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Effects of Biochar-Based Fertilizers on Energy Characteristics and Growth of Black Locust Seedlings

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Abstract: To understand ecological and energy problems in the karst area of Guizhou, China, the effects of using biochar-based fertilizers on the energy characteristics of different species of black locust were studied. To determine the most suitable species and the best rational application method of biochar, an outdoor pot experiment was performed using three species of black locust (White-flowered locust (W), Hong-sen locust (S), and Large-leaf fast-growing locust (L)). There were six treatments: control (CK), MF, RH2MF, RH4MF, W2MF, and W4MF (M—compost; F—NPK fertilizer; RH—rice husk biochar; and W—wood biochar), where the numbers represented the mass ratio of biochar to soil. Biochar-based fertilizers had significant effects on the total organic carbon (TOC), total nitrogen (TN), total potassium (TK), branch gross calorific values (GCV), and ash removal calorific values (AFCV) of seedlings. RH4MF had the best overall values. Different species had significant effects in all indicators (except for TN); the effect on S was better than that of W and L. Principal component analysis showed that RH4MF-S had the highest comprehensive scores. In summary, Hong-sen locust (S) was a high-quality energy species and RH4MF may be used as fertilization for energy forest development. This study provides a reference for future long-term energy forest research in this area.

Keywords: biochar; karst; black locust; energy characteristics; calorific value

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1. Introduction

Guizhou province is located in the center of the karst region in southwest China, which is one of the most developed karst regions in the world [1]. Along with rocky desertification, soil erosion is unceasing, the soil layer is shallow, and vegetation restoration is more difficult [2–4]. In addition, Guizhou is short on resources and there is a gap in energy product utilization, which restricts local economic development [5]. Research on biomass energy has attracted increasing attention in the field of global renewable energy research, and research on plant calorific values provides basic support for the development and utilization of biomass energy [6]. An energy forest is the most representative of biomass energy and is renewable, clean energy. The selection, development, and utilization of energy forests have been studied in developed countries, among which the United States is the most typical [7]. Developing an energy forest not only reduces the pressure of natural forest resource consumption, but also protects and improves the local ecology [8].

Plant calorific values reflect the conversion efficiency of plants to solar energy and the ability of plants to effectively use various natural resources, and are an effective index to measure plant growth status [9]. Calorific values vary with different regions, habitat conditions, and plant species [10,11]. The average calorific value of different organs of the same plant also varies significantly [12,13]. The black locust (*Robinia pseudoacacia* L.) is strongly adaptable, resistant to drought and different soil fertility conditions, has easy reproduction, and grows fast [14]. Zhu et al. (2014) have shown that the black locust adapts well to soil conditions in karst areas; thus, it may be an excellent species for cultivation as a fuel-type energy forest [15]. There are few studies on black locust energy forests in karst areas of southwest China, so planting black locust in this area may allow for both soil and water conservation and energy forest effects [16].

Biochar is a solid product produced by pyrolysis of biomass materials (rice husks, corn stalks, and bagasse) under high temperature and low oxygen conditions and is widely used as an environmentally friendly soil improvement product [17–19]. Biochar plays an important role in the improvement of soil physical and chemical properties, which can improve microbial community structure, promote plant growth and development, and improve land productivity. Biochar has become a research hotspot in the fields of environment, ecology, agriculture, and forestry in recent years [20]. Biochar has many advantages, but excessive application can also produce negative effects. Zhang et al. (2010) found that applying biochar to soil inhibits the development of crops in the early stage of growth, and the inhibition gradually decreases in the later stage [21,22]. Different biochar and different production processes will also bring different effects and benefits, so it is necessary to choose the appropriate amount of biochar application to achieve the maximum economic benefits. Biochar-based fertilizer is a green sustained-release fertilizer that is composed of biochar and organic or inorganic fertilizer [23]. Biochar-based fertilizer integrates the advantages of biochar and fertilizer, and is superior to its raw material composition; it slowly releases nutrients, maintains lasting fertilizer effects, continuously improves the soil environment, promotes plant growth, and has great potential for the application of energy forests [24]. Biochar-based fertilizer has received increased attention due to its environmental friendliness and resource-saving potential [25]. To date, there have been few studies on the effects of biochar on the calorific values of plant branches and leaves in karst areas. However, data obtained show that fertilization increases the productivity of energy forests to different degrees [26].

The purpose of this study was to explore the effects of biochar-based fertilizer application on element contents, calorific values, and biomass of three different species of black locust seedlings using an outdoor pot experiment. The goals were to characterize the influence of biochar-based fertilizer on seedlings to provide theoretical support for improving the survival rate of energy forests in karst areas, determine the appropriate ratio of biochar-based fertilizer, and the best species of black locust to use. We hypothesized that the addition of biochar-based fertilizer would (1) increase the content of NPK elements in black locust seedlings but decrease the content of ash; (2) significantly promote the calorific values of branches and leaves; (3) promote the growth of black locust seedlings; and (4) induce significant differences in the energy characteristics among different species.

2. Materials and Methods

2.1. Soil and Biochar-Based Fertilizers

The test soil was from Puding County, Guizhou Province, China (105°27′–105°58′ E, 26°9′–26°31′ N), which is a typical karst area. The soil in this area is typical lime soil with an average thickness of 25 cm [27]. The soil sampling depth of this test was 0–20 cm of the surface tillage layer, and was disinfected with a 2 mm sieve for reserve use [28]. The basic physical and chemical properties of the tested soil were as follows: pH = 7.42; soil bulk density, 1.48 g/cm^3. Soil bulk density refers to the weight per unit volume of soil in the case of undamaged natural structure, measured by the ring knife method. A ring knife (a cylindrical metal soil collector) with a volume of 100 cm^3 (V) was pressed into a naturally loose soil sample to fill it. The ring knives with soil samples were immediately brought back to the laboratory and then dried in a constant temperature drying oven at 105 °C for 24 h until a constant dry soil weight (m); the value of m/V is the soil bulk density. The soil organic matter was 14.44 g/kg; alkaline hydrolyzable nitrogen, 65.81 mg/kg; and available phosphorus, 5.40 mg/kg.

The biochar used in this experiment was purchased from Anhui Bayerfu Biotechnology Co., Ltd. (Chizhou, Anhui Province, China). The biochar was derived from two materials, namely, rice husk biochar (RH) and wood biochar (W), with a pyrolysis temperature of 500 °C for 40 min. Organic manure was swine manure compost (M), which was bought from Shanghai Shike Biotechnology Co., Ltd. (Shanghai, China) (Table S2). The NPK fertilizer (F) was urea, monoammonium phosphate, and potassium chloride. Biochar-based fertilizer was prepared using the solid–liquid adsorption method, in which NPK fertilizer, pig manure compost, and biochar were mixed in a certain proportion and dissolved in ultra-pure water. The mixture was stirred well and mixed, and let to stand for 24 h. Then, it was dried in an oven at 60 °C for 48 h to a constant weight and sealed in a valve bag for reserve [2]. The proportion of chemical fertilizer, compost, and biochar in biochar-based fertilizer was prepared according to the proportion of each component, as outlined below.

2.2. Experimental Design

Three species of black locust were purchased from the Puding nursery market. They were all annual seedlings with a height of approximately 50 cm: White-flowered locust (W), Hong-sen locust (S), and Large-leaf fast-growing locust (L). Biochar was added according to the mass ratio. The former study of our group showed that the activity of alkaline phosphatase treated by biochar-based fertilizer (RH2MF and RH4MF) with moderate amounts of biochar application was the highest, while the activity of alkaline phosphatase was inhibited by the higher amount of biochar application (RH8MF) [27]. The biomass of black locust treated by rice husk carbon increased firstly and then decreased with the increase in application amount (1%, 2.5%, 5%, 10%), and did best at RH5 [29]. In the medium biochar application rate (2.5%, 5%), the soil structure and water-holding characteristics of lime soil in a karst mountain can be improved [30]. If too much biochar is applied, the cost will be too high and it may damage microbial communities; so, we applied conservative amounts of biochar combined with the effect of manure compost to find a relatively appropriate application amount. The pot experiment consisted of six treatments: the control (CK); compost and NPK fertilizer (MF); 2% rice-husk biochar, compost, and NPK fertilizer (RH2MF); 4% rice husk biochar, compost, and NPK fertilizer (RH4MF); 2% wood biochar, compost, and NPK fertilizer (W2MF); and 4% wood biochar, compost, and NPK fertilizer (W4MF), where the numbers represented the mass ratio of biochar to soil. The fertilizer types and contents of each treatment are listed in Table 1. In January 2017, pots were installed with 9 kg soil for each pot and six repetitions for each treatment. Biochar-based fertilizer was mixed with the soil in each pot. After potting, the fertilizer and soil reacted for one week and then seedlings were planted and regularly watered. In October 2017, seedling samples were collected and brought back to the laboratory. All plant samples were green removed at 105 °C for 20 min, and then dried at 80 °C for 24 h to a constant weight. We used an electric heating constant temperature blast oven for drying the plant samples. In order to make the composition of the samples more uniform and easy to analyze, the dried plant samples were crushed with a high-speed grinder (VRera RS-300, Surui Instrument Co., Ltd., Changzhou, China) for 5 min and mixed evenly. The powder was then sifted through a sieve of 60 mesh (0.25 mm) and then packed in airtight bags and stored in a desiccator for later use. Grinding plant samples to 0.25 mm allowed for a more complete reaction with experimental reagents to obtain more reliable elemental content data. Using rough or complete samples may result in the percentage of elements being lower than the values in the text [31].

Table 1. Types and amounts of fertilizers in different treatments.

Treatments	Urea	Mono-Ammonium Phosphate	Muriate of Potash	Compost	Rice Husk Biochar	Wood Biochar
CK	-	-	-	-	-	-
MF	400	300	200	136	-	-
RH2MF	400	300	200	136	180	-
RH4MF	400	300	200	136	360	-
W2MF	400	300	200	136	-	180
W4MF	400	300	200	136	-	360

Note: The unit of soil pure nutrient content is mg/kg, and the unit of biochar and compost is g/pot. CK: control; MF: compost and NPK fertilizer; RH2MF: 2% rice-husk biochar, compost, and NPK fertilizer; RH4MF: 4% rice-husk biochar, compost, and NPK fertilizer; W2MF: 2% wood biochar, compost, and NPK fertilizer; W4MF: 4% wood biochar, compost, and NPK fertilizer.

2.3. Experimental Indicators and Determination Methods

In this study, the potassium dichromate-sulfuric acid oxidation method was used to determine the carbon content of the samples. We weighed the dried and powdered plant sample to 50 mg and poured it into a hard test tube, slowly adding 10 mL 0.4 mol·L^{-1} potassium double complex acid solution with a pipette, and then adding 10 mL sulfuric acid (98.3%). We shook the test tube to make the soil fully mixed with the solution. Then we put the test tube into the paraffin oil bath at 190 °C, adjusted the power supply to maintain the temperature at 175 °C, and started timing when the solution in the test tube boils. We removed the test tube 5 min later, and used a ferrous sulfate (0.2 mol·L^{-1}) titration system for the rest of the potassium dichromate titration, according to the consumption of oxidant calculation before and after the reaction of the organic carbon content. An elemental analyzer (Elementer, VARIO Macro, Hanau, Germany) was used to determine the total nitrogen (TN), total phosphorus (TP), and total potassium (TK) content. The ash content was determined using the direct ashing method. Firstly, the cleaned crucible was placed in the electric furnace and burned for several times to a constant weight. We put the crucible with 5 g plant samples on the electric furnace for carbonization, and slowly heated in the fume hood until smokeless, and then placed it in a muffle furnace (575 ± 25 °C) for calcination for 4–6 h. After the ashing process was completed (burn until ash was almost white), we waited for the furnace temperature to drop to about 200 °C. Then, the samples were moved into the dryer and weighed after cooling to room temperature, after which the ash content was measured.

The plant calorific values were measured using a microcomputer oxygen bomb calorimeter (IKAC200, German Instrument Science, Baden-Württemberg, Germany). The calorific value of the sample was the dry mass gross calorific value (GCV). The environmental temperature was controlled at approximately 20 °C. Each sample was measured three times, and the error between repetitions was controlled to be ±0.2 kJ/g. Benzoic acid was used as a calibration before each experiment. The ash removal calorific value (AFCV) was calculated using the dry weight calorific value and ash content, as follows:

$$AFCV = GCV/(1 - \text{ash content}). \quad (1)$$

2.4. Data Analysis

In this study, SPSS 22.0 statistical analysis software was used to analyze the measured data of each indicator. Analysis of variance (ANOVA), Duncan multiple comparison, and Pearson correlation analysis were used to test differences between each indicator and conduct correlation analysis. To explore the differences in energy characteristics among the different species, principal component analysis (PCA) was used to express the information of each character with a few comprehensive indicators, and then these comprehensive indicators were used as a new indicator to evaluate the plant energy characteristics.

3. Results

3.1. Element Content

3.1.1. C, N, P, and K Content

The total organic carbon (TOC) content of the branches and leaves of the seedlings under different treatments was studied (Figure 1a); biochar-based fertilizers had a significant effect on TOC ($p < 0.05$). TOC showed the highest value with RH4MF, which increased by 16.7–26.5% compared to that of the CK. The TN, TP, and TK content of the branches and leaves showed remarkable increases with biochar addition. As shown in Figure 1b,d, biochar-based fertilizers have significant impacts on TN and TK ($p < 0.05$); however, the TP difference was not significant (Figure 1c). Except for MF, by adding biochar and compost, leaf TN was higher than that of the CK. Leaf TN was above 2% with the RH4MF, W2MF, and W4MF treatments. Leaf TK in each treatment was significantly higher than that of the CK ($p < 0.01$). Fertilization did not increase the TP content in seedling branches.

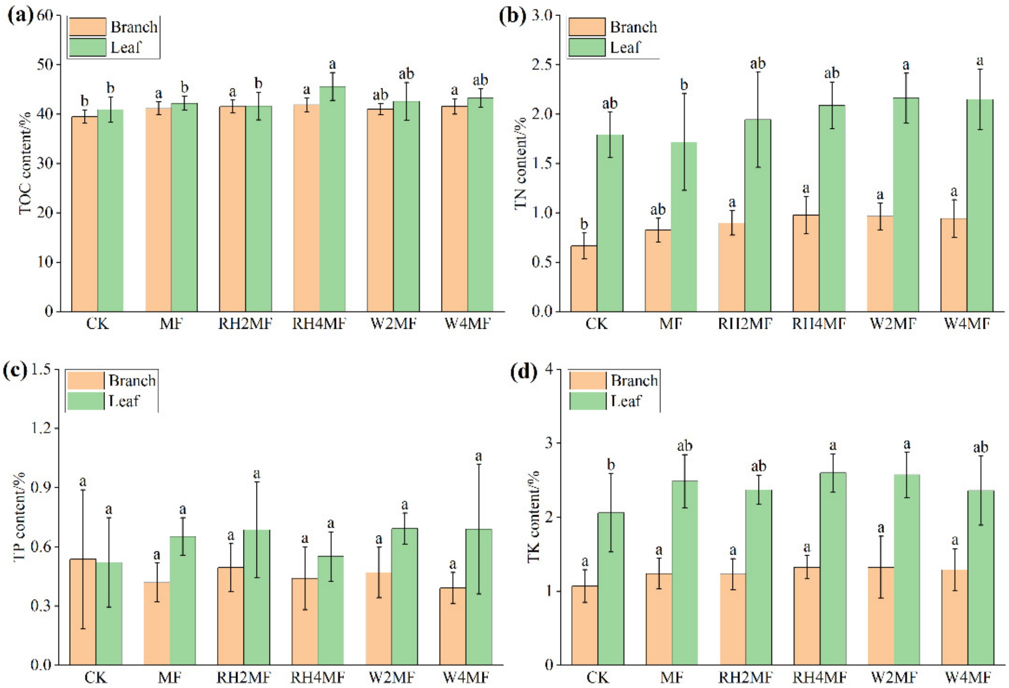

Figure 1. Effects of biochar-based fertilizers on the C, N, P, and K element content of branches and leaves. Note: (**a**): the TOC content of branches and leaves in different fertilization treatments; (**b**): the TN content of branches and leaves in different fertilization treatments; (**c**): the TP content of branches and leaves in different fertilization treatments; (**d**): the TK content of branches and leaves in different fertilization treatments. Similar letters indicate no significant difference ($p > 0.05$), while different letters indicate a significant difference ($p < 0.05$). The data are the average ± standard deviation. CK: control; MF: compost and NPK fertilizer; RH2MF: 2% rice-husk biochar, compost, and NPK fertilizer; RH4MF: 4% rice-husk biochar, compost, and NPK fertilizer; W2MF: 2% wood biochar, compost, and NPK fertilizer; W4MF: 4% wood biochar, compost, and NPK fertilizer; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus; and TK: total potassium.

Figure 2 demonstrates that different species have a different element content in branches and leaves. There was no significant difference in branch TOC (Figure 2a), but leaf TOC was significantly different ($p < 0.05$). In the three different seedling species, the maximum values of leaf TOC appeared in L, while the branch TOC in S was the highest, significantly higher than that of W ($p < 0.01$). Biochar addition effectively improved the carbon content of all three species. Notable effects on TP and TK content were observed among all species (Figure 2c,d), but the effect on TN was not significant (Figure 2b). Species L had the highest value of branch TN, which was significantly higher than that of W and S

($p < 0.01$). For branch TP, species L also had the maximum value, which was 0.65%, and was significantly higher than that of W and S ($p < 0.001$). In contrast, leaf TP in L and S was significantly higher than that of W ($p < 0.05$), and species S had the highest leaf TP content. Branch TK in W and S was significantly higher than that of L ($p < 0.001$), and leaf TK in W was significantly higher than that of S and L ($p < 0.001$).

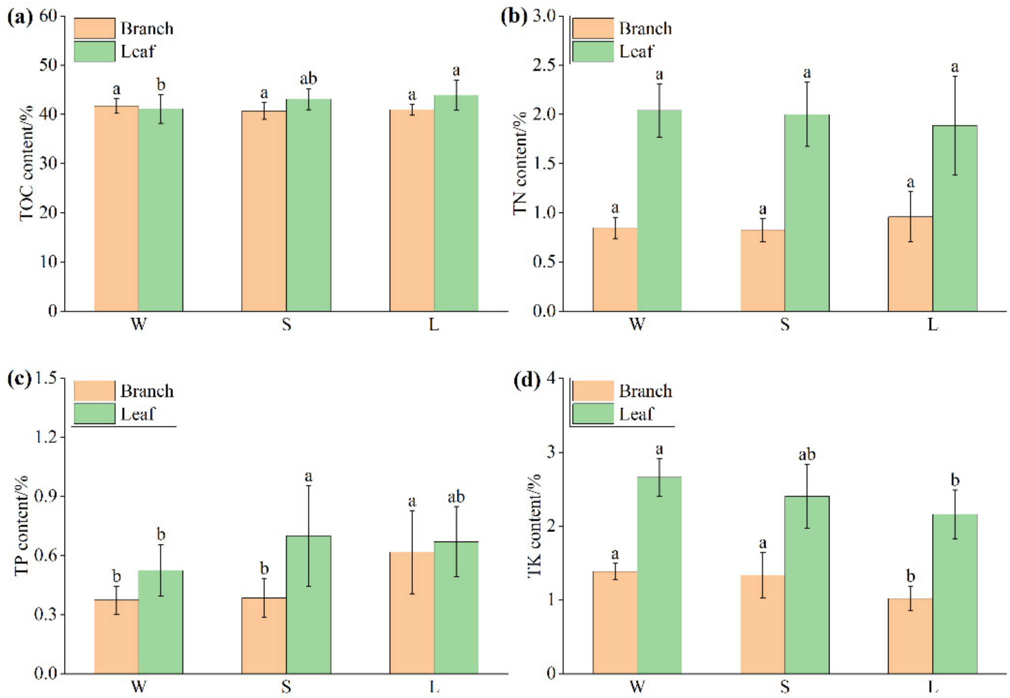

Figure 2. C, N, P, and K element content of branches and leaves in different black locust species. Note: (**a**): the TOC content of branches and leaves in different species; (**b**): the TN content of branches and leaves in different species; (**c**): the TP content of branches and leaves in different species; (**d**): the TK content of branches and leaves in different species. Similar letters indicate no significant difference ($p > 0.05$), while different letters indicate a significant difference ($p < 0.05$). The data are the average ± standard deviation. TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium; W: White-flowered locust; S: Hong-sen locust; and L: Large-leaf fast-growing locust.

3.1.2. Ash Content

Differences in ash content were not significant among the different biochar treatments (Table 2). Adding biochar-based fertilizers decreased the ash content by 1.6–12.1% (branches) and 3.0–14.3% (leaves) compared to that of the CK. The ash content in branches and leaves showed the lowest value with the WH4MF treatment, and the leaf ash content was significantly lower than that of the CK ($p < 0.05$). However, there were significant differences between different species ($p < 0.001$). Species L had the lowest branch ash content and species S had the lowest leaf ash content.

Table 2. Ash content of the different fertilizer treatments and different black locust species.

Treatments	Ash Content (Branch)/%	Ash Content (Leaf)/%
CK	5.13 ± 1.17 a	9.24 ± 1.26 a
MF	5.05 ± 0.52 a	8.52 ± 1.08 a
RH2MF	4.72 ± 0.66 a	8.97 ± 1.48 a
RH4MF	4.51 ± 0.37 a	7.92 ± 0.72 a
W2MF	4.89 ± 0.58 a	8.32 ± 1.54 a
W4MF	4.67 ± 0.76 a	8.31 ± 1.46 a
W	5.32 ± 0.63 a	9.71 ± 1.26 a
S	4.93 ± 0.63 a	7.75 ± 0.61 b
L	4.24 ± 0.44 b	8.18 ± 1.05 b

Note: Similar letters indicate no significant difference ($p > 0.05$), while different letters indicate a significant difference ($p < 0.05$). The data are the average ± standard deviation. CK: control; MF: compost and NPK fertilizer; RH2MF: 2% rice-husk biochar, compost, and NPK fertilizer; RH4MF: 4% rice-husk biochar, compost, and NPK fertilizer; W2MF: 2% wood biochar, compost, and NPK fertilizer; W4MF: 4% wood biochar, compost, and NPK fertilizer; W: White-flowered locust; S: Hong-sen locust; and L: Large-leaf fast-growing locust.

3.2. Calorific Value

The calorific value of the seedling branches and leaves under the six treatments was studied (Table 3). Biochar-based fertilizers effectively increased the calorific value of the black locust seedlings and had a significant effect on the GCV and AFCV of branches ($p < 0.05$), but had no significant effect on leaves. The branch GCV and AFCV with the RH4MF and W4MF treatments were significantly higher than that of the CK ($p < 0.05$), which were 24.79% and 24.06% and 17.11% and 16.64% higher, respectively. In addition, the GCV and AFCV of the leaves in all treatments were larger than those of the branches. Both the GCV and AFCV of the leaves were the highest with the RH4MF treatment, which were 18.93 kJ·g^{-1} and 20.55 kJ·g^{-1}, respectively, and were 16.01% and 14.48% higher than that of the CK.

Table 3. Calorific values of the different fertilizer treatments and different black locust species.

Treatments	GCV-Branch	GCV-Leaf	AFCV-Branch	AFCV-Leaf
CK	14.20 ± 1.27 b	16.32 ± 3.54 a	14.97 ± 1.31 b	17.96 ± 3.81 a
MF	16.44 ± 1.83 ab	17.33 ± 1.43 a	17.32 ± 1.98 ab	18.95 ± 1.60 a
RH2MF	15.78 ± 1.27 ab	16.94 ± 2.64 a	16.56 ± 1.31 ab	18.59 ± 2.77 a
RH4MF	17.73 ± 2.63 a	18.93 ± 2.67 a	18.57 ± 2.77 a	20.55 ± 2.88 a
W2MF	15.52 ± 2.33 ab	17.07 ± 1.92 a	16.31 ± 2.44 ab	18.61 ± 2.02 a
W4MF	16.64 ± 2.53 a	18.77 ± 2.95 a	17.45 ± 2.65 ab	20.44 ± 3.02 a

Note: units of added amounts of each ingredient are kJ/g. Similar letters indicate no significant difference ($p > 0.05$), while different letters indicate a significant difference ($p < 0.05$). GCV: gross calorific value; AFCV: ash removal calorific value; CK: control; MF: compost and NPK fertilizer; RH2MF: 2% rice-husk biochar, compost, and NPK fertilizer; RH4MF: 4% rice-husk biochar, compost, and NPK fertilizer; W2MF: 2% wood biochar, compost, and NPK fertilizer; W4MF: 4% wood biochar, compost, and NPK fertilizer.

Results of the variance analysis and multiple comparisons showed that the GCV and AFCV of the three species were significantly different ($p < 0.05$, Table 3). For branch GCV and AFCV, the values in S were 17.17 kJ·g^{-1} and 18.05 kJ·g^{-1}, respectively, which were the highest values. Species branch calorific values from high to low were S > L > W. However, the calorific values of leaves were different from those of the branches. In terms of calorific value of leaves, both GCV and AFCV were the highest values in L, and the species order from high to low was L > S > W. The seedlings with the largest total calorific values were S, and W had a relative disadvantage in terms of calorific value.

3.3. Biomass

The biomass of black locust seedlings under each treatment is listed in Figure 3. RH4MF treatment showed the highest value and a significant difference in seedling biomass ($p < 0.05$), which was 11.21% higher than that of the CK. The biomass with different treatments, in descending order, was RH4MF > MF > W4MF > RH2MF > CK > W2MF (Figure 3a). One-way ANOVA showed that there were significant differences in seedling biomass among the different black locust species ($p < 0.05$), and the biomass of S had the highest values. The biomass of the different black locust species, in descending order, was S > W > L (Figure 3b).

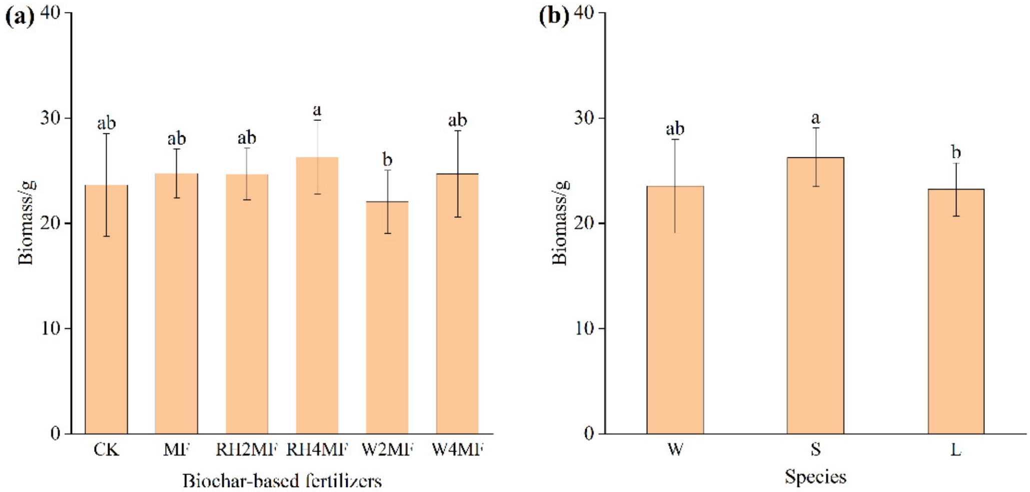

Figure 3. Effects of different biochar-based fertilizers and black locust species on seedling biomass. Note: (**a**): seedling biomass of branches and leaves in different fertilization treatments; (**b**): seedling biomass of branches and leaves in different species. Similar letters indicate no significant difference ($p > 0.05$), while different letters indicate a significant difference ($p < 0.05$). The data are the average ± standard deviation. CK: control; MF: compost and NPK fertilizer; RH2MF: 2% rice-husk biochar, compost, and NPK fertilizer; RH4MF: 4% rice-husk biochar, compost, and NPK fertilizer; W2MF: 2% wood biochar, compost, and NPK fertilizer; W4MF: 4% wood biochar, compost, and NPK fertilizer; W: White-flowered locust; S: Hong-sen locust; and L: Large-leaf fast-growing locust.

3.4. Correlation Analysis of Indicators

Correlation analysis was performed on the relationship between the branch and leaf element content, calorific values, and biomass of black locust seedlings. The results are shown in Figure 4. There was a significant positive correlation between GCV and AFCV ($p < 0.001$). A significant positive correlation appeared between biomass and GCV ($p < 0.05$) and branch biomass and AFCV ($p < 0.05$). Ash content showed different results; it was negatively correlated with calorific value, and there was a significant negative correlation between the leaf ash content and calorific values ($p < 0.01$). There was a significant positive correlation between branch TOC and branch calorific values ($p < 0.05$), and a significant positive correlation between leaf TOC and leaf calorific values ($p < 0.01$). The correlation between the TN, TP, and TK content of branches and leaves and calorific values was not significant.

3.5. PCA Analysis and Comprehensive Evaluation

Since there were many traits included in this study, and there was a certain correlation between each trait, to make the analysis simple and accurate, the PCA analysis method was used, which simplified the original high-dimensional variables and preserved the original data information to the greatest extent. Using fewer comprehensive indicators expressed the information of each trait, and then each principal component score was used as a new indicator for evaluating energy characteristics of different treatments for cluster analysis. In this study, TOC (X_1), TN (X_2), TP (X_3), TK (X_4), ash (X_5), GCV (X_6), AFCV (X_7), and biomass (X_8) were used as the PCA variables. To exclude the differences in the dimension and magnitude of the indicators and ensure the objectivity of the results, the original data were first standardized, and then the PCA was performed.

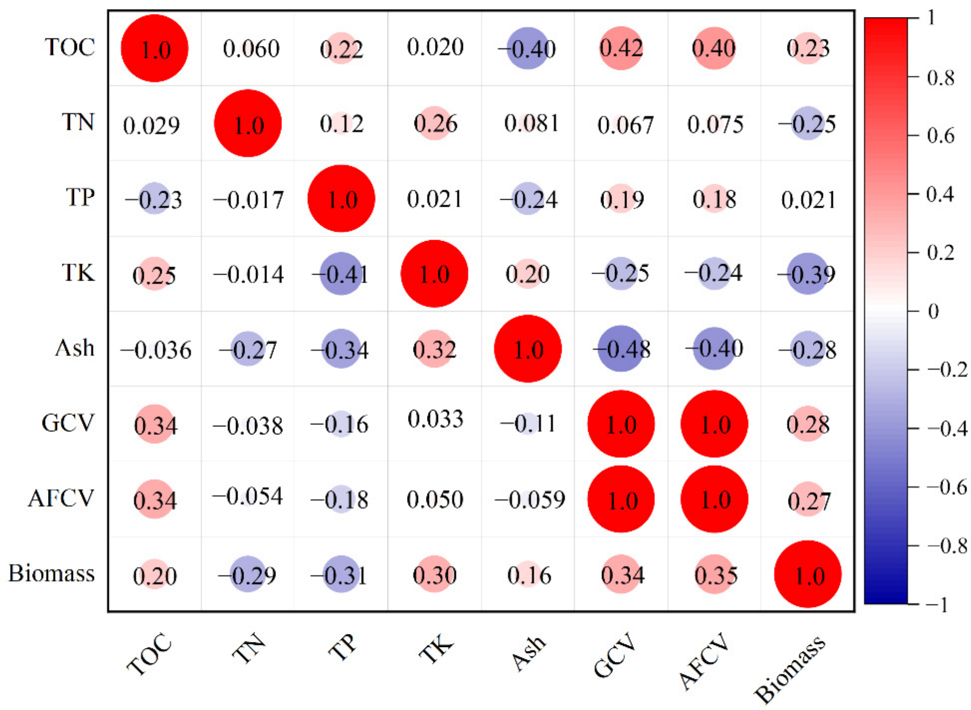

Figure 4. Correlation analysis of the element content, calorific values, and biomass of black locust seedlings. Note: Red circles indicate a positive correlation and blue circles indicate a negative correlation. The larger the circle diameter, the stronger the correlation between the two variables. The lower-left section of the table is the correlation analysis data of the element content of black locust branches and calorific values. The upper-right section of the table is the correlation analysis data of the element content of black locust leaves and calorific values. TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium; GCV: gross calorific value; AFCV: ash removal calorific value.

The eigenvalues and contribution rates of each principal component obtained through the analysis are shown in Table 4. The cumulative variance contribution rate of the extracted three principal components (eigenvalues more than 1) was 82.365%, which meant that these three principal components contained the most information of the original data. Therefore, it was feasible to use these three principal components as a comprehensive index for evaluating the energy characteristics of black locust seedlings. The principal component was the linear combination of the normalized original indexes and the eigenvectors. The eigenvectors represented the importance of the original indexes to the principal components; that is, the load value of the principal component corresponding to each index was divided by the square root of the eigenvalue corresponding to the principal component (Table S1). It can be seen from the table that TOC (X1) and TN (X2) are the main influencing factors of the first principal component; that is, when the X1 and X2 scores are high, the score value of the first principal component is large, so Y1 is called the element factor. The second principal component was ash content (X5), GCV (X6), and AFCV (X7)—as the main influencing factors—and Y2 was called the calorific value factor. The third principal component was biomass (X8)—as the main factor—and Y3 as the growth factor. According to the principal component calculation formula, the linear combination of the three principal components and the original eight indicators was obtained:

$$Y1 = 0.478\,X1 + 0.315\,X2 + 0.212\,X3 + 0.008\,X4 - 0.451\,X5 + 0.403\,X6 + 0.486\,X7 + 0.156\,X8, \quad (2)$$

$$Y2 = 0.085\,X1 + 0.471\,X2 - 0.402\,X3 + 0.577\,X4 + 0.260\,X5 + 0.304\,X6 - 0.134\,X7 - 0.312\,X8, \quad (3)$$

$$Y3 = 0.144\,X1 - 0.258\,X2 - 0.441\,X3 + 0.344\,X4 + 0.142\,X5 - 0.170\,X6 + 0.263\,X7 + 0.694\,X8. \quad (4)$$

Table 4. Eigenvalues and variance contribution rates of the principal component analysis.

Principal Component	Initial Eigenvalues			Extract Sum of Squares and Load		
	Eigenvalues	Contribution Rate %	Cumulative %	Eigenvalues	Contribution Rate %	Cumulative %
1	3.448	43.102	43.102	3.448	43.102	43.102
2	1.891	23.632	66.734	1.891	23.632	66.734
3	1.251	15.631	82.365	1.251	15.631	82.365
4	0.513	6.408	88.774			
5	0.386	4.823	93.596			
6	0.259	3.235	96.831			
7	0.174	2.176	99.007			
8	0.079	0.993	100			

Substituting the standardized raw data into the above equations, the scores of each principal component of the three species of black locust seedlings were obtained under different treatments. As shown in Table 5, the comprehensive score is the score of each principal component and its corresponding contribution rate is the sum of the products; that is, $Y = 0.431 \times Y1 + 0.236 \times Y2 + 0.156 \times Y3$ (this correlation is strictly valid for the tested conditions only). Thus, RH4MF-S had the highest score, with positive scores on the three new principal components, and the best comprehensive performance. In addition, according to the ranking, the 4% biochar fertilization treatment was the best application amount and the application of biochar-based fertilizer was more effective than the single application of compost. Additionally, the effect of rice husk biochar was better than that of wood biochar. Among the three black locust species, S had the best performance, followed by L, and W had slightly less performance than the other two species. According to the CPA results, for field applications, more attention to plant organic carbon content, calorific values and biomass can help us effectively improve the energy characteristics of black locust seedlings.

Table 5. Composite scores and rankings of various principal components of different species of black locust.

Treatments	Y1	Y2	Y3	Y	Ranking
CK-W	−4.62	1.97	−0.79	−1.65	18
CK-S	−1.52	−2.45	0.76	−1.11	16
CK-L	−1.43	−3.01	−0.94	−1.47	17
MF-W	−1.02	0.85	0.61	−0.14	10
MF-S	−0.48	−0.6	0.68	−0.24	13
MF-L	−0.66	−2.04	0.4	−0.7	15
RH2MF-W	−1.77	0.61	0.3	−0.57	14
RH2MF-S	−0.39	−0.41	0.81	−0.14	11
RH2MF-L	0.95	−1.02	−1.57	−0.08	9
RH4MF-W	0.39	0.86	1.42	0.59	5
RH4MF-S	2.58	0.1	2.11	1.46	1
RH4MF-L	2.68	0.29	−0.32	1.17	2
W2MF-W	−0.94	1.66	−0.79	−0.14	12
W2MF-S	0.61	0.82	−0.31	0.41	7
W2MF-L	1.33	0.62	−2.02	0.4	8
W4MF-W	−0.12	1.24	1.27	0.44	6
W4MF-S	2.56	0.41	−0.21	1.17	3
W4MF-L	1.85	0.11	−1.4	0.6	4

Note: CK: control; MF: compost and NPK fertilizer; RH2MF: 2% rice-husk biochar, compost, and NPK fertilizer; RH4MF: 4% rice-husk biochar, compost, and NPK fertilizer; W2MF: 2% wood biochar, compost, and NPK fertilizer; W4MF: 4% wood biochar, compost, and NPK fertilizer; W: White-flowered locust; S: Hong-sen locust; and L: Large-leaf fast-growing locust.

4. Discussion

Biomass is an important indicator to measure the level of plant energy storage and productivity [32,33]. Biochar-based fertilizers had no significant effect on black locust biomass, which was inconsistent with a previous study from our research group [29]. This may be because biochar has an adsorption effect; the fertilizer effect is released slowly, and the effect on plant growth is not very significant in the short term [34]. In addition, the plant growth status is also related to site and water conditions, and the dependence on fertilizers in the early stage of branch growth is not strong [35]. There were significant differences in biomass among different species, which was related to the specific characteristics of each species. Relevant studies have shown that the large-leaf fast-growing locust (L) has thick branches and plump leaves, but early harvest time leads to low yield [36]; in turn, Hong-sen locust (S) branches are round and grow rapidly in a short time, and the annual growth height may reach 3.8 m [37,38], which is consistent with the results of this study.

Carbon is the most important component in plants, accounting for approximately 40% of the dry weight [39]. The N, P, and K content is an important nutrient component in plants and an important indicator that affects plant growth [40–42]. The results showed that biochar-based fertilizer had a significant effect on the TOC, TN, and TK contents in black locust, but not on the TP content. Biochar is produced from carbon-rich biomass materials; it can improve and fertilize soil, increase soil carbon absorption [43], the soil organic carbon content, and the C/N ratio [44]; improve the soil absorption capacity of other nutrient elements; and finally improve the soil fertility level [45]. The direct effect of biochar-based fertilizer on carbon content is that a large amount of carbon is brought into the soil through the application of biochar-based fertilizer, which increases the carbon content in the soil and promotes the absorption of carbon by plants, thereby increasing the carbon content of plants. The indirect effect was to increase the carbon content by affecting plant biomass [46], which was positively correlated with organic carbon (Figure 4).

Some studies have shown that carbon combustion releases substantial heat, so it is positively correlated with the calorific value; these results are consistent with most relative research [47]. There is a negative correlation between the N and P content of locust branches and the calorific values, which is consistent with the results of Gao et al. [48]. The N content in the leaves was positively correlated with its calorific value. This is because most of the N accumulates in the leaves for photosynthesis to meet the needs of growth and development, so more energy is allocated to the leaves [49]. In addition, photosynthesis promotes plants to absorb more carbon, thereby indirectly increasing the calorific value of leaves [50]. Ash content is the non-combustible component left after the plant is burned; the more mineral elements in the plant, the higher the ash content [51]. Some studies have shown that compared to white flower locust (W), the ash content of large-leaf fast-growing black locust (L) is lower and the nutrients are higher [36], which is similar to the results of this study. In addition, compared to the ash content of branches and leaves of other varieties of black locust [52], the average ash content of black locust in this study was higher (4.24–9.71%), because fertilization increases the mineral element content in the soil, thereby increasing the ash content. The ash content is negatively correlated with the calorific value (Figure 4), because the ash content is not combustible, and the higher the ash content, the smaller the calorific value, a trend that is consistent with most studies [53,54].

According to the above PCA, the calorific value had a great influence on the energy characteristics of plants. The GCV and AFCV of black locust were significantly different among various species, and the calorific value of Hong-sen locust (S) was the highest. Biochar-based fertilizer had a significant effect on seedling GCV and AFCV, and the calorific value was highest with the RH4MF treatment. The effect of biochar-based fertilizer on the calorific value of plants indirectly affects the calorific value by affecting the element content in the plant; the application of biochar to the soil increases the content of soil organic carbon, thereby increasing the calorific value [55].

5. Conclusions

In summary, among the three species of black locust seedlings, the element content, growth, and calorific values of Hong-sen locust were better than those of the other two species, reflecting its fast-growing and high-quality growth characteristics and good growth potential. Thus, Hong-sen locust may be used as a suitable tree species for a karst energy forest. The biochar-based fertilizer treatment mixed with compost and chemical fertilizer under the application rate of 4% rice husk biochar (RH4MF) had the best improvement effect. Compared to wood biochar, the application of rice husk biochar better promoted the growth of seedlings and increased the calorific values. The development and utilization of biomass energy have great development potential in China. This study used different species of black locust seedlings to understand the characteristics of their growth, element content, and calorific values under different biochar-based fertilizer treatments, and explored their role as a feasible and suitable energy tree species, evaluating the characteristics of various sources of energy under different treatments. Thus, we propose the Hong-sen locust as a high-quality provenance for cultivating karst energy forests in Guizhou, using 4% rice husk biochar-based fertilizer as a more appropriate application rate. At the same time of the pot experiment, we also carried out a field experiment of similar treatments in the karst mountain area of Guizhou (from 2016 to 2018). By analyzing the soil nutrient content and soil microbial community characteristics and other indicators, combined with a pot experiment, we found that the 4% biochar + MF treatment can play an important role in local soil health and vegetation restoration [4,28,56,57]. These data provide a scientific and theoretical basis for the development of biomass energy in karst areas of China. This research was based on a small-scale pot experiment and screened out the suitable species of black locust. In the future, we should start research on a large-scale, such as optimal allocation of energy forest stand structure and sustainable harvesting, and carry out research on the transformation and utilization technology of bio-energy forest products, prepare and produce corresponding biomass energy products, realize the transformation from an ecological function chain to ecological industry chain, and finally alleviate the problem of energy shortage in karst areas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14095045/s1, Table S1: Initial factor loading matrix and eigenvectors. Table S2: The main properties of biochar and compost. Table S3: Effects of biochar-based fertilizers and species on soil nutrient contents.

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References

1. Yan, Y.; Dai, Q.; Yuan, Y.; Peng, X.; Zhao, L.; Yang, J. Effects of rainfall intensity on runoff and sediment yields on bare slopes in a karst area, SW China. *Geoderma* **2018**, *330*, 30–40. [CrossRef]
2. Zhidong, Z.; Ting, G.; Lukas, V.Z.; Qian, Z.; Taotao, Y.; Jianhui, X.; Yongbo, W. Soil Microbial Community Structure Shifts Induced by Biochar and Biochar-Based Fertilizer Amendment to Karst Calcareous Soil. *Soil Sci. Soc. Am. J.* **2019**, *83*, 398–408.
3. Liu, S.; Zhang, W.; Wang, K.; Pan, F.; Yang, S.; Shu, S. Factors controlling accumulation of soil organic carbon along vegetation succession in a typical karst region in Southwest China. *Sci. Total Environ.* **2015**, *521–522*, 52–58. [CrossRef]
4. Yan, T.; Xue, J.; Zhou, Z.; Wu, Y. Biochar-based fertilizer amendments improve the soil microbial community structure in a karst mountainous area. *Sci. Total Environ.* **2021**, *794*, 148757. [CrossRef] [PubMed]

5. Bin, Y.; Kangning, X.; Qi, W.; Qiming, W. Can agricultural biomass energy provide an alternative energy source for karst rocky desertification areas in Southwestern China? investigating Guizhou Province as example. *Environ. Sci. Pollut. Res.* **2021**, *28*, 44315–44331.
6. Dejan, M.; Zoran, S.; Veselin, D.; Ana, V.; Nevena, S. Application of renewable energy sources along motorway infrastructures on high karst plateaus: West Serbia case study. *Environ. Earth Sci.* **2016**, *75*, 859.
7. Aslan, A. The causal relationship between biomass energy use and economic growth in the United States. *Renew. Sustain. Energy Rev.* **2016**, *57*, 362–366. [CrossRef]
8. Feng, L.S.; Wei, W.W.; Zhong, J.W. Gaming Analysis among Different Participants in the Construction of Biomass Energy Forest Base. *Adv. Mater. Res.* **2012**, *512*, 416–420.
9. Kumar, R.; Pandey, K.K.; Chandrashekar, N.; Mohan, S. Study of age and height wise variability on calorific value and other fuel properties of *Eucalyptus hybrid*, *Acacia auriculaeformis* and *Casuarina equisetifolia*. *Biomass Bioenergy* **2011**, *35*, 1339–1344. [CrossRef]
10. Cha, D.S.; Ji, B. Estimating the heating value of major coniferous trees by moisture content. *Korean J. Agric. Sci.* **2011**, *38*, 619–624.
11. Yokoyama, S.; Adachi, Y. Seasonal variations in organic materials and calorific values of Japanese cedar and cypress leaves. *J. Mater. Cycles Waste* **2017**, *19*, 592–597. [CrossRef]
12. Song, G.; Hou, J.; Li, Y.; Zhang, J.; He, N. Leaf Caloric Value from Tropical to Cold-Temperate Forests: Latitudinal Patterns and Linkage to Productivity. *PLoS ONE* **2016**, *11*, e157935. [CrossRef] [PubMed]
13. Golley, F.B. Caloric Value of Wet Tropical Forest Vegetation. *Ecology* **1969**, *50*, 517–519. [CrossRef]
14. Kraszkiewicz, A. Productivity of Black Locust (*Robinia pseudoacacia* L.) Grown on a Varying Habitats in Southeastern Poland. *Forests* **2021**, *12*, 470. [CrossRef]
15. Zhu, X.Q.; Wang, C.Y.; Chen, H.; Tang, M. Effects of arbuscular mycorrhizal fungi on photosynthesis, carbon content, and calorific value of black locust seedlings. *Photosynthetica* **2014**, *52*, 247–252. [CrossRef]
16. 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]
17. Ogura, A.P.; Lima, J.Z.; Marques, J.P.; Sousa, L.M.; Silvestre Rodrigues, V.G.; Gaeta Espindola, E.L. A review of pesticides sorption in biochar from maize, rice, and wheat residues: Current status and challenges for soil application. *J. Environ. Manag.* **2021**, *300*, 113753. [CrossRef]
18. Brtnicky, M.; Datta, R.; Holatko, J.; Bielska, L.; Gusiatin, Z.M.; Kucerik, J.; Hammerschmiedt, T.; Danish, S.; Radziemska, M.; Mravcova, L.; et al. A critical review of the possible adverse effects of biochar in the soil environment. *Sci. Total Environ.* **2021**, *796*, 148756. [CrossRef]
19. Ji, M.; Wang, X.; Usman, M.; Liu, F.; Dan, Y.; Zhou, L.; Campanaro, S.; Luo, G.; Sang, W. Effects of different feedstocks-based biochar on soil remediation: A review. *Environ. Pollut.* **1800**, *294*, 118655. [CrossRef]
20. Cheng, C.; Lehmann, J.; Thies, J.E.; Burton, S.D.; Engelhard, M.H. Oxidation of black carbon by biotic and abiotic processes. *Org. Geochem.* **2006**, *37*, 1477–1488. [CrossRef]
21. Hanzhi, Z.; Yuan, H.; Gang, L.; Yanping, X.; Jinshan, L.; Qicheng, B.; Xingwu, L.; Jianguo, Z.; Zubin, X. Effects of biochar on corn growth, nutrient uptake and soil chemical properties in seeding stage. *Ecol. Environ. Sci.* **2010**, *19*, 2713–2717.
22. Liu, M.; Lai, Y.; Li, W.; Xiao, J.; Bi, Y.; Liu, M.; Li, W. Effect of Biochar and Nitrogen Application Rate on Growth Development and Yield of Soybean. *Soybean Sci.* **2015**, *34*, 87–92.
23. Chen, Z.; Pei, J.; Wei, Z.; Ruan, X.; Hua, Y.; Xu, W.; Zhang, C.; Liu, T.; Guo, Y. A novel maize biochar-based compound fertilizer for immobilizing cadmium and improving soil quality and maize growth. *Environ. Pollut.* **2021**, *277*, 116455. [CrossRef] [PubMed]
24. You, X.; Yin, S.; Suo, F.; Xu, Z.; Chu, D.; Kong, Q.; Zhang, C.; Li, Y.; Liu, L. Biochar and fertilizer improved the growth and quality of the ice plant (*Mesembryanthemum crystallinum* L.) shoots in a coastal soil of Yellow River Delta, China. *Sci. Total Environ.* **2021**, *775*, 144893. [CrossRef]
25. Thomas, F.D.; Jeffrey, M.N.; Gilbert, C.S.; James, A.I.; Hannah, C.R.; Donald, W.W.; Kristin, M.T.; Kurt, A.S.; Kenneth, C.S.; Mark, G.J. Microbial response to designer biochar and compost treatments for mining impacted soils. *Biochar* **2021**, *3*, 299–314.
26. Birk, E.M.; Turner, J. Response of flooded gum (*E. grandis*) to intensive cultural treatments: Biomass and nutrient content of eucalypt plantations and native forests. *For. Ecol. Manag.* **1992**, *47*, 1–28. [CrossRef]
27. Qian, Z.; Zhidong, Z.; Yi, S.; Yongbo, W.; Jianhui, X. Effects of biochar-based fertilizer on phosphorus content of karst calcareous soil. *Acta Ecol. Sin.* **2018**, *38*, 4037–4044.
28. Yan, T.; Xue, J.; Zhou, Z.; Wu, Y. Impacts of biochar-based fertilization on soil arbuscular mycorrhizal fungal community structure in a karst mountainous area. *Environ. Sci. Pollut. Res.* **2021**, *28*, 66420–66434. [CrossRef]
29. Sun, J.; Bu, X.; Wu, Y.; Xu, J. Effects of biochar application on the growth of *Robinia pseudoacacia* L. seedlings and soil properties in limestone soil in a karst mountain site. *Chin. J. Ecol.* **2016**, *35*, 3250–3257.
30. Qian, Z.; Taotao, Y.; Zhidong, Z.; Jiaman, S.; Jianhui, X.; Yongbo, W. Effects of biochar application on soil properties of limestone soil in karst and growth of *Robinia pseudoacacia* seedlings. *Jiangsu Agric. Sci.* **2018**, *46*, 241–245.
31. Zhidong, Z.; Ting, G.; Qian, Z.; Taotao, Y.; Dongchang, L.; Jianhui, X.; Yongbo, W. Increases in bacterial community network complexity induced by biochar-based fertilizer amendments to karst calcareous soil. *Geoderma* **2019**, *337*, 691–700.
32. Wu, S.; Zhu, Y.; Xu, J.; Lu, Z.; Chen, G.; Song, P.; Guo, W. Genetic variation and genetic gain for energy production, growth traits and wood properties in *Eucalyptus hybrid* clones in China. *Aust. For.* **2017**, *80*, 57–65. [CrossRef]

33. Illman, A.M.; Scragg, A.H.; Shales, S.W. Increase in Chlorella strains calorific values when grown in low nitrogen medium. *Enzym. Microb. Technol.* **2000**, *27*, 631–635. [CrossRef]
34. Zhao, Z.; Wu, Q.; Nie, T.; Zhou, W. Quantitative evaluation of relationships between adsorption and partition of atrazine in biochar-amended soils with biochar characteristics. *Rsc Adv.* **2019**, *9*, 4162–4171. [CrossRef]
35. Gu, L.; He, Y.; Li, F. Effects of different water and fertilizer management on the growth and calorific value of alder sprouts in Sichuan and Yunnan. *J. Yunnan Agric. Univ.* **2011**, *26*, 683–688.
36. Chang, L. A comparative experiment on the growth of tetraploid *Robinia pseudoacacia* and common *Robinia pseudoacacia*. *Gansu Agric.* **2005**, *4*. [CrossRef]
37. Hou, J.; Wang, T.; Liu, Z. Creation technology of fast-growing and high-yield forest of *Hongsen locust*. *South. Agric.* **2014**, *8*, 78–80.
38. Hou, J.; Wang, T. Excellent clone of locust tree—*Hongsen locust*. *Mod. Hortic.* **2014**, *22*. [CrossRef]
39. Armecin, R.B.; Gabon, F.M. Biomass, organic carbon and mineral matter contents of abaca (*Musa textilis* Nee) at different stages of growth. *Ind. Crop. Prod.* **2008**, *28*, 340–345. [CrossRef]
40. Jeong, K.S.; Seo, D.; Roy, S.K.; Moon-soon, L.; Boo, H.; Woo, S.; Hyun, K.H. Effects of N, P and K Fertilizers Application on Growth, Yield and Inorganic Components Content in *Codonopsis lanceolata*. *J. Korean Soc. Int. Agric.* **2016**, *28*, 379–384.
41. Lasheen, F.E.; Negm, A.H.; Hassan, S.E.; Azab, E.; Gobouri, A.A.; Hewidy, M. Nitrogen, Phosphorous, and Potassium Application Rate on the Young Seedling Growth of *Salvadora persica*. *Agriculture* **2021**, *11*, 291. [CrossRef]
42. Marx Young, J.L.; Kanashiro, S.; Jocys, T.; Tavares, A.R. Silver vase bromeliad: Plant growth and mineral nutrition under macronutrients omission. *Sci. Hortic.-Amst.* **2018**, *234*, 318–322. [CrossRef]
43. Liang, B.; Lehmann, J.; Solomon, D.; Kinyangi, J.; Grossman, J.; O'Neill, B.; Skjemstad, J.O.; Thies, J.; Luizão, F.J.; Petersen, J.; et al. Black Carbon Increases Cation Exchange Capacity in Soils. *Soil Sci. Soc. Am. J.* **2006**, *70*, 1719–1730. [CrossRef]
44. Xu, C.; Hosseini-Bai, S.; Hao, Y.; Rachaputi, R.C.N.; Wang, H.; Xu, Z.; Wallace, H. Effect of biochar amendment on yield and photosynthesis of peanut on two types of soils. *Environ. Sci. Pollut. Res.* **2015**, *22*, 6112–6125. [CrossRef] [PubMed]
45. Novak, J.M.; Busscher, W.J.; Laird, D.L.; Ahmedna, M.; Watts, D.W.; Niandou, M.A.S. Impact of Biochar Amendment on Fertility of a Southeastern Coastal Plain Soil. *Soil Sci.* **2009**, *174*, 105–112. [CrossRef]
46. Balima, L.H.; Nacoulma, B.M.I.; Bayen, P.; Dimobe, K.; Kouame, F.N.; Thiombiano, A. Aboveground biomass allometric equations and distribution of carbon stocks of the African oak (*Afzelia africanaSm*) in Burkina Faso. *J. For. Res.* **2020**, *31*, 1699–1711. [CrossRef]
47. Adamovics, A.; Platace, R.; Kakitis, A.; Ivanovs, S. Evaluation of Combustion Properties of Biomass Fuel. In Proceedings of the 18th International Scientific Conference Engineering for Rural Development, Jelgava, Latvia, 22–24 May 2019; pp. 1523–1528.
48. Gao, K.; Zhu, T.; Xu, S.; Han, G. Effects of different habitat conditions on calorific value, C, N and ash content of *Jerusalem artichoke* tubers. *Crop Mag.* **2011**, *2*, 17–19. [CrossRef]
49. Zhu, D.; Zhu, G.; Zhang, Z.; Wang, Z.; Yan, X.; Yan, Y. Effects of Independent and Combined Water-Deficit and High-Nitrogen Treatments on Flag Leaf Proteomes during Wheat Grain Development. *Int. J. Mol. Sci.* **2020**, *21*, 2098. [CrossRef]
50. Oh, K.C.; Kim, J.; Park, S.Y.; Kim, S.J.; Cho, L.H.; Lee, C.G.; Roh, J.; Kim, D.H. Development and validation of torrefaction optimization model applied element content prediction of biomass. *Energy* **2021**, *214*, 119027. [CrossRef]
51. Platace, R.; Adamovics, A. Content of ligning and ash in grass biomass depending on fertiliser type and rate. In Proceedings of the13th International Scientific Conference on Engineering for Rural Development, Jelgava, Latvia, 29–30 May 2014; pp. 444–449.
52. Zhang, J.; Ma, Y.; Yan, Z.; Li, Z.; Zhu, Y. Comparative analysis of branch biomass and calorific value of four clones of 5-year-old *Robinia pseudoacacia*. *For. Sci.* **2012**, *48*, 75–80.
53. Lieskovsky, M.; Jankovsky, M.; Trenciansky, M.; Merganic, J.; Dvorak, J. Ash Content vs. the Economics of Using Wood Chips for Energy: Model Based on Data from Central Europe. *Bioresources* **2017**, *12*, 1579–1592. [CrossRef]
54. Kai, G.; Xia, Z.T.; Xun, T.; Lin, W.; Yang, G. The influence of root-cutting radius on tuber yield and fuel characteristics of *Helianthus tuberosus* L. in a semi-arid area. *Ind. Crop. Prod.* **2018**, *115*, 202–207.
55. Schmid, M.I.; Noack, A. Analysis, distribution, implications, and current challenges. *Glob. Biogeochem. Cycles* **2000**, *14*, 777–793. [CrossRef]
56. Yan, T.; Xue, J.; Zhou, Z.; Wu, Y. Effects of biochar-based fertilizer on soil bacterial network structure in a karst mountainous area. *Catena* **2021**, *206*, 105535. [CrossRef]
57. Yan, T.; Xue, J.; Zhou, Z.; Wu, Y. Biochar and compost amendments alter the structure of the soil fungal network in a karst mountainous area. *Land Degrad. Dev.* **2022**, *33*, 685–697. [CrossRef]