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Adjusting pH of the Secondary Composting Materials to Further Enhance the Lignocellulose Degradation and Promote the Humification Process

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Abstract: Straw waste has a large amount of lignocellulose, which shows a strong resistance to biodegradation in the composting process and hinders the formation of humic substances. Therefore, the effects of pH adjustment and inoculation of degradation bacteria on the composting process, lignocellulose degradation and humus formation in secondary fermentation were explored through aerobic composting experiments. The experiment was designed with four treatment groups: CK (conventional composting), T1 (adjusting pH), T2 (inoculating *P. chrysosporium*), and T3 (adjusting pH and inoculating *P. chrysosporium*), respectively. Results showed that except for treatment CK, all other treatments met the maturation standards at the end of composting. Adjustment of the pH value and the inoculation of *Phanerochaete chrysosporium* (*P. chrysosporium*) can significantly shorten the fermentation time. Among them, the lignin content of treatment T3 was reduced significantly by 14.28% compared to treatment T2; the content of humic acid in T3 increased significantly by 51.32% and 14.04% compared with T1 (adjusting pH) and T2 ($p < 0.05$), respectively. In terms of key enzyme activity and precursor substance changes, the pH adjustment treatment was superior to other treatments after composting. This study confirmed that the change of pH conditions is an important environmental factor for microorganisms to enhance the humification process; degrading enzymes were used as a "bridge" to enhance the continuous degradation of lignocellulose by microorganisms and increase the supplementation of precursors and the synthesis of humic acid, which is the mechanism to enhance the humification process. Our findings provided a new method to enhance the humification process, which is a valuable and economical technical approach to improve organic fertilizer quality.

Keywords: composting; secondary fermentation; pH; enzyme activity; humic acid precursors; humification process

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1. Introduction

With the rapid development of social economy and population expansion, the amount of agricultural organic solid waste has increased considerably day by day in China, causing serious harm to human health, production, and the environment [1]. Composting, as the main process for treating organic agricultural waste is a dynamic process that uses microbial community succession to stabilize and treat harmlessly organic agricultural waste under the action of enzyme catalysis [2], which can convert organic materials into stable humus (HS), resulting in high-fertility organic fertilizers [3–5]. The lignin–protein theory and phenol–protein theory as the main theories of HS formation show that lignin and its degradation products (phenols and quinones) are the key components in HS formation by polymerization with nitrogen-containing compounds (proteins and amino acids) [6,7]. Straws, as one of the agricultural wastes show a strong resistance to biodegradation in composting due to their rich lignin content, which affects their fast and efficient composting [8,9], while the content and degradation rate of lignocellulose in composting are correlated significantly with the formation of HS [10,11]. In addition, the precursor substances (polyphenol and quinone-like) formed by the oxidation of lignin under the action of microorganisms can form HS through condensation or polymerization with microbial degradation products, such as amino acid reducing sugars [12–14]; HS is rich in carboxyl, quinone, hydroxyl, and other functional groups, which can be complex with a variety of pollutants and can reduce the migration and transformation of toxic substances in the environment [15,16]. Therefore, it is urgent to find a novel approach to enhance the conversion of lignocellulose to HS to improve composting efficiency and quality [10,17].

To enhance the degradation of lignocellulose, most of the studies used exogenous inoculation of microorganisms [18,19]. The *Phanerochaete chrysosporium* (*P. chrysosporium*), as a model microorganism for lignin degradation can significantly promote the degradation of lignocellulose through exogenous inoculation [20], and enhance the synthesis of HS [21,22], especially the inoculation in the secondary fermentation stage of composting [23,24]. Secondary fermentation, as the main stage of composting humus formation [25,26] involves two processes which are the decomposition of organic materials and the synthesis of HS. Firstly, microorganisms secrete specific extracellular enzymes (LiP, MnP, Lac) to produce side-chain oxidation and aromatic-ring substitution, which break the bonds and accelerate the continuous degradation of lignocellulose. Secondly, organic materials are degraded to form a large number of HS precursors (such as amino acids, sugars, polyphenols, and phenolic derivatives, etc.); the HA is formed by condensation or polymerization and promotes the process of composting humification, and microorganisms play an important role, especially fungi (Ascomycota, Basidiomycota, etc.) [23,27,28]. However, fungi as the main biological factor driving the humification process in the secondary fermentation stage of composting [29], are affected by various environmental factors, such as pH and temperature. The pH is the main abiotic factor affecting fungal activity, which can be regulated and changed [30,31]; unfavorable pH conditions can reduce or even inhibit microbial activity, resulting in slow degradation of composting organic materials [32]. It is due to this that the secondary fermentation process of composting is always going to be alkaline (pH > 7.5), most fungi prefer a neutral-to-acidic environment (pH < 7.5) [33]. The microorganisms involved are mainly mesophilic fungi, and the fungal community is easily affected by the pH environment [21]. It is speculated that the high pH environment (pH > 7.5) during the secondary fermentation may affect the reproduction and metabolism of the fungal community, and thus affect the humification process. Lignin is the source and skeleton of HS formation [34], and the existence of a three-dimensional structure makes it difficult for lignin to be completely degraded during the primary fermentation of composting [35]. Studies have shown that inoculation of fungi during the secondary fermentation of composting can enhance the degradation of lignocellulose to promote the humification process [23]. The possible microbial mechanism of the process was analyzed, and the importance of ensuring fungal microbial activity in this process was proven. Therefore, it should be considered whether adjusting the pH value of the secondary composting material can ensure the activity of microorganisms, continue to degrade lignocellulose, and promote the humification process. Based on the above, the purpose of this study is: (1) to investigate the effects of pH adjustment on the secondary composting process; and (2) to analyze the mechanism of promoting humification by microorganisms under the change of pH during secondary composting.

2. Materials and Methods

2.1. Materials and Experimental Design

A composting reactor was used to conduct composting experiments in the laboratory of Yunnan Agricultural University. The sources of raw materials used for composting, treatment methods, fermentation conditions, and operations of composting are referred to in Chen et al. [23]. The composting reactors are the same as Zhao's [11]. Based on primary fermentation, secondary fermentation was carried out when the composting temperature was reduced to about 35 °C (18 days). The definition of secondary fermentation was detailed in the study of Xu et al. [36]. The changing trend of physical and chemical properties of materials during primary fermentation of composting was consistent with the previous research results of Chen et al. [23]. Properties of primary fermentation products are as follows: pH value of 7.68 ± 0.03, C/N ratio of 21.32 ± 0.23, GI value of 53.96 ± 2.51%, cumulative degradation ratio of 13.34 ± 1.43%, humus content of 93.84 ± 0.56 g·kg^{-1}, fulvic acid content of 43.49 ± 0.69 g·kg^{-1}, and humic acid content of 50.35 ± 0.85 g·kg^{-1}, respectively. The basic physical and chemical properties of maize straw and canola residue are shown in Table 1.

Table 1. The basic physical and chemical properties of the composting materials. (n = 3).

Raw Material	Moisture Content (%)	Total C (g·kg^{-1})	Total N (g·kg^{-1})	C/N
Maize straw	9.89 ± 0.77%	485.8 ± 4.76	5.98 ± 0.25	81.24 ± 5.56
Canola residue	7.17 ± 0.29%	452.86 ± 6.32	56.37 ± 0.84	8.03 ± 0.17

Considering that inoculation of *P. chrysosporium* in the secondary fermentation stage can effectively ensure its activity, and the appropriate pH value of fungi was not higher than 7.5, four groups of composting experiments were designed for secondary fermentation in this experiment, and the fermentation time is 18 days. The experiment was designed with four treatment groups: CK (conventional composting), T1(adjusting pH), T2 (inoculating *P. chrysosporium*), and T3 (adjusting pH and inoculating *P. chrysosporium*). *P. chrysosporium* density was 10^8 cfu mL^{-1} from the Chinese Academy of Sciences. The composting materials were mixed every 3 days (days 18~24), then every 6 days (days 24~42). The 300 g samples were collected from five different areas of the mixed material, and the physical and chemical indicators were measured, respectively.

The composting temperature was measured by PT 100. The physical and chemical indicators (the pH and seed germination index GI) in the composting material were determined by the method of Yu et al. [37]. The degradation of lignin and the humification indicator were determined by Zhang ang Sun et al. [38]. The activities of the key degrading enzymes (LiP, MnP, and Lac) were determined by Zeng et al. [24]. The contents of the reduced sugar were determined according to Tursun et al. [39]. The concentration of the amino acid was determined according to the ninhydrin colorimetric method [40]. Total phenolics were assayed using the Folin–Ciocalteu reagent [41].

The seed germination index (GI) was determined using Cress seed (*Lepidium sativum* L.). The GI was calculated by the following formula:

$$\text{GI (\%)} = (\text{germination rate of seed extract} \times \text{seed root length})/(\text{control seed germination rate} \times \text{seed root length}) \times 100.$$

2.2. Statistical Analyses

The physical–chemical parameters, key enzyme activity, Maillard precursor concentration, and humic component concentration were analyzed using Excel® 2016 (Microsoft, Inc., Redmond, WA, USA), and related graphics were drawn. All statistical analyses were performed with SPSS version 21. Data were reported as means of triplicates and analyzed using ANOVA. The significant level of differences in the study was set at $p < 0.05$. Canoco (Version 5.0) software was used for redundancy analysis (RDA) to study the correlation between environmental parameters and the humification process.

3. Results and Discussion

3.1. Variations in Physicochemical Parameters during the Secondary Fermentation of Composting

The change of heap temperature was the main indicator to characterize the maturation process and microbial activity of organic materials in the process of aerobic composting, and it is also a key factor of the whole composting process [42]. At the whole secondary fermentation stage, Figure 1a plotted that although the normal composting (CK) temperature continued to decrease, the treatment of adjusting pH (T1), inoculating *P. chrysosporium* (T2), and adjusting pH and inoculating *P. chrysosporium* (T3) showed peak values (39.33 °C, 41.67 °C, and 44.67 °C, respectively) on the 20th day, indicating the high metabolic activity of composting microorganisms after adjusting pH and inoculating *P. chrysosporium* [24,43]. The reason for the slight increase in the T1 treatment temperature may be related to the adjustment of pH to meet the suitable acid–base environment of microorganisms (Figure 1b) to increase its activity. The reason for the peak in the T2 treatment temperature was consistent with the results of inoculation during the cooling period by Chen et al. and Zeng et al. [23,24]. Interestingly, the effect of T3 treatment on temperature was more significant than that of T1 and T2 treatments; the reason may be that the suitable acid–base environment provided better environmental conditions for the inoculation of *P. chrysosporium*.

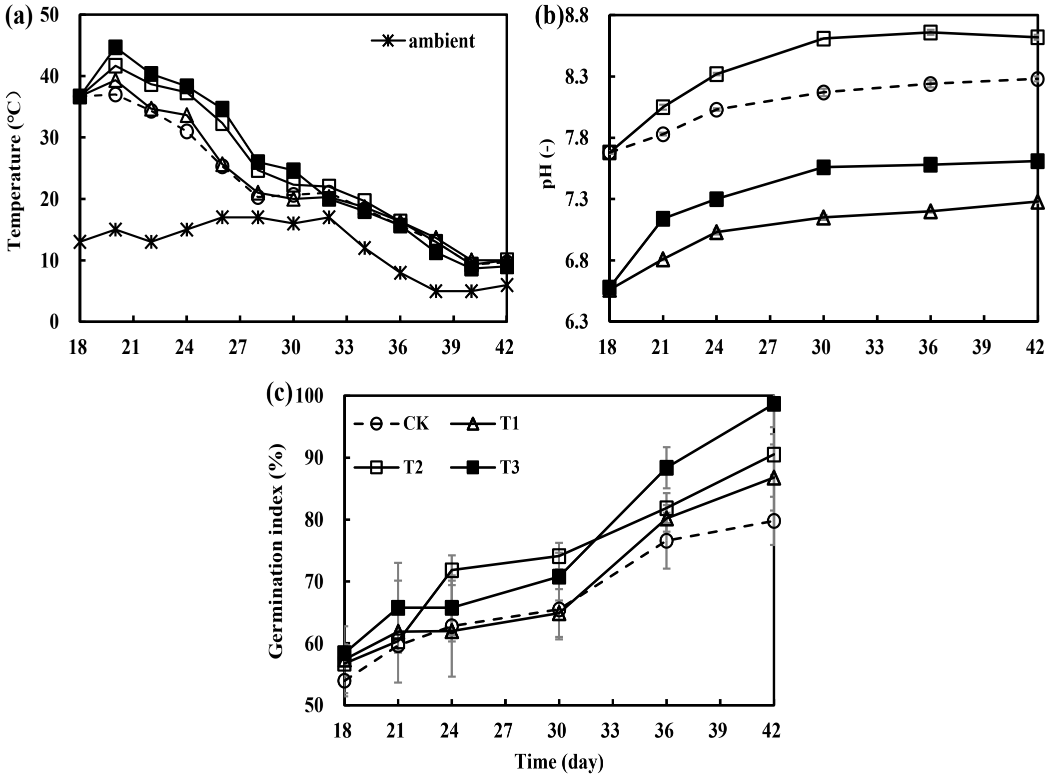

Figure 1. Changes of (**a**) temperature, (**b**) pH value, and (**c**) seed germination index.

The pH is an important factor affecting microbial activity and an important indicator that directly reflects the degree of internal acidity and alkalinity for composting [44]. As shown in Figure 1b, the pH value of all treatments showed an upward trend in the whole secondary fermentation stage. The pH values of CK, T1, T2, and T3 treatment were 8.28, 7.28, 8.62, and 7.61 ($p < 0.01$) at the end of composting, respectively. The results showed that even if the pH value of the secondary fermentation material was adjusted (the pH value was adjusted to 6.5), the pH value still showed an upward trend in the whole secondary fermentation stage (T1 and T3), although the composting material maintained a relatively low pH environment (Figure 1b) after adjusting the pH value. The inoculation of *P. chrysosporium* resulted in the massive decomposition of organic materials and the production of more nitrogen-containing compounds (Figure 2d), which were the main reasons that the pH value of T2 was higher than that of other treatments ($p < 0.05$) [45].

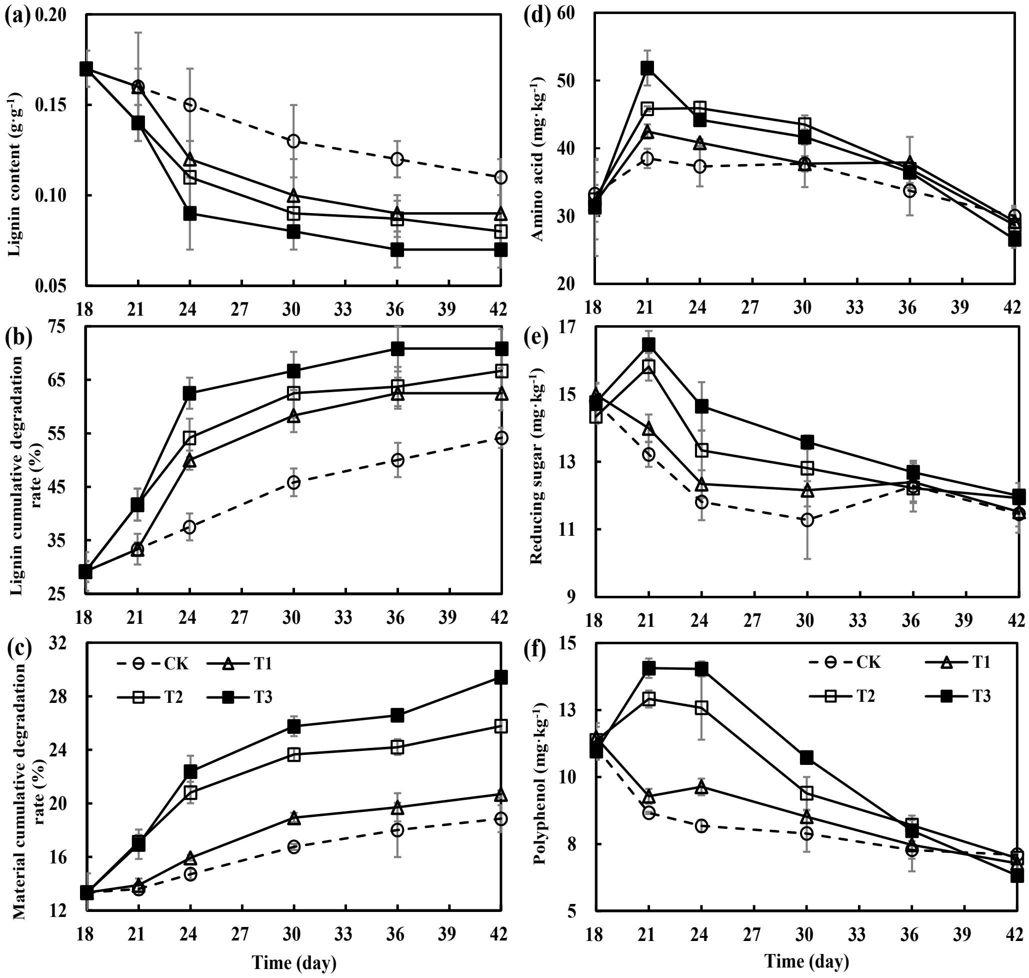

Figure 2. Changes of (**a**) lignin content, (**b**) lignin cumulative degradation rate, (**c**) material cumulative degradation rate, (**d**) amino acid, (**e**) reducing sugar, and (**f**) polyphenol during composting.

Seed germination index (GI) has been regarded as one of the most important indicators which reflects the maturity and harmlessness of a compost [46]. When GI $\geq$ 50%, the toxicity of the compost is relatively low; when the GI $\geq$ 80%, the compost has matured fully, which is beneficial to the growth and development of plants [47]. At the end of composting, the GI values of CK, T1, T2, and T3 treatments were 79.78%, 86.8%, 90.52%, and 98.7% ($p < 0.05$) (Figure 1c), respectively. Among them, T1, T2, and T3 treatments met the maturity standard (GI > 80%) 6 days, 7 days, and 9 days earlier than CK. The research found that adjusting the pH and inoculating *P. chrysosporium* during secondary fermentation of composting could effectively shorten the fermentation period and enhance the maturity degree. Among them, the composting effect of T3 treatment (adjusting the pH value and inoculating) is better. The reason may be that the adjustment of the pH value is more conducive for *P. chrysosporium* to deepen the degradation of organic materials (Figure 2) and to increase the composting fermentation intensity (Figure 3), while meeting the suitable growth environment of microorganisms (Figure 1b), which accelerates the rate of conversion from precursors, such as phenols, to mature HS. This result is consistent with the research of Wang et al. that a high degree of humification was beneficial to the reduction of toxicity of composting products [48]. Furthermore, Zhong et al. found that the composting maturity and quality were higher after biofortification [49].

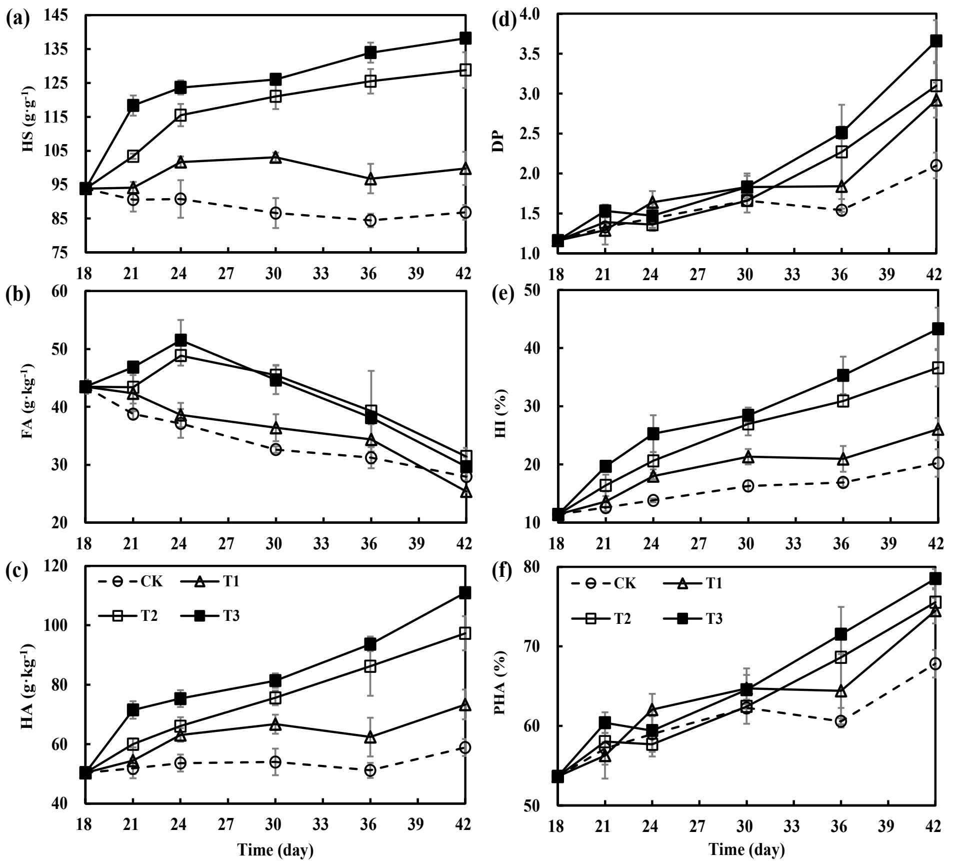

Figure 3. Changes of (**a**) humus substance, (**b**) fulvic acid, (**c**) humic acid, (**d**) degree of polymerization, (**e**) humification index, and (**f**) percent of humus acid during composting.

3.2. Variations in Decomposition of Organic Materials during the Secondary Fermentation of Composting

Although lignin, as the skeleton generated by HS [50] played an important role in the condensation of composting HS, and the existence of a three-dimensional structure made it difficult to be demanding to be degraded completely [8,51], the degradation of macromolecular organic carbon such as lignocellulose is a key factor which determined the composting maturity process [28]. In Figure 2a, the lignin content of CK, T1, T2, and T3 treatments decreased from 0.17 $g{\cdot}g^{-1}$ at the beginning of the secondary fermentation to 0.11 $g{\cdot}g^{-1}$, 0.09 $g{\cdot}g^{-1}$, 0.08 $g{\cdot}g^{-1}$, and 0.07 $g{\cdot}g^{-1}$ at the end of composting, respectively. The T1 treatment greatly outperformed the CK treatment ($p < 0.05$) in terms of the rate of decline, indicating that adjusting the pH in the secondary fermentation stage of composting was helpful to stimulate the re-degradation of lignin, the reason being directly related to the enhancement of the degrading enzyme activity (Figure 4). Since the optimum temperature of *P. chrysosporium* is around 35 °C, the inoculation can effectively ensure the activity during the cooling period, which is the main reason that the decline rate of the T2 treatment is higher than that of the T1 treatment [23,24]. The decline rate of T3 was higher than that of T2 ($p < 0.05$). At the end of composting, the cumulative degradation rate of T3 lignin reached 70.83% (Figure 2b). The results verified that inoculating *P. chrysosporium* deepened the continuous degradation of lignocellulose and enhanced the fermentation intensity. At the same time, the appropriate acid–base environment (Figure 1b) was an important environmental factor to further enhance the humification process in the secondary fermentation of composting.

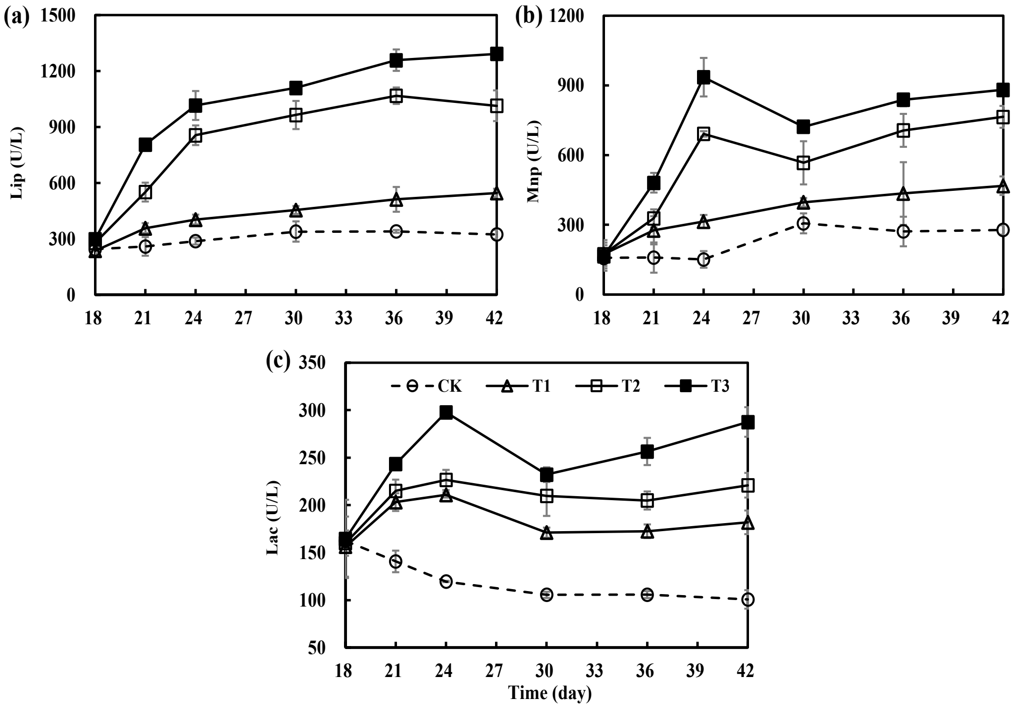

Figure 4. Changes of (**a**) lignin peroxidase, (**b**) manganese peroxidase, and (**c**) laccase during composting.

The degradation rate of materials during the composting process can reflect the maturity progress [52,53]. The cumulative degradation rate of lignin was consistent with the overall trend of the cumulative degradation rate of materials (Figure 2b,c). At the end of composting, the cumulative degradation rates of lignin in T1, T2, and T3 treatments increased by 8.33%, 12.5%, and 16.63% compared with CK, respectively (Figure 2b). The cumulative degradation rates of organic materials increased by 1.84%, 6.92%, and 10.58%, respectively (Figure 2c). The reason for the enhanced degradation ability of the T1 treatment may be directly related to the suitable acid–base environment. Furthermore, the inoculation of the functional bacteria that decompose lignin (*P. chrysosporium*) for the T2 treatment at the appropriate temperature stage can effectively ensure its activity and maximize the decomposition of organic materials [23]. The results of the T3 treatment demonstrated that the appropriate acid–base environment (Figure 1b) helped to enhance the continuous degradation of organic materials by microorganisms.

Amino acids (AA), reducing sugars (R-sugars), and polyphenols as important HS precursors can participate in the formation of HS through the Maillard reaction [50]. The variations in concentrations of precursors can reflect the formation process of HS [7]. As shown in Figure 2d, AA increased first and then decreased during the whole secondary fermentation process of straw composting, and CK, T1, T2, and T3 treatments showed peaks on the 21st day, which were 38.5 mg·kg^{-1}, 42.47 mg·kg^{-1}, 48.92 mg·kg^{-1}, and 57.1 mg·kg^{-1}, respectively. The peaks of T1 and T2 treatments were significantly higher than CK, which may be related to the adjustment of pH and the inoculation of *P. chrysosporium* which helps to stimulate metabolic activity of microorganisms, and to deepen the decomposition of organic materials (Figure 2c). This result is consistent with Zhang et al. [26], which also found that the massive degradation of lignocellulose can promote the formation of AA. The adjustment of pH and the inoculation of *P. chrysosporium* (T3) treatment further enhanced the continuous decomposition of lignocellulose by microorganisms (Figure 2b). The concentration of AA increased significantly in the early stage of secondary fermentation ($p < 0.05$), and then, its concentration decreased significantly and was in a negative phase with HA ($p < 0.05$). The decline rate of the AA concentration in each treatment was T3 > T2 > T1 > CK at the end of composting (Figure 2d), which reflects the results of the humification effect in Figure 3, indicating that adjusting pH conditions and inoculating *P. chrysosporium* in the secondary fermentation process of composting has the most significant effect on enhancing the humification process of composting (Figure 3c).

The change trends of R-sugar and polyphenol were similar (Figure 2e,f), and both T2 and T3 treatments increased in the early stage of humification, which was again related to the deepening degradation of lignocellulose in the secondary fermentation stage; then, it decreased rapidly, and was negatively correlated with the change of HA ($p < 0.05$), indicating that a large amount of R-sugar and polyphenol formed HS by the Maillard reaction [12]. Other studies have shown that polyphenol can affect the formation of HS by polymerization with AA or sugar compounds during composting [40]. The considerable decrease in phenols and the increase in HS in this research support the idea that phenols are used as precursors in HS synthesis [13]. In addition, the inoculation of *P. chrysosporium* stimulated the polymerization of more carbohydrate components to form HS (Figure 3), inoculation of *P. chrysosporium* after pH adjustment enhanced the polymerization of polyphenol precursors during the secondary fermentation (Figure 5d), and the significant decrease of phenolic compounds and the increase of HA in T3 treatment emphasized that the regulation of the pH value for the secondary composting material promotes the formation of HA. In conclusion, these findings indicated that adjusted pH promoted the polymerization of polyphenols and amino acids, thereby forming more HS in secondary composting.

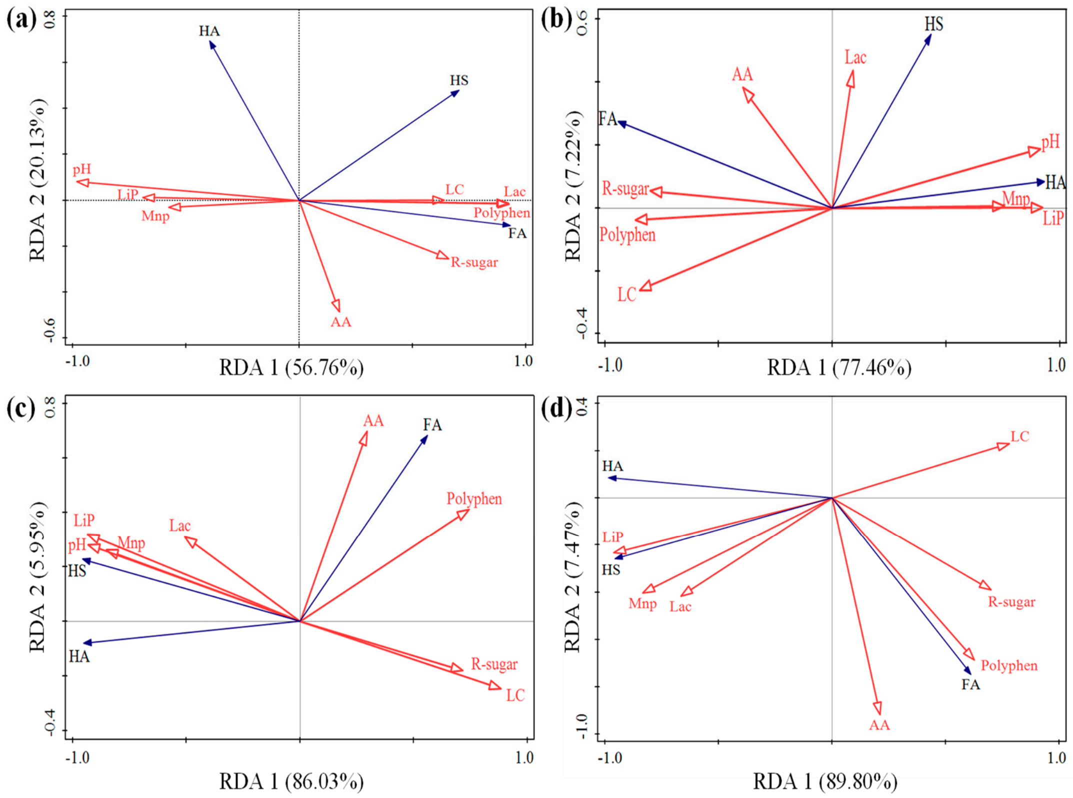

Figure 5. Redundancy analysis (RDA) of the relationship between environmental parameters and humification process in (**a**) CK, (**b**) TI, (**c**) T2, and (**d**) T3. Red and blue arrows represent environmental and humic acid parameters, respectively.

3.3. Variations in Humification Parameters during Secondary Fermentation of Composting

As depicted in Figure 3a, the changing trend of the total humic acid content (HS) in the CK treatment was consistent with the research of Chen et al. [23] during secondary fermentation, which might be because when the available OM was insufficient, the unstable HS was degraded by microorganisms to meet the energy required for their growth and metabolism. The T1 treatment showed an increasing and then a decreasing trend; the final

HS content was 99.76 g·kg^{-1}, an increase of 15.17% compared with CK ($p < 0.05$), indicating that the adjusted pH could promote the production of HS during composting. The reason may be that a suitable acid–base environment (Figure 1b) enhanced the decomposition rate of organic materials by microorganisms (Figure 2c) and the degree of polymerization of precursors to HS (Figure 3). The T2 and T3 treatments showed a consistent change trend with a significant increase in the whole secondary fermentation stage, which was consistent with the change trend of HS for composting inoculated *P. chrysosporium* in the secondary fermentation stage by Chen et al. [23].The reason is that inoculation with *P. chrysosporium* in the secondary fermentation stage can effectively ensure its activity (the appropriate temperature was about 35 °C), promote the conversion of lignin into phenols and quinones (HS precursors) (Figure 2f), and promote the formation of HS [54]. The final content of HS for T3 treatment increased by 7.28% ($p < 0.05$) compared with the T2 treatment, which verified that the effect of adjusting pH for materials was an enhancement to the humification process of composting.

Fulvic acid (FA) has a relatively small molecular weight, simple structure, and is decomposed easily by microorganisms [22,40,47]. During the secondary fermentation stage of composting, it showed a downward trend as a whole (Figure 3b). On the one hand, it is easy to be decomposed, and on the other hand, it was directly related to the fact that microorganisms could use FA as an energy substance to form more stable HA [55,56]. The FA content of CK and T1 treatments decreased continuously in the whole humification stage; the FA content of T2 and T3 treatments decreased from 24 days, which was 6 days later than the CK and T1 treatments (Figure 3b). The reason may be that new FA was produced in the process of lignin re-decomposition, and FA was the precursor of HA [22].

According to Figure 3c, the humic acid (HA) content of each treatment showed an increasing trend in the whole secondary fermentation stage, which was consistent with the changes of normal composting [40]. The final HA content of CK, T1, T2, and T3 treatments reached 58.85 g·kg^{-1}, 73.34 g·kg^{-1}, 97.31 g·kg^{-1}, and 110.98 g·kg^{-1} ($p < 0.01$), respectively. A suitable acid–base environment can stimulate microbial activity and accelerate the decomposition of composting materials (Figure 2c) to generate new precursors (Figure 2d–f), which can well explain the HA content of the T1 treatment being higher than the CK treatment; the result of the HA content increasing significantly in the T2 treatment was consistent with the research of Chen et al. [23]. Compared with that in T2 treatment, the HA content of the T3 treatment increased significantly by 14.05% (Figure 3c), which proved that adjusting the pH value of the secondary fermentation material is an important environmental factor for the microbial enhancement of the humification process of composting.

The humification indices (DP, HI, and PHA) are the ratios between C_{HS}, C_{HA}, and TOC, and to a certain extent, these indices can better reflect the changes in the properties of humic components and the maturity degree of the compost [57]. The DP value can be widely used to present the relative chelation rate between HA and FA [26]. As the fermentation process continued and DP, HI, and PHA increased, on the one hand, this reflected the organic carbon that experienced the strong humification (Figure 3), and on the other hand, the conversion of unstable FA to stable HA expedited the compost's stabilization and maturation process. In contrast, the final DP, HI, and PHA of each treatment was T3 > T2 > T1 > CK ($p < 0.05$), and the humification indices increased rapidly in the whole late composting period, which may be related to the formation of HS concentrated in secondary fermentation and the maturity stage [25,26]. The research found that adjusting the pH in the secondary fermentation period (T1) was beneficial to the humification of lignin-based biomass and the sequestration of organic carbon. Among the humification indices of the different treatments inoculated with *P. chrysosporium*, the indices differed slightly, with T3 being slightly higher than T2 in the whole secondary fermentation stage. The results showed that adjusting pH and inoculating *P. chrysosporium* was more beneficial to composting maturity during lignin-based biomass composting [58].

3.4. Variations in Key Degrading Enzymes during Secondary Fermentation of Composting

Previous studies had confirmed that manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase (Lac) were the main enzymes to degrade lignin [59,60]. As shown in Figure 4a,b, the activities of LiP and MnP showed an overall upward trend in the secondary fermentation stage of composting. The change trend of T2 and T3 treatment was consistent with the change in enzyme activity after inoculation with *P. chrysosporium* during the secondary fermentation of composting [23,24]. Compared with the CK treatment, the reason for the significantly increased enzyme activity of the T1 treatment may be related to the suitable acid–base environment of microorganisms; inoculation of *P. chrysosporium* stimulated the metabolism and activity of other microorganisms, and a suitable acid–base environment (Figure 1b) satisfied the conditions for its reproduction and metabolism, which was the main reason for the rapid increase in enzyme activity for the T3 treatment. The increase in enzyme activity enhanced the ability of microorganisms to degrade lignocellulose, which may have led to a rapid decline in the lignin content of the T3 treatment (Figure 2a) and the rapid increase in the HA content (Figure 3c).

Laccase (Lac), as a polyphenol oxidase containing four copper ions, compared with MnP and LiP can oxidize phenols, carboxylic acids, their derivatives, etc., and small molecular phenols derived from lignin are easily polymerized by Lac [61] and involved in the process of humification during composting [62]. The activity of Lac was relatively low compared to LiP and MnP (Figure 4c), which was consistent with the previous research results of inoculating *P. chrysosporium*, and also proved the possibility that *P. chrysosporium* does not secrete Lac [23,24], but this result does not rule out the possibility that Lac and other species participate in lignocellulose degradation in composting. The final Lac activities of CK, T1, T2, and T3 treatments were 100.73 U/L, 181.97 U/L, 220.9 U/L, and 287.51 U/L, respectively. Compared to conventional composting (CK), the increase in Lac activity in the T1 treatment may be directly related to the change in the pH value for composting. The inoculation of *P. chrysosporium* stimulated the metabolism of Lac-secreting microorganisms inside the pile, which was the main reason for the significant increase in Lac activity in the T2 and T3 treatments. Among them, adjusting the pH and inoculating *P. chrysosporium* (T3) further increased Lac activity. The reason may be that a suitable acid–base environment can stimulate other microorganisms to synergistically produce more Lac.

3.5. The Relationship between Physicochemical Parameters and Humification Process

Generally speaking, the humification process of composting is affected by changes in environmental parameters [63]. In addition, key enzymes are important for driving the formation and polymerization of HS precursor species, because they can act as a "bridge" for HS formation. To study the key factors affecting the humification process, a redundancy analysis (RDA) can be adopted to explore the correlation between precursor species, degrading enzymes, and HS formation (Figure 5). In the CK treatment, the pH value showed a significant correlation with the humification process (Figure 5a), followed by R-sugar and LiP, and the results verified the regulatory effect of pH on the humification process, which was consistent with the research results of He et al. and Zhang et al. [26,35]). In the T1 and T2 treatments, the effect of pH and R-sugar on HS formation was reduced. In contrast, LiP and AA were the biggest factors affecting humus formation (Figure 5b). The results indicated that adjusting pH or inoculating *P. chrysosporium* would increase the decomposition of organic materials by LiP to a certain extent and enhance the effect of AA in the humification process. Polyphenol is significantly related to the formation of HA in the T3 treatment. Studies have shown that the degradation products of lignin are mainly polyphenols [60], indicating that adjusting the pH value and inoculating *P. chrysosporium* was beneficial to the continuous degradation of a large amount of lignin. In addition, the role of changes in Lac activity and lignin content cannot be ignored (Figure 5c). The results of this study clearly showed that regulating the pH value of the secondary fermentation process of composting promoted the polymerization of phenolic compounds to form stable HA during the process of lignocellulosic biomass composting. In summary, by adjusting the pH value of the secondary fermentation process of composting, using degrading enzymes as a "bridge" to strengthen the continuous degradation of organic materials (lignocellulose) by microorganisms, and increasing the supplement of HS precursors may be a possible way to strengthen the humification process and enhance the formation of HS.

4. Conclusions

Inoculation of *P. chrysosporium* can accelerate the maturity process of lignin-based biomass composting, enhancing the humification degree. Adjusting pH and inoculating *P. chrysosporium* in secondary composting can promote more thorough lignocellulose decomposition, which is more beneficial to the humification process. Adjusting the pH of the secondary fermentation of composting is an important environmental factor for microorganisms to enhance the humification process. Degrading enzymes are used as a "bridge" to enhance the continuous decomposition of lignocellulose by microorganisms, by increasing the supplementation of HS precursors and the synthesis of HA, which is an important mechanism for enhancing the humification process. Our results provide new insight into the role of the pH value in the formation of HS and has practical significance to promote the continuous degradation of lignocellulose by microorganisms and strengthen the composting humification process by regulating the pH value of the secondary composting materials.

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