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Relationship between Leaf Scorch Occurrence and Nutrient Elements and Their Effects on Fruit Qualities in Chinese Chestnut Orchards

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Abstract: Chinese chestnut (*Castanea mollissima*) is a multipurpose tree providing nuts and timbers, which holds an important position in the mountainous villages in China. However, leaf scorch disease is becoming more and more serious in the chestnut orchards of Yanshan Mountain areas, but the cause of occurrence is still unclear. In this study, the nutrient elements were analyzed from the leaves, roots, and surrounding soils of roots as well as the nut qualities in the healthy and scorched trees from two adjacent chestnut orchards. The results showed that the elements of nitrogen (N), iron (Fe), boron (B), and zinc (Zn) in leaves significantly increased in the scorched trees as well as N and B in roots, and potassium (K), and available potassium (AP) in soils, but leaf magnesium (Mg), root manganese (Mn), and soil Mg, copper (Cu), Fe, and B significantly decreased. Correlation analysis demonstrated that B, Zn, Mg, and Fe had a greater influence on the status of leaf health, and soil AK, K, Fe, B, and Cu had an impact on leaf B concentration. In addition, the occurrence of leaf scorch affected the nut sizes, contents of total soluble proteins and ascorbic acid as well as the catalase activity in the nuts. These results indicated that the disruption of soil-element balance may be one of the main causes resulting in the occurrence of leaf scorch, which would provide a theoretical basis and practical guidance for the prevention of chestnut leaf scorch disease.

Keywords: chestnut; leaf scorch; soil elements; leaf elements; nut quality

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1. Introduction

Chestnut trees (*Castanea*), belonging to the family Fagaceae, are economically important fruit trees known as woody grain plants in Asia, North America, and Europe [1]. In China, chestnut cultivation has a long growing history of over 3000 years, which is particularly widespread, being distributed in arid, semi-arid mountainous areas. Its nuts have high contents of nutrients, such as carbohydrates, proteins, and vitamin C, so it is also called a source of nutritional health care [2,3]. In recent years, however, leaf scorch disease is becoming severe and has spread in the chestnut orchards from the Yanshan Mountains, which has threatened the sustainable development of the chestnut industry.

Leaf scorch is a common type of leaf disease in herbaceous or woody plants. Its etiology is very complex, resulting not only from pathogens, but also from environmental factors such as nutrient abundance, temperature, and drought [4–6]. At present, most studies on leaf scorch disease focus on the identification of pathogenic bacteria, while there has not been a systematic investigation of the aspects of cultivation management and nutrient elements [7]. Leaf scorch can be caused by *Neoscytalidium dimidiatum*, and then dieback of twigs, branches, and even whole trees of olive [8], as well as by *Xylella fastidiosa* in olive [9] and almond [10]. Maydis leaf blight, caused by *Cochliobolus heterostrophus*, appears as small lesions with a dark brown margin and a straw to light brown colored center to absolute foliage blight [11]. A serious leaf disease in Chinese chestnut (*C. mollissima*), referred to as the brown margin leaf blight with irregular dry rot in a large area, is caused by *Phomopsis castaneae-mollissimae* [12] coordinated with *Ophiognomonia castaneae* [13], which is different from the yellow or brown wilted leaves caused by *Cryphonectria parasitica* in chestnut blight in Iran [14]. In addition, leaf scorch is also caused by a combination of environmental factors. For example, zinc (Zn) deficiency causes leaf mottled yellowing in citrus trees, which is more serious when citrus trees grow in the sun [15], and the effective Zn content in soils is affected by the joint influence of pH, organic matter, and soil structure [16]. The low potassium (K) content in leaves is related to leaf scorch of *Ginkgo biloba*, and comprehensive environmental factors such as high temperature and drought also cause its physiological scorching phenomena [17], while excessive sodium content in soils may lead to the scorched death of apple leaf margins [18]. High temperature and low rainfall lead to an increase in the content of available phosphorus (P) in soils, which in turn leads to a significant increase in Cl^- and Ca^{2+} in leaves, and a significant decrease in Mg^{2+}, resulting in scorched leaves [19]. However, at present, there are no studies concerning the effect of leaf scorch on fruit quality.

In recent years, field investigation found that a kind of leaf disease, showing the scorching symptom between leaf margins and leaf veins (Figure 1), has become increasingly serious in chestnut orchards in the Yanshan Mountains, which is completely different from the previously reported leaf scorch [12,14]. However, as described by our previous study, no pathogenic bacteria were detected in the isolation and identification of microorganisms in the tested leaf-scorched orchards from five experimental locations in the Yanshan Mountains in 2019 [20]. Therefore, we analyzed and compared (1) the differences in the nutrients of leaves, roots, and soils, and (2) changes in nut phenotypes, nutrients, and antioxidants, from healthy and leaf-scorched trees, respectively, in the same chestnut orchards, to investigate the main factors leading to leaf scorch, and its effects on nut characteristics. This study would provide a basis for the prevention and control of chestnut leaf scorch disease and the scientific management of chestnut orchards in the future.

Figure 1. Healthy and scorched leaves of chestnut trees. ZF: Yanshanzaofeng; ZQ: Zhongqing No. 1.

2. Materials and Methods

2.1. Study Sites and Sample Collection

Sample collections were conducted in two adjacent chestnut orchards where no pathogenic bacteria resulting in leaf scorch were detected in 2019 [20], located on the halfsunny slope of Xiaolugou village (118°45′ E, 40°21′ N), Qinglong Manchu Autonomous County, Hebei province, in early September 2020, and in which there were healthy and scorched trees, respectively. The soil type is leaching cinnamon soil, the texture of the soil is sandy loam, and the pH is 5.9–6.1. The surrounding area is characterized by a temperate climate with an annual precipitation of 715 mm and an average temperature of 8.9 °C. Both orchards were established in 2006 and grafted with the cultivars Yanshanzaofeng (ZF) and Zhongqing No. 1 (ZQ) for 14 years, respectively. The spacing between plants and rows of chestnut trees was approximately 4 m × 5 m. Both orchards were managed using a similar measure by the same farmer, including the application of approximately 450 kg of a compound fertilizer of potassium sulfate (N:P:K = 15:15:15) and 22,500 kg of compost consisting of sheep manure for each hectare after autumn harvest, the spraying of herbicide (glufosinate ammonium) in the early of June and August, and the extra supplement of boron element. In recent years, leaf scorch symptoms were found within the chestnut trees of these two orchards, which occurred piecemeal with an increasing trend in the number of diseased trees.

The chestnut trees were divided into two treatments (groups) of healthy and diseased when collecting samples. The trees with more than 1/3 scorched leaves were identified as the symptomatic (diseased) ones, while the trees without obvious scorched leaves were identified as the asymptomatic (healthy) ones. Three sampling sites for healthy and diseased trees, respectively, were randomly selected in each orchard, with each sampling site consisting of three healthy or scorched trees. The samples were randomly collected from the east, west, south, and north sides of each tree. Leaves and fine roots (<3 mm diameter) and nuts were collected in the selected trees as well as the corresponding soils surrounding the roots. The collected samples in each sampling site were mixed and brought back to the laboratory.

2.2. Determination of Nutrient Elements

The collected leaves and roots were rinsed with tap water and deionized water, in turn, heated at 105 °C for 30 min, and then dried continuously at 75 °C in an oven until a constant weight was reached. Soil samples were air-dried naturally, crushed, and passed through an 80-mesh sieve prior to the element analysis. Total N was determined by Kjeldahl's method [21]. Available potassium (AK), available phosphorus (AP), phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), sulfur (S), boron (B), and molybdenum (Mo) were subjected to wet mineralization by treating 0.5 g of dry sample with 6 mL of hydrochloric acid (37%), 2 mL of nitric acid (65%) and 2 mL of hydrogen peroxide (30%). After the mixed solutions were filtered, the element concentrations were determined by ICP-OES (Ametek Spectro, Arcos, Kleve, Germany) [22].

2.3. Determination of Nut Qualities

Morphological traits: The transverse diameter, vertical diameter, and lateral diameter of the fresh nuts were determined with vernier calipers. Every treatment included three replicates, each consisting of 30 nuts, and the measurement accuracy is 0.1 mm. The fruit shape index was calculated by the formula: nut shape index = vertical diameter/transverse diameter, and the nut volume was calculated by the formula: nut volume = transverse diameter × vertical diameter × lateral diameter. Single seed fresh was calculated by randomly weighing 10 fresh nuts, which were then used for the determination of the water content. The formula of water content = (fresh weight − dry weight)/fresh weight × 100%.

Nutrient compositions: Total soluble sugars and starch were analyzed according to the anthrone-sulfuric acid method [23,24], and total amino acids were analyzed according to the Ninhydrin hydrate method [25], by using commercial assay kits (Comin Biotechnology, Suzhou, China). Total soluble proteins were determined by using the Bradford Protein Assay Kit (Sangon Biotech, Shanghai, China).

Antioxidant compounds: Total polyphenols were determined by using the Folin–Ciocalteu colorimetric method [26]. Total flavonoids were determined by using the aluminum nitrate method [27,28]. Ascorbic acid (ASA), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and polyphenol oxidase (PPO) activities were determined by using commercial assay kits (Comin Biotechnology, Suzhou, China).

2.4. Statistical Analysis

The statistical analysis was performed by one-way ANOVA and factor analysis at a 5% level of significance using the SPSS software (v25.0). GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) was used for plotting. Correlation heatmaps and redundancy analysis (RDA) were performed using the OmicStudio tools at https://www.omicstudio.cn/tool, accessed on 18 August 2022.

3. Results

3.1. Leaf Phenotypes of Healthy and Diseased Chestnut Trees

The leaves of the two cultivars, ZF (Yanshanzaofeng) and ZQ (Zhongqing No. 1), in the areas of the Yanshan Mountains, showed different degrees of yellowish brown color after scorching (Figure 1), and the main symptoms of leaf margins and interveinal tissues showed a continuous brown dry state, but this did not occur in the leaf veins and petioles. Other than brown and green, there was no obvious transition color.

3.2. Relationship between the Occurrence of Leaf Scorch and Nutrients in Chestnut Orchards

3.2.1. Comparison of Nutrient Elements of Healthy and Disease Trees

The variance analysis of nutrient elements from soils (Table S1), roots (Table S2), and leaves (Table S3), demonstrated that there were no significant differences in the contents of most elements between healthy and diseased trees of ZF and ZQ cultivars, indicating that the varieties were not the main factor of leaf scorch symptoms. Thus, the difference in elements between the two varieties was ignored in this study.

The coefficients of variation (C.V.) between the elements varied greatly in leaves (Table 1). Compared with the healthy trees, the contents of leaf N, Fe, B, and Zn in diseased trees significantly increased by 27.61%, 20.74%, 61.66%, and 31.37%, respectively, while the Mg content significantly decreased by 16.92%, but no significant changes were detected in other elements between healthy and diseased trees.

Table 1. Leaf nutrient elements in healthy and diseased chestnut trees.

Elements	Healthy Trees (g/kg)			Diseased Trees (g/kg)		
	Range of Variations	The Average ± Standard Error	C.V.%	Range of Variations	The Average ± Standard Error	C.V.%
N	20.105–24.285	22.582 ± 0.606	6.572	26.693–31.619	28.816 ± 0.769 ***	6.535
P	1.189–2.289	1.698 ± 0.166	23.910	1.564–2.44	2.061 ± 0.143	16.982
K	4.073–6.644	5.465 ± 0.436	19.543	5.643–11.475	7.793 ± 0.976	30.694
Ca	13.193–22.272	16.617 ± 1.306	19.257	11.338–16.533	14.781 ± 0.816	13.517
Mg	6.561–9.128	7.493 ± 0.37	12.091	5.098–7.705	6.225 ± 0.4 *	15.727
Fe	0.136–0.202	0.172 ± 0.011	15.698	0.172–0.261	0.217 ± 0.015 *	17.051
B	0.082–0.196	0.143 ± 0.019	32.867	0.311–0.425	0.373 ± 0.015 ***	9.920
Cu	0.006–0.007	0.007 ± 0	14.286	0.007–0.01	0.008 ± 0	12.500
Mn	0.88–2.368	1.499 ± 0.207	33.756	0.706–2.737	1.509 ± 0.332	53.943
Zn	0.027–0.046	0.035 ± 0.003	20.000	0.036–0.059	0.051 ± 0.003 **	15.686
Mo	0.001–0.001	0.001 ± 0	0.000	0.001–0.001	0.001 ± 0	0.000

Note: "***": $p < 0.001$; "**": $p < 0.01$; "*": $p < 0.05$. The same below in Tables 2 and 3.

Table 2. Root nutrient elements in healthy and diseased chestnut trees.

Elements	Healthy Trees (g/kg)			Diseased Trees (g/kg)		
	Range of Variations	The Average ± Standard Error	C.V.%	Range of Variations	The Average ± Standard Error	C.V.%
N	1.571–10.164	7.448 ± 1.247	41.018	7.733–13.87	11.439 ± 0.897 *	19.206
P	0.819–1.54	1.036 ± 0.107	25.193	0.558–1.154	0.896 ± 0.085	23.103
K	3.876–5.202	4.62 ± 0.201	10.671	4.172–6.88	5.576 ± 0.384	16.876
Ca	9.342–13.696	11.964 ± 0.676	13.850	10.537–13.369	12.305 ± 0.469	9.330
Mg	2.277–3.635	2.871 ± 0.201	17.102	2.218–2.863	2.496 ± 0.107	10.457
Fe	0.62–1.991	1.198 ± 0.236	48.247	0.334–1.595	0.88 ± 0.207	57.727
B	0.009–0.049	0.024 ± 0.006	62.500	0.051–0.114	0.081 ± 0.011 **	34.568
Cu	0.007–0.012	0.01 ± 0.001	20.000	0.007–0.009	0.008 ± 0	12.500
Mn	0.054–0.103	0.079 ± 0.008	25.316	0.035–0.061	0.051 ± 0.004 *	19.608
Zn	0.02–0.04	0.028 ± 0.003	28.571	0.018–0.024	0.022 ± 0.001	13.636
Mo	0.001–0.002	0.001 ± 0	100.000	0–0.002	0.001 ± 0	100.000

Note: "**": $p < 0.01$; "*": $p < 0.05$.

Table 3. Soil nutrient elements in healthy and diseased chestnut trees.

Elements	Healthy Trees (g/kg)			Diseased Trees (g/kg)		
	Range of Variations	The Average ± Standard Error	C.V.%	Range of Variations	The Average ± Standard Error	C.V.%
N	10.198–11.346	10.765 ± 0.183	4.171	10.283–12.068	11.14 ± 0.316	6.957
P	0.367–3.299	1.847 ± 0.538	71.305	0.613–1.427	0.99 ± 0.121	29.798
K	16.968–23.042	20.643 ± 1.051	12.464	20.207–40.026	29.372 ± 3.035 *	25.306
AK	0.041–0.109	0.07 ± 0.01	35.714	0.099–0.187	0.136 ± 0.017 *	30.882
AP	0.008–0.087	0.042 ± 0.014	83.333	0.015–0.06	0.045 ± 0.007	37.778
Na	11.104–16.145	12.452 ± 0.759	14.937	6.827–15.256	11.156 ± 1.49	32.718
Ca	6.81–22.967	14.939 ± 2.389	39.173	5.807–12.167	9.236 ± 0.932	24.708
Mg	12.736–21.32	17.506 ± 1.171	16.383	12.344–16.328	14.092 ± 0.607 *	10.545
Cu	0.025–0.056	0.041 ± 0.004	24.390	0.013–0.02	0.017 ± 0.001 ***	17.647
Zn	0.086–0.156	0.124 ± 0.011	21.774	0.088–0.127	0.104 ± 0.006	13.462
Fe	45.268–64.584	54.392 ± 2.728	12.283	37.756–48.599	44.177 ± 1.529 *	8.480
Mn	0.456–1.075	0.754 ± 0.087	28.117	0.511–0.759	0.63 ± 0.034	13.016
B	0.048–0.072	0.060 ± 0.004	15.000	0.04–0.053	0.048 ± 0.002 *	10.417
S	0.085–0.32	0.174 ± 0.035	50.000	0.058–0.741	0.26 ± 0.103	96.923

Note: "***": $p < 0.001$; "*": $p < 0.05$.

Similarly, the C.V. values between the elements varied greatly in roots (Table 2). Compared with the healthy trees, the contents of root N and B in diseased trees significantly increased by 34.89% and 70.37%, respectively, while the Mn content significantly decreased by 35.44%, but no significant changes were found in other elements between healthy and diseased trees.

The C.V. values between the elements also varied greatly in soils (Table 3). Compared with the healthy trees, the soil K and AK content in diseased trees significantly increased by 29.72% and 48.53%, respectively, while the Mg, Cu, Fe, and B content significantly decreased by 19.50%, 58.54%, 18.78%, and 20.00%, but no significant changes were shown in other elements between healthy and diseased trees.

3.2.2. Correlation Analysis between Elements in Various Parts

There was a significant positive correlation between soil Mg and root Mn as well as soil AK and root B, while soil Cu, B, and Fe were significantly negatively correlated with root B. (Figure 2a). The root N was significantly positively correlated with the leaf Zn, Fe, and N, and root B was significantly positively correlated with the leaf Zn, Fe, N, and B, but root Mn was significantly negatively correlated with the leaf Zn, N, and B (Figure 2b). The soil AK was significantly positively correlated with leaf B, and soil K was significantly positively correlated with leaf N and B, but negatively correlated with leaf Mg. The soil Cu was significantly negatively correlated with leaf Zn, Cu, N, and B, but positively correlated with leaf Mg. The soil B and Fe were significantly negatively correlated with leaf B (Figure 2c).

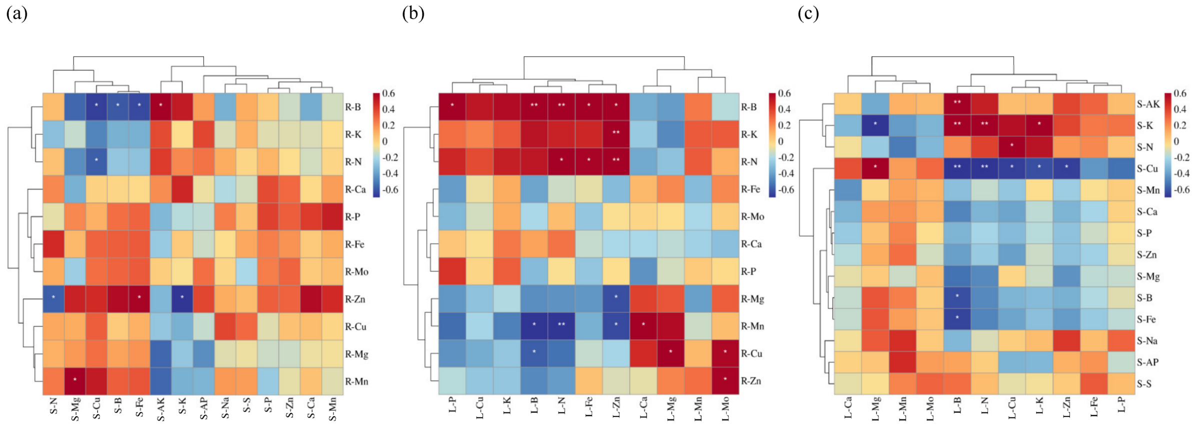

Figure 2. Clustering correlation heatmap with signs in various parts: (**a**) Soil (S) and root (R); (**b**) Root and leaf (L); (**c**) Soils and leaf. "**": $p < 0.01$; "*": $p < 0.05$.

3.2.3. Chestnut Leaf Factor Analysis

Factor analysis was performed based on the results of 11 determined elements in healthy leaves (Table 4) and diseased leaves (Table 5). In healthy leaves, it can be seen that the cumulative variance contribution rate of the first four main factors accounted for 96.78% of the total variance of the original variables, which largely retains the characteristics, differences, and interrelationships of the original 11 variables (Table 4), so the complex relationships between the 11 elements in the healthy leaves can be converted into 4 unrelated comprehensive indicators. From the loading point of view of the factors, the first main factor is mainly determined by N, K, and B, the second main factor is mainly determined by Fe, Mn, and Zn, the third main factor is mainly determined by P and Cu, and the fourth main factor is mainly determined by Mo.

Table 4. Analysis of nutrient factors in healthy leaves.

Characters	Principal Component 1	Principal Component 2	Principal Component 3	Principal Component 4
N	0.991	0.035	0.037	−0.116
P	0.469	0.035	0.674	−0.397
K	0.847	−0.479	0.208	0.043
Ca	−0.963	0.024	−0.222	−0.121
Mg	−0.918	0.083	−0.348	0.132
Fe	−0.019	0.814	0.131	0.525
B	0.841	0.014	−0.184	−0.500
Cu	0.120	0.199	0.940	−0.128
Mn	−0.182	0.940	0.058	−0.258
Zn	0.065	0.752	0.594	0.277
Mo	−0.109	0.065	−0.261	0.932
Eigenvalues	5.129	2.915	1.597	1.005
Variance contribution rate/%	46.631	26.500	14.515	9.138
Cumulative contribution rate/%	46.631	73.130	87.645	96.784

In diseased leaves, the cumulative contribution rate of the 11 variances of the first 4 main factors accounted for 90.63% of the total variance of the original variables (Table 5). Similarly, the relationship between the 11 elements in the scorched leaf can be converted into 4 unrelated comprehensive indicators. The first main factor is mainly determined by N, K, and Zn, the second main factor is mainly determined by Mg and Mn, and the third main factor is mainly determined by P, Fe, and Cu. In this way, the contribution of 11 elements to the scorched leaves of chestnut leaves can be divided into three categories: {N, K, Zn}, {Mg, Mn}, {P, Fe, Cu}.

Table 5. Analysis of nutrient element factors of diseased leaves.

Characters	Principal Component 1	Principal Component 2	Principal Component 3
N	0.718	−0.596	0.306
P	0.408	0.584	0.668
K	0.741	−0.405	0.469
Ca	−0.861	0.359	−0.169
Mg	−0.303	0.865	0.093
Fe	−0.207	0.500	0.810
B	−0.983	0.023	0.136
Cu	0.131	−0.301	0.913
Mn	0.033	0.990	0.022
Zn	0.815	0.431	0.056
Mo	−0.109	−0.055	−0.802
Eigenvalues	4.624	3.376	1.969
Variance contribution rate/%	42.037	30.693	17.903
Cumulative contribution rate/%	42.037	72.730	90.633

3.3. Effects of Leaf Scorch Occurrence on Nut Qualities of Chestnuts

3.3.1. Changes in Nut Phenotypic Index

Leaf scorch showed significant effects on nut phenotypes (Figure 3), including a significant reduction in the transverse diameter in both cultivars, the fruit shape index in ZQ, and the vertical diameter, nut volume, and single seed fresh in ZF. Compared with those in the healthy ones, the nut volume and single seed fresh considerably decreased by 59.80% and 36.78%, respectively, indicating that the occurrence of leaf scorch severely affected the nut production of the ZF cultivar.

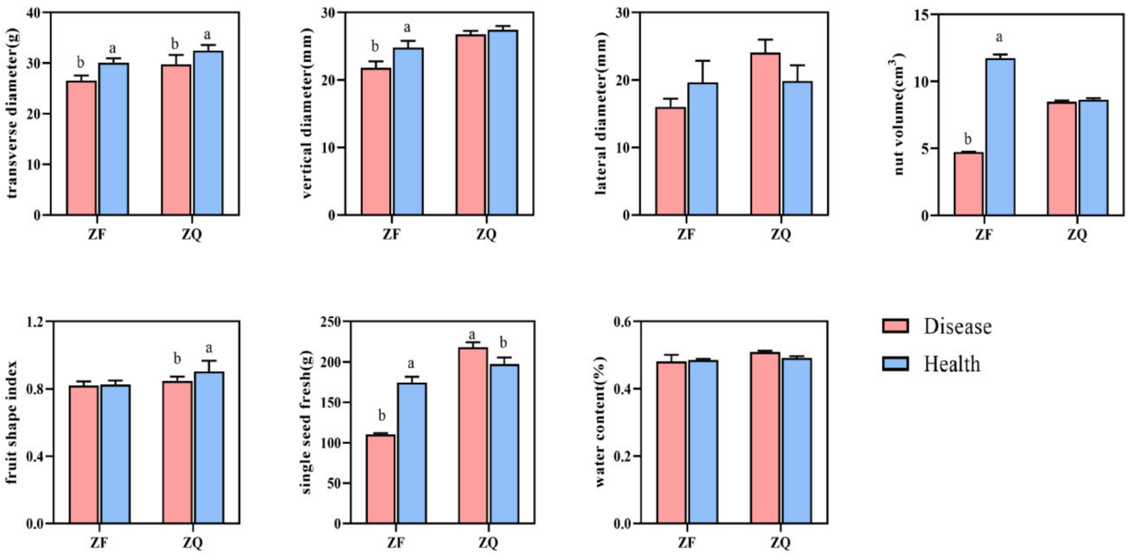

Figure 3. Changes in nut phenotypic traits in healthy and diseased chestnut trees. The significance of difference was labeled as 'a' and 'b' between healthy and diseased trees.

3.3.2. Changes in Nut Nutrients and Antioxidants

At the nutrient level, leaf scorch had almost no significant effect on starch, soluble sugars, and total amino acids, as well as total soluble proteins, except for its significant decrease in ZQ (Figure 4). Similarly, leaf scorch had no significant influences on the total polyphenols, total flavonoids, SOD, and POD in the two cultivars, but significantly increased the CAT activity in ZF and the ASA content in ZQ, compared with the healthy trees, indicating that the different cultivars may resist leaf scorch disease through different antioxidants.

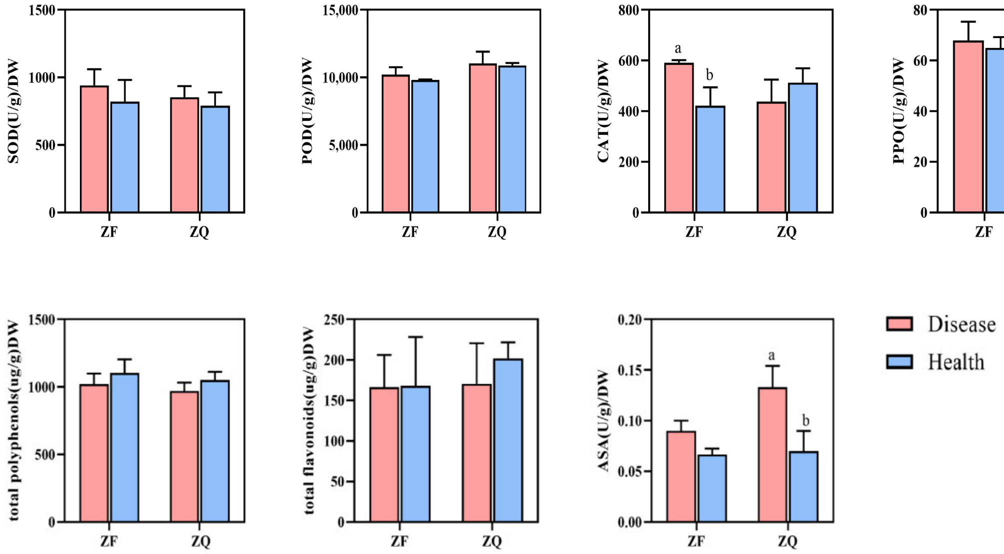

Figure 4. Changes in nut nutrients and antioxidants in healthy and diseased chestnut trees. The significance of difference was labeled as 'a' and 'b' between healthy and diseased trees.

3.3.3. Correlation Analysis of Soil Elements and Nut Qualities

Redundancy analysis (RDA) showed that RDA1 and RDA2 explained 33.88% and 18.39% of the total variations, respectively (Figure 5a). In healthy trees, Mg, Fe, B, Cu, Zn, Ca, P, total amino acids, total polyphenols, total flavonoids, and CAT were closely related to each other, revealing their importance in the health of chestnuts, while N, K, AK, AP, PPO, SOD, ASA, POD, and soluble sugars were closely related to each other in diseased trees, indicating that these parameters played important roles in the response to the occurrence of leaf scorch. The Pearson correlation analysis (Figure 5b) demonstrated that N was significantly positively correlated with total soluble proteins, but AP was contrary; N was negatively correlated with POD, but AK and K were significantly positively correlated with ASA and CAT, respectively, indicating the application of N may increase growth but decrease the resistance, but K can increase the resistance by enhancing the activities of antioxidants. Mg was positively correlated with starch but negatively correlated with ASA, as were Cu, Fe, and B.

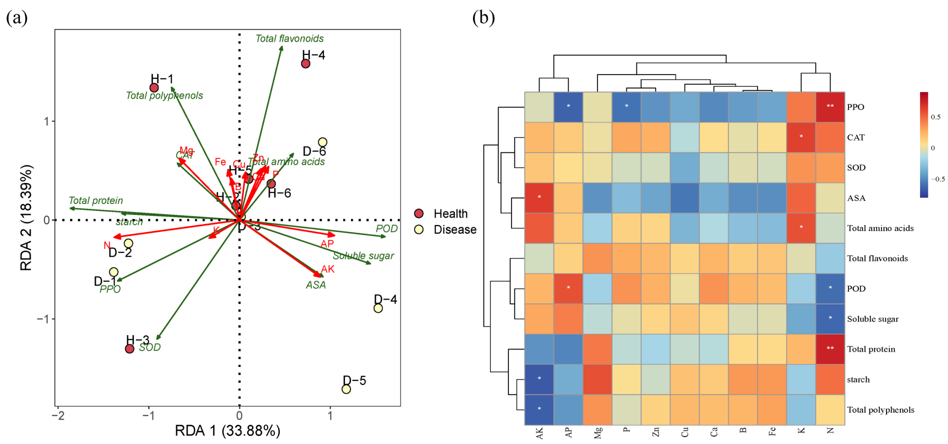

Figure 5. Correlation of nut characteristics with soil elements. (**a**) Redundancy analysis (RDA). The color ranges of red and green lines (**a**) represented positive and negative correlation, respectively; (**b**) Pearson correlation heatmap. * and ** represented the significance at 0.05 and 0.01 levels, respectively.

3.3.4. Correlation Analysis of Leaf Elements and Nut Qualities

The analysis of RDA showed that RDA1 and RDA2 explained the total change of 33.88% and 18.39% respectively (Figure 6a). The nut characteristics of healthy trees were significantly different from those of leaf-scorched trees, which were initially separated by axis 2 and negatively correlated with B, Mn, Fe, and Zn. In addition, B and Mg had a significant positive correlation with ASA and soluble sugars, but a negative correlation with CAT and total soluble proteins. Zn was significantly positively correlated with SOD and ASA, but significantly negatively correlated with CAT, total polyphenols, and total flavonoids. The Pearson correlation analysis showed that Zn was significantly positively correlated with SOD, and Fe was significantly negatively correlated with total polyphenols, furthermore, B was significantly positively correlated with ASA, and Mg was negatively correlated with CAT (Figure 6b).

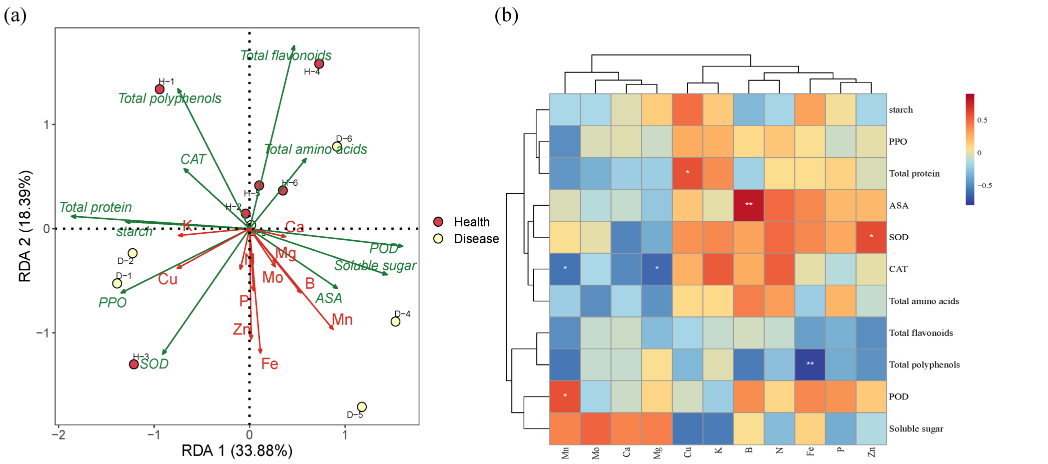

Figure 6. Correlation of nut characteristics with leaf elements. (**a**) Redundancy analysis (RDA). The color ranges of red and green lines (**a**) represented positive and negative correlation, respectively; (**b**) Pearson correlation heatmap. * and ** represented the significance at 0.05 and 0.01 levels, respectively.

4. Discussion

During the management of chestnut orchards, B is the most important trace element because of its role in pollination and fertilization; thus, farmers often apply extra B fertilizer to reduce the empty percentage of chestnut fruits. However, it is very easy to cause B toxicity due to the excessive application in practice because farmers have not mastered scientific fertilization methods. It was also found in our field investigation that excessive application of B fertilizer by chestnut farmers caused the whole leaves to wither. B toxicity is regarded as one of the limiting factors affecting plant growth [29]. After an overdose, B is first concentrated in the old leaves, starting from the leaf tip and leaf margin of the plants. A common symptom of B overdose is the appearance of yellow borders along the leaf margins, and symptoms of loss of green color, scorching, and necrosis occur at sites with high B concentrations [30–32]. In this study, leaf B was higher by over 160.8% in scorched trees compared with healthy trees. Excess B can arouse leaf scorch on old leaves and delay development in spinach [33], significantly inhibit chlorophyll content and cause the tips of leaves to undergo chlorosis and necrosis in rice seedlings [34,35], and lead to yellow areas at the top of the leaves and their margins in citrus plants [36]. The symptoms described in these studies are similar to the symptoms of leaf scorch in the leaf-scorched ZF and ZQ cultivars. In addition, B toxicity can induce membrane damage, altering the plant's antioxidant defense system [37] as, for example, in the increase in CAT activity of sunflowers leaves [38], *Cucurbita pepo* and *Cucumis sativus* [39] and pepper plants [40], and the increase in ASA of citrus plants [41], which are consistent with results in this study. It is also interesting to note that leaf B in the healthy trees reached 0.143 g/kg, far higher than that in roots (0.024 g/kg) and soils (0.060%), while B contents in leaf, root, and soils are all lower than 0.100 g/kg in other chestnut orchards without leaf scorch occurrence (our unpublished data), further indicating the excessive B application in the selected chestnut orchards. The nut phenotypes of scorched chestnut trees were also inhibited to a certain extent in this study, which was similar to the previous results in wheat [42,43], tomatoes, and cucumbers [44].

Magnesium is one of the key elements that make up chlorophyll and has an important role in photosynthetic organs [45,46]. At the beginning of leaf Mg deficiency, the inter-vein color of the leaf tip and leaf margin fades, while the veins remain green, forming clear veins on the leaves; the leaves even dry and fall off in severe cases [47]. In this study, a lower Mg content of soils (Table 3) and roots (Table 2) may result in a significant decrease in leaf Mg content (Table 1), which may be one of the key reasons for the occurrence of leaf scorch in the diseased chestnut trees.

Zinc and Fe are essential elements required for chestnut growth, but excess Zn and Fe can inhibit growth and affect the colors of leaves [42,43,45,46]. In this study, however, the ranges of Zn and Fe contents in both leaves and roots were within the normal level of Zn of between 0.030 and 0.200 mg/kg DW [48,49] and Fe of between 64 and 250 mg/kg (DW) [50], which were also around or lower than the values of Zn and Fe in other chestnut orchards without leaf scorch occurrence (our unpublished data), although both were higher in scorched leaves compared to healthy ones. This indicated that the symptom of chestnut leaf scorch in the present work may be not caused by the levels of zinc or Fe.

Overall, the difference in B and Mg contents in the scorched leaves, based on this study, suggested that synergistic and/or antagonistic effects on micro- and macro-nutrients existed in soils and chestnut trees (Figure 7). Interactions among mineral elements affect plant nutrient status and plant health and yield [51]. Previous studies showed that applying K fertilizer in soils can lead to an increase in B concentration in soybean leaves [52,53], accompanied by a reduction in soil Mg concentration, and thus, decrease the accumulation and concentration of Mg in leaves [45,52]. In this work, the correlation analysis showed that the increase in K and AK in soils had a promotive role in the accumulation of B in leaves and roots by sacrificing B in soils, but had an inhibiting role in leaf Mg content (Figure 7; Tables 1–3), which was consistent with a previous report [52].

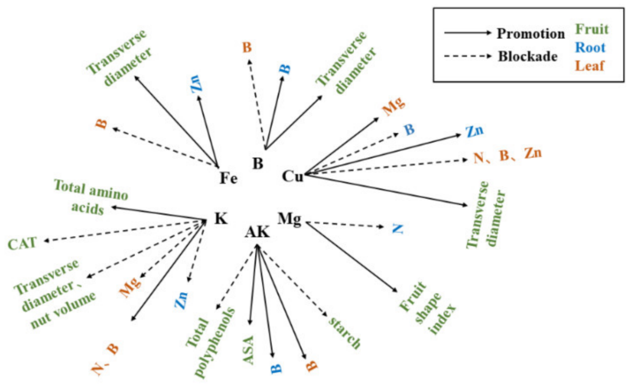

Figure 7. An action model of the altered soil elements on root elements, leaf elements, and nut characteristics.

An increasing N content prompts the demand for B in the plant [51], and the N content is significantly increased in roots (Table 2), while the Mn content in the roots, which is not conducive to boron increase [51], is significantly reduced (Table 2), coupled with the accumulation of B in roots, resulting in an extremely significant difference in the B content in leaves. The Zn content can be affected by fertilization and the balance of nutrient elements in plants [51]. The application of N fertilizer increases Zn concentration and bioavailability in wheat [54]. In this study, an increase in leaf Zn may be caused by the increase in root N of diseased trees, which may result from the excessive application of N fertilizer in order to improve the yield of chestnut orchards.

Soil is the carrier of tree growth, and the nutritional status of soil directly affects the growth, yield, and quality [55]. The available B content in soils was significantly positively correlated with citrus fruit diameter [56], similar to the results in this study. The AK content in soils is the determinant of ASA content in peppers [57], and excess K can significantly increase the ASA content in apples and *Prunus persica* [58,59]. In this study, the AK content was also positively correlated with the ASA content in the fruit. In addition, excess K leads to a decrease in the CAT activity in rapeseed but increases the free amino acid content in wheat [60,61], consistent with the results of this work. In general, leaf scorch increased the ASA content and CAT activity in chestnut nuts, which can enhance fruit quality to a certain extent, although these changes were the consequence of tree adaptation to the occurrence of leaf scorch.

5. Conclusions

This study showed that chestnut leaf scorch may be caused by excessive boron content and Mg deficiency in the leaves, which may be caused by the alteration of the AK, B, Mg, Cu, and Fe balances in soils, of which the reduction of Mg is most likely caused by AK. In addition, leaf scorch had a significant impact on the phenotypes and characteristics of the chestnut tree nuts. Thus, we would hope that the present work could provide a theoretical basis and practical guidance for the prevention of chestnut leaf scorch disease. In addition, it is also urgent to conduct a deep investigation into the specific factors causing leaf scorch in the future.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f14010071/s1, Table S1: Analysis of leaf element variance of ZF and ZQ; Table S2: Analysis of root element variance of ZF and ZQ; Table S3: Analysis of soil element variance of ZF and ZQ.

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