchards. *Sci. Total Environ.* **2016**, *551–552*, 357–366. https://doi.org/10.1016/j.scitotenv.2016.01.182.
56. Repullo-Ruibérriz De Torres, M.A.; Ordóñez-Fernández, R.; Giráldez, J.V.; Márquez-García, J.; Laguna, A.; Carbonell-Bojollo, R. Efficiency of four different seeded plants and native vegetation as cover crops in the control of soil and carbon losses by water erosion in olive orchards. *Land Degrad. Dev.* **2018**, *29*, 2278–2290. https://doi.org/10.1002/ldr.3023.
57. Márquez-García, F.; Sánchez, E.J.G.; Castro-Garcia, S.; Ordóñez-Fernández, R. Improvement of soil carbon sink by cover crops in olive orchards under semiarid conditions. Influence of the type of soil and weed. *Span. J. Agric. Res.* **2013**, *11*, 335. https://doi.org/10.5424/sjar/2013112-3558.
58. Rodrigues, M.; Dimande, P.; Pereira, E.L.; Ferreira, I.Q.; Freitas, S.; Correia, C.M.; Moutinho-Pereira, J.; Arrobas, M. Early-maturing annual legumes: An option for cover cropping in rainfed olive orchards. *Nutr. Cycl. Agroecosyst.* **2015**, *103*, 153–166. https://doi.org/10.1007/s10705-015-9730-5.
59. Nicholls, C.I.; Parrella, M.P.; Altieri, M.A. Reducing the abundance of leafhoppers and thrips in a northern California organic vineyard through maintenance of full season floral diversity with summer cover crops. *Agric. For. Èntomol.* **2000**, *2*, 107–113. https://doi.org/10.1046/j.1461-9563.2000.00054.x.
60. Irvin, N.A.; Bistline-East, A.; Hoddle, M.S. The effect of an irrigated buckwheat cover crop on grape vine productivity, and beneficial insect and grape pest abundance in southern California. *Biol. Control* **2016**, *93*, 72–83. https://doi.org/10.1016/j.biocontrol.2015.11.009.
61. De Notaris, C.; Rasmussen, J.; Sørensen, P.; Olesen, J.E. Nitrogen leaching: A crop rotation perspective on the effect of N surplus, field management and use of catch crops. *Agric. Ecosyst. Environ.* **2018**, *255*, 1–11. https://doi.org/10.1016/j.agee.2017.12.009.
62. Rodrigues, M.A.; Arrobas, M. Cover cropping for increasing fruit production and farming sustainability. In *Fruit Crops: Diagnosis and Management of Nutrient Constraints*; Srivastava, A.K., Hu, C., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 279–295.

63. Arrobas, M.; Rodrigues, M.A. *Fertilização do Pomar Baseada no Método de Otimização Dinâmica. Revista Voz do Campo*; Voz do Campo Editora, Lda.: Castelo Branco, Portugal, 2021; pp. 61–63.
64. Arrobas, M.; Belotto, L.B.; Marchetti, J.A.; Barroso, V.; Raimundo, S.; Cassol, L.C.; Correia, C.M.; Rodrigues, M.Â. Excessive delay in nutrient release by controlled-release fertilizers can reduce chestnut yield. *Horticulturae* **2022**, *8*, 1067. https://doi.org/10.3390/horticulturae8111067.