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Relative Contribution of Fungal Communities to Carbon Loss and Humification Process in Algal Sludge Aerobic Composting

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Abstract: Harmful algal blooms in eutrophic lakes pose significant challenges to the aquatic environment. Aerobic composting is an effectively method for processing and reusing dewatered algal sludge. The fungal communities are the main driver of composting. However, their relationship with carbon loss and the humification process during algal sludge composting remains unclear. In this study, the succession of fungal communities in algal sludge composting was investigated via internal transcribed spacer (ITS) rRNA amplicon sequencing analysis. Overall, no significant differences were observed with the α-diversity of fungal communities at different stages. The composition of the fungal communities changed significantly before and after compost maturation and became more stable after the compost maturation. Redundancy analysis showed that the fungal communities were significantly correlated with physicochemical properties, including humic acid (HA)/fulvic acid (FA), temperature, pH, humic acid, microcystins, and CO_2. The co-occurrence network showed that different fungal community modules had different relationships with physicochemical properties. Structural equation modeling further revealed that different metabolic or transformation processes may be mainly driven by different fungi modules. The microcystin degradation, carbon loss, and humification during composting were mainly mediated by fungal communities which were mainly influenced by temperature. Humification was influenced not only by fungal communities but also by the microcystin levels. These results show that changes in the fungal community composition and interaction and their relationship with physicochemical properties could represent a useful guide for optimizing the composting process.

Keywords: algal sludge; aerobic composting; fungal communities; carbon loss; microcystins; humification process

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1. Introduction

The eutrophication of the lake ecosystem can lead to the growth of harmful algae, which severely damages aquatic ecosystems and poses great risks to humans [1]. Direct salvaging, followed by mechanical pressure filtration, is the main algae pre-treatment method used in Tai Lake, the third largest freshwater lake in China [2]. On the one hand, because biomass is easily decomposed, algal sludge can easily cause additional environmental pollution if not treated in time [3]. On the other hand, algal sludge may have good prospects for resource recovery because it is rich in organic matter, nitrogen, phosphorus, and other substances. Aerobic composting, characterized by low cost and high cost-effectiveness, can be considered an environmentally friendly method for algal sludge disposal and utilization.

Aerobic composting is a natural process that involves the conversion of organic waste by microbial communities into stable humus [4]. During the composting, the degradation of organic matter leads to a large amount of carbon loss, and humification plays a critical role

in carbon sequestration [5]. Humification is a slow and inefficient process that promotes the complete degradation of organic matter, resulting in carbon losses and greenhouse gas emissions [6]. According to Zhang et al. (2021) and Hwang et al. (2020), nearly half of the biomass in feedstocks was lost during pig and chicken manure composition [2,7]. Some carbon loss is inevitable during composting. However, excessive loss can hinder humus formation and reduce compost yield and quality [8]. Microbial communities are an indispensable driving force in the composting process. Fungi can survive under extremely adverse habitats and play important roles in the degradation and humification of organic matter. Some studies have shown that many thermophilic fungi, especially Basidiomycota and Ascomycota, play crucial roles in the composting process of lignocellulose [9]. Duan et al. (2019) showed that the thermophilic fungi demonstrated a strong potential to degrade carboxylic acids and polymers in the mature stage during composting [10]. Xie et al. (2021) found that the members of saprotrophic fungi played important roles in the degradation of hemicellulose and cellulose through their cellulolytic activities to promote humus formation [11]. Therefore, identifying the composition of the fungal communities during composting is crucial for elucidating biomass degradation and humification mechanisms and promoting pollutant degradation. Current studies mainly focus on bacterial communities but ignore the information of the fungal communities during the aerobic composting of algal sludge.

Fungal communities play essential roles in the transformation of organic matter during composting, and their structure and function are closely related to the substrates and conditions of composting, such as different treatments and physicochemical conditions [12]. Duan et al. (2019) found that the concentration of straw biochar in the feedstock significantly affected the composition of the fungal communities, which ultimately affected the quality of the poultry manure compost product [10]. Huang et al. (2022) reported that fungal communities enhanced humification and were influenced by heavy metals in municipal sludge composting under hyperthermophilic conditions [9]. However, few studies have investigated the factors influencing microbial community structure, especially the fungal communities, in algal sludge composting. Microbial interactions, such as symbiosis or competition, significantly affect the stability and function of the communities [13]. Meng et al. (2022) found that lignocellulose degradation was dominated by microbial co-occurrence networks rather than community diversity during corn stover and cow manure composting [14]. Wu et al. (2022) found that the selective modulation of environmental factors effectively controlled control compost maturation by regulating the growth of microorganisms and the establishment of microbial interactions [15]. Therefore, a thorough understanding of fungal community interactions is essential to optimize both community composition and function in algal sludge composting.

In this study, a mushroom substrate was selected for co-composting with algal sludge substrates to alleviate the problems of low C/N ratio and high moisture content in the algal sludge [16]. Mushroom substrate is the residual waste generated from mushroom harvesting that contains disintegrated lignocellulosic biomass (e.g., corn cobs and straw) that provides a suitable growth environment for fungal communities [17]. This study mainly aimed (1) to analyze the dynamics of the carbon loss, humification, and microcystin parameters; (2) to investigate the changes in fungal community composition and co-occurrence patterns; and (3) to examine the relevance of fungal communities and carbon loss, humification, and microcystin degradation.

2. Material and Methods

2.1. Experimental Materials, Design, and Sampling Collection

The raw materials for composting were obtained from freshly collected and filtered (belt press) algal sludge from the algae–water separation station in Wujin District, Changzhou City, China. The initial physicochemical properties of the raw materials are presented in Table S1. The composting test was conducted in an aerobic composting bioreactor made of polyethylene materials with an effective volume of more than 100 L (Figure S1).

Aeration air from the bottom of the reactor provided oxygen for the composting process. The initial volume ratio of algal sludge, mushroom residue, and mature compost was set at 15:7.5:1. Samples were conducted on days 0, 0.5, 1, 2, 3, 4, 5, 7, 9, 14, 19, 25, 31, 39, and 49. In addition, the pile was manually turned for 5 and 16 d. Three parallel samples were collected from 10 cm below the surface of the pile on each sampling day. Compost samples were stored for physicochemical analysis and for DNA extraction (at −20 °C).

2.2. Determination of Compost Samples

The temperature of the compost pile was measured with an electronic thermometer at 10:00 AM. The gases were collected at 8:00 PM on each sampling day. CO_2 and CH_4 were collected from the reactor and quantified using a gas chromatograph (Agilent Technologies, Wilmington, DE, USA). Fresh samples and distilled water were added and pumped for 30 min (mass ratio 1:10). After the precipitation of the sample, the supernatant was extracted, and its pH was determined using a water quality meter (86031 AZ Waterproof IP67 Combo Water Quality Tester, AZ Instrument Corp., Taichung City, China). The presence of microcystin in algal sludge aerobic compost was determined using Beacon Microcystin Plate Kits (Beacon Analytical Systems, Saco, ME, USA). HA and FA were isolated with NaOH-$Na_4P_2O_7$ and determined using an organic carbon analyzer following the previously described procedure [5].

2.3. Bioinformatic Analyses

Total community DNA was extracted from the compost samples using the FastDNA SPIN Kit (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. The DNA samples were stored at −80 °C for further use. The fungal ITS region was amplified using ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) pair primers. PCR products from three replicate reactions were pooled and gel purified using an AxyPrep™ DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The purified PCR products were sequenced using Illumina Miseq Sequencing at Shanghai BIOZERON Biotechnology Co., Ltd. (Shanghai, China) after quality control and the removal of primer fragments. The remaining sequences were clustered into operational taxonomic units (OTUs) with a 97% nucleotide identity cutoff using search software on the QIIME2 platform [9].

2.4. Statistical Analyses

The α-diversity of the fungal communities, including the ACE, Simpson, and Pielou's evenness indices, was estimated using the vegan package in R (version 4.3.2). The fungal community structure was visualized through a principal coordinate analysis (PCoA) based on Bray–Curtis distance using the vegan package in R. Permutational multivariate analysis of variance (PERMANOVA) was used to test the significance of the differences in fungal communities between different stages using the R package vegan. Redundancy analysis (RDA) assuming a linear relationship was conducted to examine the relationship between physicochemical properties and the fungal communities using the vegan package in R, with 999 permutations.

Co-occurrence correlations of the fungal network were determined using the hmisc package in R based on Spearman rank coefficients, with $|r| \geq 0.6$ and $p \leq 0.05$ as thresholds. The nodes, edges, and modules of the co-occurrence network were calculated using the igraph package in R. The co-occurrence network was visualized using the Gephi 0.9.2 software. The Mantel test was performed using the vegan and dplyr packages to assess the Pearson correlation between each module of the co-occurrence network and each physicochemical property.

Structural equation modeling (SEM) was used to evaluate the direct and indirect relationship between physicochemical properties and fungal communities. The model was developed and tested based on the analytical results of this study and existing literature, and was performed using Amos 24.0 software (SPSS, Chicago, IL, USA). The chi-squared

test (χ^2/DF), incremental fit index (IFI), and comparative fitting index (CFI) were calculated to test if the model represented the theoretical hypotheses [18]. Principal component analysis (PCA) was used to reduce the dimensionality of the observed variables and fungal community composition using SPSS 22.0. The first principal component with a cumulative variance equal to or greater than 50% was used for further analysis, and the first principal components were selected for the fungal communities. CO_2 and CH_4 were used as a group to represent carbon loss. Models 4 and 6 (selected from co-occurrence network analysis) represent the fungal communities.

3. Results and Discussion

3.1. Physicochemical Changes during Composting

Temperature is a crucial parameter that influences microbial activity during the composting process and is one of the key criteria used to measure the maturity of aerobic composting [10]. In this study, the value of the germination index exceeded 80% on day 25, reaching the maturity standard (>80%). According to the dynamic changes in compost temperature, the composting process was divided into three typical stages, thermophilic stage A (0.5 d, 1 d, 2 d, 3 d, and 4 d), cooling stage B (5 d, 7 d, 9 d, 14 d, and 19 d), and mature stage C (25 d, 31 d, 39 d, and 49 d) (Figure 1b). The temperature initially increased to 75 °C at day 0.5 and then gradually decreased. The thermophilic phase (>50 °C) lasted for 4 days, which ensured composting safety standards by killing pathogens and weed seeds [19]. The temperature steadily decreased after the peak and stabilized at approximately 25 °C after day 25, indicating a decrease in microbial activity associated with the depletion of easily degradable organic materials [20]. In aerobic composting, microbial communities decompose and use large amounts of easily degradable carbon compounds through intense biochemical activity [21].

Generally, optimal microbial activity during composting occurs within the pH range of 6.7 to 9.0 [22]. In this study, pH rapidly increased from day 0 to 8.74 within 0.5 days and then decreased, stabilizing at approximately 7.5 after 14 days (Figure 1c). The increase in pH at the beginning of composting can be attributed to the mineralization of nitrogen-rich compounds in algal sludge to produce NH_3, which in turn neutralizes organic acids. Meanwhile, the organic acids continue to accumulate in the later composting [23,24]. After the composting of the sample, the pH was 7.44, which was lower than 8.5, meeting the maturity requirements of the final product [17].

CO_2 production and emission during aerobic composting are mainly caused by the metabolic activities of microorganisms involved in the degradation of organic matter. Two prominent peaks of CO_2 emission were observed during the composting, which occurred on days 2 and 7 (Figure 1d). CO_2 emissions were highest on day 2 due to organic matter degradation and microbial respiration [7]. The CO_2 emissions increased on day 7, probably because the turning of the pile further facilitated the degradation of unused organic matter in the material. CH_4 emissions, as another major form of carbon loss in compost, is mainly produced by methanogens using CO_2 and acetic acid under anaerobic conditions [7]. The CH_4 emissions during the composting peaked on day 1 (Figure 1e). Microorganisms rapidly degraded organic matter and consumed the oxygen supplied by the aeration system at the beginning of composting [25]. Then, as the oxygen content in the reactor increased, the CH_4 emissions rapidly decreased and eventually approached zero.

Microcystins are the main obstacle to the effective use of algal waste. Microcystins steadily decreased during the composting process (Figure 1f). During the first five days of composting, the degradation efficiency of microcystins was 77.08%, indicating that the thermophilic stage was the suitable period for the degradation of microcystins. At the end of composting, 93.7% of microcystins were degraded, indicating that most of the microcystins in the algal sludge were efficiently degraded through composting.

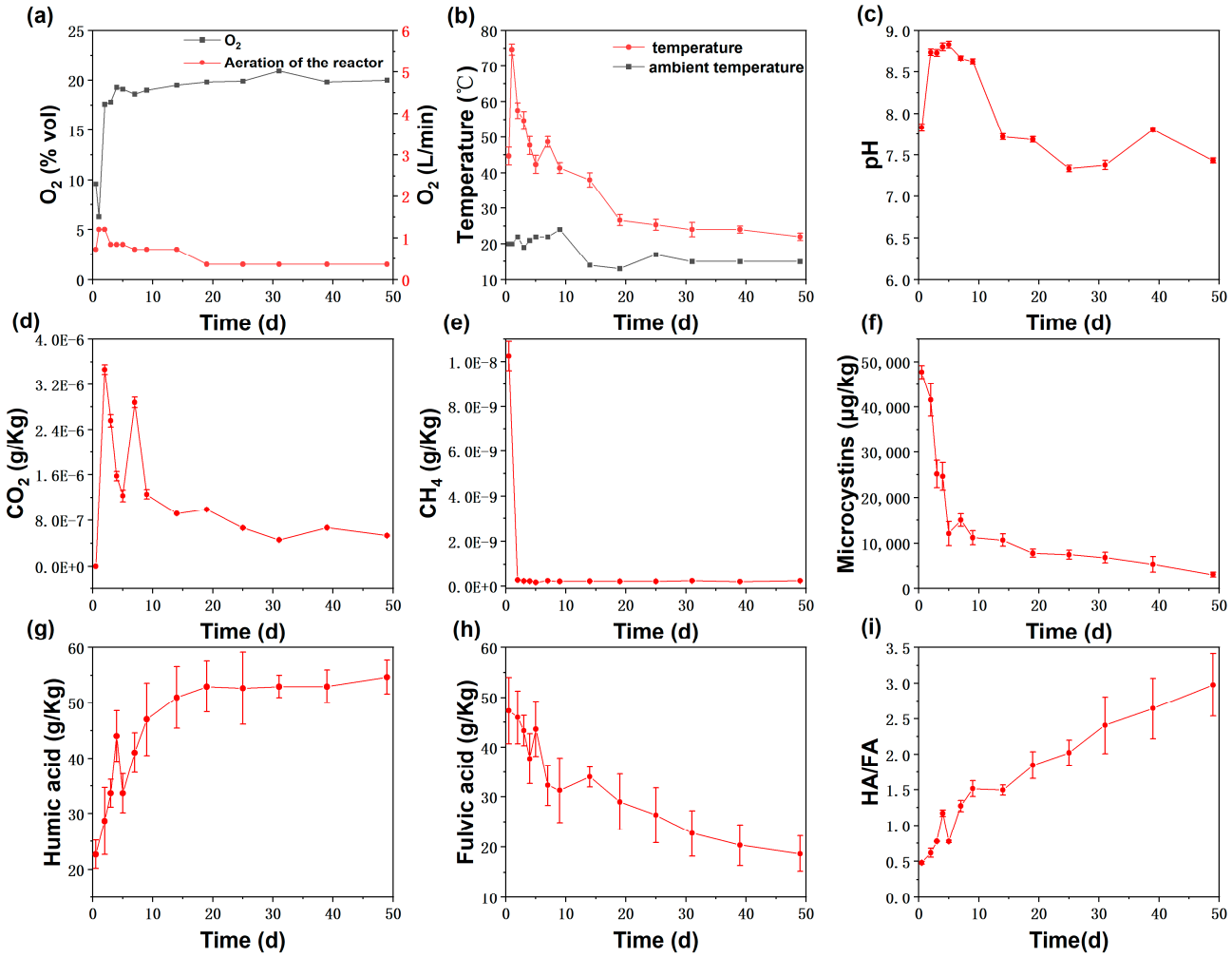

Figure 1. Changes in O_2 (**a**), temperature (**b**), pH (**c**), CO_2 (**d**), CH_4 (**e**), microcystins (**f**), humic acid (**g**), fulvic acid (**h**), and HA/FA (**i**) during the algal sludge aerobic composting. Error bars indicate standard deviation.

Both the algal sludge and the mushroom residue contain abundant fibrous structural components that are important for composting, such as cellulose and lignin, which provide essential starting materials for the humification process [17,26]. Humic substances are key indicators of compost maturity. Efficient conversion of organic matter into humic substances is of great importance for improving composting efficiency and product quality [27]. HA rapidly increased during the initial composting phase. The decomposition of organic matter led to the rapid generation of HA during the composting process (Figure 1g). The final HA content in the compost was 54 g/kg. Conversely, FA continuously decreased during composting (Figure 1h). FA with low molecular weight has relatively high acid functional groups and high water solubility [28]. The content of FA was relatively high in the initial composting phase. Microorganisms used FA for metabolism and participated in the conversion of organic matter into HA [29]. The ratio of HA to FA is a key indicator for describing the relative rates of humic acid and fulvic acid conversion and assessing the final maturity of the compost (Figure 1i). When HA/FA > 1.9, it indicated that the compost maturity was relatively stable. The degree of humification increased with the increase in this ratio [29]. This phenomenon indicated that the compost product was highly humified when algal sludge aerobic composting matured (day 25, HA/FA = 2.04; day 49, HA/FA = 2.82).

3.2. Dynamics of Fungal Communities

A total of 567 fungal OTUs were detected from 15 composting samples with 97% sequence identity. The Simpson index gradually decreased during composting, indicating that the diversity of the fungal communities gradually increased (Figure 2a). This phenomenon may be due to the inhibition of the growth of some fungal communities during the thermophilic stage [30]. Chen et al. (2022) found that microbial community diversity decreased, whereas microbial activity increased during the thermophilic stage. This phenomenon occurred because the microorganisms that could adapt to moderate temperature were obviously more than thermophilic microorganisms [31]. In summary, no significant differences were observed in α-diversity for the three stages of composting (ACE, Simpson, and Pielou evenness index), as indicated by PERMANOVA ($p > 0.05$).

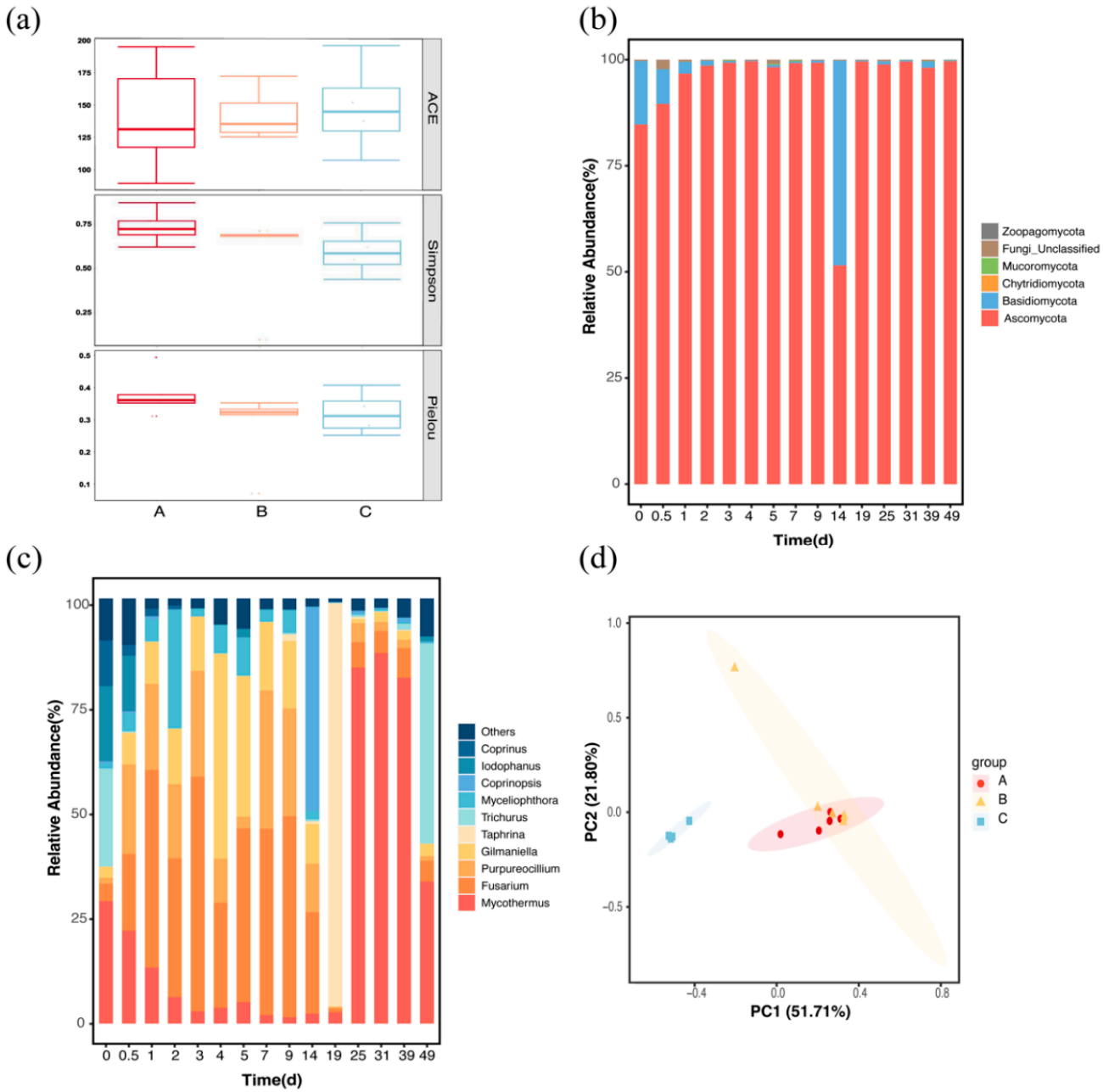

Figure 2. Changes in fungal community α-diversity (**a**), composition ((**b**), phylum level; (**c**), genus level), and structure (**d**) during the algal sludge aerobic composting.

Five phyla were detected during the composting of the samples (Figure 2b), including Ascomycota, Basidiomycota, Chytridiomycota, Mucoromycota, and Zoopagomycota. Ascomycota (84.8–99.6%) and Basidiomycota (0.2–48.3%) were the dominant fungal phyla during composting. They were the main thermophilic fungal phylum with a strong ability to degrade lignocellulose. Additionally, they are often found in the compost of mushrooms, straw, and other materials with a high lignocellulosic content. Ascomycota can maintain their activity and competitiveness in composting because they are well adapted to high temperatures, low humidity, and low nutrients, and they secrete thermostable cellulases and hemicellulases [32].

The top 10 dominant fungi at the genus level were Mycothermus, Fusarium, Purpureocillium, Gilmaniella, Taphrina, Trichurus, Myceliophthora, Coprinopsis, Iodophanus, and Coprinus (Figure 2c). The relative abundance of Fusarium, Purpureocillium, and Gilmaniella was significantly higher in the thermophilic and cooling stages than in the mature stage ($p < 0.5$). They all belong to the Ascomycota and show a strong adaptation to high temperatures. Fusarium secretes a variety of lignocellulosic degrading enzymes to support its basic metabolic needs and inhibit the growth of pathogens [33]. Bilgrami and Khan (2022) showed that Purpureocillium exhibited a strong defense against harmful species [34]. Gilmaniella promotes carbon fixation, carbohydrate metabolism, and energy metabolism [35]. The relative abundance of Coprinopsis during the cooling stage was significantly higher than that of the other two stages ($p < 0.5$). Coprinopsis is suitable for growth in lignin-containing matrices and belongs to the Basidiomycota. Coprinopsis has a strong ability to decompose the refractory substances in the composting materials and promotes carbohydrate metabolism and amino acid metabolism [36,37]. In the mature phase, Mycothermus was the dominant fungal genus (33.41–83.79%). Mycothermus produces cellulases that degrade carboxylic acids and polymers, and it belongs to the Ascomycota [38]. Trichurus had a high relative abundance prior to the start of composting, but sharply declined after the start of composting and recovered after day 49. It has been reported to degrade the cellulose of plant residues [39].

The changes in the fungal community structure during algal sludge aerobic composting were further analyzed using the PCoA (based on the Bray–Curtis distance matrix) (Figure 2d). The first two axes explained a high proportion of the variance (72.79%). The PCoA revealed that the compositions of the fungal community in different stages were distributed in different positions before and after the maturity of the compost. Therefore, apparent differences in fungal community composition were observed before and after the maturity. In addition, the composition of fungal communities in the mature stage was more similar, indicating that the fungal community composition might be more stable after the compost matured [30]. In conclusion, there were significant differences in the composition of the fungal community before and after the maturation of algal sludge aerobic composting.

3.3. Relationship between Physicochemical Properties and Fungal Communities

Redundancy analysis revealed the relationship between compost physicochemical properties and fungal community structure, and the validity of the interpretation was performed through screening indicators and correlation analysis. The physicochemical properties of compost in the two RDA axes explained 28.22% and 23.03% in the total variation for fungal communities ($p = 0.001$). Seven physicochemical properties (HA/FA, FA, temperature, pH, humic acid, microcystins, and CO_2) were significantly associated with the fungal community composition ($p < 0.05$, explaining 14.97, 14.06, 13.40, 13.11, 12.27, 11.09, and 9.05% of community variance, respectively) (Figure 3). Microcystins, HA, temperature, pH, and CO_2 were positively correlated with the fungal community composition in the thermophilic and cooling stages. Many studies have shown that temperature is the most important factor affecting the succession of the fungal communities, and it will gradually decrease as microbial activity decreases [40]. The microbial decomposition of organic matter produced a large amount of organic acid and CO_2 during the thermophilic stage [41]. The

thermophilic stage was the main period for the degradation of microcystins. HA/FA and HA were positively correlated with fungal community composition in the maturity stage. The microorganisms degraded organic matter in the thermophilic and cooling stages and gradually formed HA with a more complex structure and more stable molecules in the maturity stage. In summary, there was a significant relationship between physicochemical properties and fungal community composition during algal sludge composting.

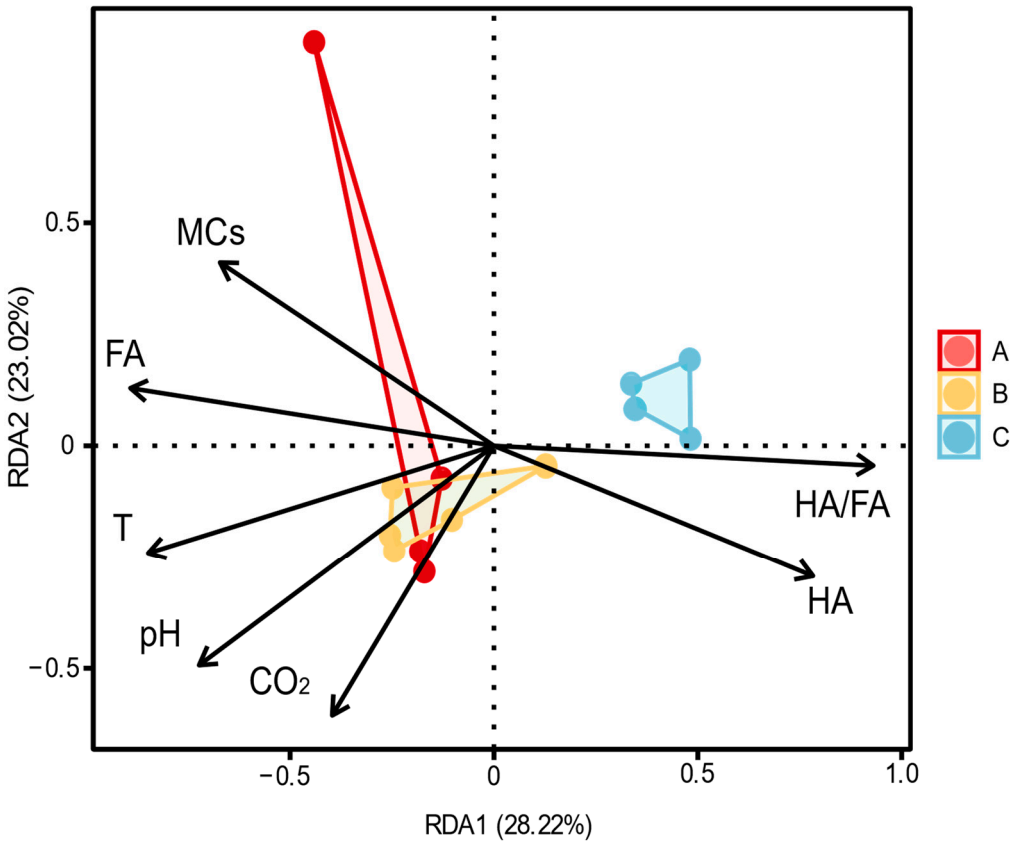

Figure 3. Redundancy analysis between physicochemical factors and fungal community during the algal sludge aerobic composting. HA/FA ($p < 0.001$), fulvic acid (FA) ($p < 0.001$), temperature (T) ($p < 0.001$), pH ($p < 0.005$), humic acid (HA) ($p < 0.005$), microcystins (MCs) ($p < 0.01$), and CO_2 ($p < 0.05$).

The network analysis was conducted to reveal interactions between fungal communities. The fungal co-occurrence network was based on Spearman correlation, with a threshold set at $|r| > 0.6$ and $p < 0.05$ [42]. The connectivity followed a power law distribution ($R^2 = 0.87$, $p = 0.001$). The modularity, average path distance, and average clustering coefficient of the real network (0.666, 3.108, and 0.11, respectively) were higher than those of the random network (0.484, 2.24 ± 0.0029, and 0.071 ± 0.0027, respectively), suggesting that the construction of the fungal community network was non-random. The fungal co-occurrence network comprises 63 nodes and 120 edges, clustered into six modules: module 6 (28.57%), module 1 (20.63%), module 3 (19.05%), module 4 (17.46%), module 2 (7.94%), and module 5 (6.35%) (Figure 4a). Each module can be viewed as a sub-community of the compost fungal communities, where each node interconnected more frequently with others within the same module than with nodes in other modules. The fungal network had a higher proportion of positive connections (67.5%) than negative connections (32.5%). Positive connections between communities can be attributed to ecological niche overlap and mutualistic symbiosis [37,43] and play a crucial role in degrading recalcitrant materials in the composting process [13]. Meng et al. (2022) reported that the enhancement of positive connections of microbial communities during composting reflected environmental changes

and organic matter degradation [14]. All modularity values of the fungal network exceeded 0.4, indicating significant modularity and strong resistance to environmental changes [44].

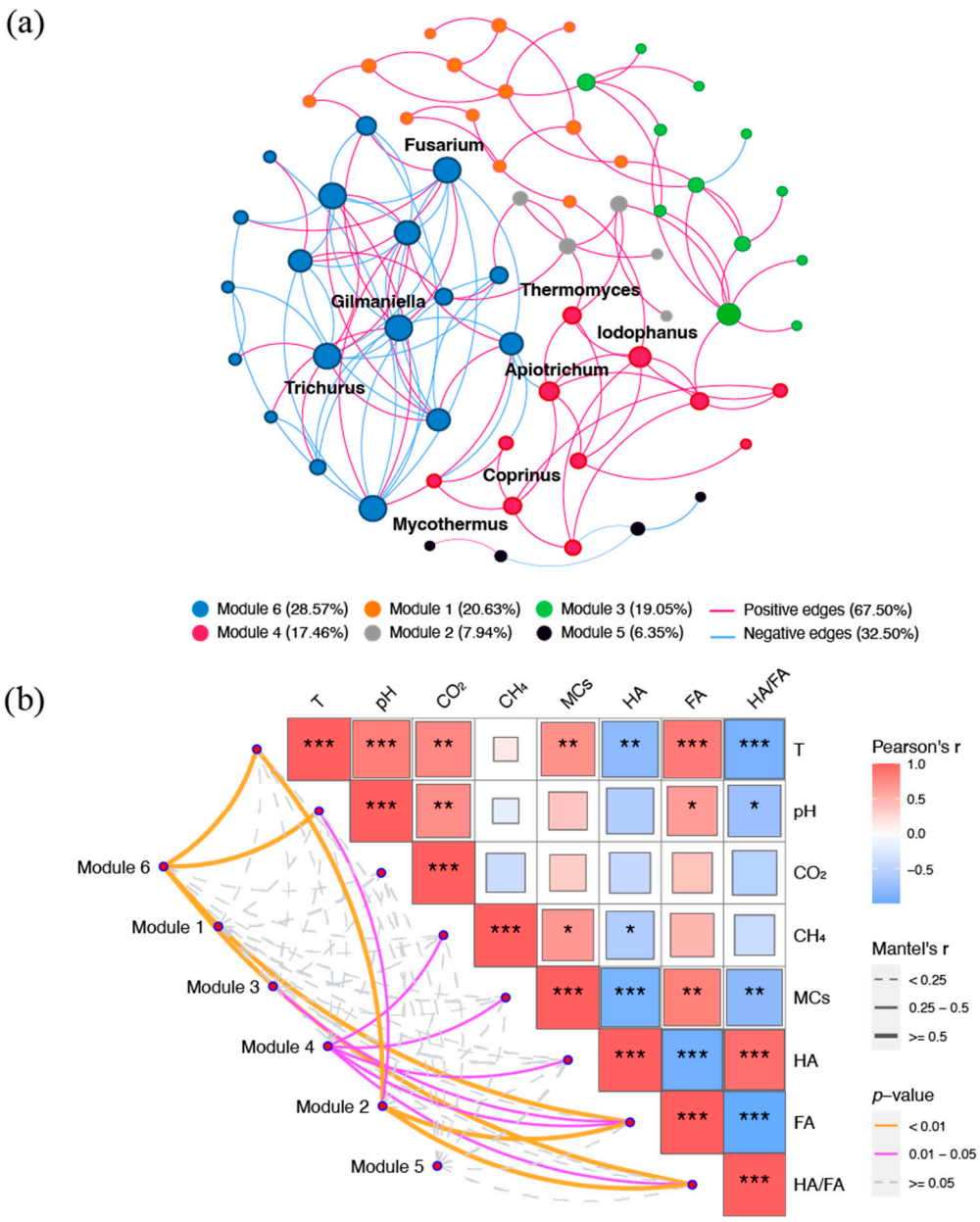

Figure 4. Co-occurrence networks of compost fungi (**a**) and the relationship between modules and physicochemical factors (**b**). "*" means there is no significant correlation between the two (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$).

The correlation between the fungal community interactions and the physicochemical properties of the compost was further analyzed using the Mantel test. There were differences in the relationships between different modules and physicochemical factors, suggesting that fungi in different modules might be mainly involved in different transformation processes. Module 6 was significantly correlated with temperature, pH, HA/FA, and HA. Among the fungal genera included in module 6, *Fusarium* and *Purpureocillium* can tolerate high temperatures and acidity changes during algal sludge composting. *Gymnoascus* and *Penicillium* can tolerate unfavorable conditions, including high temperatures and high pollutant concentrations [9,45]. *Mycothermus*, *Fusarium*, *Trichurus*, and *Coprinopsis* can degrade organic matter and promote the humification of compost. *Aspergillus* and

Penicillium can secrete substrate-degrading enzymes, which have a remarkable decomposition effect on organic matter and cellulose [46]. *Myceliophthora* is often found in composts made from solid cultures, including agricultural residues, such as wheat bran [47]. High temperatures promote the growth of *Myceliophthora*, which secretes enzymes responsible for breaking down cellulose hydrolysis and is a highly active cellulose decomposer [48]. Therefore, module 6 was closely related to the humification process during composting.

Module 4 showed a significant correlation with microcystins, CH_4, FA, HA, and HA/FA. Among the fungal genera included in module 4, *Apiotrichum* can tolerate high concentrations of toxins in the environment and has the ability to degrade toxins [49]. *Epicoccum* was significantly correlated with CH_4 emissions, as found in previous studies on pig manure compost modified with pine leaf biochar [46]. *Coprinus* is a member of the *Basidiomycota* family, which include major lignin-degrading organisms in nature, degrading lignin faster than any other organisms [50,51]. *Coprinus* can be inoculated after the thermophilic phase to enhance lignin degradation [52]. Nitrogen or carbon loss in the soil can affect the activity of *Coprinus* and lignocellulosic degradation [53]. *Trichosporon* is resistant to high temperature environments and degrades toxic substances and lignocellulose [24]. Therefore, module 4 was closely related to the degradation of microcystins, the carbon loss, and the humification of the composting process during composting.

3.4. Relative Contributions of Potential Factors to Carbon Loss and Humification Process

An SEM analysis was conducted to further evaluate the direct and indirect comprehensive relationship between physicochemical properties and fungal communities (Figure 5). The SEM results exhibited a satisfactory fit (χ^2/DF = 2.000 IFI = 0.965, CFI = 0.961).

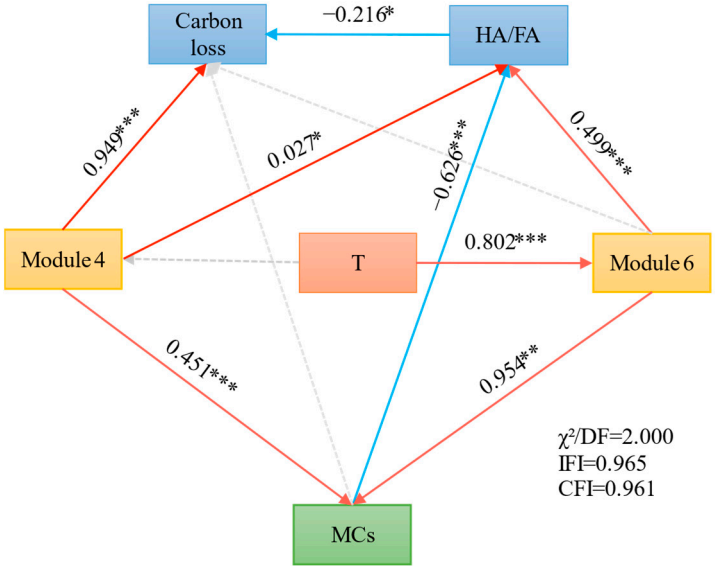

Figure 5. Structural equation modeling explains the hypothesized causal relationship between fungi communities (two main functional modules: modules 4 and 6), carbon loss (CO_2 and CH_4), compost humification (HA/FA), temperature (T), and microcystins (MC) during the algal sludge aerobic composting. Red and blue lines indicate significant positive and negative pathways (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$), respectively. The line outlined in gray indicates no significance ($p > 0.05$). Numbers on arrows are standardized path coefficients.

The SEM revealed that microcystin degradation, carbon loss, and humification during composting were mainly mediated by fungal communities. This result also showed that temperature was the main factor influencing fungal communities during the composting process, while some fungal communities could tolerate high temperatures. Microbial activity is closely related to temperature and can also influence the temperature of the compost pile through the heat generated by the aerobic degradation of organic matter [54].

As expected, module 4 had a significant direct effect on the degradation of microcystins, the carbon loss, and the humification process, and module 6 had a significant direct effect on the humification process during composting. Moreover, module 6 also had a significant direct effect on the degradation of microcystins, which was not found in the single relationship test, i.e., the Mantel test. Thus, different metabolic or transformation processes might be mainly driven by different modules. In addition, the SEM showed that humification was negatively correlated with carbon loss. This correlation may arise from the fact that humification can prevent carbon loss and greenhouse gas emissions by converting the intermediate products of degradation into humic substances through various mechanisms [6].

The humification was influenced not only by fungal communities but also by the microcystin levels. Microcystins are toxins that are unique to algae sludge compost, and their residues in mature fertilizers can be toxic to crops [55]. The stable structure of microcystin hinders its degradation under natural conditions, and it is resistant to high temperature, acid, and alkali [3]. High concentrations of microcystins can affect microbial activity, inhibit the humification process of compost, and reduce composting efficiency. Adding external functional microbial inoculants and increasing the microbial activity of compost are feasible methods to enhance the degradation of microcystins [56]. Considering that different modules may have different functions, the selection of functional microbial groups should be more targeted.

4. Conclusions

This study revealed the relationship between the succession of fungal communities and the carbon loss as well as humification process during algal sludge composting. Overall, based on the germination index, the algal sludge aerobic compost matured in 25 days. No significant difference was observed between the α- diversity of fungal communities at different stages. However, the composition of the fungal communities significantly changed before and after compost maturation, and it may be more stable after compost maturity. RDA revealed that the fungal communities were significantly correlated with physicochemical properties, i.e., HA/FA, fulvic acid, temperature, pH, humic acid, microcystins, and CO_2. The co-occurrence network showed that different fungal community modules had different relationships with physicochemical properties. The SEM revealed that microcystin degradation, carbon loss, and humification during composting were primarily mediated by fungal communities, which were mainly affected by temperature. Different metabolic or transformation processes were mainly driven by different modules. Module 4 had a significant direct effect on the degradation of microcystins, the loss of carbon, and the humification process. Module 6 had a significant direct effect on the humification process and the degradation of microcystins during composting. The humification was affected not only by fungal communities, but also by the content of microcystins. Considering these results, the optimization of microbial community interactions by adding functional microbes or adjusting composting conditions is a promising approach for improving the efficiency of microcystin degradation and the quality of the compost product.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16081084/s1, Figure S1: Diagram of compost reactor; Table S1: The characteristics of the raw materials.

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