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Combined Addition of Bovine Bone and Cow Manure: Rapid Composting of Chestnut Burrs and Production of a High-quality Chestnut Seedling Substrate

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Abstract: In China, chestnut burrs (CB) are produced at a rate of a million tons per year as the major byproduct of chestnut orchards. It is necessary to utilize the chestnut forest green waste and convert it into a valuable seedling media for the sustainable cultivation of chestnut seedlings. In this study, we composted CB with two waste products of cattle farming, namely cow manure (CM) and bovine bone (BM). We also evaluated the potential of CB compost application in chestnut forest sustainability. Results indicated that the best combination was the addition of 15% BM and 55% CM. This combination significantly improved the composting environment by increasing pH, enhancing phosphorus concentration and mineral elements such as Ca, Na, Mg and Zn, and shortened the composting period to 38 days. This combination also resulted in the highest content of citric acid-P (109.20 times than the control treatment) and the lowest content of NH_4^+–N (0.28 times than control treatment) indicating a better N and P structure of the final compost product. This combination achieved a greater degradation rate of CB cellulose (61.45%), hemicellulose (37.87%), and a more significant degradation of outer epidermis structure. When CB compost was used as a growing media, a significant decrease in photosynthesis stress of chestnut seedlings was observed, which was mainly manifested as a decrease in photochemical quenching (qP) and an increase of the maximum efficiency of PSII photochemistry under dark-adaption (Fv/Fm). Addition of 10% CB compost (in volume basis) is suggested, which resulted in the tallest chestnut seedlings (59.83 cm) with a stem diameter of 0.91 cm after six months of growth. In summary, this research provides an environmentally friendly strategy for chestnut orchard sustainability: rapid composting of CB, then immediate application as a high-quality substrate for chestnut seedlings.

Keywords: chestnut burrs; cattle farming waste; waste-to-resource; composting; spicular structure rapid degradation; fluorescence parameters; plant biomass; circular economy; sustainable agriculture

1. Introduction

China is the largest chestnut producer in the world with more than 1.94 million tons of chestnuts produced every year [1,2]. Consequently, a large volume of chestnut burrs (CB), leaves, and stems, are generated every year and have become the major forest green waste (FGW) in most chestnut growing regions. Different from other FGW, CB wraps tightly around the nut and occupies 40–50% of the whole chestnut fruit on a fresh weight basis. Artificial separation of chestnut burrs and nuts is a necessary

step for chestnut production. The popularization of burr removal equipment has resulted in the need for intensive management of CB. Due to CB recalcitrant biodegradability and knotty spicular structure, incineration is still the main method of dealing with CB waste [3,4]. Since CB, as FGW, is rich in organic matter and minerals, it is desirable to convert it into a high value byproduct such as compost.

Composting is a popular and clean method to dispose of FGW [5]. Mature FGW compost has high porosity and high organic carbon content and can be utilized as a high-quality soil amendment [6]. Shrestha et al. [7] reported that the application of compost increased soil organic carbon content, reduced CO_2 and N_2O emissions, and increased CH_4 uptake. Chehab et al. [8] indicated that the olive FGW compost amendment resulted in an increased performance in mature olive trees and in a maximum yield of photosystem II (Fv/Fm). Composting recycles organic matter, nitrogen, phosphorous, and mineral elements in the FGW when growing seedlings [9]. In a recent study, Shalizi et al. [10] increased the biomass of *Eucalyptus benthamii* seedlingsby adding a compost product that was rich in nutrients, especially nitrogen.

Previous studies have indicated the difficulty of completely composting CB in a short time because of its high C/N ratio and lignocellulose content [11,12]. Therefore, adding supplements during composting is necessary to speed up the composting process. Cattle farming waste (CFW) might be the ideal additive in CB composting because of the close geographical location of cattle farms and because of the nature of the cattle farming waste. Chestnut orchards and cattle farms are in similar geographical regions, which saves on transportation costs. Additionally, FGW and CFW possess complementary attributes in terms of nutrient composition and microbial species [13]. Cow manure (CM) and bovine bone (BM) are the major two cattle farming wastes. Previous research has indicated that CM is a suitable additive in FGW composting. Addition of CM adjusts the C/N ratio in the composting pile to a more suitable range and introduces microbial communities and enzymes into the composting materials [13]. However, there is little research on the benefits of BM on FGW composting. BM is rich in mineral content such as Ca, Mg, and Zn, which are lacking in CB and CM [14]. BM is rich in phosphorus and has a suitable pH and electrical conductivity (EC), indicating its suitability as a composting additive.

In China, topsoil is the primary component of media used in the production of chestnut seedlings. However, topsoil is an unsustainable resource with poor nutritional content and compaction. It is necessary, therefore, to convert CB waste into a valuable seedling media that will add value to chestnut FGW and improve the sustainable cultivation of chestnut seedlings. In this work, we composted chestnut burrs with various rates of bone meal and cow manureto determine: (1) the biodegradability potential of CB and the ideal ratio of CB, CM, and BM; (2) the physicochemical properties and nutrient composition of the final product; and (3) feasibility of CB compost as a substrate for chestnut seedling production.

2. Materials and Methods

2.1. Chestnut Burrs (CB), Cow Manure (CM), Bovine Bone (BM), and Other Materials

Chestnut burrs (CB) were collected in Hunan Province, China. The CB were chopped using a multifunctional shredder (model RT-34, Beijing, China) to a 1 cm particle size [15]. Fresh cow manure was collected in Ningxiang County, Hunan Province, China. Fresh cow manure, produced within a few days before the initiation of this study, was used. Bovine bone was purchased from the Xingtai Hebei Sheng Feng Co. Ltd., China, and ground into a powder form. Urea was purchased from Xuzhou Jiangsu Heng Sheng Co. Ltd., China, and used to adjust the C/N ratio prior to composting. In order to study the effect of CM and BM during composting, no additional microbial inoculum was added in this study. The quantities of CM and BM added in the nine treatments were calculated according to the percentages indicated by the orthogonal design presented in Table 1. Selected properties of the raw materials are listed in Tables 2–4.

2.2. Experiment Design and Procedure

The C/N ratio (dry weight basis) was calculated by summing the total carbon content of each substrate multiplied by its molecular weight and dividing by the sum of the total Kjeldahl nitrogen content of each substrate multiplied by its molecular weight. Different quantities of CM and BM were added to the CB and mixed evenly to yield nine treatments. The C/N ratio of all treatments was close to 25, while the initial moisture content varied between 60% and 70% (w/w) and was monitored weekly throughout the composting period.

About 45 L of the mixture from each mixing ratio or treatment was left for 38 days in 50 L compost bins, according to Li et al. [16]. Samples were collected each time the compost mixtures were turned on 0, 4, 8, 12, 16, 20, 24, 31, and 38 days. At each sampling date, a homogeneous sample of 200 g was taken from the top, middle, and bottom of each compost bin using the quartering method [15,17]. The three samples were combined to yield a composite sample. The composite samples were then divided into three parts. The first part was air-dried to 3–5% water content level, and was used for the determination of physical properties, pH, EC, total Kjeldahl nitrogen (TKN), total organic carbon (TOC), total phosphorus (TP), water-soluble P, and citric acid-P. The second part was oven-dried at 65 °C until it reached a stable weight and was used for the determination of macro- and micronutrients. The third part was kept in a sealed bag at 4 °C and used to quantify NH_4^+–N and in the phytotoxicity test. All analyses were performed on three replicate subsamples from each composite sample.

Table 1. The proportion of cow manure (CM) and bovine bone meal (BM) in % dry weight added to the various treatments of this study.

Treatment	CM Content in CB (% Dry Weight)	BM Content in CB (% Dry Weight)
T1	0	0
T2	0	10
T3	0	15
T4	35	0
T5	35	10
T6	35	15
T7	55	0
T8	55	10
T9	55	15

CB = chestnut burrs; CM = cow manure; BM = bovine bone meal.

2.3. Analytical Methods

2.3.1. Physical Properties:

Throughout the composting process, temperature in the top, middle, and bottom portion of each composting mixture was measured daily with a mercury thermometer before the composting mixture was turned and watered [18,19]. The three temperature readings per composting mixture were averaged [15]. Bulk density (BD), total porosity (TPS), and aeration porosity (APS) of the final compost product were determined by the ring knife method [15]. The particle-size distribution of the finished compost was determined according to the sieve method of Gabhane et al. [20]. Analysis of the CB outer epidermis structure was analyzed using the stereomicroscope and scanning electron microscope (SEM) imagery structural analysis [21]. According to Wang et al. [22], x-ray diffraction (XRD) was used to estimate the microstructure changes of CB cellulose.

2.3.2. Chemical Properties

A water extract was prepared by adding 100 mL of distilled water to a 10 g sample (1:10 ratio of sample to water on a weight by volume basis), and mixing thoroughly on a shaker for 30 min, followed by filtering through filter paper. pH and EC were measured by a PHS-3E pH meter (Shanghai, China)

and DDS-307A EC meter (Shanghai, China), respectively. TOC was measured using the dichromate wet combustion method and a spectrometer [23]. Mineral N was extracted with 2 M KCl followed by colorimetric analysis of NH_4^+–N, which were expressed per g of dry weight of composting sample. Total Kjeldahl nitrogen (TKN) was determined by the Kjeldahl method. Total P was determined using the Automated Discrete Analyzers Model Smart Chem 200 after digestion with H_2SO_4–H_2O_2. Water-soluble P contents were determined by the method of Maluf et al. [24]. Citric acid-P was extracted by stirring the mixture with a 2% citric acid solution for 30 min, in a sample to solution ratio of 1:20 (w/v), according to the Chinese standard of organic fertilizers [25]. After digestion of samples with HNO_3–H_2O_2 in a microwave, total potassium (TK), total calcium (Ca), total sodium (Na), total magnesium (Mg), and total iron (Fe) were determined using an atomic absorption spectrophotometer model TAS-990 (Beijing, China), while other metals were measured by inductively coupled plasma mass spectrometry (ICP-MS) model ICAP Q. According to the methodology of Zhang and Sun [26], microbial biomass P was estimated based on the difference between TP in chloroform-fumigated vs. non-fumigated samples. The contents of lignin, cellulose, and hemicellulose were determined according to the method of Yu et al. [27].

2.3.3. Phytotoxicity Test

The phytotoxicity of the compost product was assessed by testing the effects of a compost extract on the seed germination of Pak Choi [17]. After incubation in the dark at 25 °C for 72 h, the percentage of germinated seeds and their root lengths were determined. Relative seed germination (RSG), relative root elongation (RRE), and seed germination index (GI) were calculated according to Zucconi et al. [28].

2.3.4. Pot Assay

After the 38-day composting period, the product was used immediately in an assay to quantify the compost quality on the growth of chestnut seedlings. Different dosages of compost mixed with red soil were used in this assay. Compost was blended with soil at dosages of 0%, 5%, 10%, and 15% (by volume). Chestnut seeds were grown in the four substrate mixtures, with 60 replicates per treatment. Seedling height and stem diameter were measured from 6-month old plants. Photosynthesis was measured using a chlorophyll fluorometer (Mini-PAM, Heinz Walz, Effeltrich, Germany). PSII photosynthetic efficiency (Fv/Fm) was measured on leaves that were dark-adapted for 20 min [29].

2.4. Analytical Methods

One-way analyses of variance (ANOVAs) were used to determine how treatments affected the physical and chemical changes during composting with or without the addition of CM and BM. When ANOVAs were significant for the main effects, means were separated with an least significant difference method (LSD) test. Pearson correlative analysis was used to test for significance between fluorescence parameters and the chestnut seedlings' biomass. All statistical analyses were performed using SPSS19.0 software (IBM, Armonk, NY). All line charts were made by Origin 8.5 software (Origin Laboratory, Northampton, MA).

3. Results and Discussion

3.1. Composting Temperature

Changes in composting temperatures are shown in Figure 1a. All treatments went through a sharp initial heating, then a cooling down stage. A slow temperature rise was observed after each turning of the compost, followed by a final drop to reach room temperature. In the initial heating stage, temperatures of T3, T6, and T9 increased rapidly and peaked on day 1. At the second heating stage, maximum temperature was once again achieved earlier in T9 than in the other treatments. During composting, peak temperatures were observed in T4, T5, T6, T8, and especially in T9 (55% CM and 15% BM). Exposure to temperatures of 50–60 °C for at least three consecutive days is sufficient to produce a

sanitary compost [30]. In this study, the thermophilic phase of T4, T5, T6, T8, and T9 was sustained for more than three consecutive days during the composting process.

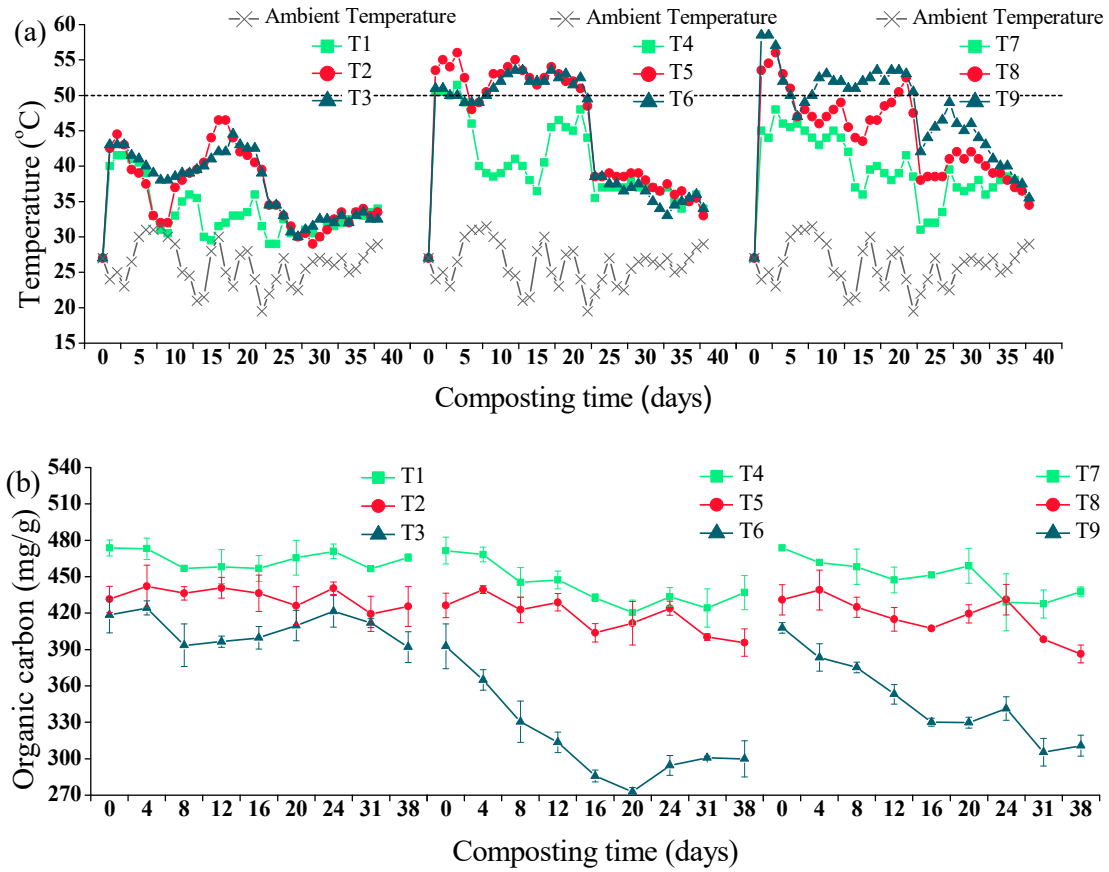

Figure 1. Variation in composting temperature (**a**) and organic carbon content (**b**) during the composting process. Treatments T1–T9 are described in Table 1. The part above the dotted line in (**a**) represents the time of the thermophilic period during composting.

The addition of CM and BM significantly increased the maximum composting temperature and extended the thermophilic phase. The increase in composting temperature was mainly caused by the microbial decomposition of organic matter [15] due to the large number and variety of microorganisms in the CM [31]. Consequently, the degradation of organic matter was promoted, which in turn resulted in the generation and release of metabolic heat. The abundant pore and high surface area of the CM contributed to the high porosity of the composting mass and gas exchange, which likely increased microbial activity [26,32]. On the other hand, the total P content of CB and CM was very low (Table 2), but the addition of BM greatly increased the P content of the composted products. This exogenous phosphorus in the composting could increase the microbial activity and, consequently, heat generation [26,30].

3.2. Organic Carbon Content

The initial change in organic carbon content directly reflects changes in organic matter content during composting. After 38 days of composting, the organic carbon content fluctuated slightly among all treatments (Figure 1b). The initial total organic carbon content was 47.37%, 43.14%, 41.84%, 47.15%, 42.63%, 39.27%, 47.37%, 43.10%, 40.79%, and decreased to 46.57%, 42.55%, 39.20%, 43.70%, 39.57%, 30.00%, 43.78%, 38.64% and 31.07% at the completion of the composting period, respectively. As the process of composting advances, the organic carbon is converted to inorganic carbon such as CO_2, which is emitted into the compost mass [33,34]. A lower depletion rate of total organic carbon

was observed in T1 (1.69%) and T2 (1.37%) compared to T6 (23.61%) and T9 (23.82%) with a higher depletion rate.

As shown in Figure 1b, the addition of CM and BM increased the mineralization rate of organic carbon. Previous studies have shown that the addition of CM could introduce a different and diverse microbial community and enzymes into the composting materials, which could enhance the degradation rate of the organic carbon [35,36]. However, they found different results of the degradation rate of organic carbon by adding different phosphorus sources to the composting. Some studies have suggested that the addition of mineral P sources would decrease the organic matter decomposition rate during composting [37,38], while other studies obtained the opposite result [26,30]. Lee et al. [39] and Maluf et al. [24] added different forms of phosphorus during composting, and both concluded that the addition of phosphorus increased the organic matter decomposition rate during composting, but the addition of a high concentration of soluble salt to the compost piles inhibited the activity of the decomposers. In this study, when the addition ratio of BM reached 15%, the mineralization rate of organic carbon had a dramatic increase, which resulted in the lowest amounts of total carbon measured for treatments T6 and T9 (Figure 1b). The above results indicate that BM was a very effective phosphorus type additive during the CB composting. The combined addition of CM and 15% BM could significantly promote the decomposition of organic carbon during CB composting. This increase could be due to the low content of soluble phosphate in BM [40] and the generous microbial communities and enzymes in CM [13].

3.3. Nitrogen Changes

Change in TKN and NH_4^+–N concentrations during composting are shown in Figure 2a. TKN concentrations of all treatments decreased greatly at the start of the composting process and then increased by the end of the process (Figure 2a). As previously reported by Awasthi et al. [41], the initial decrease in TKN is attributed to ammonia (NH_3) emissions, and the subsequent increase in TKN probably resulted from the decomposition of organic matter. Among the nine treatments, TKN increases were the earliest and greatest in treatment T9 (55% CM and 15% BM), while the TKN decreases were greatest in treatment T6 (35% CM and 15% BM).

The change in ammonia and total nitrogen showed the opposite curve (Figure 2a). At the beginning of composting, aminating bacteria converted organic nitrogen (protein, urea, uric acid, etc.) into ammonia nitrogen, which lead to an increased NH_4^+–N concentration and enhanced NH_3 emissions. In addition, the decrease in ammonia nitrogen content and a corresponding increase in TKN content after the eighth day can be partly attributed to the formation of humus, which will promote the transformation of mineral nitrogen to organic nitrogen [42]. According to Tiquia [43], a high concentration of NH_4^+–N will lead to a toxicity in plants. Our results indicated that combining CM and BM significantly decreased NH_4^+–N content and increased the growth rate of TKN in the final product. Treatment T9 (with 55% CM and 15% BM) had the lowest content of NH_4^+–N and the highest rate of TKN, which indicated that the addition of 55% CM and 15% BM in CB composting could greatly decrease the toxicity of ammonia in the final product, and also result in a more favorable N transformation.

3.4. Phosphorus Changes

Phosphorus (P) is an essential nutrient for plant growth, thus, it is a valuable indicator when evaluating compost maturity and its nutritive value as a biofertilizer. Changes in total phosphorus (TP) concentration, citric acid-soluble P concentration, and water-soluble P concentration during composting are presented in Figure 2b,c. Addition of BM significantly increased TP content at the start of composting. Then, TP concentrations increased in all treatments during composting (Figure 2b). After 38 days of composting, TP concentration of the final product was the highest in T9 and lowest in T1. The final TP concentration of T9 was 19.32 times higher than that of T1 (Table 2). Addition of a phosphorus source can significantly increase the compost TP content. Similar results were observed

by Zhang and Sun [26]. In their research, the addition of rock phosphate in the green waste compost markedly increased the TP content of the final product.

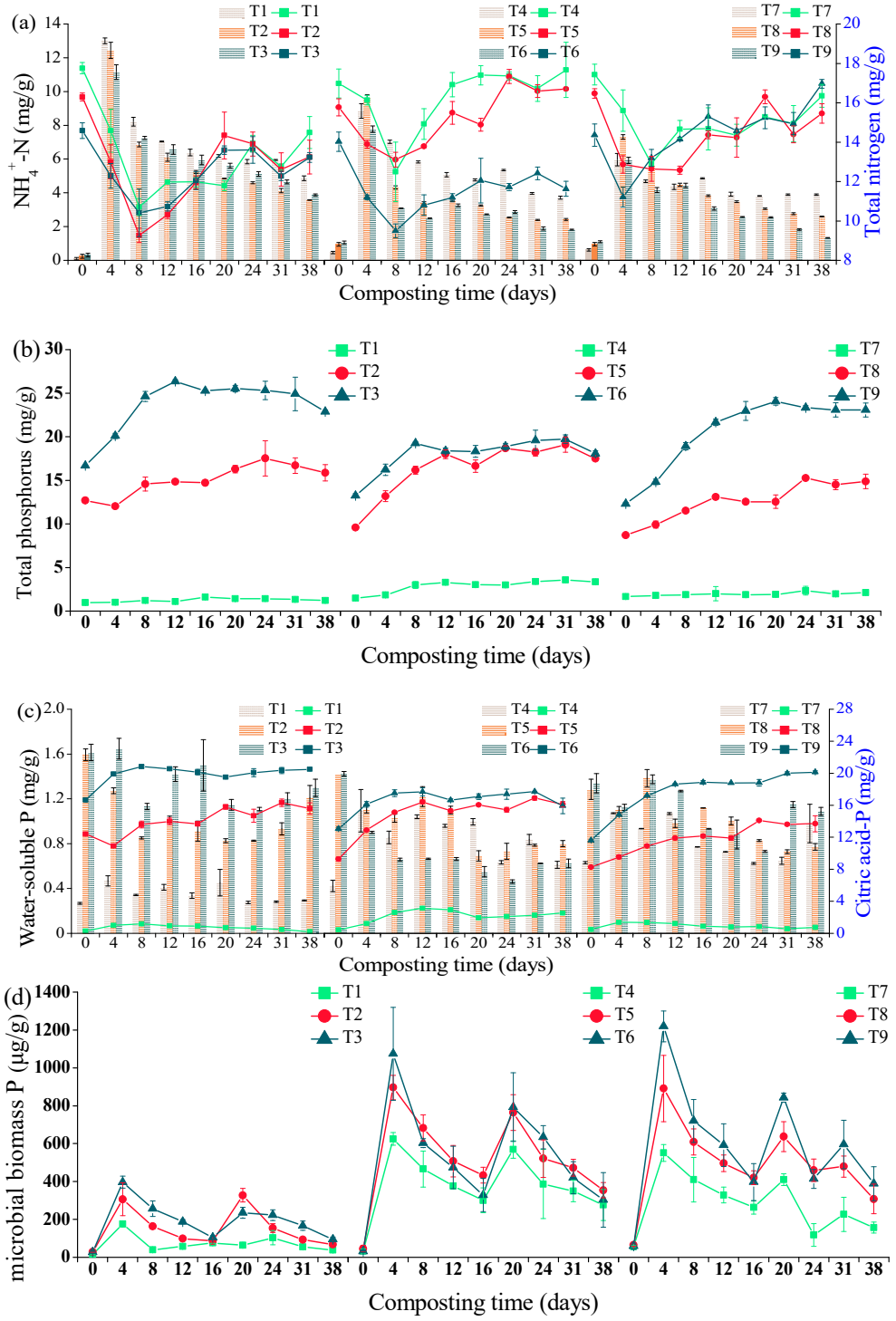

Figure 2. Temporal variation in NH_4^+–N content and total Kjeldahl nitrogen content (**a**), water-soluble P and citric acid-P (**c**) during the composting process (bar diagram is represented by primary *y*-axis and line diagram by secondary *y*-axis (blue)). Variation in total phosphorus content (**b**) and microbial biomass P (**d**) during the composting process. Treatments T1–T9 are described in Table 1.

Citric acid-P is insoluble or slightly soluble in water, but plants can dissolve and absorb it through organic acids secreted by roots [44]. Changes in citric acid-P concentration (Figure 2c) was similar

to changes in TP concentration, with greater citric acid-soluble P concentration of the final compost product in T3 and T9 than in the other treatments. The final citric acid-soluble P concentration of T9 was 109.20 times higher than that of T1. The addition of BM significantly increased the water-soluble P concentration at the start of composting (Figure 2c). During the composting process, the concentration of water-soluble P increased in T1, T4, and T7 (without additional BM), but decreased in the other treatments with added BM. The reduction in water-soluble P may be related to metal cations (such as Ca^{2+}) reacting with phosphorus to form a more stable phosphate compound during composting [44,45], which would reduce the water solubility of phosphorus [46]. In our study, the water-soluble P concentration was highest in T3 and lowest in T1 at day 38 of composting (Figure 2c).

The trends of microbial biomass phosphorus (P) were similar to those for temperature (Figure 1a). Treatments T7, T8, and T9 (all with 55% CM) had three peaks of microbial biomass P, while the other treatments had only two peaks. Throughout the composting process, microbial biomass P was higher in T5, T6, T8, and T9 (with the combined addition of CM and BM) than in the other treatments; it was highest in T9 (55% CM and 15% BM) and lowest in treatment T1. CM and BM addition greatly increased microbial biomass P during composting. This increase may result from the substantial quantities of N (Table 2) and could also be due to the increased P concentration when BM was added to the composting process (Figure 2b,c). In addition, 55% CM (highest rate among all treatments) could increase the number of fluctuations in microbial biomass P during composting (Figure 2d), which may also be a reason for the longer thermophilic phase in these treatments.

The above results indicate that the addition of BM dramatically increased the concentrations of TP and citric acid-soluble P of the final product. However, the water-soluble P concentrations were reduced when BM was added. In general, compost would increase the availability of phosphorus [47]; however, this increase in phosphorus activity is bound to cause phosphorus leaching when the product is added to the soil [48]. In other words, adding BM to the CB compost could significantly increase the plant-available P content in the final product, and could reduce P leaching when added to the soil [16]. These results also indicated that adding BM to CB composting could greatly increase microbial biomass P during composting, thus improve the composting efficiency. By adding 55% CM to CB composting could prolong the active period of the microorganisms, which would lead to a longer period of high-temperature and enhanced degradation of organic carbon.

3.5. The Degradation of Spicular Structure

As shown in Figure 3a–e, the breakdown of CB is mainly caused by the degradation of its internal structure; therefore, the difficulty in CB composting lies in the degradation of its outer epidermis. In this work, we selected the residual CB in the final compost product of each treatment and, more specifically, the tips of their outer epidermis for observation using microscopy. Figure 3f shows the scanning electron microscope (SEM) images of the outer epidermis of CB tips before composting. It has a smooth, flat, and dense epicuticular wax. After a 38-day composting period, the epidermis structure of CB tips in each treatment changed to various degrees (Figure 3g–o). The SEM images in T1, T2, T4, and T7 only showed a degradation of the epicuticular wax in CB epidermis. In addition, the combined addition of CM and BM, especially in T6 and T9, showed more significant degradation of the outer epidermis of CB, which was manifested by a disordered flat surface, a looser cellular structure, and the appearance of uneven hole sizes.

Figure 4a shows the cellulose, hemicellulose, and lignin content in the remaining CB at the end of the composting process. According to Figure 4a, the lignin content of CB in all treatments did not significantly decrease after 38-days of composting. However, obvious changes were observed in the cellulose and hemicellulose content due to composting. The cellulose and hemicellulose content tended to increase in T1, T2, T4, and T7. This is probably due to the rapid degradation of proteins, lipids, and starches by microorganisms [49]. In contrast, T3, T5, T6, T8, and T9 showed different degrees of decrease in cellulose and hemicellulose content, with higher degradation in T5, T6, T8, and T9 than in T3.

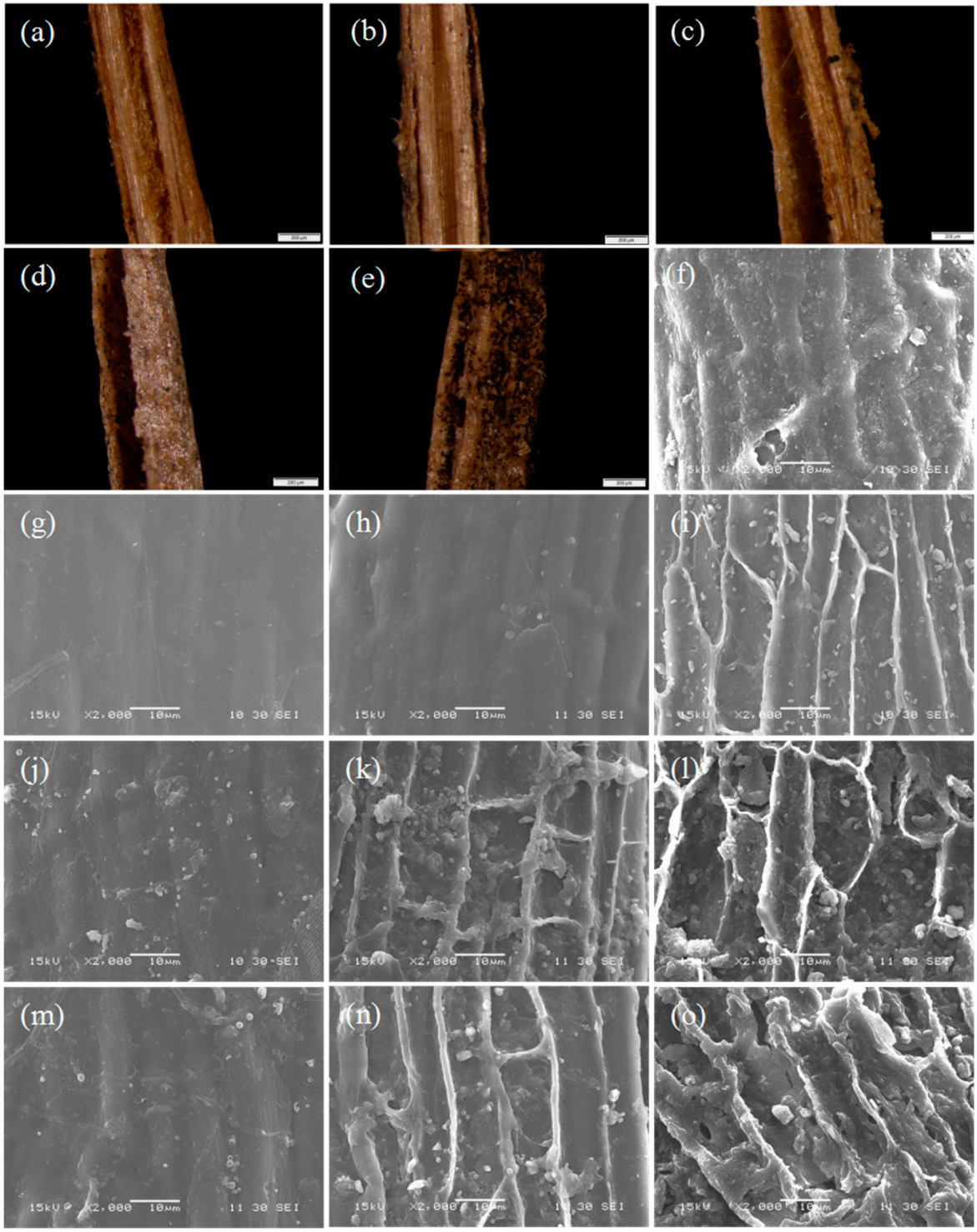

Figure 3. (**a**–**e**) are the longitudinal cross-sections of the five degradation processes of chestnut burrs (CB) during composting. (**a**) initial stage, mainly composed of three layers: surface layer, skeleton layer and innermost layer; (**b**) degradation of the innermost layer in treatment T9 on day 8; (**c**) degradation of the skeleton layer in treatment T9 on day 20; (**d**) a hollow structure in treatment T9 on day 31; (**e**) collapse of the CB spiny structure in treatment T9 on day 38. (**f**–**o**) show the scanning electron microscope (SEM) images of the tips of the CB outer epidermis at the end of composting of different treatments. (**f**) SEM micrograph of the tip of the initial CB, (**g**–**o**) SEM micrographs of the tips of the CB outer epidermis.

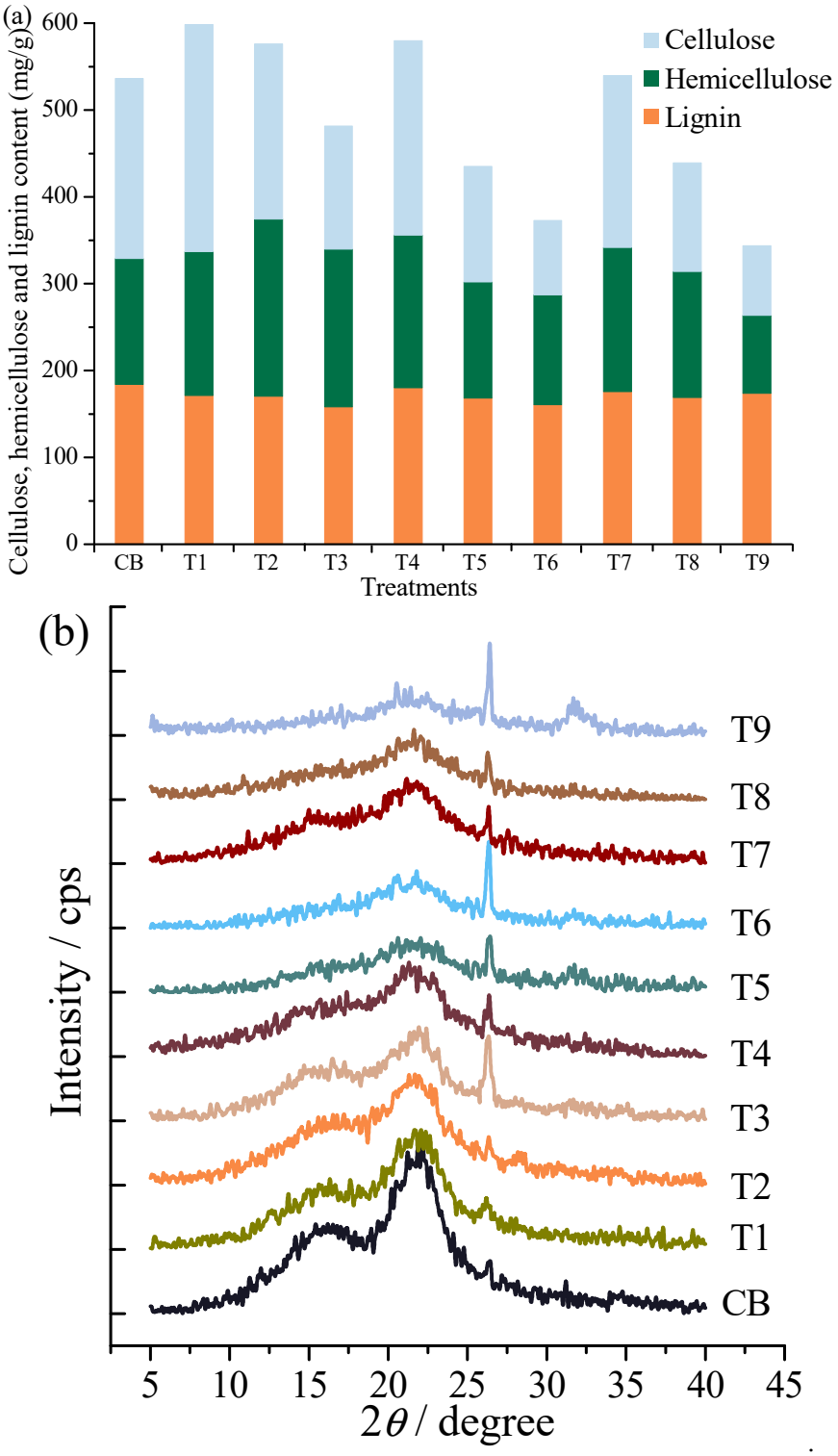

Figure 4. The contents of cellulose, hemicellulose, and lignin (**a**) and the x-ray diffractions (**b**) of the remaining chestnut burrs (CB) at the end of composting (CB: initial chestnut burrs, T1–T9: the remaining CB in treatments T1–T9).

To estimate the microstructure changes of cellulose, samples were further analyzed using x-ray diffraction of the crystalline structure (Figure 4b). Cellulose is a complex system mostly crystalline and amorphous, with the high content of crystal structure the main reason to reduce or prevent cellulose degradation [22]. The samples in all treatments had crystalline regions of cellulose near $2\theta = 22°$. Compared with the initial CB, the diffraction peak intensity at 22° of each treatment was decreased,

suggesting that the degree of polymerization of CB cellulose was reduced. The diffracted intensity at 22° of the nine treatments were (from highest to lowest) as follows: T1 (541), T2 (499), T3 (417), T4 (402), T7 (362), T8 (279), T5 (218), T6 (206), and T9 (186). In this work, the combined addition of CM and BM significantly reduced the diffracted intensity at 22°, with T9 having the lowest diffracted intensity of 186, indicating that the crystalline structure of CB cellulose was effectively destroyed. In addition, a stronger peak appeared in the vicinity of $2\theta = 26.6°$ for each treatment, which is the diffraction peak of SiO_2 [50]. Composting significantly increased the diffracted intensity in the peak at 26.6°, which could be due to the degradation of organic matter and the increase in ash content.

The substantial quantities of crystalline cellulose and the recalcitrant outer epidermis in CB make it difficult to compost completely. In our study, the combined addition of CM and BM significantly increased the degradation rate of cellulose and hemicellulose in CB. The addition of CM and BM significantly improved the composting environment by increasing pH (Table 4), enhancing phosphorus concentration (Figure 2b–c), adding mineral elements (Table 2), and so on. This CM and BM combination could also introduce a new and diverse microbial community and enzymes to the composting materials, which can enhance its degradation rate [35,36]. SEM images further indicate that the addition of CM and BM significantly increased the degradation of the outer epidermis structure of the CB tips. These results confirm that the combined addition of CM and BM significantly increased the degradation of CB, which was mainly manifested in the decrease of cellulose and hemicellulose content, and the destruction of the outer epidermis.

3.6. Physicochemical Properties of the Final Compost

Table 3 shows the bulk density (BD), total porosity (TPS), aeration porosity (AP) and water-holding porosity (WHP) of the final compost products. The total porosity was highest in T5 and the water-holding porosity was highest in T9. Bulk density is a simple and useful tool to evaluate composting [51]. According to Zhang and Sun [13], an ideal BD range in the final product is about 0.4 g/cm^3. In our study, T6 and T9 had a significantly higher and more suitable BD than the other treatments (Table 3), indicating that the combined addition of CM and 15% BM produced an ideal compost product for use as a growing media.

Particle-size is also an important physical property of composting because it directly influences porosity and water-holding capacity [52]. According to Zhang and Sun [49], a high percentage of particles between 0.1 and 0.5 mm in the final compost mixture could increase the water-holding porosity. As shown in Figure 5, the addition of CM and BM (especially 55% CM and 15% BM) significantly increased the percentage of these particles and resulted in a final product with higher WHP, compared to the control T1. In addition, composting significantly improved the aeration porosity by increasing the proportion of large particles (≥2 mm) (Figure 5), which could be due to the humus binding properties resulting in the aggregation of small particles into larger particles (≥2 mm) [13].

Nitrogen, phosphorus, and potassium are essential nutrients for plant growth and are thus important for evaluating compost maturity as well as its nutritive values as a biofertilizer. The addition of CM significantly increased the total potassium (TK) content of the final compost (Table 2). Additionally, the TK content of the final compost product was significantly higher in T4, T5, T6, T7, T8, and T9 (all with additional CM) than in the other treatments (Table 2). On the other hand, the addition of BM significantly increased the total phosphorus (TP) content of the final compost. Regarding secondary macronutrients of the final compost, the addition of BM significantly increased the Ca and Mg content of the final compost (Table 2), and the content of Ca was significantly higher in T9 (55% CM and 15% BM) than in the other treatments. The Mg content was significantly higher in T6 and T9 than in the other treatments. The micronutrient (Fe, Na, Mn, Se, and Zn) concentrations of the final compost product are shown in Table 2. After a 38-day composting period, the final compost of all treatments (especially T6 and T9) was rich in micronutrient concentrations (Table 2).

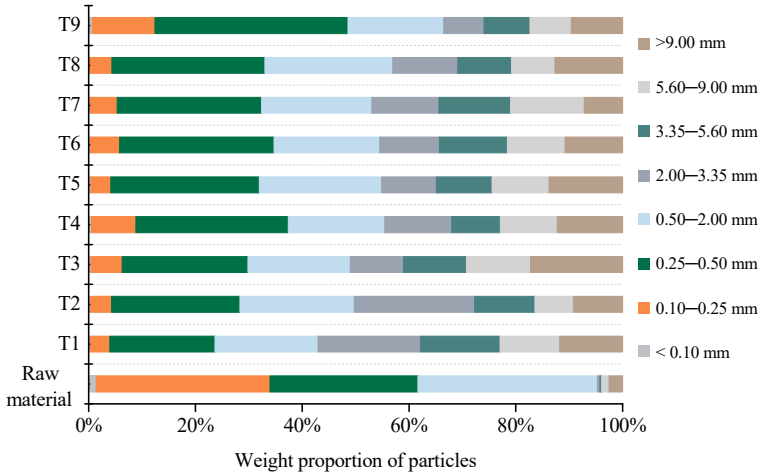

Figure 5. Variation in particle size distribution of the initial CB and final products (Raw material: initial CB, Treatments T1–T9 are described in Table 1).

The above results indicate that CB could be composted to be a high-quality product with good ventilation and higher water-holding capacity when BM and CM are added. Furthermore, the addition of CM and BM to the composting mass, especially at a rate of 55% CM and 15% BM, increased the macronutrient and micronutrient content of the final compost product by increasing the mineralization of the initial composting mixture. This was due to the direct addition of nutrients like TP, TK, Ca, Mg, Na, etc.; and by reduced nutrient leaching [53].

Table 2. Selected macronutrient and micronutrient content of chestnut burrs (CB), cow manure (CM), bovine bone meal (BM), and of the final composts of various mixture treatments. Treatments T1–T9 are described in Table 1.

Treatment	TOC (%)	TKN (%)	TP (%)	TK (%)	Ca (%)	Na (%)
CB	45.20 ± 2.05	0.29 ± 0.04	0.10 ± 0.00	1.18 ± 0.06	3.48 ± 0.06	0.17 ± 0.02
BM	4.23 ± 0.88	1.12 ± 0.01	12.84 ± 0.49	0.52 ± 0.03	25.66 ± 0.45	1.49 ± 0.08
CM	34.69 ± 0.51	2.01 ± 0.13	0.30 ± 0.02	2.08 ± 0.03	3.61 ± 0.03	1.42 ± 0.03
T1	46.57 ± 0.30 d	1.45 ± 0.08 bc	0.12 ± 0.01 a	1.24 ± 0.01 b	3.37 ± 0.3 a	0.36 ± 0.05 a
T2	42.55 ± 1.65 c	1.32 ± 0.09 b	1.59 ± 0.09 b	1.12 ± 0.02 a	4.34 ± 0.19 b	0.40 ± 0.08 ab
T3	39.20 ± 1.26 b	1.32 ± 0.02 b	2.29 ± 0.02 b	1.12 ± 0.01 a	4.83 ± 0.59 bc	0.42 ± 0.02 ab
T4	43.70 ± 1.42 c	1.77 ± 0.14 e	0.33 ± 0.01 a	1.60 ± 0.05 c	3.67 ± 0.31 a	0.51 ± 0.10 bc
T5	39.57 ± 1.12 b	1.67 ± 0.01 de	1.75 ± 0.01 b	1.60 ± 0.03 c	4.64 ± 0.16 bc	0.54 ± 0.03 c
T6	30.00 ± 1.50 a	1.16 ± 0.04 a	1.81 ± 0.04 b	1.98 ± 0.11 e	4.88 ± 0.00 bc	0.51 ± 0.02 bc
T7	43.78 ± 0.39 c	1.64 ± 0.06 de	0.21 ± 0.01 a	1.65 ± 0.03 c	3.82 ± 0.12 a	0.40 ± 0.01 ab
T8	38.64 ± 0.73 b	1.55 ± 0.05 cd	1.49 ± 0.08 b	1.79 ± 0.03 d	5.10 ± 0.04 c	0.51 ± 0.02 bc
T9	31.07 ± 0.87 a	1.70 ± 0.02 de	2.31 ± 0.08 b	2.04 ± 0.06 e	6.35 ± 0.03 d	0.70 ± 0.01 d
Treatment	**Mg (%)**	**Fe (%)**	**Mn (×10^{-3}%)**	**Zn (×10^{-3}%)**	**Se (×10^{-3}%)**	
CB	0.18 ± 0.01	0.38 ± 0.08	54.47 ± 1.54	1.35 ± 1.25	1.14 ± 1.05	
BM	0.64 ± 0.00	0.24 ± 0.04	3.83 ± 0.92	4.52 ± 1.31	1.73 ± 0.84	
CM	0.26 ± 0.02	0.38 ± 0.09	54.01 ± 7.53	1.64 ± 0.95	1.51 ± 0.65	
T1	0.15 ± 0.05 a	0.36 ± 0.01 bc	47.74 ± 3.10 a	1.50 ± 1.07 a	1.98 ± 0.96 a	
T2	0.18 ± 0.01 ab	0.15 ± 0.02 a	31.27 ± 17.06 a	3.53 ± 1.38 ab	1.64 ± 1.35 a	
T3	0.21 ± 0.02 bc	0.40 ± 0.11 c	34.65 ± 4.14 a	4.78 ± 1.94 ab	1.33 ± 0.92 a	
T4	0.20 ± 0.01 bc	0.21 ± 0.09 ab	30.67 ± 16.64 a	2.29 ± 1.37 ab	1.35 ± 0.89 a	
T5	0.23 ± 0.01 c	0.36 ± 0.03 bc	50.43 ± 4.43 a	6.45 ± 1.03 ab	1.38 ± 0.89 a	
T6	0.31 ± 0.01 d	1.46 ± 0.06 e	103.62 ± 8.04 c	17.20 ± 2.98 c	1.72 ± 0.13 a	
T7	0.17 ± 0.01 ab	0.40 ± 0.14 c	49.74 ± 7.86 a	3.10 ± 2.09 ab	0.89 ± 0.15 a	
T8	0.21 ± 0.03 bc	0.28± 0.03 abc	45.06 ± 1.70 a	7.06 ± 2.10 ab	0.96 ± 0.47 a	
T9	0.29 ± 0.00 d	1.08 ± 0.03 d	85.63 ± 3.28 b	7.72 ± 3.29 b	1.81 ± 0.47 a	

Values are means ± SD; $n = 3$. TOC = total organic carbon; TKN = total Kjeldahl nitrogen; TP = total phosphorus; TK = total potassium. On the last day of composting, means in a column followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

Table 3. Bulk density (BD), total porosity (TPS), aeration porosity (APS), water-holding porosity (WHP), and the amount of urea added to the nine separate or various mixtures of chestnut burrs (CB), cow manure (CM), bovine bone meal (BM), and of the final compost mixtures.

Treatment	BD (g/cm^3)	TPS (%)	APS (%)	WHP (%)	Amount of Urea Added (% Dry Weight)
CB	0.188 ± 0.007	80.83 ± 1.30	45.43 ± 4.76	35.40 ± 7.81	–
BM	0.572 ± 0.004	35.76 ± 1.31	6.20 ± 0.31	29.56 ± 0.54	–
CM	0.364 ± 0.042	33.42 ± 3.97	6.49 ± 0.20	26.93 ± 2.21	–
T1	0.188 ± 0.002 a	82.05 ± 2.04 ab	65.49 ± 2.98 c	16.57 ± 0.94 a	3.43
T2	0.204 ± 0.003 ab	77.46 ± 2.22 ab	61.42 ± 1.45 abc	16.05 ± 0.77 a	2.91
T3	0.229 ± 0.003 b	79.14 ± 1.91 ab	61.15 ± 2.28 abc	17.99 ± 0.37 ab	2.73
T4	0.245 ± 0.004 c	82.06 ± 1.65 ab	61.92 ± 1.58 bc	20.14 ± 0.06 bc	2.46
T5	0.242 ± 0.012 c	83.46 ± 0.54 b	62.98 ± 0.65 c	20.48 ± 0.11 bc	2.02
T6	0.362 ± 0.003 d	75.23 ± 1.21 a	55.19 ± 1.51 a	20.04 ± 0.30 bc	1.71
T7	0.205 ± 0.000 ab	78.68 ± 0.40 ab	59.15 ± 0.76 abc	19.53 ± 0.37 bc	2.13
T8	0.233 ± 0.018 bc	80.20 ± 0.23 ab	59.98 ± 0.11 abc	20.22 ± 0.11 bc	1.74
T9	0.344 ± 0.006 d	77.21 ± 3.22 ab	56.27 ± 1.36 ab	20.95 ± 1.86 c	1.53
IR[a]	≈0.400	70.00–85.00	–	–	–

Values are means ± SD; n = 3. [a] IR = ideal range, according to Zhang and Sun [26]. Means in a column followed by the same letter are not significantly different at $p \leq 0.05$ by least significant difference method (LSD).

3.7. Compost Maturity Assessment

The C/N ratio of a compost is conventionally used to evaluate compost maturity. A final C/N ratio of <20 is normally considered as satisfactory for compost maturity [54]. In our study, the initial C/N ratio of all treatments was adjusted to near 25 through the addition of urea. At the end of the composting process, only T9 had a standardized C/N ratio of less than 20 (Table 4). The rapid decrease in C/N ratio of T9 during composting could be due to a rapid carbon degradation (Figure 1b) and a greater increase in nitrogen concentration (Figure 2a).

Table 4. Criteria used to evaluate the maturity of the final compost products or mixtures. Treatments T1–T9 are detailed in Table 1.

Treatment	Number of Thermophilic Phases	C/N	pH	EC (mS/cm)	RSG (%)	RRE (%)	GI (%)
CB	–	157.86 ± 20.03	5.25 ± 0.19	0.43 ± 0.06	–	–	–
BM	–	3.77 ± 0.68	6.81 ± 0.24	0.53 ± 0.06	–	–	–
CM	–	17.31 ± 1.00	7.50 ± 0.38	3.60 ± 0.01	–	–	–
T1	0	32.21 ± 1.57 f	6.38 ± 0.29 b	2.13 ± 0.00 f	71.23 ± 4.78 a	108.40 ± 9.37 a	76.38 ± 8.62 a
T2	0	32.21 ± 2.14 f	6.71 ± 0.55 cd	1.61 ± 0.01 b	89.88 ± 3.45 c	100.26 ± 7.54 a	90.11 ± 7.22 b
T3	0	29.64 ± 0.95 e	6.82 ± 0.43 d	1.68 ± 0.01 c	84.80 ± 3.36 b	105.68 ± 8.00 a	89.61 ± 7.27 b
T4	4	24.84 ± 1.77 bc	6.42 ± 0.52 bc	2.73 ± 0.02 h	89.88 ± 3.45 c	102.98 ± 6.75 a	92.56 ± 6.67 bc
T5	19	23.69 ± 0.59 b	6.49 ± 0.55 bcd	1.87 ± 0.00 d	89.88 ± 5.59 c	100.26 ± 8.22 a	92.35 ± 8.66 b
T6	18	25.81 ± 1.34 cd	6.76 ± 0.59 cd	1.57 ± 0.00 a	96.67 ± 2.50 d	100.26 ± 8.02 a	96.34 ± 7.79 d
T7	0	26.79 ± 0.86 d	6.69 ± 0.47 cd	3.01 ± 0.02 i	91.58 ± 2.37 c	102.97 ± 7.64 a	95.39 ± 6.86 c
T8	7	25.02 ± 0.81 bc	6.73 ± 0.42 cd	2.04 ± 0.01 e	91.58 ± 2.37 c	108.39 ± 6.31 a	100.49 ± 6.20 e
T9	20	18.32 ± 0.58 a	6.02 ± 0.19 a	2.20 ± 0.02 g	98.36 ± 3.60 d	105.68 ± 5.72 a	104.21 ± 6.57 f
IR [a]	≥10	<20	6.0–8.0	<2.5	–	–	>80

Values are means ± SD; n = 3. EC = electrical conductivity; RSG = relative seed germination; RRE = relative root elongation; GI = germination index. [a] IR = ideal range, according to NY525-2012 [55], Huang et al. [56]. Means in a column followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

Seed germination index (GI) is another method to assess compost maturity. GI > 80% of final compost products indicate an absence of compost phytotoxicity and a mature and stable compost [28]. After 38 days of composting, GI values were >80% for all treatments except for T1 (Table 4). According to Table 4, T9 had the highest RSG (98.36) and GI (104.21) values, which could be due to the lowest NH_4^+–N concentration (Figure 2a) and a thorough degradation of organic matter (Figure 1b) [43].

3.8. Quality Evaluation of the Final Production

The maturity parameters confirmed that a compost mixture with 15% BM and 55% CM (treatment T9) became mature and stable within 38 days of CB composting initiation. Therefore, we selected the final product of T9 as the CB composting product (CBC) for use in the pot assay.

Growth parameters of chestnut plants are presented in Table 5. With the addition of CBC, chestnut seedlings had significantly higher plant biomass (Table 5) (Figure 6a). Maximum plant biomass was observed with the addition of 10% CBC, but biomass gradually decreased with 15% CBC.

Table 5. Electron transport rate (ETR), non-photochemical quenching coefficient (NPQ), photochemical quenching (qP), non-photochemical quenching (qN), maximum efficiency of PSII photochemistry under dark-adaption (Fv/Fm), plant height, and stem diameter in different dosages of CBC addition.

Treatment	ETR	NPQ	qP	qN	Fv/Fm	Plant Height (cm)	Stem Diameter (cm)
Control (Soil)	69.83 ± 1.80 a	2.05 ± 0.21 a	1.05 ± 0.16 a	0.80 ± 0.02 a	0.68 ± 0.06 a	38.97 ± 3.95 a	0.66 ± 0.05 a
Soil + CBC (5%)	77.03 ± 14.92 a	1.57 ± 0.68 a	0.84 ± 0.03 b	0.69 ± 0.14 a	0.77 ± 0.02 ab	55.67 ± 4.73 b	0.82 ± 0.06 b
Soil + CBC (10%)	81.80 ± 9.08 a	1.43 ± 0.36 a	0.84 ± 0.02 b	0.68 ± 0.08 a	0.78 ± 0.03 b	59.83 ± 4.75 b	0.91 ± 0.03 c
Soil + CBC (15%)	74.17 ± 4.71 a	1.24 ± 0.30 a	0.81 ± 0.02 b	0.65 ± 0.06 a	0.74 ± 0.03 ab	53.50 ± 4.77 b	0.73 ± 0.03 a

Values are means ± SD. CBC = chestnut burrs compost. means in a column followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

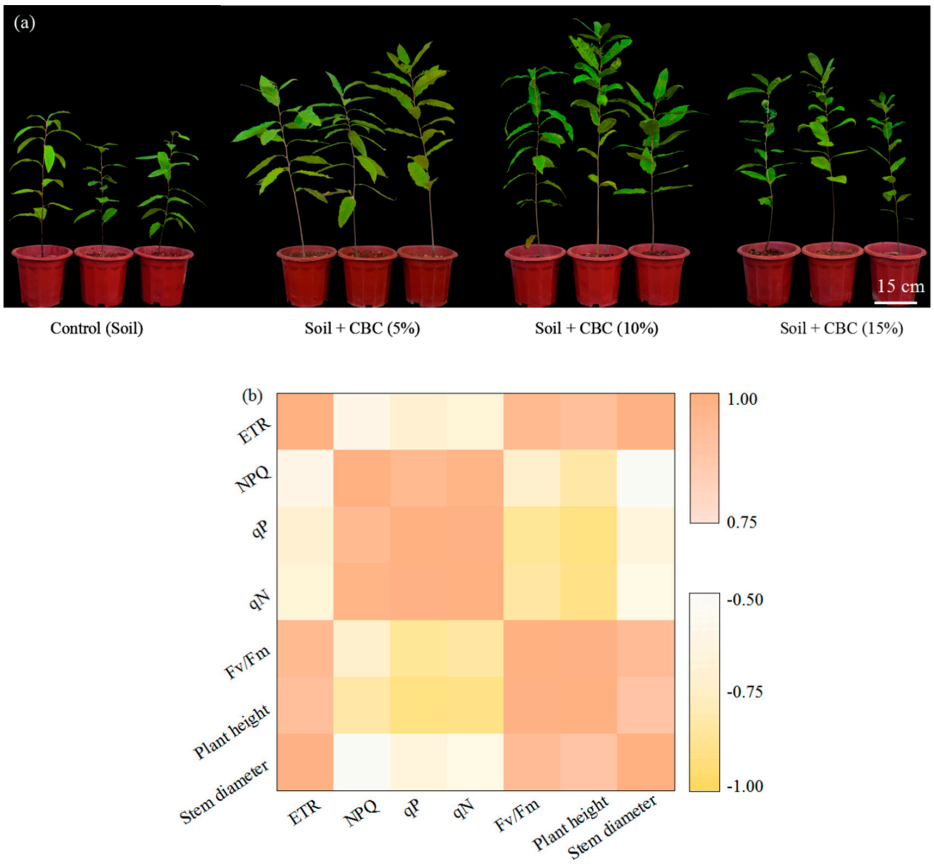

Figure 6. Growth of chestnut plants with different dosages of CBC addition (**a**), and the Pearson correlation matrix (**b**) between the fluorescence parameters and chestnut plant biomass (CBC: chestnut burrs compost, ETR: Electron transport rate, NPQ: non-photochemical quenching coefficient, qP: photochemical quenching, qN: non-photochemical quenching, Fv/Fm: maximum efficiency of PSII photochemistry under dark-adaption.

The rapid light response curve of chlorophyll fluorescence is a quick and sensitive method to detect the effects of stress on plant photosynthesis. Electron transport rate (ETR) can rapidly reflect the internal characteristics of the photosynthetic system and measure the photosynthesis of plants under different conditions [57], while qP and qN reflect the light energy absorbed by PSII antenna pigments. The above indices represent the degree of injury to photosynthetic organs due to stress. In this study, there was a significant reduction of both qN and qP after CBC addition. In other words, chestnut seedlings had lower plant stress when grown in containers with additional CBC. Fv/Fm ratio is another estimate of stress on PSII integrity [58]. Table 5 indicates that the maximum Fv/Fm (0.78) and ETR (81.80) were observed with the application of 10% CBC, but gradually decreased by adding 15% CBC.

In our experiments, a strong Pearson correlation between fluorescence parameters and plant biomass indicated that the addition of CBC greatly enhanced the growth measurements of chestnut plants by decreasing plant photosynthetic stress (Figure 6b). This could be due to an improvement in the soil environment, especially in the increased availability of nutrients such as N, P, K, Ca, Fe, and so on (Table 2). Many researchers have shown that low-nitrogen [59], low-potassium [29], low-phosphorus [60], or low-calcium [61] would lead to stress in plant photosynthesis. In addition, studies have indicated that a lower soil porosity would lead to a significant decrease in plant biomass, which may due to a restriction in root elongation [62]. CBC has a rich concentration of macro- and micronutrients and a higher aeration porosity, qualities not found in poor, degraded soils.

The pot experiments indicated that CBC could be used as a high-quality planting substrate for chestnut cultivation. More importantly, this CBC product was composted from the fibrous spicular waste in chestnut orchard and was produced in only 38 days.

4. Conclusions

Our research demonstrated that: (1) the combined addition of bovine bone (BM) and cow manure (CM) achieved a faster and more thorough degradation of chestnut burrs (CB); (2) a quality compost product was produced in 38 days with the highest levels of macro- and micronutrients and lowest phytotoxicity; and (3) using this product greatly decreased photosynthetic stress and increased the biomass of 6-month chestnut seedlings. In this work, the combined addition of 15% BM and 55% CM produced a high-quality CB compost product that can be used immediately for the production of new chestnut seedlings, which in turn maintains orchard sustainability.

Author Contributions: W.C. and F.Z. conceived the experiment and developed the experimental design. W.C.; L.H., and S.T. developed and carried out the specific methodology. W.C. and L.H. contributed to the data analysis. D.Y. secured funding for the research and was responsible for overall project administration. F.Z., D.Y. and R.Z. supervised the research of W.C. W.C., L.H.; F.Z., and J.M. contributed to the preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

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