



Article

Excessive Delay in Nutrient Release by Controlled-Release Fertilizers Can Reduce Chestnut Yield

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Abstract: Farmers are increasing the use of fertilizers in chestnut, the only cash crop produced in the mountainous areas of northeastern Portugal. This calls for more studies to guide them towards a more ecological intensification. The effects of three controlled-release fertilizers, two that release nutrients over three months (BoskGrow 20:05:20_3m, Exactyon 18:05:13_3m) and one over six months (Exactyon 18:05:12_6m), and an organic amendment authorized for organic farming (Humix 12:03:05) were compared with an untreated control during a three-year field trial (2019–2021). BoskGrow 20:05:20_3m, Exactyon 18:05:13_3m and Humix 12:03:05 gave significantly higher nut yields (90.6 to 97.0 kg tree⁻¹, average 2019 + 2021) than Exactyon 18:05:12_6m (66.3 kg tree⁻¹) and the control (69.5 kg tree⁻¹). Leaf concentrations of nitrogen and potassium tended to be higher in the BoskGrow 20:05:20_3m and Exactyon 18:05:13_3m treatments, and they were stated as the most important causes in the establishment of the two productive groups. Humix 12:03:05, although less concentrated in nutrients, led to a chestnut yield at the level of the most productive treatments, possibly due to the multiple positive effects of organic matter on soil and plants. Under the conditions of this experiment, where rainfall is low in the summer, fertilizers whose nutrient release takes a long time, such as Exactyon 18:05:12_6m, seem not to be a good fertilization option due to reduced nutrient uptake and increased levels of soil inorganic nitrogen at the end of the growing season. Humix 12:03:05 emerged as a possible solution for organic producers.

Keywords: *Castanea sativa*; soil fertilization; organic farming; plant nutritional status; soil inorganic nitrogen

1. Introduction

In recent years, sweet chestnut (*Castanea sativa* Mill.) has experienced a higher incidence of severe disease and pests. Chestnut blight (*Cryphonectria parasitica* (Murrill) Barr.) and the invasive gall wasp (*Dryocosmus kuriphilus* Yasumatsu) have severely weakened trees, and chestnut ink disease (*Phytophthora* sp.pl.) has decimated entire orchards all over the world [1–3]. However, the worst is expected to be over. Currently, there are treatments that can reduce the incidence of chestnut blight, provided they are properly applied [4], and others that can mitigate the severity of ink disease [5,6]. Persistent programs to control the gall wasp, consisting of the release of *Torymus sinensis* Kamijo in infested orchards, are also providing promising results [7]. Out of this tenuous balance between orchards damaged by pests and diseases and favorable market prices, world chestnut production has increased.

Between 2010 and 2020, world chestnut production rose from 2.0×10^6 t to 2.3×10^6 t [8]. In the same period, the rise in chestnut production in Europe was even higher, increasing from 130.6×10^3 t to 333.5×10^3 t [8]. While cultivated areas in Europe have also increased (108,035 ha in 2010 and 150,385 ha in 2020), and this has likely accounted for part of the continent's increased production, the improvement in phytosanitary conditions and other cultivation practices has also been the basis of such a large increment in crop yield. Portugal has followed the European trend, showing a large increase in crop productivity (22.5×10^3 t in 2010 and 42.2×10^3 t in 2020) and also in cultivated areas (34,616 ha in 2010 and 51,700 ha in 2020) [8].

Several social transformations have taken place in recent decades in many municipalities of the mountain areas of northern Portugal [9]. These have made chestnut virtually the only cash crop available and the one capable of stemming the severe depopulation of these territories [10]. While in other parts of the world, chestnut stands are seen as agroforestry systems of reduced intensification [11], chestnut producers in northern Portugal are intensifying the cropping system in healthy orchards to stimulate crop productivity [12–14]. Considering that chestnut is usually grown in fragile mountain ecosystems, it is of utmost importance to optimize cropping practices in order to reduce environmental impacts. However, currently available data on chestnut orchard management are still poor, which calls for more studies as the crop grows in social importance and territorial scale.

Fertilizing is one of the cropping practices that chestnut producers are devoting more attention to as a way of increasing the productivity of healthy trees [15]. Crop fertilization has been one of the basic pillars of the green revolution that took place after World War II and is currently a widespread practice among farmers, due to its easily visible effects on increasing crop productivity [16,17]. However, the excessive use of fertilizers, in addition to being economically inefficient, can lead to environmental contamination. This is mainly caused by nitrogen (N) fertilizers, due to leaching of nitrates into groundwater and aquifers [18,19] and emission of N oxides into the atmosphere [20,21]. It is therefore necessary to learn how to fertilize chestnut trees by using fertilization strategies that guarantee high productivity, while reducing the risk of environmental contamination. Currently, these strategies are often called “ecological intensification” and result from the understanding that crop productivity must be high to ensure food for a growing world population while, at the same time, being efficient in the use of production factors [22,23].

The Mediterranean climate also raises particular problems in the fertilization of rainfed tree crops, such as chestnut. Under these conditions, precipitation is concentrated in winter, with summers being dry [24]. In this type of climate, precipitation also presents high inter-annual variability, a feature that will worsen in the context of climate change [25,26]. The irregularity of spring precipitation, with reference to the months of April to June, represents a great challenge in the decision making about the best date for the application of fertilizers to tree species [27]. Farmers prefer to apply fertilizer very early in the winter. Researchers tend to recommend slightly later applications, mainly because of the high mobility of N in the soil and the risk of N loss to the environment [28,29].

An alternative that can help to overcome the problem of the application date is to use slow- and/or controlled-release fertilizers and to apply them earlier. These fertilizers, containing nutrients in less soluble or less bioavailable forms after application, can reduce the risks of nutrient loss [30–33] and may offer a viable alternative to conventional fertilization. Thus, in order to increase the available data on the chestnut tree response to fertilizer application and to seek solutions for farmers trying to intensify the production system, four commercial fertilizers were included in this study. These have some mechanism of delaying nutrient bioavailability, and one of them is authorized for organic farming. They were applied at a single rate in late winter in chestnut orchards grown under rainfed conditions. The effect of fertilizers was evaluated by monitoring the nutritional status of trees and their photosynthetic performance and nut yield. The effect of the fertilizers on soil properties was also assessed, particularly their effect on the availability of inorganic N in the soil at the end of the growing season.

2. Materials and Methods

2.1. Experimental Conditions

The field trial was carried out in a chestnut orchard of cv. Judia, located in Carragosa (41°52'31.3" N 6°47'34.4" W, 800 m above sea level), in the municipality of Bragança, northeastern Portugal. The trees were 30 years old and were spaced at 10 m × 10 m.

According to the Köppen–Geiger classification, the region benefits from a warm-summer Mediterranean climate (Csb). The mean annual temperature is 12.3 °C, and annual precipitation is 758.3 mm [24]. Mean monthly temperatures and accumulated monthly precipitation during the experimental period are presented in Figure 1. The precipitation at the beginning of 2019 was lower, and the month of December was rainier than the climatological normal. In 2020, the spring/summer period was notably warmer than normal. The year 2021 was characterized by little rain in the spring and early summer.

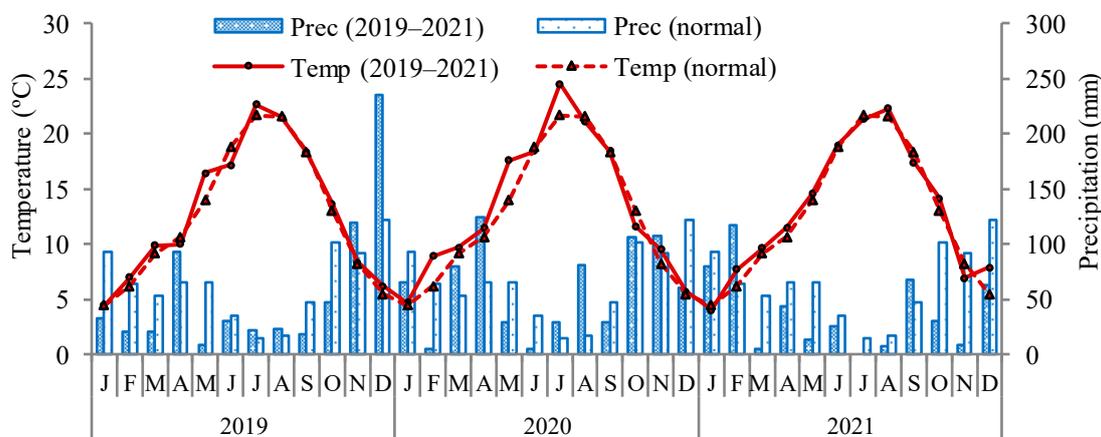


Figure 1. Average monthly temperatures (Temp) and accumulated precipitation (Prec) during the experimental period and values of the climatological normal from the meteorological station of Bragança.

The plot where the orchard is planted has a slope of less than 2%. The soil is a dystric Cambisol of a sandy loam texture (18.2% clay, 12.8% silt and 69.0% sand). Composite soil samples of the experimental plot, taken at a depth of 0–0.2 m at the start of the trial, indicated average values of organic carbon (C) of 19.5 g kg⁻¹, pH (1:2.5 soil:water *w/v*) of 5.2, extractable phosphorus (P) of 48.2 mg P₂O₅ kg⁻¹ and extractable potassium (K) of 198.0 mg K₂O kg⁻¹.

2.2. Experimental Design and Plot Management

Twenty trees with a similar canopy size were selected, separated from one another by an unmarked tree, and were drawn into five groups (treatments), with four individual trees per group (four replicates of individual trees per treatment), in a completely randomized design [34]. The five treatments, composed of four compound NPK fertilizers and a control treatment, were as follows: BoskGrow 20:05:20[®] (Boskgrow20:5:20_3m); Humix 12:03:05[®] (Humix12:3:5); Exactyon AG 18:05:13[®] (Exactyon18:5:13_3m); Exactyon AG 18:05:12[®] (Exactyon18:5:12_6m); and the control (Control). All the fertilizers used in this study were manufactured in Portugal by Atlantusi Europe, Lda., and are in common use in the region.

BoskGrow 20:05:20 is a compound NPK (20% N, 5% P₂O₅ and 20% K₂O) fertilizer. N is found in nitric (1.2%), ammoniacal (3.2%) and urea (15.6%) forms. The fertilizer is a blend of granules from which the release of N lasts three months, being partially controlled by a biodegradable thin polyurethane coating (27.6% N) and urea coated by ammonium nitrate (50% N). In addition to N, P and K, the fertilizer also contains relevant amounts of sulfur (6.2% SO₃), calcium (4.5% CaCO₃), magnesium (0.4% MgO) and boron (0.16% B₂O₃).

Humix 12:03:05 is an organic fertilizer, authorized for organic farming in Portugal, obtained from condensed liquid molasses of cassava (*Manihot esculenta* Crantz) and sugarcane (*Saccharum officinarum* L.), containing 13% N (10% organic N), 3% P₂O₅ and 5% K₂O. The fertilizer contains 74% organic matter, 16% amino acids, 8.9% humic acids and 32.8% fulvic acids. Other important minerals in the fertilizer are Ca (2.5% CaO), Mg (0.4% MgO) and sulfur (3.1% SO₃).

Exactyon 18:05:13 is a controlled-release fertilizer, also lasting 3 months (47% N encapsulated by a polymer and 28.8% N present as urea coated by ammonium sulfate). It contains 18% N (0.5% ammoniacal N and 17.5% ureic N), 5% P₂O₅ and 13% K₂O. The fertilizer also contains relevant amounts of Ca (14.9% CaCO₃), Mg (1.4% MgO), S (2.4% SO₃) and B (0.64% B₂O₃).

Exactyon 18:05:12 is a controlled-release fertilizer, lasting 6 months. It contains 18% N (0.6% nitric N, 1.7% ammoniacal N and 15.7% ureic N), 5% P₂O₅ and 12% K₂O. The controlled-release mechanism (94.9% of N) is based on a sulfur-coated urea and polyurethane coating. Other important nutrients in the fertilizer are Ca (8.3% CaCO₃), Mg (1.4% MgO), S (13.6% SO₃) and B (0.64% B₂O₃).

The fertilizers were selected based on the content of macronutrients (NPK) and the time taken for the release of N, the nutrient most vulnerable to be lost from the soil [18–21]. They were applied manually and spread homogeneously under the canopy at a rate of 4 kg of fertilizer per tree (~400 kg of fertilizer per ha), a common fertilizer rate among local farmers. Thus, in the BoskGrow 20:05:20_3m treatment, 80, 20 and 80 kg ha⁻¹ of N, P₂O₅ and K₂O were applied, respectively. In the Humix 12:03:05 treatment, the values for the same nutrients were, respectively, 48, 12 and 20; in the Exactyon 18:05:13_3m treatment, they were 72, 20 and 52; and in the Exactyon 18:05:12_6m treatment, they were 72, 20 and 48.

The fertilizers were applied annually in the second half of March and incorporated into the soil with a cultivator. This is the most common procedure of managing the fertilization of chestnut trees in the region. The soil was tilled again at the end of May as part of weed control. During the experimental period, the trees were not pruned, nor did they receive any other cropping practice. At harvest, the ripe fruit fell to the ground, usually from late October to late November, being picked up by hand usually in three successive passes. The fruits of each pass were weighed, and at the end of the harvesting period, the results were reported as chestnut yield per tree.

2.3. Field Measurements

In the field, optical properties of the leaves related to the nutritional status of the trees and chlorophyll fluorescence variables related to the photochemistry of photosynthesis were determined.

Leaf greenness was measured by using a SPAD (soil plant analysis development)-502 Plus[®] chlorophyll meter (Spectrum Technologies, Inc., Aurora, IL, USA). The device provides adimensional values, proportional to the chlorophyll content of the leaves, by measuring the transmittance of light through the leaves in two wavelengths (650 nm red light, absorbed by chlorophyll, and 940 nm infrared light, not absorbed by chlorophyll). It has been widely used as a N nutritional index [35,36]. To obtain the mean value of each tree, 30 readings were taken in all quadrants around the canopy using fully grown young leaves.

The normalized difference vegetation index (NDVI) was determined by using the portable FieldScout CM 1000[®] (Spectrum Technologies, Inc., Aurora, IL, USA) meter. The tool senses and measures the ambient light at the wavelength of 660 nm and the reflected light (not absorbed by leaf chlorophyll) at an 840 nm wavelength. Light having a wavelength of 840 nm is unaffected by the chlorophyll content and serves as an indication of how much light is reflected [36,37]. The NDVI values (between -1 and 1) are calculated from the equation $[(\% \text{Near Infrared} - \% \text{Red}) / (\% \text{Near Infrared} + \% \text{Red})]$. To obtain the readings, the light ray emitted by the device, after pressing a trigger that activates the tool, was directed towards the blade of young fully expanded leaves, trying to avoid recording

values from the midribs and major veins. The average value of each tree was the result of 30 readings around the canopy as reported for leaf greenness measurements.

Chlorophyll a fluorescence and OJIP transient were determined by using the dark adaptation protocols FV/FM and FV/F₀ and the advanced OJIP test by using the OS-30p+[®] fluorometer (Opti-sciences, Inc., Hudson, NH, USA). F_M, F₀ and F_V are, respectively, maximum, minimum and variable fluorescence from dark-adapted leaves. F_V/F_M is estimated as (F_M – F₀)/F_M and F_V/F₀ as (F_M – F₀)/F₀. The OJIP test provides origin fluorescence at 20 μs (O), fluorescence at 2 ms (J), fluorescence at 30 ms (I) and maximum fluorescence (P, or F_M). The fluorometer uses a pulse-modulated detection system to allow for a variety of tests with capability for measuring plant stresses affecting photosystem II [38,39]. Measurements were taken between 11:00 h and 12:30 h from young fully expanded leaves after a period of dark adaptation longer than 35 min.

Following harvest, the fruits of each pass were weighed, and at the end of the harvesting period, the results were reported as chestnut yield per tree. In 2020, the COVID-19 pandemic restrictions did not allow the completion of harvest records, and therefore only the values for 2019 and 2021 are available.

2.4. Sampling Plant Tissues and Soils

In addition to three composite samples taken at the beginning of the experiment for plot characterization, the soil was sampled again in October 2021 to evaluate the effect of the treatments on soil fertility. Each composite sample resulted from soil collection at 10 different sampling points per replicate and treatment. Sampling was carried out at a 0.0–0.20 m soil depth.

By the end of July, in each of the three years, samples of 30 young fully developed leaves were taken from that year's shoots around the canopy, for elemental analysis and monitoring of the nutritional status of the trees.

After harvesting, samples of 50 nuts per replicate were randomly taken to evaluate their size and also for elemental analysis. After counting and weighing, the kernel was separated from the shell and pellicle and the two parts analyzed separately.

2.5. Laboratory Analysis

The samples of leaves, fruit kernels, shells and pellicles were oven-dried at 70 °C until they reached a constant weight, ground (1 mm mesh) and analyzed for elemental composition. The tissue N concentration was determined in a Kjeltec Auto 8400 analyzer, after sample digestion with sulfuric acid and selenium as a catalyst. For the determination of boron (B), the samples were incinerated in calcium oxide and the ash diluted with sulfuric acid. In the extract, B was determined by colorimetry using the azomethine-H method. For the other nutrients, the samples were digested in nitric acid in a microwave. P was determined by colorimetry, using the blue ammonium molybdate method with ascorbic acid as a reducing agent. Potassium, calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) were determined by atomic absorption spectrophotometry. For more details on all of these analytical procedures, the reader is referred to Temminghoff and Houba [40].

Soil samples were well-mixed and oven-dried at 40 °C. Thereafter, the samples were analyzed for pH (H₂O 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). Soil B was extracted using hot water and determined using the method of azomethine-H. For more details on these analytical procedures, the reader is referred to Van Reeuwijk [41]. 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 first described by Lakanen and Erviö [42]. Soil inorganic N was determined in soil extracts prepared from 20 g of soil and 50 mL of 2 M KCl. Briefly, 50 mL of 2 M KCl was added to 20 g of soil and placed in an oven at 100 °C for 4 h. After cooling, the suspension was filtered. The procedure was repeated

using cold KCl, shaken for 1 h. The suspensions were filtered through Watmann #42 filter paper. Hydrolyzable NH_4^+ was estimated by the difference between NH_4^+ extracted hot and cold. NO_3^- was determined in a cold KCl solution [43]. Nitrate (ultraviolet spectrophotometric screening method) and ammonium (phenate method) concentrations in the extracts were analyzed by UV-Vis spectrophotometry [44].

2.6. Data Analysis

The data were analyzed for normality and homogeneity of variance using the Shapiro-Wilk and Bartlett tests, respectively. Analysis of variance was performed according to the experimental design 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 test ($\alpha = 0.05$).

3. Results

3.1. Nutrient Concentration in Plant Tissues

The leaf N concentration varied significantly between fertilization treatments over the three years (Figure 2). Mean values for the BoskGrow 20:05:20_3m (22.2 to 23.7 g kg⁻¹) treatment appeared at the top of the results, while those for the control (17.5 to 20.1 g kg⁻¹) treatment were at the bottom. Exactyon 18:05:12_6m displayed low values on the first sampling date (17.5 g kg⁻¹), in comparison to the other treatments, and moderately high values on the last sampling date (20.9 g kg⁻¹), showing a consistent increase over the years. The leaf P concentration also varied significantly between fertilization treatments in all the years. However, it was not possible to observe such a coherent trend in the relative position of the treatments, with the values of each one appearing above or below on different sampling dates. The results of the K concentration in the leaves showed significant differences on two of the three sampling dates. What stands out most in these results are the highest mean values of the BoskGrow 20:05:20_3m treatment. The Ca content in the leaves also varied significantly between treatments on two sampling dates. For this nutrient, consistently higher values were recorded in the Exactyon 18:05:13_3m treatment. The leaf Mg concentration did not vary significantly with the fertilization treatments on any sampling date.

Leaf B levels varied significantly between fertilization treatments on the three sampling dates (Figure 3). The values in the plots treated with Exactyon 18:05:13_3m and Exactyon 18:05:12_6m increased markedly from the first to the last sampling date, from average values close to 25 mg kg⁻¹ to values around 80 mg kg⁻¹. The values of the BoskGrow 20:05:20_3m treatment also showed a slight increase on the third sampling date in comparison to those of the Humic 12:03:05 and control treatments. The Fe concentration in the leaves showed an increasing trend over the years, but without a clear consistency in the relative position of the different fertilization treatments. The control showed consistently lower leaf Mn levels on the three sampling dates (873 to 1363 mg kg⁻¹) compared to any of the fertilized treatments (1739 to 2533 mg kg⁻¹). Leaf Zn and Cu levels did not vary significantly between treatments.

The N concentration in the kernel did not vary among treatments in the sampling of 2019 (Table 1). In 2021, the differences between treatments fertilized with higher and lower amounts of N seem to have been accentuated, with significant differences having occurred. For the concentrations of P, K, Mg, Zn and Cu in the kernel, no significant differences or noticeable trends between treatments were found for any of the sampling dates. Significant differences between treatments were found for kernel Ca concentrations, as well as for B, Fe and Mn. However, in the values for Ca and Fe, no consistency was observed between the sampling dates. In the case of B, although in 2021 there were no significant differences, the average values indicate the formation of two groups of treatments, with the lowest values recorded in the Humix 12:03:05 and control treatments. In the case of Mn, there also seems to be consistency between the two sampling dates, with significantly lower average values in the control compared to the fertilized treatments.

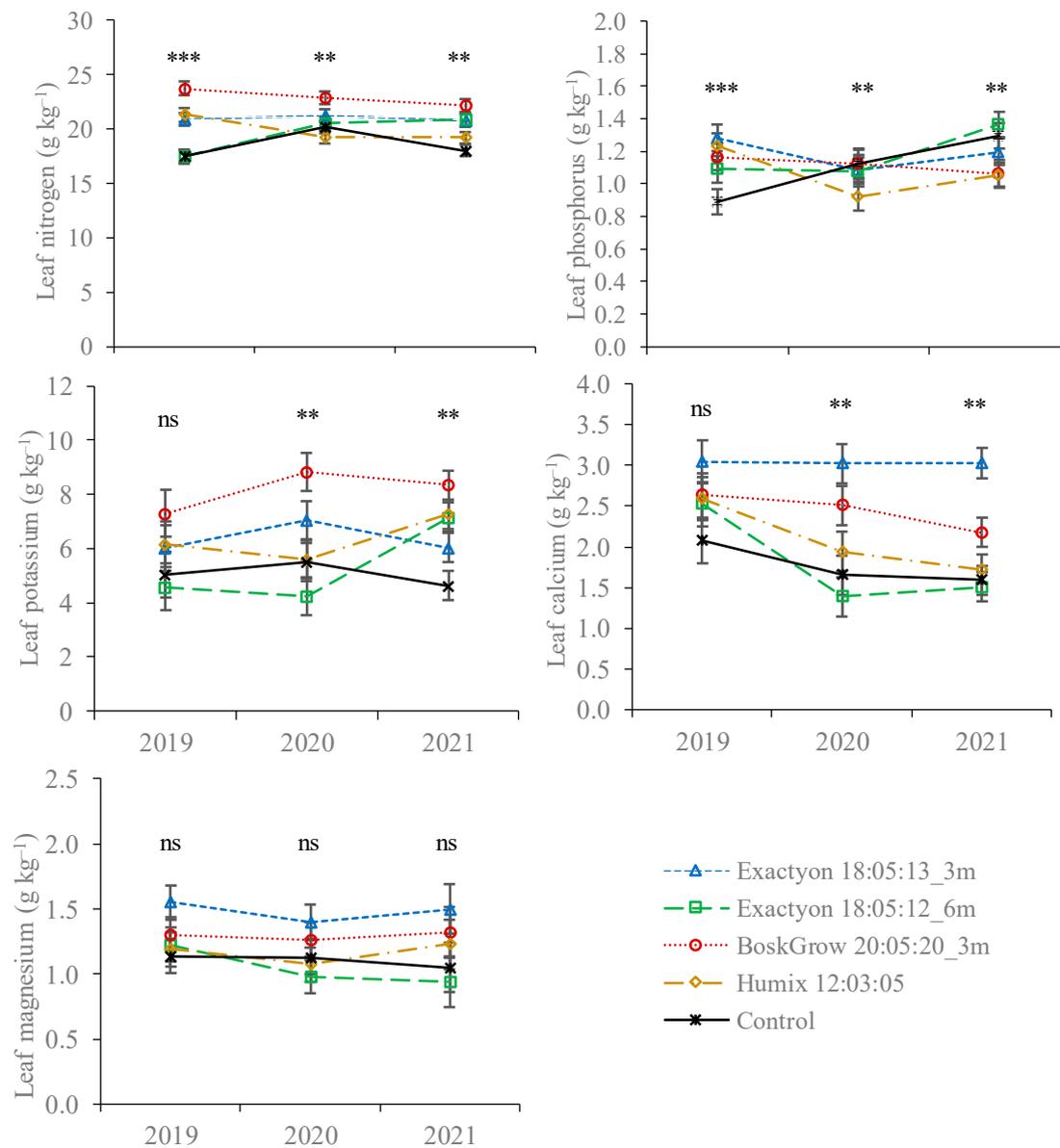


Figure 2. Average concentration and standard error of the mean of nitrogen, phosphorus, potassium, calcium and magnesium of chestnut leaves collected in July of 2019, 2020 and 2021 as a function of fertilization treatments. ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant.

Table 1. Nutrient concentration in the kernels from the harvests of 2019 and 2021 as a function of the fertilization treatments.

Treatments	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Boron	Iron	Manganese	Zinc	Copper
	g kg ⁻¹					mg kg ⁻¹				
2019										
Exactyon 18:05:13_3m	10.16 a *	1.00 a	4.31 a	0.48 ab	0.50 a	13.4 b	125.6 ab	183.0 ab	15.5 a	8.6 a
Exactyon 18:05:12_6m	9.56 a	1.02 a	3.92 a	0.39 b	0.44 a	16.3 a	109.9 ab	166.9 ab	15.3 a	9.1 a
BoskGrow 20:05:20_3m	10.33 a	0.89 a	4.40 a	0.58 a	0.57 a	13.1 bc	148.8 a	140.3 bc	15.9 a	8.9 a
Humix 12:03:05	9.94 a	1.04 a	3.96 a	0.47 ab	0.51 a	11.4 bc	112.2 ab	204.4 a	17.0 a	8.9 a
Control	9.29 a	1.01 a	4.64 a	0.48 ab	0.51 a	10.7 c	93.6 b	102.6 c	15.7 a	8.5 a
Probability	0.4689	0.3460	0.4929	0.0375	0.3473	<0.0001	0.0114	0.0006	0.8209	0.9231
Standard error	0.44	0.05	0.32	0.04	0.04	0.60	9.48	12.97	1.07	0.55

Table 1. Cont.

Treatments	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Boron	Iron	Manganese	Zinc	Copper
	g kg ⁻¹					mg kg ⁻¹				
2021										
Exactyon 18:05:13_3m	10.07 ab	1.10 a	7.16 a	0.43 a	1.10 a	23.1 a	197.7 a	178.1 a	17.7 a	8.5 a
Exactyon 18:05:12_6m	12.20 a	1.21 a	6.37 a	0.43 a	0.82 a	25.5 a	194.9 a	162.2 a	18.9 a	9.2 a
BoskGrow 20:05:20_3m	12.73 a	1.06 a	6.41 a	0.40 a	0.85 a	26.2 a	150.8 a	187.1 a	17.3 a	8.5 a
Humix 12:03:05	8.53 b	1.14 a	7.70 a	0.49 a	0.90 a	13.5 a	183.2 a	209.6 a	17.5 a	8.5 a
Control	9.02 b	1.08 a	6.52 a	0.40 a	0.42 a	16.7 a	198.1 a	94.6 b	18.6 a	9.8 a
Probability	0.0048	0.7401	0.1104	0.6146	0.2731	0.0928	0.3138	0.0030	0.5420	0.5831
Standard error	0.47	0.08	0.32	0.04	0.19	2.93	16.01	9.89	0.74	0.65

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

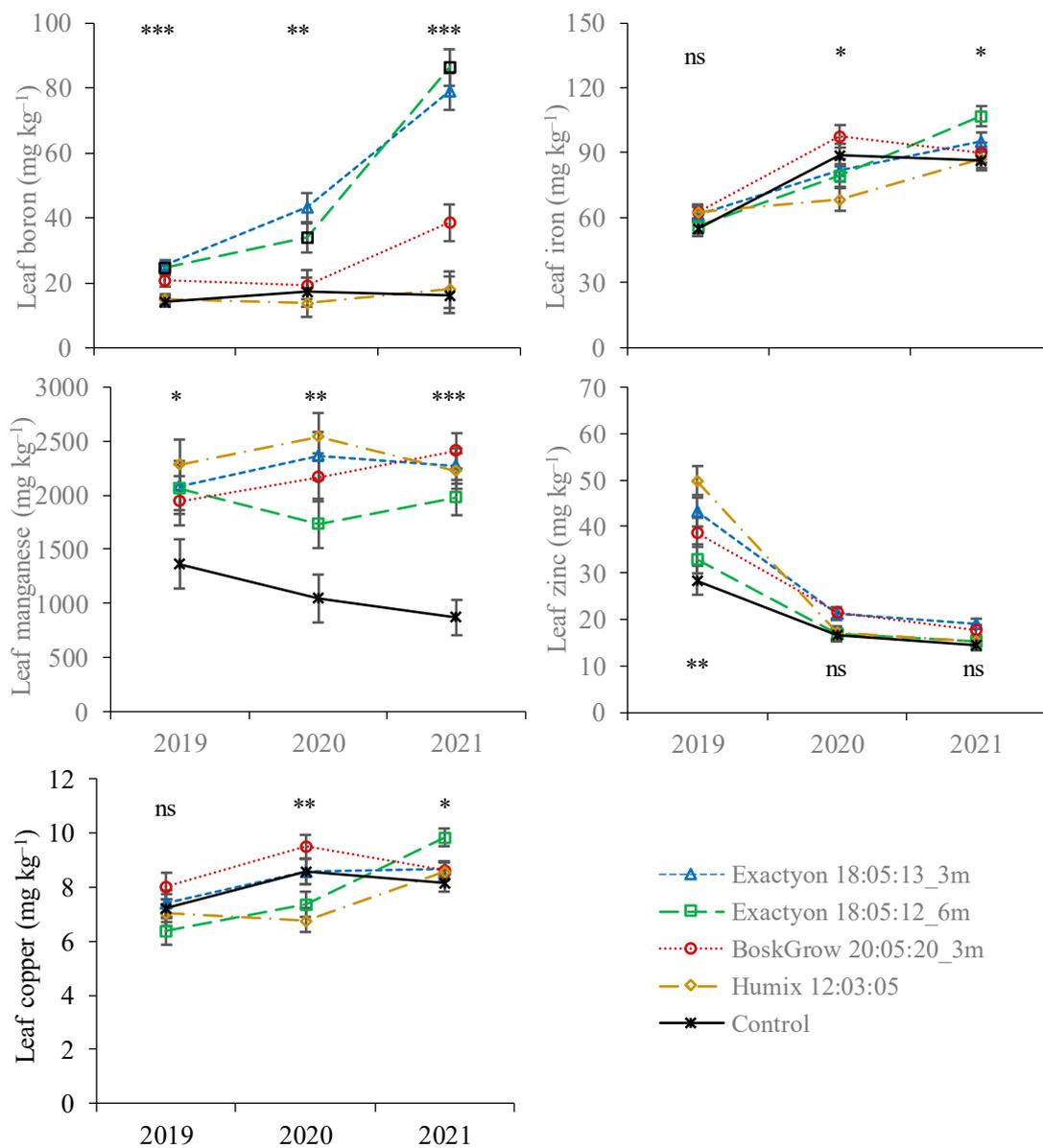


Figure 3. Average concentration and standard error of the mean of boron, iron, manganese, zinc and copper concentrations in chestnut leaves collected in July of 2019, 2020 and 2021 as a function of fertilization treatments. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant.

The concentration of B in the chestnut shells did not differ significantly between treatments in the 2019 harvest (Table 2). In 2021, significantly higher values were recorded in Exactyon 18:05:13_3m (35.3 mg kg⁻¹) and Exactyon 18:05:12_6m (34.1 mg kg⁻¹) than in the Humix 12:03:05 (20.8 mg kg⁻¹) and control (22.6 mg kg⁻¹) treatments. For all the other nutrients, no significant differences or noticeable trends between treatments were found.

Table 2. Boron concentration in the chestnut shells (pericarps) from the harvests of 2019 and 2021 as a function of the fertilization treatments.

Treatments	2019	2021
	mg kg ⁻¹	
Exactyon 18:05:13_3m	22.0 a *	35.3 a
Exactyon 18:05:12_6m	20.3 a	34.1 a
BoskGrow 20:05:20_3m	22.4 a	28.6 ab
Humix 12:03:05	21.9 a	20.8 b
Control	18.8 a	22.6 b
Probability	0.4728	0.0050
Standard error	1.34	1.67

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

3.2. SPAD Readings, FieldScout NDVI and Chlorophyll a Fluorescence

SPAD values varied significantly between treatments in two of the three readings (Table 3). In 2019, the values of the Exactyon 18:05:13_3m (45.0) and BoskGrow 20:05:20_3m (44.9) treatments were significantly higher than those of Exactyon 18:05:12_6m (42.4) and the control (42.5). In 2021, the average values of the Exactyon 18:05:12_6m treatment increased, but significant differences were only found between the Exactyon 18:05:13_3m (49.2) and control (45.9) treatments. The NDVI was significantly higher in the Exactyon 18:05:13_3m (0.798) treatment than in the control in 2019 (0.763). In 2020, no significant differences were found between treatments. In 2021, the NDVI was significantly higher in the Exactyon 18:05:13_3m (0.820) and BoskGrow 20:05:20_3m (0.818) treatments than in the control (0.785). FV/FM did not vary significantly between treatments in any of the measurements. However, in 2021, the mean values in the control treatment showed a noticeable downward trend, although without significant differences for the fertilized treatments ($p = 0.1054$). Many other measured and estimated variables by the OS-30p+ fluorometer did not vary with the fertilization treatments.

Table 3. SPAD (soil plant analysis development) values, NDVI (normalized difference vegetation index) and F_V/F_M (ratio of variable fluorescence/maximum fluorescence) in the summers of 2019, 2020 and 2021 as a function of the fertilizer treatments.

Treatments	SPAD			NDVI			F _V /F _M		
	2019	2020	2021	2019	2020	2021	2019	2020	2021
Exactyon 18:05:13_3m	45.0 a *	42.1 a	49.2 a	0.798 a	0.783 a	0.820 a	0.819 a	0.803 a	0.803 a
Exactyon 18:05:12_6m	42.4 b	40.4 a	47.3 ab	0.775 ab	0.770 a	0.808 ab	0.807 a	0.811 a	0.815 a
BoskGrow 20:05:20_3m	44.9 a	42.3 a	48.7 ab	0.793 ab	0.785 a	0.818 a	0.830 a	0.800 a	0.794 a
Humix 12:03:05	43.8 ab	41.8 a	46.9 ab	0.780 ab	0.780 a	0.798 ab	0.803 a	0.808 a	0.819 a
Control	42.5 b	41.4 a	45.9 b	0.763 b	0.765 a	0.785 b	0.807 a	0.799 a	0.730 a
Probability	0.0350	0.8880	0.0208	0.0261	0.1001	0.0430	0.2759	0.9636	0.1054
Standard error	0.67	1.40	0.68	0.007	0.006	0.008	0.009	0.016	0.022

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

3.3. Soil Properties

Soil organic C and pH did not vary among treatments (Table 4). Soil extractable P varied significantly between treatments, with the highest values recorded in the BoskGrow 20:05:20_3m and Exactyon 18:05:13_6m treatments and the lowest in the Humix 12:03:05

and control treatments. Soil extractable K also varied significantly between treatments, with the highest values recorded in the BoskGrow 20:05:20_3m treatment and the lowest in the Humix 12:03:05 treatment, followed by the control treatment. Exchangeable Ca did not vary between treatments, and Mg values were significantly higher in the Exactyon 18:05:13_3m treatment than in the control treatment. Soil B levels were markedly different between treatments. The highest values were recorded in the Exactyon 18:05:13_3m treatment, followed by Exactyon 18:05:12_6m, BoskGrow 20:05_3m, Humix 12:03:05 and, finally, the control, without significant differences between them. Soil Mn levels varied between treatments, with the lowest average value found in the control treatment.

Table 4. Selected soil properties determined from composite soil samples (n = 4) taken at 0–0.20 m in October 2021 in the plots of the different fertilization treatments.

Treatments	Organic Carbon g kg ⁻¹	pH _(H₂O)	Phosphorus mg P ₂ O ₅ kg ⁻¹	Potassium mg K ₂ O kg ⁻¹	Calcium cmol ₊ kg ⁻¹	Magnesium cmol ₊ kg ⁻¹	Boron mg kg ⁻¹	Manganese mg kg ⁻¹
Exactyon 18:05:13_3m	18.6 a *	5.29 a	55.2 ab	261.3 ab	2.00 a	0.63 a	1.90 a	124.0 ab
Exactyon 18:05:12_6m	20.1 a	5.29 a	63.9 a	245.3 bc	1.73 a	0.56 ab	1.32 b	122.4 ab
BoskGrow 20:05:20_3m	21.1 a	5.16 a	65.3 a	329.3 a	1.52 a	0.54 ab	0.80 c	137.2 a
Humix 12:03:05	18.4 a	4.93 a	44.1 b	187.0 c	1.20 a	0.51 ab	0.30 d	147.6 a
Control	19.0 a	5.31 a	46.1 b	220.7 bc	1.57 a	0.47 b	0.47 d	76.6 b
Probability	0.1270	0.1456	0.0004	0.0006	0.1369	0.0288	<0.0001	0.0090
Standard error	1.15	0.11	2.59	14.89	0.20	0.03	1.16 b	10.94

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

Soil NH₄⁺ extracted using hot KCl varied significantly with the fertilization treatments, with the highest (68.6 mg kg⁻¹) and lowest (23.1 mg kg⁻¹) average values being recorded in the Exactyon 18:05:12_6m and control treatments, respectively (Table 5). When extracted with cold KCl, the NH₄⁺ values did not vary significantly with the fertilization treatments. Hydrolyzable NH₄⁺, representing the difference between NH₄⁺ extracted using hot and cold KCl, showed significantly higher values in Exactyon 18:05:12_6m (29.3 mg kg⁻¹) in comparison to the other treatments (<20 mg kg⁻¹). Soil NO₃⁻ levels were significantly higher in Exactyon 18:05:13_3m in comparison to all the other treatments, and the values in the BoskGrow 20:05:20_3m and Exactyon 18:05:12_6m plots were significantly higher than in the Humix 12:03:05 and control plots.

Table 5. Soil ammonium (NH₄⁺) extracted using hot and cold potassium chloride, hydrolyzable NH₄⁺ (Hyd) (NH₄⁺ hot – NH₄⁺ cold) and nitrate extracted using cold KCl determined from composite soil samples (n = 4) taken at 0–0.20 m in October 2021 in the plots of the different fertilization treatments.

Treatments	NH ₄ ⁺ Hot	NH ₄ ⁺ Cold	NH ₄ ⁺ Hyd mg kg ⁻¹	NO ₃ ⁻ Cold
Exactyon 18:05:13_3m	48.0 ab *	28.3 a	19.7 b	213.5 a
Exactyon 18:05:12_6m	68.6 a	39.4 a	29.3 a	131.7 b
BoskGrow 20:05:20_3m	42.8 ab	26.4 a	16.3 b	146.6 b
Humix 12:03:05	37.8 ab	20.4 a	17.5 b	50.1 c
Control	23.1 b	10.7 a	12.4 b	62.6 c
Probability	0.0120	0.0813	0.0007	<0.0001
Standard error	6.97	6.24	1.81	12.81

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

3.4. Nut Yield

In 2019, significant differences were recorded in chestnut production between the fertilizer treatments (Figure 4). The Exactyon 18:05:13_3m treatment presented the highest mean values and the Exactyon 18:05:12_6m and control treatments the lowest. In 2021, no significant differences were found between treatments. When comparing the results of the sum of the two years, the response of chestnut to the fertilization treatments appeared in two groups, with significant differences between them. Exactyon 18:05:13_3m, BoskGrow 20:05:20_3m and Humix 12:03:05 showed average nut yields above 90 kg tree⁻¹, while Exactyon 18:05:12_6m and the control gave cumulative yields of less than 70 kg tree⁻¹.

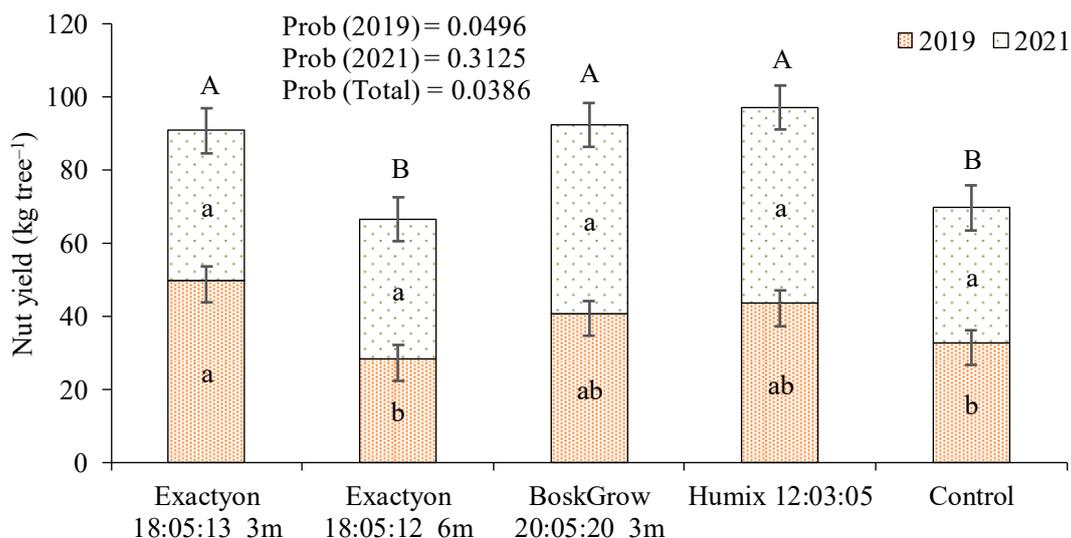


Figure 4. Average chestnut yield and standard errors in 2019 and 2021 as a function of fertilization treatments. Within each year (lowercase) and in total (uppercase), means followed by the same letter are not significantly different according to the Tukey HSD test ($\alpha = 0.05$).

4. Discussion

The cumulative fruit production (2019 + 2021) appeared to be separated into two treatment groups, with significant differences between them: the most productive composed of Exactyon 18:05:13_3m, BoskGrow 20:05:20_3m and Humix 12:03:05, and the least productive composed of the Exactyon 18:05:12_6m and control treatments. Although it is difficult to attribute this grouping to a single cause, the determined variables clearly point to the effect of some of the nutrients applied as fertilizer.

The supply of N by fertilizers may have been an important cause. Two of the fertilizers that contain more N, BoskGrow 20:05:20_3m and Exactyon 18:05:13_3m, appeared in the most productive treatment group and were associated with higher tissue N concentrations as well as higher SPAD values and NDVIs. In general terms, leaf N levels were low, with less productive treatments often showing values below the sufficiency range that has been used for this species (20–28 g kg⁻¹) [45,46]. As far as we know, there are no SPAD or NDVI reference values in the international literature for this crop, but they are indices that often show a good relationship with plant nutritional status, in particular with N nutrition [36,47,48]. N can increase the chlorophyll content in the leaves and their greenness. SPAD-502 measures the transmittance of light through the leaf at 650 nm (red light, absorbed by chlorophyll pigments) and at 940 nm (infrared light, not absorbed by chlorophylls), so the higher the chlorophyll content, the higher the SPAD values [36]. The FieldScout CM 1000 senses the light at wavelengths of 660 nm and 840 nm. Chlorophylls absorb only 660 nm light, so the less the reflected light, the higher the NDVI value [36]. FV/FM is the most used chlorophyll fluorescence variable since values have been found to correlate with several plant stress types that affect photosystem II [49–51]. For most plant species, 0.78–0.83 is the optimal response range, with lower values indicating plant

stress [52]. In this study, only the last reading of 2021 showed an average value below the above range in the control treatment. Although the trend was similar to the SPAD values and NDVI, significant differences between treatments were not found. It seems that FV/FM and the other measured and estimated chlorophyll fluorescence variables displayed by the OS-30p+ fluorometer have low sensitivity to nutritional stresses, as previously reported in other studies [53–55].

The group of less productive treatments, Exactyon 18:05:12_6m and the control, showed a tendency to present lower tissue K levels than the most productive treatments. Moreover, leaf K levels were often below the lower limit of the sufficiency range, which makes K a factor that may have been relevant in the establishment of the productivity groups. In the control treatment, lower values of K in the soil were also observed. Soil K levels were high at the beginning of the experiment and increased in treatments receiving K. However, it seems that high levels of K in the soil did not ensure high levels of K in the leaves, as these values were often found below the lower limit of the sufficiency range. K can be difficult to be taken up by plants in dry soils due to the restrictions of its movements by mass flow and diffusion [56], which, in this region, may occur in the summer (Figure 1). Thus, the supply of K to the soil may not ensure adequate amounts of K in the leaves, since in summer, the absorption is restricted and the fruits accumulate important amounts of K [10,46]. Under these conditions, the K remobilized to the fruits is not compensated for by further absorption, which results in a reduction in the K concentration in the leaves. Exactyon 18:05:12_6m, delaying the availability of nutrients for six months, due to the polyurethane coating, might have reduced K absorption in the spring, which is when the moisture conditions are adequate (Figure 1), leading to lower levels of K in the leaves than the other fertilizers with equivalent amounts of K in their composition.

Exactyon 18:05:12_6m and the control also showed a tendency to have lower tissue Ca and Mg concentrations than the most productive treatments. This soil is acidic, and plants showed lower levels of Ca and Mg in the leaves than those reported as the sufficiency ranges for this species (5–15 and 1.5–6.0 g kg⁻¹, respectively) [10,45,46]. Ca and Mg are two essential plant nutrients. The former has functions mainly in the apoplasm, where a part is firmly bound in structures, while another part is exchangeable Ca at the cell walls and at the exterior surface of the plasma membrane [57]. The functions of Mg in plants are also diverse, including its roles as the central atom of the chlorophyll molecule, in chlorophyll and protein synthesis and in enzyme activation, phosphorylation and photosynthesis [57]. All fertilizers provided Ca and Mg, albeit in different amounts, which may explain the differences found for the control. Exactyon 18:05:12_6m showed a type of behavior closer to the control than to the other fertilized treatments, probably due to difficulties in nutrient uptake caused by the delay in nutrient release. Although the total amounts of Ca and Mg supplied by fertilizers were low, their effect on crop production may have been important because tissue concentrations were clearly below the sufficiency ranges, especially for Ca.

Leaf B concentrations increased significantly in treatments with fertilizers more concentrated in this micronutrient. The concentration of B in the shells also clearly indicates an increase in soil B availability in those treatments. B has a central role in the formation and functioning of the cell wall [58], with these tissues being concentrated in B, which is not normally the case for most other nutrients. Leaf B concentrations in the control treatment were close to, or below, the lower limit of the sufficiency range, which could indicate a relevant role for the nutrient in the present results. In previous studies, the importance of B in the productivity of chestnut was found to be clear [33,46], as well as in several other crops in the region cultivated in soils with similar properties [29,59,60]. However, the fact that the Exactyon 18:05:12_6m treatment showed high levels of B in the tissues and low productivity seems to reduce the influence of this element on crop productivity in this study.

Tissue Mn concentrations were much lower in the control than in the fertilized treatments. These results were also consistent with soil Mn levels. Tissue Mn values in the most productive treatments were very high and were found to be close to, or above, the upper limit of the sufficiency range (2000 mg kg⁻¹), levels at which there may be a risk

of toxicity [57]. This shows a high tolerance of chestnut to the concentration of Mn in the tissues, probably because it is a plant adapted to acidic soils. The bioavailability of Mn increases with soil acidity [57]. However, in this study, soil pH did not change significantly with the treatments. Nonetheless, all fertilizers have some ammoniacal N that undergoes nitrification, with a tendency to acidify the soil [17]. Although at the time of soil sampling, the pH did not show a significant difference between treatments, perhaps after the application of fertilizers, small fluctuations in soil pH were sufficient to increase the bioavailability of Mn. The bioavailability of Mn is also very sensitive to the redox potential of the soil [61]. Fertilization, increasing the biological and enzymatic activity of the soil, may have contributed to the consumption of oxygen, reducing the redox potential and increasing the bioavailability of Mn. Reduction conditions may increase soluble forms of Mn in the soil due to the dissolution of Mn oxides, which can result in a strong uptake of Mn by plants [57]. In the Humix 12:03:05 treatment, the average values of Mn in the tissues and in the soil were among the highest of all treatments. Perhaps the organic component of the fertilizer had a more acidic effect on the soil, due to mineralization and nitrification, and also a reduction in the redox potential, due to greater biological activity, with increased Mn bioavailability.

Humix 12:03:05, although tending to be less concentrated in N, P and K than the other fertilizers, and having registered a tendency to reduce the concentration of these nutrients in the tissues, maintained chestnut yield at a level equivalent to BoskGrow 20:05:20_3m and Exactyon 18:05:13_3m. The reason may be due to multiple beneficial effects beyond the availability of nutrients attributed to organic amendments. As previously reported, there is always to be expected a certain “manuring effect” on plants of organic materials that is difficult to achieve by providing nutrients exclusively through the application of mineral fertilizers [55,62].

5. Conclusions

The fertilizers BoskGrow 20:05:20_3m, Exactyon 18:05:13_3m and Humix 12:03:05 significantly increased chestnut yield compared to the control treatment, probably because they provided N and K, but also Ca and Mg, which are also part of their composition. N, Ca and Mg may have positively influenced crop productivity as they are very limiting elements in the ecosystem, in addition to K as there is a limitation in uptake due to the restriction of nutrient movement by mass flow and diffusion during the summer period. The Exactyon 18:05:12_6m fertilizer, whose nutrient availability lasted six months, had an effect on crop productivity not significantly different from the unfertilized control. The delay in the release of nutrients makes their uptake difficult, as they are not available in the spring while there is still moisture in the soil, which was demonstrated by the lack of improvement of the indices of the nutritional status of the plant and by the increase in inorganic N in the soil in the winter. Humix 12:03:05, although less concentrated in nutrients, had an effect on production at the level of BoskGrow 20:05:20_3m and Exactyon 18:05:13_3m, a result attributed to some “manuring effect” that is sometimes associated with organic amendments.

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