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Effect of Biochar Using N, P, and K Fertilisers on Growth and Quality of *Lithocarpus litseifolius*

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Abstract: Objective: In this study, we aimed to investigate the effects of facile combinations of biochar and N, P, and K fertilisers on the growth and physiological characteristics of *Lithocarpus litseifolius* seedlings, and to optimise the biochar/NPK ratio of *Lithocarpus litseifolius*. Method: A four-factor three-level orthogonal method was used to conduct a field experiment using 2-year-old *Lithocarpus litseifolius.* Nine fertiliser treatments and one control treatment (CK, i.e., no fertiliser) were established in a completely randomised block group with six replications of ten treatments. The plants were planted in October 2020 and harvested in November 2021, and fertiliser was applied once in April 2021 and once in July 2021. Results: Rational application could effectively promote the growth of *Lithocarpus litseifolius*, and T4($C_2N_1P_2K_3$) produced the highest increase in plant height growth (17.03 cm), diameter growth (5.47 mm), soluble sugar (94.60 mg/g), soluble protein (4.59 mg/g), and phlorizin (old leaf: 16.00%; tender leaf: 15.13%); T8($C_3N_2P_1K_3$) resulted in the highest chlorophyll a content (1.46 mg/g), chlorophyll b content (0.62 mg/g), and total chlorophyll content (2.08 mg/g), and T1($C_1N_1P_1K_1$) resulted in the highest contents of starch (11.60 mg/g) and trilobatin (old leaf: 0.29%; tender leaf: 2.28%). The indicators corresponding to the above three treatments were significantly higher than those under the other treatments ($p < 0.05$). The results as analysed by the affiliation function method show that the highest mean value of the affiliation function was 0.645 after T4($C_2N_1P_2K_3$) treatment. Conclusions: The combination of biochar and nitrogen, phosphorus, and potassium fertilisers can effectively increase the biomass and active components of *Lithocarpus litseifolius* while reducing the amount of chemical fertiliser applied. A comprehensive analysis of the results showed that the T4 treatment (biochar: 20 g/plant; urea: 10 g/plant; superphosphate: 9 g/plant; potassium chloride: 12 g/plant) resulted in the highest comprehensive score, with the highest increase in plant height growth, ground diameter growth, root–crown ratio, soluble sugar, soluble protein, and phlorizin, as well as other indicators.

Keywords: *Lithocarpus litseifolius*; biochar; formulated fertilisation; growth indices; physiological indices

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1. Introduction

Lithocarpus litseifolius (Hance) Chun is a plant of the genus Lithocarpus in the Fagaceae family, widely distributed in the south of the Qinling Mountains in China. Young leaves of *Lithocarpus litseifolius*, when brewed, have a sweet taste, making so-called sweet tea [1]; they are also used for other teas, medicine, and sugar, and have other functions. They are also known as "Cordyceps sinensis on a tree" [2]. It has been proven [3] that *Lithocarpus litseifolius* has three main medicinal effects, namely high antioxidant, antibacterial, and anti-inflammatory effects, with people's understanding of the nutritional and health values of *Lithocarpus litseifolius* growing as it becomes more popular, but it is difficult to meet the demand for wild resources, and large-scale cultivation is likely to become an issue in the future. Many farmers apply a high yield and large number of chemical fertilisers, causing soil acidification and sclerosis and subsequently reducing the rate at which fertiliser is utilised; this impairs plant yield and quality, seriously affecting the sustainable development of the *Lithocarpus litseifolius* industry. Therefore, it is urgent to improve fertiliser application measures for *Lithocarpus litseifolius* production.

To compensate for the lack of soil fertility (low N, P, and K), farmers across the globe often overapply chemical fertilisers in the expectation of increasing crop yields [4]. Urea is the most commonly used low-cost nitrogen fertiliser, and when it is applied to soil, it is rapidly converted to ammonium and carbon dioxide by microbially generated urease enzymes, thus providing essential nutrients for plant growth and development [5]. However, overapplication of urea may lead to several problems, such as soil acidification and poor fertiliser utilisation. Among them, ammonia volatilisation is the main source of N losses and the main cause of N use inefficiency [6,7]. Phosphorus (P) is a key essential element for plant growth and development and is involved in some fundamental biochemical processes, such as energy conversion and transmission of genetic information [8]. Phosphorus fertilisers commonly used in agriculture are usually in the form of plant-absorbable phosphates, which are derived from phosphate rock deposits and converted through a series of physical and chemical processes so that they can be effectively absorbed by the plant's root system. However, the overapplication of phosphorus fertiliser can encourage overexuberant plant respiration, resulting in greater dry matter consumption than accumulation, which can lead to premature maturity and lower yields [9]. Potassium (K), one of the important nutrients that regulate various physiological and biochemical processes in plants [10], increases photosynthesis and biomass when applied, but it also increases transpiration in trees and may exacerbate water stress in plants, resulting in problems such as increased water stress [11]. Therefore, how to increase crop yields while reducing the use of nitrogen, phosphorus, and potassium fertilisers has become an urgent question for farmers.

In view of the many drawbacks associated with inorganic fertilisers, researchers have turned their attention to biochar, a soil amendment derived from organic matter. In recent years, biochar has been widely adopted in agroforestry for its ability to significantly increase soil fertility and improve soil structure [12]. Biochar is known for its rich pore structure and stable physicochemical properties, which can effectively retard the release and loss of nutrients from fertilisers, thereby promoting the enhancement of effective nutrients in the soil and significantly optimising the composition of the soil microbial community [13]. Thus, the use of biochar is considered one of the excellent strategies to curb nutrient losses and enhance plant uptake of key nutrients, such as N, P, and K [14]. In addition, biochar can indirectly promote better nutrient uptake by plants by modifying the composition of soil microorganisms and their enzyme activities [15]. Therefore, the application of biochar to soil not only enhances the efficacy of nutrient uptake by plant, but also significantly improves the efficiency of uptake.

This experiment aimed to discover the optimal ratio of Lithocarpus litseifolius fertiliser with biochar to provide sound theoretical support for the application of biochar in agriculture, as well as to provide technical support for the efficient production of Lithocarpus litseifolius. By enabling the use of agricultural resources to be optimised and agricultural pollution to be reduced, this study will provide a solid theoretical foundation for sustainable and green development.

2. Materials and Methods

2.1. Overview of the Test Site

The experimental site was Longta Work Area (118°11′ E, 25°51′ N), Gekeng State Forestry Farm, Dehua County, Quanzhou City, with an altitude of 600–1000 m. The average annual temperature was 17 °C, and the annual precipitation ranged from 1800 to 2000 mm, with a meso-subtropical oceanic monsoon climate. The soil in the test site was loamy, with field water-holding capacity of 253.2 g/kg, maximum water-holding capacity of 413.0 g/kg, bulk density of 1.03 g/cm^3, capillary porosity of 26.92%, total potassium of 4.27 g/kg, fast-acting potassium of 23.03 mg/kg, alkaline dissolved nitrogen of 24.43 mg/kg, effective phosphorus of 32.33 mg/kg, total phosphorus of 0.68 g/kg, and organic matter 4.95 g/kg, pH 5.66.

2.2. Test Materials

The test seedlings were taken from 2-year-old *Lithocarpus litseifolius* cuttings with an average height and ground diameter of 32.59 cm and 2.72 cm, respectively. They were purchased from the Zhijiang Shengkang *Lithocarpus litseifolius* family farm in Huaihua, Hunan Province, China. The seedlings were robust and uniform in appearance.

The biochar used in the experiment was corn stover charcoal with the following nutrients: organic carbon content of 404.78 g/kg; total nitrogen, total phosphorus, and total potassium contents of 8.45 g/kg, 2.31 g/kg, and 15.56 g/kg, respectively; moisture coefficient of 1.03; and pH of 9.78. Fertiliser: urea (total nitrogen $\geq$ 46.0%), calcium superphosphate ($P_2O_5 \geq 12\%$), and potassium chloride ($K_2O \geq 60\%$). When applying biochar, 10 g/plant, 20 g/plant, and 30 g/plant were applied according to the experimental design. (According to the amount of nitrogen fertiliser, plant type and growth stage, soil type, and pH, combined with comprehensive literature consideration.)

2.3. Experimental Design

$L_9(3^4)$, i.e., a 4-factor 3-level orthogonal test, was used to design a completely randomised block design with 9 fertiliser treatments and 1 control treatment (CK, i.e., no fertiliser), resulting in a total of 10 treatments with 6 replications, 60 plots, and 5 plants per plot, with a spacing of 1 m $\times$ 1 m. The *Lithocarpus litseifolius* plants were planted in October 2020 and harvested in November 2021. They were fertilised once in April 2021 and once in July 2021, and the levels of fertilisation for each treatment are shown in Table 1.

Table 1. $L_9(3^4)$ orthogonal experimental factors and levels.

Serial Number	Treatment	Biochar g/Plant	CH_4N_2O g/Plant	$Ca(H_2PO_4)_2$ g/Plant	KCI g/Plant
T1	$C_1N_1P_1K_1$	10	10	6	6
T2	$C_1N_2P_2K_2$	10	15	9	9
T3	$C_1N_3P_3K_3$	10	20	12	12
T4	$C_2N_1P_2K_3$	20	10	9	12
T5	$C_2N_2P_3K_1$	20	15	12	6
T6	$C_2N_3P_1K_2$	20	20	6	9
T7	$C_3N_1P_3K_2$	30	10	12	9
T8	$C_3N_2P_1K_3$	30	15	6	12
T9	$C_3N_3P_2K_1$	30	20	9	6
CK	$C_0N_0P_0K_0$	0	0	0	0

Note: Digital subscripts, with C, N, P, and K indicating the fertilisation level for each fertiliser.

2.4. Measurement Items and Methods

A random sampling method was adopted in each experimental area, with 3 groups of 10 plants selected at random. In November 2021, the survival rate and growth indicators of each treatment were measured by measuring the plant height with a ruler and the ground diameter with a micrometer. New leaves sprouted in spring, summer, and autumn, and fresh and tender leaves were harvested from March to October. Mature leaves were harvested from November to February of the following year. The fresh weight was determined using an electronic balance. Ten seedlings with consistent growth levels were selected from each treatment, completely dug out, washed, and wiped dry, and the leaves, stems, and roots were killed green separately at 105 °C for 30 min. After drying at 75 °C to constant weight, their dry weight was determined after cooling using an electronic balance (accurate to 0.01 g).

The chlorophyll content was determined using ethanol extraction; the soluble sugar content and starch content were determined using anthrone colorimetry [16]; the total flavonoid content was determined using ethanol ultrasonic extraction [17]; and the soluble protein content was measured using a Caulmers Brilliant Blue assay [18]. The nitrogen content was determined using a fully automatic micro-carbon and -nitrogen element analyser (ELEMENTAR, Germany), and the phosphorus and potassium contents were determined using an inductively coupled plasma emission spectrometer (ICP). The phlorizin and trilobatin contents were determined via high-performance liquid chromatography [19,20]. [The chromatographic column was Shim-pack VP-ODS (250 × 4.6 mm, 5 µm), mobile phase: acetonitrile–0.04% formic acid (28:72), flow rate: 0.8 mL B7·min^{-1}, detection wavelength: 285 nm, column temperature: room temperature, and injection volume: 5 µL.]

2.5. Data Processing

The experimental results were analysed and statistically and graphically compared using Excel 2019 and Origin 2021 software, and they were evaluated using SPSS19.0 software for the one-way analysis, significance analysis based on a 5% probability level, the LSD multiple comparison test, the analysis of variance, and the affiliation function method. The formula for the calculation of the affiliation function of each indicator was $U(X_i) = (X_i - X_{min})/(X_{max} - X_{min})$, where X_i is the measured value of the indicator, and X_{max} and X_{min} are the maximum and minimum values of each treatment, respectively.

3. Results

3.1. Plant Height and Ground Diameter of Lithocarpus litseifolius

As shown in Figure 1, the treatments ranked as follows in terms of their effects on plant height growth: T4 > T5 > T3 > T6 > T1 > T9 > T8 > T2 > T7 > CK. Compared with the control, the highest level of growth was seen with the T4($C_2N_1P_2K_3$) treatment, which was 3.63 times that obtained with CK($C_0N_0P_0K_0$); the second was with T5($C_2N_2P_3K_{13}$), which was 3.62 times that observed with CK($C_0N_0P_0K_0$); and the lowest amount of growth in plant height was seen with T7($C_3N_1P_3K_2$), which was 23%, and this difference was significant. The treatments ranked as follows in terms of their effects on the growth in diameter, in order of largest to smallest: T4 > T6 > T7 > T3 > T2 > T8 > T5 > T9 > T1 > CK. Except for the T1($C_1N_1P_1K_1$) treatment, all the treatments differed significantly from CK, and there was no highly significant difference; among them, T4($C_2N_1P_2K_3$) resulted in the largest growth in diameter, which was 5.47 mm, and this was 3.94 times that achieved with CK($C_0N_0P_0K_0$); the smallest growth in the diameter with T1($C_1N_1P_1K_1$) was 1.62 mm, and this increase was 16.55%. The above results showed that the application of biochar with chemical fertiliser helped to increase the plant height and diameter of *Lithocarpus litseifolius*, and these two parameters were highest with the T4 treatment.

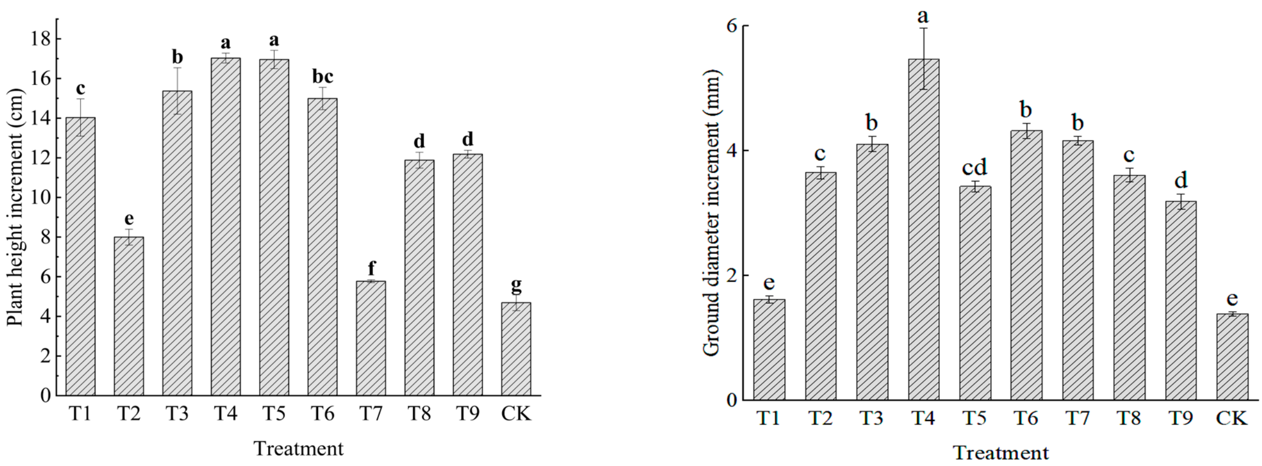

Figure 1. Effects of different fertilisation treatments on the growth of the plant height and ground diameter of *L. liteseifolius*. Note: Different lowercase letters indicate significant differences between treatments ($p < 0.05$). The same applies to the tables and figures below.

3.2. Biomass of Lithocarpus litseifolius

As shown in Table 2, among the nine fertiliser treatments, total fresh weight biomass, leaf fresh weight, root fresh weight, and stem fresh weight were not significantly different among the treatments with biochar dosing. There were significant differences in the total dry weight biomass, with the T2($C_1N_2P_2K_2$) treatment resulting in the highest total dry weight biomass of 57.74 g/plant, which was 55.42% higher than that obtained with the CK($C_0N_0P_0K_0$) treatment. This was followed by T1($C_1N_1P_1K_1$), T9($C_3N_3P_2K_1$), and T4($C_2N_1P_2K_3$), which produced increases of 47.73, 34.54, and 20.16% over the control (CK), respectively. The highest leaf dry weight of 14.27 g/plant was obtained with T2($C_1N_2P_2K_2$), followed by the weights obtained with T1($C_1N_1P_1K_1$), T9($C_3N_3P_2K_1$), and T4($C_2N_1P_2K_3$), which showed 75.09%, 69.20%, 69.57%, and 46.75% increases over the control (CK), respectively. The root dry weight was the highest at 20.59 g/plant with the T2($C_1N_2P_2K_2$) treatment, which showed a 90.30% increase over the control, followed by the weights obtained with T1($C_1N_1P_1K_1$), T4($C_2N_1P_2K_3$), and T9($C_3N_3P_2K_1$), which showed 58.87%, 56.47%, and 56.47% increases over the control (CK), respectively. The highest stem dry weight was observed with T2($C_1N_2P_2K_2$), at 22.88 g/plant, which was 25.85% higher than that obtained with the CK($C_0N_0P_0K_0$) treatment, followed by that obtained with T9($C_3N_3P_2K_1$), which was 19.53% higher than the control. By looking at the comprehensive biomass indices, it can be seen that T2($C_1N_2P_2K_2$) resulted in the highest values for all factors, except leaf fresh weight, which was slightly lower than with the T1($C_1N_1P_1K_1$) treatment; thus, it can be preliminarily concluded that T2($C_1N_2P_2K_2$) is the optimum fertiliser ration for improving *Lithocarpus litseifolius* biomass, followed by T1($C_1N_1P_1K_1$), T9($C_3N_3P_2K_1$), T4($C_2N_1P_2K_3$), and T5($C_2N_2P_3K_1$).

Table 2. Analysis of biomass of *L. litseifolius* from different fertiliser treatments.

Treatment	Total Biomass		Root–Crown Ratio	
	Fresh Weight (g)	Parts Dry (g)	Fresh Weight (%)	Parts Dry (%)
T1	90.44 b	51.88 b	0.516 c	0.495 bc
T2	100.61 a	57.74 a	0.597 b	0.554 ab
T3	60.50 de	35.20 e	0.483 c	0.435 cd
T4	81.61 b	44.64 c	0.707 a	0.606 a
T5	71.03 c	42.18 cd	0.449 c	0.437 cd
T6	63.76 cd	37.15 de	0.484 c	0.410 d
T7	47.49 fg	26.37 fg	0.502 c	0.474 cd
T8	52.36 ef	29.78 f	0.476 c	0.428 cd
T9	87.18 b	49.98 b	0.460 c	0.408 d
CK	39.88 g	23.00 g	0.496 c	0.275 e

Note: Values within a column followed by different lowercase letters indicate significant differences ($p < 0.05$).

3.3. Physiological indices of Lithocarpus litseifolius

As can be seen from Table 3, the total chlorophyll content and chlorophyll a/b values obtained with each fertilisation treatment were enhanced compared to the values obtained with CK($C_0N_0P_0K_0$). The total chlorophyll content and the chlorophyll a/b value obtained with T8($C_3N_2P_1K_3$) were the highest, at 23.91 mg/G and 2.83, respectively, which were significantly different from those obtained with other treatments. The total chlorophyll content obtained with T8($C_3N_2P_1K_3$) was 2.31 times that obtained with CK($C_0N_0P_0K_0$), under which chlorophyll a/b increased by 23.04%. Therefore, it can be concluded that the application of chemical fertiliser combined with biochar can improve the chlorophyll content of *Lithocarpus litseifolius* leaves, promote photosynthesis, and increase yield, and that T8 is the best fertiliser ratio to use to increase the chlorophyll content in *Lithocarpus litseifolius* leaves.

Table 3. Comparison of the physiological indices obtained with different fertilisation treatments.

Treatment	Total Chlorophyll	Chlorophyll a/b	Soluble Protein	Soluble Sugar	Starch
T1	10.87 b	2.48 bcd	2.20 c	74.12 g	5.80 e
T2	12.59 b	2.51 bcd	1.10 d	74.98 f	4.06 g
T3	14.69 b	2.58 b	3.73 b	73.97 g	5.28 f
T4	13.32 b	2.45 bcd	4.59 a	94.6 a	7.14 d
T5	12.42 b	2.54 bc	0.22 e	60.09 i	11.03 a
T6	10.55 b	2.34 de	0.49 de	84.74 c	8.68 b
T7	14.31 b	2.60 b	2.86 c	82.57 d	6.01 e
T8	23.91 a	2.83 a	0.75 d	78.78 e	7.79 c
T9	13.57 b	2.58 b	3.98 ab	87.84 b	8.28 b
CK	10.34 b	2.30 e	3.94 ab	72.65 h	5.66 ef

Note: Values within a column followed by different lowercase letters indicate significant differences ($p < 0.05$).

Soluble sugars include the vast majority of monosaccharides and oligosaccharides. They can store energy and act as mediators of transfer, structural substances, and ligands for functional molecules, such as glycoproteins in plants. Soluble proteins are important osmoregulatory substances and nutrients, and their increase and accumulation can improve the water retention capacity of cells and play a protective role for cellular vital substances and biofilms, which are among the important indicators of plant drought resistance. The treatments ranked as follows in terms of their ability to increase soluble protein content: T4 > T9 > CK > T3 > T7 > T1 > T2 > T8 > T6 > T5. The content of soluble protein obtained with the T4($C_2N_1P_2K_3$) treatment was the highest (4.59 mg/g), which was significantly higher than that obtained with the other treatments. The treatments ranked as follows regarding the soluble sugar contents obtained with them: T4 > T9 > T6 > T7 > T8 > T2 > T1 > T3 > CK > T5. The soluble sugar contents obtained with T4($C_2N_1P_2K_3$) and T9 were significantly higher than those obtained with the other treatments (30.21% and 20.91% higher than those obtained with CK($C_0N_0P_0K_0$), respectively). The order of the treatments regarding the increases in starch content was T5 > T6 > T9 > T8 > T4 > T7 > T1 > CK > T3 > T2. The highest starch content was achieved with the T5($C_2N_2P_3K_1$) treatment, 11.03 mg/g, which was 94.88% higher than that obtained with CK($C_0N_0P_0K_0$), and all the other treatments resulted in higher contents than the CK($C_0N_0P_0K_0$) treatment. The soluble protein contents under all the treatments except T4($C_2N_1P_2K_3$) and T9($C_3N_3P_2K_1$) were lower than those under CK($C_0N_0P_0K_0$). T4($C_2N_1P_2K_3$) and T9($C_3N_3P_2K_1$) were the most favourable treatments in terms of soluble protein and soluble sugar accumulation, and T5($C_2N_2P_3K_1$) and T6($C_2N_3P_1K_2$) were the most favourable treatments for increasing the starch content.

3.4. Active Constituents of *Lithocarpus litseifolius*

The effects of different fertilisation formulations on the phlorizin, trilobatin, and flavonoid contents of *Lithocarpus litseifolius* are shown in Table 4. The content of phlorizin in *Lithocarpus litseifolius* was significantly higher with all the treatments than with CK($C_0N_0P_0K_0$), and the three treatments resulting in the highest phlorizin content were T4($C_2N_1P_2K_3$), T1($C_1N_1P_1K_1$), and T7($C_3N_1P_3K_2$), in which the contents were increased by 152, 147, and 111%, respectively, in comparison with the content observed with CK($C_0N_0P_0K_0$). All three treatments had the same urea level; the most significant effect on the increase in the phlorizin content in *Lithocarpus litseifolius* was observed when 10 g of urea was applied. The mean phlorizin values were 10.71% for T1($C_1N_1P_1K_1$), T2($C_1N_2P_2K_2$), and T3($C_1N_3P_3K_3$); 12.31% for T4($C_2N_1P_2K_3$), T5($C_2N_2P_3K_{13}$), and T6($C_2N_3P_1K_2$); and 11.60% for T7($C_3N_1P_3K_2$), T8($C_3N_2P_1K_3$), and T9($C_3N_3P_2K_1$), showing a trend of increasing and then decreasing. This suggests that, for the accumulation of phlorizin, more biochar does not mean better results; rather, the appropriate amount of biochar can effectively promote increases in phlorizin.

Table 4. The analysis of active constituents of *L. litseifolius* (%).

Treatment	Phlorizin (%)	Trilobatin (%)	Flavonoids (%)
T1	13.75 b	1.29 a	3.47 bc
T2	11.39 d	0.61 b	3.93 a
T3	6.99 f	0.01 e	3.34 c
T4	15.57± a	0.10 d	3.53 b
T5	10.55 e	0.01 e	3.98 a
T6	10.83 de	0.02 e	1.68 f
T7	12.78 c	0.02 e	3.11 d
T8	11.06 de	0.09 d	3.09 d
T9	10.97 de	0.29 c	3.60 b
CK	5.64 g	0.03 e	2.92 e

Note: Values within a column followed by different lowercase letters indicate significant differences ($p < 0.05$).

The results for the content of trilobatin are shown in Table 4. Among all the treatments, the three treatments resulting in the highest contents of trilobatin were T1($C_1N_1P_1K_1$), T4($C_2N_1P_2K_3$), and T2 ($C_1N_2P_2K_2$), with which the contents were significantly higher than those observed with the CK($C_0N_0P_0K_0$) treatment; these treatments were 9.86, 7.55, and 5.41 times more effective than the control, respectively. The rest of the treatments were not statistically significantly different from CK(C0N0P0K0) in their effects. The treatments resulting in a lower content than CK($C_0N_0P_0K_0$) were T3($C_1N_3P_3K_3$), T5($C_2N_2P_3K_{13}$), T6($C_2N_3P_1K_2$), and T7($C_3N_1P_3K_2$); the contents were reduced by 60%, 80%, 60%, and 20%, respectively. In the T3($C_1N_3P_3K_3$), T5($C_2N_2P_3K_{13}$), and T7($C_3N_1P_3K_2$) treatments, the same level of calcium superphosphate fertiliser was applied (12 g/plant), which might indicate that the excessive application of calcium superphosphate leads to a reduction in *Lithocarpus litseifolius* trilobatin content. Similarly, the content of trilobatin varied at different urea levels, with the trichothecene content in *Lithocarpus litseifolius* at low urea levels being higher than the trilobatin content at high urea levels.

As can be seen from Table 4, the total flavonoid content obtained with the remaining treatments was significantly higher than that obtained with the CK($C_0N_0P_0K_0$) treatment, except for that achieved with the T6($C_2N_3P_1K_2$) treatment. The size order of the total flavonoid content achieved with each treatment was T5 > T2 > T9 > T4 > T1 > T3 > T7 > T8 > CK > T6. T5($C_2N_2P_3K_{13}$) and T2($C_1N_2P_2K_2$) produced significantly higher contents than the rest of the treatments, while T9($C_3N_3P_2K_1$), T4($C_2N_1P_2K_3$), and T1($C_1N_1P_1K_1$) produced significantly higher contents than the remaining treatments. These five treatments resulted in contents that were 36.30%, 34.59%, 23.29%, 20.89%, and 18.84% higher than those obtained with CK($C_0N_0P_0K_0$), respectively, while T6($C_2N_3P_1K_2$) produced a content 42.47% lower than that obtained with CK($C_0N_0P_0K_0$). On the whole, the highest total flavonoid content in *Lithocarpus litseifolius* was obtained when 10 g of biochar was applied, followed by the application of 15 g of biochar, and the lowest content was obtained when 20 g of biochar was applied. The reason the total flavonoid content in *Lithocarpus litseifolius* was lower with T6($C_2N_3P_1K_2$) than with the CK($C_0N_0P_0K_0$) treatment may be due to the combined effect of a high urea level and low calcium superphosphate and potassium chloride levels. Overall, chemical fertilisers reasonably matched with biochar were advantageous in increasing the contents of phlorizin and trilobate, as well as total flavonoids, in *Lithocarpus litseifolius*.

3.5. N, P, and K of Lithocarpus litseifolius Leaves

As shown in Figure 2, compared with the control, all the treatments using chemical fertiliser with biochar significantly enhanced plant total nitrogen, and the leaf nitrogen contents obtained with T2($C_1N_2P_2K_2$), T4($C_2N_1P_2K_3$), and T9($C_3N_3P_2K_1$) were significantly higher than those obtained with the other treatments; the lowest nitrogen content was obtained with T8($C_3N_2P_1K_3$), which was only 7.88% higher than that obtained with CK($C_0N_0P_0K_0$). For plant total phosphorus, the T7($C_3N_1P_3K_2$) treatment resulted in the highest content, 1.53 times higher than that obtained with CK($C_0N_0P_0K_0$), followed by those with T9($C_3N_3P_2K_1$) and T4($C_2N_1P_2K_3$), which were both significantly higher than the content obtained with CK($C_0N_0P_0K_0$), and the rest of the treatments resulted in lower contents than CK($C_0N_0P_0K_0$). For plant total potassium, the formulated fertiliser resulted in a lower content, and CK($C_0N_0P_0K_0$) resulted in the highest content of 5.21 g/kg. The potash contents observed with the treatments differed significantly, but they were all lower than the content observed for CK($C_0N_0P_0K_0$). Overall, the fertiliser formulated with biochar had a significant effect on *Lithocarpus litseifolius* plants. Overall, the application of biochar with chemical fertiliser promoted the accumulation of total nitrogen and total phosphorus in Xylocarpus indicus plants, especially nitrogen, while it was unfavourable for the accumulation of potassium.

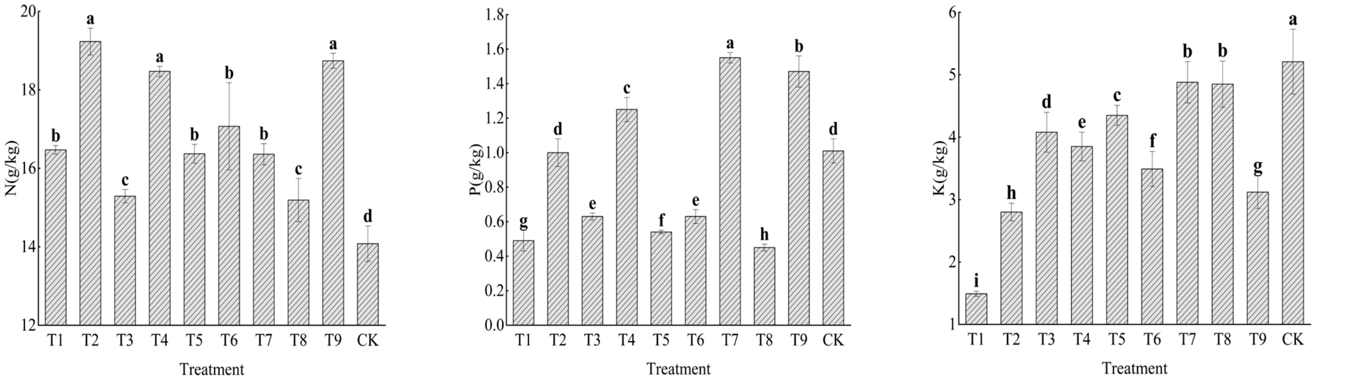

Figure 2. Effects of different fertiliser formulae on N, P, and K in *Lithocarpus litseifolius* leaf. Note: Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

3.6. Comprehensive Evaluation of Different Formulated Fertiliser Treatments

The growth indices, physiological indices, and active ingredients of *Lithocarpus litseifolius* were comprehensively evaluated using the fuzzy affiliation function, and the effects of each fertilisation treatment on the growth and development of *Lithocarpus litseifolius* were observed. As can be seen from Table 5, the treatments in descending order of the affiliation function values obtained were T4 > T9 > T2 > T1 > T7 > T8 > T5 > T3 > T6 > CK. The T4($C_2N_1P_2K_3$) treatment resulted in the highest affiliation function value, indicating that the parameters for *Lithocarpus litseifolius* were optimised under the application of biochar: 20 g/plant; urea: 10 g/plant; calcium superphosphate: 9 g/plant; and potassium chloride: 12 g/plant.

Table 5. Value of membership function and comprehensive ranking.

Indicators	Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9	CK
plant height		0.76	0.27	0.87	1.00	0.99	0.83	0.09	0.58	0.61	0.00
Ground diameter		0.06	0.55	0.67	1.00	0.50	0.72	0.68	0.54	0.44	0.00
Total biomass	Fresh weight	0.83	1.00	0.34	0.69	0.51	0.00	0.13	0.21	0.78	0.39
	Dry weight	0.83	1.00	0.35	0.62	0.55	0.00	0.10	0.20	0.78	0.41
Root–crown ratio	Fresh weight	0.26	0.58	0.13	1.00	0.00	0.14	0.21	0.11	0.05	0.18
	Dry weight	0.67	0.84	0.48	1.00	0.49	0.41	0.60	0.46	0.40	0.00
Total chlorophyll		0.04	0.17	0.32	0.22	0.15	0.02	0.29	1.00	0.24	0.00
Chlorophyll a/b		0.34	0.39	0.53	0.28	0.45	0.07	0.57	1.00	0.54	0.00
Soluble protein		0.41	0.43	0.40	1.00	0.00	0.71	0.65	0.54	0.80	0.36
Soluble sugar		0.45	0.20	0.80	1.00	0.00	0.06	0.60	0.12	0.86	0.85
Starch		0.25	0.00	0.17	0.44	1.00	0.66	0.28	0.54	0.61	0.23
Phlorizin		0.82	0.58	0.14	1.00	0.50	0.52	0.72	0.55	0.54	0.00
Trilobate		1.00	0.54	0.02	0.76	0.00	0.03	0.07	0.30	0.17	0.08
Flavonoid		0.78	0.98	0.72	0.80	1.00	0.00	0.62	0.61	0.83	0.54

Table 5. *Cont.*

Indicators \ Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9	CK
N	0.46	1.00	0.23	0.85	0.45	0.58	0.44	0.21	0.90	0.00
P	0.03	0.50	0.16	0.73	0.09	0.16	1.00	0.00	0.92	0.51
K	0.00	0.35	0.70	0.64	0.77	0.54	0.91	0.90	0.44	1.00
Average	0.47	0.55	0.41	0.77	0.44	0.32	0.47	0.46	0.58	0.27
Sequence	4	3	8	1	7	9	5	6	2	10

4. Discussion

A large number of studies have shown that a combination of biochar and nitrogen, phosphorus, and potassium fertilisers can improve the growth and quality of forest trees, which is important for improving plant yield [21,22]. XiaoLi B et al. [23] found that the addition of 1–2% biochar had a significant positive effect on the seed germination, aboveground, and root growth of *Robinia pseudoacacia* L. Li H et al. [24] found that the combination of biochar and chemical fertilisers significantly improved the quality and yield of apples. Biochar has a unique structure and adsorption capacity, which is important in improving soil properties, plant yield, and quality [25]. In recent years, more research into biochar application has been conducted, but the application effects have not been improved [26]. This study showed that the appropriate formula of biochar, combined with nitrogen (N), phosphorus (P), and potassium (K) fertilisers, was beneficial for increasing the plant height, ground diameter, and biomass of *Lithocarpus litseifolius* compared with the application of 10 g/plant (C_1: T1, T2, T3) and 30 g/plant (C_3: T7, T8, T9) of biochar; the application of 20 g/plant (C_2: T4, T5, T6) had a more significant effect, indicating that an appropriate amount of biochar was more beneficial for promoting the growth of *Lithocarpus litseifolius* seedlings. This may be because applying biochar improves the soil structure of the arable layer as well as soil fertility and fertiliser efficiency, thus improving the growth and development of *Lithocarpus litseifolius*, but too much biochar will adsorb and fix some of the nutrients, meaning that *Lithocarpus litseifolius* nutrients cannot be replenished quickly, so a higher dosage of biochar does not mean better results; rather, the dosing needs to be within a certain range for optimal benefit.

There is a close relationship between chlorophyll content and photosynthesis, and photosynthesis is one of the important factors affecting plant biomass accumulation [27]. Therefore, the accumulation of chlorophyll has direct significance for plant growth and development. In this study, we found that biochar dosing was able to increase the chlorophyll content of the leaves of *Lithocarpus litseifolius* seedlings. Wang Y et al. [28] found that the addition of biochar could increase the chlorophyll content of the leaves of Pingyi sweet tea seedlings, which led to a decrease in the decline in Fv′/Fm′, repaired the optical activity of leaf photosystem II, and increased the photosynthetic rate, but the mechanism of action remains to be further investigated. Kan Z et al. [29] showed that biochar has a strong adsorption capacity and can adsorb a variety of ions after entering the soil, among which Mg2+ is the raw material for chlorophyll synthesis, so it helps to improve chlorophyll content. Zhang L [30] showed that biochar with nitrogen fertiliser could significantly increase the chlorophyll content, net photosynthetic rate, and transpiration rate of *Eucalyptus grandis x urophylla* seedlings and reduce the concentration of intercellular carbon dioxide. The increase in plant chlorophyll content upon biochar application may be because biochar improves soil fertility, enhances nutrient uptake and translocation, and accelerates photosynthetic physiological processes. On the other hand, it may be because biochar helps to protect photosynthetic pigments and slows down the senescence process of *Lithocarpus litseifolius* leaves.

Soluble sugars and soluble proteins are important osmoregulatory substances in plants that not only supply nutrients and energy to the plant body for growth and development, but also maintain the stability of cellular osmotic pressure and improve plant stress tolerance [31]. Pluchon N et al. [32] in a comparative study of biochar feedback in seedlings of various trees, found that the feedback of biochar varied among different species of seedlings. The addition of biochar to soil can effectively improve the physical and chemical properties of the soil and affect the growth and physiological characteristics of plants. Khan W M T et al. [33] found that the physiological indices of photosynthesis pigments and soluble sugars of treated seedlings were increased to a certain extent when different ratios of biochar were used on Fujian cypress and rhododendron. The results of Zhu YH et al. [34] showed that the application of biochar could increase the content of soluble protein, which tended to increase and then decrease with an increase in the amount of biochar applied, and this tendency was more or less the same as the results of this experiment. The starch content in this experiment was lower than that in the control group, except for T1($C_1N_1P_1K_1$) and T5($C_2N_2P_3K_{13}$), which may be due to the fact that there is a certain variability in the starch content under different amounts of biochar application, which leads to a tendency to promote and then inhibit the starch content, and the greater the amount applied, the stronger the inhibition. It has been shown that the contents of soluble proteins, starch, and soluble sugars in plants increase under adversity stress [35]. In this experiment, with an increase in the application of biochar, the contents of soluble protein and starch were subsequently reduced or were even lower than those of the control group, which may be because biochar improves the soil environment, enabling plants to live in a relatively low-stress environment, which, to a certain extent, protects them from adversity stress.

Trilobatin is a natural sweetener extracted from dihydrochalcones in *Lithocarpus litseifolius*. Phlorizin has a variety of important biological activities, such as lowering blood glucose, improving memory, and antioxidant and anticancer properties. Flavonoids are strong antioxidants, which can effectively scavenge oxygen radicals in the body; this antioxidant effect can stop the degradation of cells and aging, and also prevent the occurrence of cancer. DongHwan L et al. [36] studied the growth characteristics and active components of *Cephalotaxus fortunei Hook* and found that appropriate cultivation measures could effectively increase the contents of flavonoids and other active components in plants. Szakiel A et al. [37] found that the application of organic fertilisers increased the accumulation of active ingredients in medicinal plants more than inorganic fertilisers did. In this study, applying fertiliser with biochar significantly increased the contents of phlorizin, trilobate, and flavonoids in *Lithocarpus litseifolius*, which may be attributed to the fact that the application of biochar with nitrogen, phosphorus, and potash fertilisers can increase the fertiliser utilisation rate, which can improve the soil environment, create a good environment for plant root microorganisms, and increase the efficiency of nutrient conversion in plants, thus facilitating the accumulation of active components.

In agricultural practices, the method of fertiliser application has a significant impact on the nutrient composition of leaf tissue [38]. The existing literature highlights the significant impact of mineral fertiliser use on nutrient elements in plant leaves. It is noteworthy that the application of nitrogen fertilisers has been reported to increase nitrogen concentration in the leaves of apple trees [39]. However, Kowalczyk et al. [40] showed that N (a key element for plant growth) content in leaves did not necessarily change significantly after N fertiliser application. This unexpected result may be due to the interaction of external environmental factors that play an important role in determining the mineral composition of plant leaves. Moreover, the application of biochar has been acknowledged for its beneficial physiological and biochemical properties, particularly in improving the nutrient status and increasing the biomass of *Eucalyptus* spp. [41], thus supporting the outcomes of this investigation. Our study reveals that an increase in nitrogen application does not always correlate with raised nitrogen levels in *Lithocarpus litseifolius* leaves. On the contrary, there appears to be a trend toward decreased nitrogen content under certain treatment protocols. This counterintuitive observation may be linked to an elevated carbon–nitrogen ratio induced by the excessive use of biochar, which could result in competitive absorption between biochar and the plants for available nitrogen, as well as a concurrent decline in soil nitrogen availability. These dynamics could lead to a reduction—or even cessation—of plant growth [42]. Consequently, it is apparent that variations in the foliar nitrogen content of *Lithocarpus litseifolius* are not solely dependent on an increased input of fertilizers or biochar. Furthermore, this study has detected a decrease in both phosphorus and potassium levels in *Lithocarpus litseifolius* under most treatment scenarios relative to the control group (CK). This outcome is potentially tied to a dilution effect that accompanies leaf maturation and nutrient redistribution to other plant organs [43]. This hypothesis is supported by data indicating that apple plants grown in soils devoid of fertilisation exhibit higher phosphorus and potassium levels in their leaves than those receiving varying nitrogen fertiliser treatments [40]. In synthesising insights from a comprehensive review of pertinent scholarly work, it is inferred that the increased use of nitrogen fertilisers negatively affects the phosphorus and potassium contents of plant leaves. Conversely, heightened doses of phosphorus and potassium fertilisers negatively impact nitrogen uptake by the foliage. As such, the diligent pursuit of optimal fertiliser compositions that maximise plant growth dynamics remains a critical avenue for research.

5. Conclusions

The results of the field experiment in this study showed that the synergistic application of biochar and nitrogen, phosphorus, and potassium (NPK) fertilisers significantly promoted the growth of *Lithocarpus litseifolius*, which was not only conducive to the accumulation of medicinal actives, but also important for the enhancement of fertiliser utilisation efficiency and economic benefits. Specifically, the T4 treatment programme (20 g/plant of biochar, 10 g/plant of urea, 9 g/plant of calcium superphosphate, and 12 g/plant of potassium chloride) showed the most significant growth-promoting effect. Appropriate application of biochar not only effectively reduces the use of chemical fertilisers but also significantly improves plant growth and further reduces the negative impact of agricultural production on the environment.

Combining biochar with reduced fertiliser application is not only an effective strategy to promote the growth of *Lithocarpus litseifolius*, but also an initiative to reduce environmental pollution and comply with the concept of sustainable agriculture. The implementation of this strategy is not only expected to improve the sustainability between plant growth and the environment, but also provides an important reference value for future agricultural practices. Based on the in-depth analysis of this study, future research should further explore optimal biochar and fertiliser ratios to maximise growth and economic benefits while reducing environmental impacts.

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References

1. Zhang, Y.; Ning, Z.; Dong, H.; Gao, J. Continuous countercurrent extraction of total flavonoids from *Cocos nucifera*. *Mod. Food Sci. Technol.* **2011**, *27*, 550–552.
2. Peng, Y.; Zhou, J.; Huang, Y.; Cao, X. Bibliometric analysis of the current status of domestic research on *Lithocarpus litseifolius*. *Jiangxi Sci.* **2020**, *38*, 607–610.
3. Wang, M.; Liu, X.; Zhang, Z.; Yu, J.; Liu, J.; Wu, Y. Phytochemicals and bioactive analysis of different sweet tea (*Lithocarpus litseifolius* [Hance] Chun) varieties. *J. Food Biochem.* **2021**, *45*, e13183. [CrossRef]

4. Arif, M.; Ilyas, M.; Riaz, M.; Ali, K.; Shah, K.; Haq, I.U.; Fahad, S. Biochar improves phosphorus use efficiency of organic-inorganic fertilizers, maize-wheat productivity and soil quality in a low fertility alkaline soil. *Field Crops Res.* **2017**, *214*, 25–37. [CrossRef]
5. Akbar, W.A.; Rahim, H.U.; Irfan, M.; Alatalo, J.M.; Mudasssir, M.; Bundschuh, J. Effects of incorporating pine-woodchip biochar with reduced NP fertilizer on calcareous soil characteristics, organic carbon, NPK availability, and maize productivity. *J. Soils Sediments* **2024**, *24*, 177–188. [CrossRef]
6. Feng, Z.; Zhu, L. Impact of biochar on soil N_2O emissions under different biochar-carbon/fertilizer-nitrogen ratios at a constant moisture condition on a silt loam soil. *Sci. Total Environ.* **2017**, *584–585*, 776–782. [CrossRef] [PubMed]
7. Dawar, K.; Fahad, S.; Jahangir, M.M.R.; Munir, I.; Alam, S.S.; Khan, S.A.; Mian, I.A.; Datta, R.; Saud, S.; Banout, J.; et al. Biochar and urease inhibitor mitigate NH_3 and N_2O emissions and improve wheat yield in a urea fertilized alkaline soil. *Sci. Rep.* **2021**, *11*, 17413. [CrossRef] [PubMed]
8. Ding, Z.; Kheir, A.M.; Ali, M.G.; Ali, O.A.; Abdelaal, A.I.; Lin, X.E.; Zhou, Z.; Wang, B.; Liu, B.; He, Z. The integrated effect of salinity, organic amendments, phosphorus fertilizers, and deficit irrigation on soil properties, phosphorus fractionation and wheat productivity. *Sci. Rep.* **2020**, *10*, 2736. [CrossRef] [PubMed]
9. Gondwe, R.L.; Kinoshita, R.; Sano, M.; Suminoe, T.; Aiuchi, D.; Koaze, H.; Palta, J.; Tani, M. Lack of yield response in potato (*Solanum tuberosum* L.) to phosphate fertilizer under contrasting soil types varying in phosphate absorption coefficient and available phosphate. *Soil Sci. Plant Nutr.* **2017**, *63*, 171–177. [CrossRef]
10. Tshewang, S.; Rengel, Z.; Siddique, K.H.; Solaiman, Z.M. Nitrogen and Potassium Fertilisation Influences Growth, Rhizosphere Carboxylate Exudation and Mycorrhizal Colonisation in Temperate Perennial Pasture Grasses. *Agronomy* **2020**, *10*, 1878. [CrossRef]
11. Asensio, V.; Domec, J.; Nouvellon, Y.; Laclau, J.P.; Bouillet, J.P.; Jordan-Meille, L.; Lavres, J.; Rojas, J.D.; Guillemot, J.; Abreu-Junior, C.H. Potassium fertilization increases hydraulic redistribution and water use efficiency for stem wood production in Eucalyptus grandis plantations. *Environ. Exp. Bot.* **2020**, *176*, 104085. [CrossRef]
12. Sakhiya, K.A.; Anand, A.; Kaushal, P. Production, activation, and applications of biochar in recent times. *Biochar* **2020**, *2*, 253–285. [CrossRef]
13. Rahim, H.U.; Akbar, W.A.; Alatalo, J.M. A Comprehensive Literature Review on Cadmium (Cd) Status in the Soil Environment and Its Immobilization by Biochar-Based Materials. *Agronomy* **2022**, *12*, 877. [CrossRef]
14. Dey, D.; Mavi, M.S. Co-application of biochar with non-pyrolyzed organic material accelerates carbon accrual and nutrient availability in soil. *Environ. Technol. Innov.* **2022**, *25*, 102128. [CrossRef]
15. Song, X.; Razavi, B.S.; Ludwig, B.; Zamanian, K.; Zang, H.; Kuzyakov, Y.; Dippold, M.A.; Gunina, A. Combined biochar and nitrogen application stimulates enzyme activity and root plasticity. *Sci. Total Environ.* **2020**, *735*, 139393. [CrossRef] [PubMed]
16. Chen, J.S.; Wang, X.F. *Guide to Plant Physiology Experiments*; South China University of Technology Press: Guangzhou, China, 2006; pp. 68–69.
17. Tang, J.; Zhu, C.; Gao, L.; Zou, R.; Xiong, Z.; Jiang, Y. Optimisation of total flavonoid extraction process and antioxidant activity of coca multiflora by response surface methodology. *Anhui Agric. Sci.* **2020**, *48*, 181–185.
18. Qu, C.; Shen, S.; Wang, X.; Cui, Y.; Song, W. Determination of soluble proteins in plant crude extracts by using Caulmers Brilliant Blue. *J. Soochow Univ. Nat. Sci. Ed.* **2006**, *2*, 85–88.
19. Wang, Y.; Wang, L.; Zhang, D.; Li, H.; Yan, R. Determination of phlorizin, trilobatin and rhizopin in *Lithocarpus litseifolius* by high-performance liquid chromatography. *Tianjin Sci. Technol.* **2019**, *46*, 66–68.
20. Wang, K.; Li, K.; Chen, J.; Huang, J.; Ma, J. Changes in the main active components and their contents of wild *Polygonum multiflorum*. *Econ. For. Res.* **2016**, *34*, 96–100+122.
21. Vannini, A.; Carbognani, M.; Chiari, G.; Forte, T.G.W.; Lumiero, F.; Malcevschi, A.; Rodolfi, M.; Ganino, T.; Petraglia, A. Effects of Wood-Derived Biochar on Germination, Physiology, and Growth of European Beech (*Fagus sylvatica* L.) and Turkey Oak (*Quercus cerris* L.). *Plants.* **2022**, *11*, 3254. [CrossRef]
22. Thomas, S.C.; Gale, N. Biochar and forest restoration: A review and meta-analysis of tree growth responses. *New For.* **2015**, *46*, 931–946. [CrossRef]
23. Bu, X.; Xue, J.; Wu, Y.; Ma, W. Effect of Biochar on Seed Germination and Seedling Growth of *Robinia pseudoacacia* L. In Karst Calcareous Soils. *Commun. Soil Sci. Plant Anal.* **2020**, *51*, 352–363. [CrossRef]
24. Li, H.; Li, W.; Wang, Y.; Wang, G. Research on the effect of biochar on water and fertiliser conservation and apple growth in sandy soil. *J. Hortic.* **2019**, *46*, 559–566.
25. Marta, M.; Urbana, B.; Davide, I.; Giacomo, L.; Francesca, M.; Nelson, M.; Elena, M. Structural and Functional Features of Chars from Different Biomasses as Potential Plant Amendments. *Front. Plant Sci.* **2018**, *9*, 375024.
26. Ghorbani, M.; Konvalina, P.; Neugschwandtner, R.W.; Marek, K.; Elnaz, A.; Daniel, B.; Anna, W.A. Interaction of Biochar with Chemical, Green and Biological Nitrogen Fertilizers on Nitrogen Use Efficiency Indices. *Agronomy* **2022**, *12*, 2106. [CrossRef]
27. Yu, Y.; Ruihan, H.; Song, C.; Zhang, H.; Zhang, X.; Wang, X.; Liu, Z.; Li, Z.; Wang, Y.; Liu, W. The B-box transcription factor PabBBX27 in the regulation of chlorophyll biosynthesis and photosynthesis in poplar (*Populus alba* × *P. berolinensis*). *Ind. Crops Prod.* **2023**, *203*, 117159. [CrossRef]
28. Wang, Y.; Shen, X.; Chen, X.; Wu, S.; Mao, Z. Effects of biochar on alleviating parabens injury to Pingyi sweet tea seedlings. *Chin. Agric. Sci.* **2014**, *47*, 968–976.
29. Kan, Z.; Ma, S.; Qi, J.; Pu, C.; Wang, X.; Zhao, X.; Zhang, H. Effects of biochar application on photosynthetic potential and seed yield of winter wheat. *J. Wheat Crops* **2019**, *39*, 719–727.

30. Zhang, L. Effects of Biochar with Nitrogen Fertiliser on Growth Physiology and Nutrient Absorption and Utilisation of Eucalyptus Megacephalus Seedlings. Master's Thesis, Central South Forestry University of Science and Technology, Changsha, China, 2017.
31. Wang, H.; Sun, Y.; Yang, L.; Chen, C.; Zhou, X. Annual changes of soluble sugar and soluble protein contents in leaves of three species of *Camellia sinensis*. *Jiangsu Agric. Sci.* **2017**, *45*, 105–107.
32. Pluchon, N.; Gundale, M.J.; Nilsson, M.C.; Kardol, P.; Wardle, D.A. Stimulation of boreal tree seedling growth by wood-derived charcoal: Effects of charcoal properties, seedling species and soil fertility. *Funct. Ecol.* **2014**, *28*, 766–775. [CrossRef]
33. Khan, W.M.T.; Lili, F.; Yueqin, C.; Muhammad, T.; Lingyan, C.; Tianyou, H.; Jundong, R.; Yushan, Z. Biochar amendment regulated growth, physiological, and biochemical responses of conifer in red soil. *iForest-Biogeosci. For.* **2020**, *13*, 490–498.
34. Zhu, Y.H.; Zhu, Y.L.; Cao, X.; Lv, F. Effects of biochar on physiological characteristics of lily. *North Hortic.* **2017**, *7*, 92–98.
35. Fang, Z.H.; Dong, K.H. Effects of NaCl stress on soluble sugar and soluble protein content of *Artemisia alkali*. *Chin. Agron. Bull.* **2010**, *26*, 147–149.
36. Lee, D.H.; Son, Y.H.; Jang, J.H.; Lee, S.Y.; Kim, H.J. The Growth Characteristics and the Active Compounds of Cudrania Tricuspidata Fruits in Different Cultivation Environments in South Korea. *Plants* **2023**, *12*, 2107. [CrossRef] [PubMed]
37. Szakiel, A.; Pączkowski, C.; Henry, M. Influence of environmental abiotic factors on the content of saponins in plants. *Phytochem. Rev.* **2011**, *10*, 471–491. [CrossRef]
38. Vitor, M.M.L.; Maria, G.F.A.; Victor, O.D.P.; Antônio, J.L.D.R.C.; Rodrigo, P.M.; Serrão, R.B.S.D.; Bruno, B.L.; João, N.C.R.; Klynger, A.L.S.D. Positive biochemical, physiological and nutritional evidence from the use of biochar in the growth of eucalyptus plants. *Bot. Lett.* **2022**, *169*, 337–350.
39. Fallahi, B.; Baird, R.C.; Fallahi, E.; Chun, I.J.; Colt, W.M. Influence of Nitrogen and Bagging on Fruit Quality and Mineral Concentrations of 'BC-2 Fuji' Apple. *HortTechnology* **2001**, *11*, 462–466. [CrossRef]
40. Kowalczyk, W.; Wrona, D.; Przybyłko, S. Effect of Nitrogen Fertilization of Apple Orchard on Soil Mineral Nitrogen Content, Yielding of the Apple Trees and Nutritional Status of Leaves and Fruits. *Agriculture* **2022**, *12*, 2169. [CrossRef]
41. Cao, Y.; Shen, Y.; Chen, Y.; Wang, Z.; Mou, Z.; Xu, G.; Zhang, D.; Sun, Y.; Mao, X. Effects of Biochar Application on Soil Organic Nitrogen Component and Active Nitrogen in Eucalyptus Plantations after Five Years in Northern Guangxi. *Huan Jing Ke Xue Huanjing Kexue* **2023**, *44*, 6235–6247.
42. Tomo, M.; Nebojša, M.; Jelena, M. The influence of organic, organo-mineral and mineral fertilizers on tree growth, yielding, fruit quality and leaf nutrient composition of apple cv. 'Golden Delicious Reinders'. *Sci. Hortic.* **2022**, *297*, 110978.
43. Dževad, L.; Mirjana, R.; Mirko, K.; Dejan, Z.; Velibor, S.; Shuraik, K.; Branislav, D.; Ruby, N.M.; Jennifer, C.; Lizny, J.; et al. Influence of cultivar and fertilization treatment on the yield and leaf nutrients content of apple (*Malus domestica* Borkh.). *Heliyon* **2023**, *9*, e16321.