



Article Effects of C/N Ratio on Lignocellulose Degradation and Enzyme Activities in Aerobic Composting

Huizhen Yang ^{1,2,3}, He Zhang ^{1,2}, Huizhen Qiu ^{1,2,3,*}, Dominic Kwadwo Anning ^{1,2}, Mengchan Li ^{1,2,3}, Youling Wang ^{1,2,3} and Chunhong Zhang ^{1,2,3}

- ¹ College of Resources and Environmental Sciences, Gansu Agricultural University, Lanzhou 730070, China; huizhen_yang@yahoo.com (H.Y.); zhanghe744200@sina.com (H.Z.); dominicanning@gmail.com (D.K.A.); limc@gsau.edu.cn (M.L.); wangyouling@gsau.edu.cn (Y.W.); zhangch@gsau.edu.cn (C.Z.)
- ² Gansu Provincial Key Laboratory of Aridland Crop Science, Gansu Agricultural University, Lanzhou 730070, China
- ³ Gansu Provincial Engineering Research Center for the Resource Utilization of Livestock and Poultry Wastes, Lanzhou 730070, China
- * Correspondence: hzqiu@gsau.edu.cn

Abstract: Lignocellulosic materials have a complex physicochemical composition and structure that reduces their decomposition rate and hinders the formation of humic substances during composting. Therefore, a composting experiment was conducted to evaluate the effects of different C/N ratios on lignocellulose (cellulose, hemicellulose and lignin) degradation and the activities of corresponding enzymes during aerobic composting. The study had five C/N ratios, namely, T1 (C/N ratio of 15), T2 (C/N ratio of 20), T3 (C/N ratio of 25), T4 (C/N ratio of 30) and T5 (C/N ratio of 35). The results showed that treatments T3 and T4 had the highest rate of degradation of cellulose and hemicellulose, while treatment T3 had the highest rate of degradation of lignin. Among the five treatments, treatment T3 enhanced the degradation of the lignocellulose constituents, indicating a degradation rate of 6.86–35.17%, 15.63–44.08% and 31.69–165.60% for cellulose, hemicellulose and lignin, respectively. The degradation of cellulose and lignin occurred mainly at the thermophilic and late mesophilic phases of composting, while hemicellulose degradation occurred at the maturation phase. Treatment T3 was the best C/N ratio to stimulate the activities of manganese peroxidase, lignin peroxidase, polyphenol oxidase and peroxidase, which in turn promoted lignocellulose degradation.

Keywords: C/N ratio; aerobic composting; lignocellulose; degradation rate; enzyme activity

1. Introduction

Livestock and poultry management constitute one of the major agricultural activities in China. Large quantities of agricultural waste are produced every year, including 3.8 billion tons of livestock waste, 900 million tons of crop straw and 120 million tons of vegetable waste [1]. Most agricultural wastes are not fully utilized, which does not only increase the amount of waste generated, but also leads to serious environmental pollution to the atmosphere, water source and soil. In addition, arable lands in China now face the problem of low fertility and high pollution risk due to organic wastes that are improperly treated. Meanwhile residues from livestock and poultry contain a large amount of organic matter, nitrogen, phosphorus, potassium and other nutritional elements. The inability to transform and use the raw materials would be a "misplaced resources" due to their enormous quantities and an excellent biomass source for organic fertilizer production [2]. Aerobic composting is suitable for the conversion of the resources into stable, harmless and useful compost products [3], which would not only be a solution to reduce and recycle a large amount of waste, but also reduce environmental pollution [4,5], with significant economic, social and environmental benefits [6]. The formation of humus during composting is a key indicator for determining the quality of composting products



Citation: Yang, H.; Zhang, H.; Qiu, H.; Anning, D.K.; Li, M.; Wang, Y.; Zhang, C. Effects of C/N Ratio on Lignocellulose Degradation and Enzyme Activities in Aerobic Composting. *Horticulturae* **2021**, *7*, 482. https://doi.org/10.3390/ horticulturae7110482

Academic Editor: Francisco Garcia-Sanchez

Received: 27 September 2021 Accepted: 8 November 2021 Published: 10 November 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and is dependent on the content, type, degree of degradation and humification process of the lignocellulose materials. Agricultural wastes such as straw contain a large amount of refractory organics (lignocellulosic materials), which are mainly composed of cellulose (about 40%), hemicellulose (about 20–30%) and lignin (about 20–30%) [7]. These biopolymers have complex structures that are difficult to decompose, while their degradation is closely related to the production of humus. Accelerating the degradation of lignocellulose is considered as key to the rapid maturation of compost [8].

Composting is a biochemical process promoted by enzymes synthesized by microorganisms [9]. The enzymatic reaction process directly reflects the number and activities of microorganisms [10], while the initial C/N ratio of the compost pile is a vital factor affecting the efficiency of composting [11,12]. Adjusting the initial C/N ratio of the compost pile to an optimal level improves microbial activities while reducing the difficulties associated with substrate decomposition [11]. In the same manner, adjusting the C/N ratio promotes the degree of humification and enhances the maturity of the compost [13]. Therefore, it is important to study the effect of different C/N ratios on enzymatic activities and the underlying mechanisms associated with lignocellulose degradation during composting. In this study, cow dung and corn straw were used as raw materials to investigate the effects of different C/N ratios on the degradation of lignocellulose (cellulose, hemicellulose and lignin) and related enzyme activities during aerobic composting, to provide basis for efficient resource utilization of agricultural waste and industrialized production of compost.

2. Materials and Methods

2.1. Test Materials

The composting experiment was carried out at the composting factory of Xinhao Biotechnology Co., Ltd., Baiyin District, Baiyin City, Gansu Province. The study area has a continental semi-desert climate, which is arid and windy, with large temperature difference between day and night. The annual average temperature is 8.5 °C and the average temperature from July to August is 28 °C. The cow dung and corn straw were obtained from the dairy and corn farm, respectively, of Xinhao Biotechnology Co. Ltd. The raw materials were subjected to natural air drying until a constant mass was obtained. The corn straw was mechanically crushed into small segments of 2–5 cm, while the cow dung was pressed by hand. Samples were taken from the raw materials and subjected to physicochemical analysis before composting. The raw materials were spread on a tarpaulin and thoroughly mixed with distilled water ($65 \pm 1\%$) to obtain a uniform mixture. The basic chemical composition of the tested raw materials are summarized in Table 1.

Table 1.	Basic	chemical	comp	position	of	corn	straw	and	cow	dung	before	com	postir	g
														0

Raw	Moisture	Total Organic	Total	Cellulose	Hemicellulose	Lignin	C/N
Material	Content (%)	Carbon (%)	Nitrogen (%)	(%)	(%)	(%)	Ratio
Cow dung	66.57	44.26	2.44	22.98	21.37	9.88	18.14
Corn straw	4.28	49.76	1.01	31.43	26.65	15.3	49.27

2.2. Composting Treatments

The study explored five levels of C/N ratios to investigate their underlying effect on lignocellulose degradation and enzyme activities during composting of corn straw and cow dung. All the treatments received the same amount of cow dung; however, the amount of corn straw was different in each treatment. The C/N ratio of all treatments was obtained directly from the mixture of corn straw and cow dung, except for the C/N ratio at 15, which was obtained by adding urea (3.70 kg) to the cow dung. The treatments were designated as T1, T2, T3, T4 and T5 for C/N ratio at 15, 20, 25, 30 and 35, respectively (Table 2). Treatments were laid in a completely randomized design with three replicates. Temperature readings, pile turning and sample collection were manually conducted during the period of composting. The piles were turned during periods (day) of high temperature (>55 $^{\circ}$ C) and when temperatures were below 35 $^{\circ}$ C. The entire composting period lasted for 45 days.

Table 2. Treatment description.

Treatment	T1	T2	T3	T 4	T5
Cow dung (kg)	1000	1000	1000	1000	1000
Corn straw (kg)	3.70	53.29	233.5	512.4	979.6
C/N ratio	15 *	20	25	30	35

* means the C/N ratio was adjusted by 3.70 kg of Urea (46% N).

2.3. Test Sample Collection

The samples were collected on days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42 and 45 from five locations within the composting heaps. After stratified sampling, the samples were evenly mixed and divided into two parts. One part was stored at 4 °C for the analysis of enzyme activity, while the other part was dried, ground and sieved through 1 mm mesh for the determination of lignocellulosic content.

2.4. Determination Index and Method

During the test, a time frame of 8:00–8:15 a.m., 14:00–14:15 p.m. and 20:00–20:15 p.m. were used. The thermometer pt14320 was used to measure the temperature at the height of 30, 60 and 90 cm from the bottom of the composting heaps. Five different points of the heaped materials were measured with the thermometer and the average values recorded for analysis. Ambient temperatures of the composting site were also measured. The pH of the composting materials was measured with pH meter (Shanghai Leici phs-3c, Shanghai, China), while the content of lignocellulose (cellulose, hemicellulose and lignin) was determined using the Van der Waals fiber analysis [14]. Lignin peroxidase (LIP) and manganese peroxidase (MNP) were determined by UV spectrophotometer (Shimadzu uv-2550, Tokyo, Japan) [15,16], while peroxidase (POD) and polyphenol oxidase (PPO) were determined by colorimetry [17].

2.5. Calculation Method of Lignocellulose Degradation Rate

Calculation Method of Lignocellulose Degradation Rate

$$M\% = X_1 / X_2 \times (1 - X_3) \times 100 \tag{1}$$

where, M = lignocellulose content, mg/g; X_1 = weight of lignocellulose in the sample, mg; X_2 = sample weight, g; X_3 = water content of the sample

Degradation (%) =
$$(M_0 - M_1)/M_0 \times 100$$
 (2)

where, M_0 is the initial lignocellulose content in the sample (mg/g) and M_1 is lignocellulose content in the sample at a given sampling time (mg/g).

2.6. Data Processing

All measurements were performed in triplicate and the data were processed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington, DC, USA). The data were subjected to SPSS software version 22.0 for Windows (IBM Corp., Chicago, IL, USA) to determine the analysis of variance (ANOVA) between the treatments at 5% probability level. Differences between the treatment means were separated using Duncan's multiple range test.

3. Results

3.1. Changes in Temperature and pH during Aerobic Composting

The changes in temperature by the different C/N ratios are shown in Figure 1. The number of days by which the treatments entered a high-temperature period differed significantly from each other. The number of days by which the treatments entered a high-temperature period varied significantly from each other. Treatments T2 and T3 had the highest temperature in the thermophilic phase (>55 °C) on the 2nd day of composting, while treatments T4, T5 and T1 entered the thermophilic phase at the 3rd, 4th and 5th day of composting, respectively. The duration of the thermophilic phase increased with increasing C/N ratio, i.e., 3, 10, 19, 19 and 23 days of composting for treatments T1, T2, T3, T4 and T5, respectively. Treatment T5 had the highest total temperature (2492.0 °C), followed by treatments T4 (2322.8 °C), T3 (2252.8 °C), T2 (2029.9 °C) and T1 (1874.8 °C. The temperature produced by treatment T3 was significantly higher than that of the other treatments, and the time required to enter the thermophilic period was shorter. For all the treatments, the temperature was significantly higher than the average atmospheric temperature throughout the composting period, except for treatment T1, where the temperature was statistically equal to the average atmospheric temperature from day 42 to day 45 of composting.



Figure 1. Changes in temperature during composting of corn straw and cow dung under different C/N ratios.

The pH of each treatment pile differed from the beginning, especially in the first 10 days of composting (Figure 2). The pH of treatments T1 and T2 increased rapidly to 8.96 and 8.94 on the 6th day and was consistently higher than the other treatments throughout the composting process. Treatments T1 and T2 had their highest pH on the 6th day of composting, while treatments T4 and T5 had their highest pH on 15th day of composting. The pH remained constant from the 15th to the 45th day of composting.



Figure 2. Changes in pH during composting of corn straw and cow dung under different C/N ratios.

3.2. Degradation of Lignocellulose during Aerobic Composting

The degradation rate of the lignocellulose constituents after the entire composting process was ranked as cellulose > hemicellulose > lignin. The rate of cellulose degradation differed significantly between the treatments and increased with the duration of composting (Figure 3). Treatment T3 had the highest cellulose degradation rate (54.4%), followed by treatments T4 (48.9%), T2 (41.9%), T5 (40.5%) and T1 (39.1%), accounting for 97.3%, 96.8%, 94.5%, 93.5% and 92.0% of the total degradation rate, respectively. There was no significant difference in cellulose degradation among treatments T1, T2 and T5. With the increase in C/N ratio, the rate of cellulose degradation also increased and vice versa. The results further showed that there was no significant difference between treatments T3 and T4, but the cellulose degradation rate of treatments T3 and T4 were significantly higher than the other treatments.



Figure 3. Changes in cellulose degradation rate during composting of corn straw and cow dung under different C/N ratios.

The rate of hemicellulose degradation varied significantly by the different C/N ratios (Figure 4). The rate of hemicellulose degradation significantly differed at the maturity phase (18 to 42 days of composting). The rate of hemicellulose degradation increased with the duration of composting. Treatment T3 had the highest hemicellulose degradation rate (66.36%), followed by treatment T4 (64.05%), T5 (63.31%) and T2 (56.32%), while treatment T1 had the lowest hemicellulose degradation rate (49.35%). As the composting process increased, the degradation rate of treatments T3 and T4 rapidly increased. At the end of

composting, the degradation rate of hemicellulose was relatively higher in treatments T3 and T4, increasing by 24.5–44.08% and 7.67–24.61%, respectively.



Figure 4. Changes in hemicellulose degradation rate during composting of corn straw and cow dung under different C/N ratios.

Lignin degradation rate was significantly affected by the different C/N ratios and increased with increasing the duration of composting (Figure 5). The rate of lignin degradation mainly occurred from the thermophilic phase to the maturation stage (9–42 days) of composting, reaching 93.17–97.01% of the total degradation rate of lignin. Treatment T3 had the highest rate of lignin degradation while treatment T1 had the lowest degradation rate. There was no significant difference in lignin degradation rate between treatments T4 and T5 throughout the composting period, but they were statistically higher than treatments T1 and T2 from day 18 to day 45 of composting. Relative to the treatment, the lignin degradation rate was higher with T3. The degradation rate of treatment T3 showed 165.60%, 96.46%, 31.69% and 44.17% increase over T1, T2, T4 and T5, respectively.



Figure 5. Changes in lignin degradation rate during composting of corn straw and cow dung under different C/N ratios.

3.3. Changes in Enzyme Activity during Aerobic Composting

Lignin peroxidase (LIP) is a vital enzyme for lignin degradation. The activity of LIP was significantly affected by the C/N ratio during the composting process (Figure 6). The LIP activity increased with the duration of the composting process up to a certain point

and then decreased until the end of the experiment (45 days). The LIP activity under treatment T3 peaked on day 25 (25.73 U/g), treatments T4 and T5 on day 15 (18.11 and 17.09 U/g, respectively), while treatments T1 and T2 peaked on day 12 (14.32 and 16.00 U/g, respectively) of composting. Treatment T3 had the highest LIP activity throughout the composting period, which was significantly different from the other treatments.



Figure 6. Changes of lignin peroxidase activity during composting of corn straw and cow dung under different C/N ratios.

Polyphenol oxidase (PPO) activity varied significantly by the C/N ratio during the composting period (Figure 7). The trend of PPO activity showed an inconsistent trend among the treatments and peaked at two different periods (thermophilic phase and maturation phase). The two peaks of PPO activity of treatment T3 occurred on the 15th and 36th day of composting and were 8.32 U/g and 6.17 U/g, respectively. Treatments T4 and T5 reached their first peak on day 18 (5.98 and 4.77 U/g, respectively) and later on day 36 (4.28 and 3.96 U/g, respectively) of composting. Moreover, the first and second peak of treatments T1 and T2 occurred on day 12 (3.88 and 4.32 U/g, respectively) and day 33 (2.84 and 3.17 U/g, respectively) of composting. The PPO activity in treatment T3 was significantly higher than that of the other treatments except on day 0 and day 24, which had statistically similar PPO activity to treatment T4.



Figure 7. Changes of polyphenol oxidase activity during composting of corn straw and cow dung under different C/N ratios.

Manganese peroxidase (MNP) activity was significantly affected by the C/N ratio during the composting process (Figure 8). The MNP activity increased with the duration of the composting process up to a certain point and then decreased until the end of the experiment (45 days). Treatment T3 had the highest MNP activity throughout the experiment and peaked on day 15 (59.37 U/g) of composting. Treatments T4 and T5

peaked on day 18, while treatments T1 and T2 peaked on day 12 of composting. On the 12th day of composting, the MNP activity of each treatment was 34.54 U/g, 38.32 U/g, 45.29 U/g, 38.08 U/g and 34.67 U/g, respectively. The activity of MNP in treatments T3, T4 and T5 increased rapidly and was significantly higher than that observed in treatments T1 and T2.



Figure 8. Changes of manganese peroxidase activity during composting of corn straw and cow dung under different C/N ratios.

Peroxidase (POD) mainly oxidizes organic matter during composting and it was significantly varied by the C/N ratio during the composting process (Figure 9). Similar to the MNP activity, POD activity increased with the duration of the composting process up to a certain point and then decreased until the end of the experiment (45 days). Treatments T3, T4, and T5 peaked on day 15 of composting (0.82, 0.53 and 0.46 U/g, respectively), while treatments T1 and T2 peaked on day 12 of composting (0.33 and 0.38 U/g, respectively). Treatment T3 had the highest POD activity throughout the composting period, while treatment T1 had the lowest activity throughout the composting period.



Figure 9. Changes of peroxidase activity during composting of corn straw and cow dung under different C/N ratios.

3.4. Correlation Analysis

3.4.1. Correlation Analysis between Enzyme Activity and Lignocellulose Degradation

The relationship between enzyme activity and lignocellulose degradation is shown in Table 3. Lignin peroxidase showed a significant and positive correlation with the rate of degradation of cellulose (r = 0.442), hemicellulose (r = 0.288) and lignin (r = 0.403). Polyphenol oxide correlated positively with the rate of degradation of cellulose (r = 0.436), hemicellulose (r = 0.293) and lignin (r = 0.432). In addition, manganese peroxidase showed a significant and positive relationship with lignin degradation rate (r = 0.219), but an insignificant and positive correlation with cellulose (r = 0.290) and hemicellulose (r = 0.111) degradation rates. There was an insignificant and negative correlation between peroxidase and the rate of degradation of hemicellulose (r = -0.036), while it was positively correlated with the rates of degradation of cellulose and lignin.

Table 3. The correlation coefficient between enzyme activity and degradation rate of cellulose, hemicellulose and lignin.

	Cellulose	Hemicellulose	Lignin
Lignin peroxidase (LIP)	0.442 **	0.288 **	0.403 **
Polyphenol oxidase (PPO)	0.436 **	0.293 **	0.432 **
Manganese peroxidase (MNP)	0.290	0.111	0.219 **
Peroxidase (POD	0.122	-0.036	0.163
Note: ** indicates $n < 0.01$			

Note: ** indicates p < 0.01.

3.4.2. Correlation Analysis between Enzyme Activity, Temperature and pH

The relationship between enzyme activity, temperature and pH is shown in Table 4. Lignin peroxidase activity showed significant and positive correlation with polyphenol oxidase (r = 0.916), manganese peroxidase (r = 0.902), peroxidase (r = 0.850), temperature (r = 0.282) and pH (r = 0.486). There was a significant and positive relationship between polyphenol oxidase activity and manganese peroxidase activity (r = 0.856), peroxidase activity (r = 0.843), temperature (r = 0.275) and pH (r = 0.435). Manganese peroxidase activity was significantly and positively correlated with peroxidase activity (r = 0.855), temperature (r = 0.472) and pH (r = 0.450). Moreover, peroxidase enzyme activity showed significant and positive correlation with temperature (r = 0.524), while it was only slightly correlated with pH (r = 0.147).

Table 4. The correlation analysis of enzyme with temperature, pH and enzyme activity.

	Lignin Peroxidase (LIP)	Polyphenol Oxidase (PPO)	Manganese Peroxidase (MNP)	Peroxidase (POD)
Lignin peroxidase (LIP)	_			
Polyphenol oxidase (PPO)	0.916 **	_		
Manganese peroxidase (MNP)	0.902 **	0.856 **	_	
Peroxidase (POD)	0.850 **	0.843 **	0.855 **	-
Temperature (T)	0.282 *	0.272 *	0.472 **	0.524 **
pH	0.486 **	0.435 **	0.450 **	0.147

Note: * indicates *p* < 0.05, ** *p* < 0.01.

4. Discussion

4.1. Effect of C/N Ratio on Temperature and pH in Aerobic Composting

Different initial C/N ratios had different effects on the aerobic composting process. It was observed that too high or too low C/N ratio prolonged the time required for the compost piles to reach the thermophilic phase and that the duration of the high-temperature phase is directly proportional to the initial C/N ratio. The microorganisms that degrade lignocellulose are mainly thermophilic actinomycetes and thermophilic fungi [18]. Treatments with C/N ratio greater than 25 (T3, T4 and T5) were more conducive to the degradation of lignocellulose. The temperature produced by treatment T3 was significantly higher than that of the other treatments due to the increased rate of biochemical reaction by microorganisms, which concurs with Cárdenas-González et al. [19]. It can be inferred that an appropriate C/N ratio effectively improves the temperature of the compost pile and promotes the decomposition of organic matter [20]. The low C/N ratio (T1) delayed the composting process, which could be attributable to an insufficient amount of carbon

and nitrogen of the compost pile. According to Ren et al. [21], an insufficient amount of carbon and nitrogen inhibits microbial growth and activity, resulting in a lower enzymatic activity in the composting system. However, an excessively high C/N ratio prolongs the time required by the compost piles to enter the high-temperature phase, which affects the decomposition of organic matter [22]. This could explain why treatment T3 accelerated the maturation process of the compost. pH is one of the most important factors affecting the growth and reproduction of microorganisms. Microbial decomposition ability is strongly enhanced when the pH of the compost pile is in the range of 7.5–8.5 [23], indicating that the compost ended its rapid degradation period and entered the maturation period. Compost piles with a low initial C/N ratio (T1) released large amount of NH₃ from the stockpile, causing the pH of the pile to increase rapidly. This phenomenon slows down the growth rate of microorganisms and decreases the assimilation of organic nitrogen [24]. The pH values of compost piles with high C/N ratio were within the range required by microorganisms for growth and reproduction [25].

4.2. Effects of C/N Ratio on Degradation of Lignocellulose and Enzyme Activity in *Aerobic Composting*

The degradation rate of the lignocellulosic constituents after the entire composting process was ranked as follows: cellulose > hemicellulose > lignin. Compared to lignin, cellulose and hemicellulose were degraded and utilized more efficiently by the microorganisms, which is consistent with previous studies [26,27]. In the early stage of composting, microorganisms might have used degradable organic substances as sources of energy and nutrients for their reproduction and growth. In this study, the degradation of cellulose and lignin mainly occurred during the thermophilic phase and the maturation phase, while the degradation of hemicellulose occurred mainly in the thermophilic phase. The rate of degradation of cellulose, hemicellulose and lignin was significantly higher in treatment T3 than in the other treatments, which was due to the adequate carbon and nitrogen content that met the energy and nutrient requirements of the microorganisms and thus promoted the degradation of lignocellulose. The degradation rate of lignin was highest in treatment T3, indicating that the degradation of lignin was influenced by C/N ratio of 25. The high temperature duration and nitrogen content, which was influenced by the high C/Nratio, affected the microbial composition and degradation of lignocellulose. This finding is consistent with previous studies that low nitrogen content facilitates the degradation of lignocellulose [28]. Eiland et al. [29] postulated that C/N ratio is an important factor that affects the degradation of lignocellulose. However, an excessively high or low C/N ratio is not conducive for lignocellulose degradation.

During the composting process, the enzyme activities were in the order: POD < PPO < LIP < MNP. Compared to the other treatments, the enzyme activity of POD, PPO, LIP and MNP in treatment T3 was higher than the other treatments throughout the composting process, indicating that the C/N ratio of 25 was more favorable for the microorganisms to secrete enzymes through the decomposition of organic matter. Microorganisms need to assimilate 5 parts of carbon and 1 part of nitrogen to form their body cells, but they can only absorb and utilize 1 part of carbon when they consume 4 parts of organic carbon. The ratio of uptake and utilization of carbon to nitrogen by microbes is 25:1 [30]. Therefore, C/N ratio of 25 is suitable for microbial growth, metabolism and decomposition of organic matter. This could explain why treatment T3 had the highest enzyme activity throughout the composting period. The difference in initial C/N ratio differs in the timing of the activity peaks. The higher the initial C/N ratio of the pile, the longer it takes the enzyme activity to peak. This is because microorganisms first use the easily degradable organic matter to ensure growth and reproduction in the compost heap, and then secrete the appropriate degradative enzymes to break down the lignocellulose. As C/N ratio increases, microorganisms first consume the excess carbon source through multiple metabolic cycles until they reach the C/N range suitable for microbial growth and reproduction, and then secrete secondary metabolites [31]. As the C/N ratio increases, the content of lignocellulose in the raw materials increases. Throughout the composting process, the degradation of

lignocellulose mainly occurred at high-temperature and maturation phases, while enzyme activities increased rapidly in the thermophilic phase (3rd day). This is because organic matter degradation is dependent on enzymes produced by microorganisms [32], and it takes time for microorganisms to proliferate, metabolize and produce enzymes to degrade lignocellulose [33].

During composting, microorganisms degrade organic matter and further convert it into humus by secreting various enzymes. The ability of microorganisms to decompose and metabolize organic matter depends on enzyme activity. Enzyme reaction is a comprehensive and complex biochemical process [34]. The increase in the activity of lignin peroxidase (LIP) and polyphenol oxidase (PPO) correlated with the degradation of lignocellulose under different C/N ratios composting (Table 3). Manganese peroxidase (MNP) participated only in the degradation of lignin, which is consistent with the study by Wang [35] who documented that MNP is a key functional enzyme for phenolic lignin degradation. However, the proportion of different enzyme components directly affects lignin degradation, resulting in changes in the correlation between enzyme activity and lignin degradation [36]. The correlation between the activity of peroxidase (POD) and the degradation of lignocellulose and other organic substances, resulting in insignificant degradation of lignocellulose by the enzyme activity.

5. Conclusions

This study investigated the effects of five levels of C/N ratio on lignocellulose degradation and enzyme activities during composting of corn straw and cow dung. The lignocellulose degradation and enzyme activities were influenced by the duration of composting and the different C/N ratios of the pile. On average, treatment T3 ensured 55.86%, 50.93% and 17.09% degradation of cellulose, hemicellulose and lignin, respectively. Relative to the lignocellulosic constituents, cellulose degradation was highest (39.07–54.35%), followed by hemicellulose (35.35–50.93%) and lignin (6.13–16.58%). The degradation of cellulose and lignin occurred mainly in the thermophilic and maturation phases, whereas the degradation of hemicellulose was higher in the maturation phase. The activities of the four enzymes involved in lignocellulose degradation differed significantly among treatments and the time required to reach the activity peak. The higher the initial C/N ratio, the longer it took the enzyme activities to reach their peak. Treatment T3 was the best C/N ratio to stimulate the activities of manganese peroxidase, lignin peroxidase, polyphenol oxidase and peroxidase, which in turn promoted lignocellulose degradation.

Author Contributions: Conceptualization, H.Y., H.Q. and C.Z.; investigation, H.Y., H.Z., M.L. and Y.W.; methodology, H.Y., H.Z., Y.W. and C.Z.; formal analysis, M.L., Y.W. and D.K.A.; data curation, H.Y., Y.W., H.Z. and M.L.; writing—original draft preparation, H.Y., H.Q. and C.Z.; writing—review and editing, H.Y., H.Q. and D.K.A.; software, H.Y. and D.K.A.; supervision, H.Q.; funding acquisition, H.Q. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by: Research and Development of Comprehensive Prevention and Remediation Technology for Agricultural Non-point Source and Heavy Metal Polluted Farmland: 2017YFD0800200; National Key Basic Research and Development Plan: 2015cb150501; National Natural Science Foundation of China: 31360500.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Yao, X.; Zhou, H.; Meng, H.; Ding, J.; Shen, Y.; Cheng, H.; Zhang, X.; Li, R.; Fan, S. Amino acid profile characterization during the co-composting of a livestock manure and maize straw mixture. *J. Clean. Prod.* 2021, 278, 123494. [CrossRef]
- Medina, J.; Monreal, C.M.; Antilen, M.; Calabi-Floody, M.; Velasco-Molina, M.; Meier, S.; Borie, F.; Cornejo, P.; Knicker, H. Influence of inorganic additives on wheat straw composting: Characterization and structural composition of organic matter derived from the process. J. Environ. Manag. 2020, 260, 110137. [CrossRef] [PubMed]
- 3. Yang, X.; Li, Q.; Tang, Z.; Zhang, W.; Yu, G.; Shen, Q.; Zhao, F.-J. Heavy metal concentrations and arsenic speciation in animal manure composts in China. *Waste Manag.* **2017**, *64*, 333–339. [CrossRef] [PubMed]
- 4. Bernal, M.P.; Alburquerque, J.; Moral, R. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* **2009**, *100*, 5444–5453. [CrossRef] [PubMed]
- 5. Ayilara, M.S.; Olanrewaju, O.S.; Babalola, O.O.; Odeyemi, O. Waste management through composting: Challenges and potentials. *Sustainability* **2020**, *12*, 4456. [CrossRef]
- 6. Bi, S.; Hong, X.; Yang, H.; Yu, X.; Fang, S.; Bai, Y.; Liu, J.; Gao, Y.; Yan, L.; Wang, W.; et al. Effect of hydraulic retention time on anaerobic co-digestion of cattle manure and food waste. *Renew. Energy* **2019**, *150*, 213–220. [CrossRef]
- Ghanney, P.; Kugbe, J.X.; Anning, D.K. Role of Microbial Biomechanics in Composting with Special Reference to Lignocellulose Biomass Digestion. *Asian J. Biotechnol. Bioresour. Technol.* 2021, 7, 30–46. [CrossRef]
- Lu, Q.; Zhao, Y.; Gao, X.; Wu, J.; Zhou, H.; Tang, P.; Wei, Q.; Wei, Z. Effect of tricarboxylic acid cycle regulator on carbon retention and organic component transformation during food waste composting. *Bioresour. Technol.* 2018, 256, 128–136. [CrossRef] [PubMed]
- 9. Zhang, X.Q.; Xu, X.H.; Wang, J.; Liu, J. Effect of inoculating lignin degradation strains on enzymic activities in composting. *J. Agro-Environ. Sci.* 2012, *31*, 843–847.
- Wan, W.; Wang, Y.; Tan, J.; Qin, Y.; Zuo, W.; Wu, H.; He, H.; He, D. Alkaline phosphatase—Harboring bacterial community and multiple enzyme activity contribute to phosphorus transformation during vegetable waste and chicken manure composting. *Bioresour. Technol.* 2020, 297, 122406. [CrossRef]
- 11. Huang, G.F.; Wong, J.W.C.; Wu, Q.T.; Nagar, B.B. Effect of C/N on composting of pig manure with sawdust. *Waste Manag.* 2004, 24, 805–813. [CrossRef]
- 12. Iqbal, M.K.; Nadeem, A.; Sherazi, F.; Khan, R.A. Optimization of process parameters for kitchen waste composting by response surface methodology. *Int. J. Environ. Sci. Technol.* **2015**, *12*, 1759–1768. [CrossRef]
- 13. Yang, Y.; Du, W.; Ren, X.; Cui, Z.; Zhou, W.; Lv, J. Effect of bean dregs amendment on the organic matter degradation, humification, maturity and stability of pig manure composting. *Sci. Total Environ.* **2020**, *708*, 134623. [CrossRef] [PubMed]
- 14. Peng, G.; Xiaofen, W.; Wanbin, Z.; Hongyan, Y.; Cheng, X.; Zongjun, C. Degradation of corn stalk by the composite microbial system of MC1. *J. Environ. Sci.* 2008, 20, 109–114.
- Zhao, M.; Zeng, G.; Huang, D.; Feng, C.; Huang, C.; Hu, S.; Su, F.; Lai, C.; Wei, Z. Research on sorption and transport characteristics of ligninolytic enzymes in different compost substances. *Environ. Sci.* 2010, *31*, 1647–1654.
- 16. Pickard, M.; Roman, R.; Tinoco, R.; Vazquez-Duhalt, R. Polycyclic aromatic hydrocarbon metabolism by white rot fungi and oxidation by coriolopsis gallica UAMH 8260 Laccase. *Appl. Environ. Microbiol.* **1999**, *65*, 3805–3809. [CrossRef]
- 17. Aquino-Bolaños, E.N.; Mercado-Silva, E. Effects of polyphenol oxidase and peroxidase activity, phenolics and lignin content on the browning of cut jicama. *Postharvest Biol. Technol.* **2004**, *33*, 275–283. [CrossRef]
- 18. Yu, H.; Zeng, G.; Huang, H.; Xi, X.; Wang, R.; Huang, D.; Huang, G.; Li, J. Microbial community succession and lignocellulose degradation during agricultural waste composting. *Biodegradation* 2007, *18*, 793–802. [CrossRef]
- Cárdenas-González, B.; Ergas, S.J.; Switzenbaum, M.S. Characterization of compost biofiltration media. J. Air Waste Manag. Assoc. 1999, 49, 784–793. [CrossRef]
- 20. Zhang, L.; Sun, X. Influence of bulking agents on physical, chemical, and microbiological properties during the two-stage composting of green waste. *Waste Manag.* 2016, 48, 115–126. [CrossRef]
- 21. Ren, L.M.; Schuchardt, F.; Shen, Y.J.; Li, G.X.; Li, C.P. Impact of struvite crystallization on nitrogen losses during composting of pig manure and cornstalk. *Waste Manag.* 2010, *30*, 885–892. [CrossRef]
- 22. Wang, Q.; Awasthi, M.K.; Ren, X.; Zhao, J.; Li, R.; Wang, Z.; Chen, H.; Wang, M.; Zhang, Z. Comparison of biochar, zeolite and their mixture amendment for aiding organic matter transformation and nitrogen conservation during pig manure composting. *Bioresour. Technol.* **2017**, 245, 300–308. [CrossRef]
- 23. Hua, D.; Liu, F.; Li, G.; Jiang, T. Effect of turning and covering techniques on pig manure—Straw composting property. *Trans. Chin. Soc. Agric. Eng.* **2011**, *27*, 210–216.
- 24. Wang, L.; Li, Y.; Prasher, S.O.; Yan, B.; Ou, Y.; Cui, H.; Cui, Y. Organic matter, a critical factor to immobilize phosphorus, copper, and zinc during composting under various initial C/N ratios. *Bioresour. Technol.* **2019**, *289*, 121745. [CrossRef]
- Zhang, L.; Dong, H.; Zhang, J.; Chen, Y.; Zeng, G.; Yuan, Y.; Cao, W.; Fang, W.; Hou, K.; Wang, B.; et al. Influence of FeONPs amendment on nitrogen conservation and microbial community succession during composting of agricultural waste: Relative contributions of ammonia-oxidizing bacteria and archaea to nitrogen conservation. *Bioresour. Technol.* 2019, 287, 121463. [CrossRef]
- Dashtban, M.; Schraft, H.; Qin, W. Fungal bioconversion of lignocellulosic residues; opportunities & perspectives. Int. J. Biol. Sci. 2009, 5, 578–595.

- 27. Deng, H.; Wang, C.; Lu, H.; Wang, F.; Tu, Q.; Wu, W. Research progress in succession of actinomycetal communities and their capacity of degrading lignocellulose during composting process. *Chin. J. Appl. Environ. Biol.* **2013**, *19*, 581–586. [CrossRef]
- 28. Xi, B.D.; Liu, H.L.; Bai, Q.Z.; Huang, G.H.; Zeng, G.M.; Li, Y.J. Study on current status of lignin and cellulose biodegradation in composting process. *Technol. Equip. Environ. Pol. Cont.* 2002, *3*, 19–23.
- 29. Eiland, F.; Klamer, M.; Lind, A.M.; Leth, M.; Bååth, E. Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. *Microb. Ecol.* **2001**, *41*, 272–280. [CrossRef]
- 30. Barrington, S.F.; El Moueddeb, K.; Porter, B. Improving small—Scale composting of apple waste. *Canad. Agric. Eng.* **1997**, *39*, 9–16.
- Mathur, S.P. Composting processes. In *Bioconversion of Waste Materials to Industrial Products*, 1st ed.; Martin, A.M., Ed.; Springer Publishing: New York, NY, USA, 1991; pp. 147–183.
- 32. Kumar, M.; Ou, Y.L.; Lin, J.G. Co-composting of green waste and food waste at low C/N ratio. *Waste Manag.* 2010, *30*, 602–609. [CrossRef]
- 33. Pedersen, M.; Meyer, A.S. Lignocellulose pretreatment severity—Relating pH to biomatrix opening. *New Biotechnol.* **2010**, 27, 739–750. [CrossRef]
- Shi, M.; Zhao, Y.; Zhu, L.; Song, X.; Tang, Y.; Qi, H.; Cao, H.; Wei, Z. Denitrification during composting: Biochemistry, implication and perspective. *Int. Biodeterior. Biodegrad.* 2020, 153, 105043. [CrossRef]
- 35. Wang, H.; Li, Z. Three important enzymes for lignin degradation. J. Biol. 2003, 20, 9–11.
- 36. Reddy, C.A. An overview of the recent advances on the physiology and molecular biology of lignin peroxidases of *Phanerochaete chrysosporium*. *J. Biotechnol.* **1993**, *30*, 91–107. [CrossRef]