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Seedling Establishment Test for the Comprehensive Evaluation of Compost Phytotoxicity

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Abstract: Application of non-phytotoxic compost is important for soil conservation and crop production. In this study, two treatments were set up to compare the effects of compost water extract on the phytotoxicity of compost based on the dry weight and wet weight of samples and explore the feasibility of seedling establishment test in compost phytotoxicity evaluation—without (CM treatment) and with the addition of a microbial agent (ACM treatment)—based on the addition of corn straw and spent mushroom substrate in cow manure composting. The compost water extracts were prepared as per the wet weight (1:10, w/v) and dry weight (1:20, w/v) of fresh samples. The physicochemical characteristics of the compost water extracts, relative radicle growth, and chlorophyll content of the seed cotyledons of Chinese cabbage were determined. The results demonstrated that the highest electrical conductivity value of 3.95 mS·cm^{-1} was obtained for the CM treatment, based on the dry weight of the samples. The contents of nitrate-nitrogen, ammonium-nitrogen, total organic carbon, and total nitrogen under different extraction methods were significantly different between the different extraction methods. The addition of microbial agent effectively promoted compost maturity and increased the relative radicle growth and chlorophyll content of the cotyledons. At the end of composting, the relative radicle growth based on the wet weight of samples was higher—74.69% for the ACM treatment and 71.05% for the CM treatment, respectively. The chlorophyll content of the cotyledons demonstrated a similar pattern. Consequently, the phytotoxicity of the compost may be underestimated when the moisture content of the sample is high. The preparation of compost water extract based on the dry weight of the samples can therefore reflect phytotoxicity more accurately. Seedling establishment tests may be used to comprehensively evaluate compost phytotoxicity.

Keywords: phytotoxicity; seedling establishment; compost water extraction; radicle length; chlorophyll content of cotyledons

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1. Introduction

With the acceleration of agricultural processes, the amount of organic solid waste in China has increased in recent years [1]. This may lead to hygiene hazards, odor pollution, and ground and surface water pollution if not treated properly [2]. As a sustainable, environmentally friendly method, composting is widely used to detoxify, reduce, and utilize organic solid waste, including livestock manure, crop straw, and plant residues, which can effectively convert bio-waste into soil amendment or organic fertilizer [3]. Mature compost with long-term nutrient-releasing properties may be applied to improve soil fertility and plant growth [4]. However, applying fresh compost with high phytotoxicity may restrict plant growth through competition for oxygen and nutrients in the rhizosphere and release toxic substances, including available heavy metals, pathogenic bacteria, parasites, salts, and ions [5]. Owing to these potential negative consequences, the evaluation of phytotoxicity is a primary issue when compost is applied to agricultural soils [6,7].

Seed germination tests are commonly used to evaluate the phytotoxicity of composts [8]. Evaluation indicators generally include relative seed germination rate (RSG), relative radical growth (RRG), and seed germination index (GI). GI is widely used as an authoritative biological indicator for evaluating the phytotoxicity of composts. The newly revised standard of organic fertilizer (NY/T 3442-2019) in China introduced GI as an evaluation index of compost maturity and added a requirement of GI $\geq$ 70% for the first time [9]. However, previous studies have primarily focused on the fate of different types of composts rather than on the evaluation process and method [8].

In general, an aqueous extract of compost prepared by mixing fresh solid compost with deionized water (1:10, w/v) is easy to operate, with a fine reflection on the changes in phytotoxicity of materials during composting [10,11]. However, this approach did not consider the differences in the moisture content of the samples collected from different composting stages. To accurately evaluate the change in the phytotoxicity of samples during composting and increase the comparability of the phytotoxicity of samples with different moisture contents, it is necessary to eliminate the interference of moisture content from the test results [8]. If not, this may otherwise lead to the underestimation of compost phytotoxicity when the moisture content of the sample is high and overestimation when the moisture content is low.

Although the seed germination test was rapid and reliable, only the potential impact of the compost on the underground parts of the plants was considered. The process of seedling establishment begins with seed germination, passes through radicle elongation, and is completed when chlorophyll is synthesized in cotyledons, which then develops into the autotrophic growth stage as the seedling gains photosynthetic ability [12]. Based on the environmental conditions required for development, the establishment of seedlings may be divided into two developmental stages—skotomorphogenesis and photomorphogenesis. Skotomorphogenesis occurs in the absence of light, whereas photomorphogenesis occurs in the presence of light [13]. In the former stage, plants grow in the dark, and the largest change observed is characterized by the rapid elongation of radicles. Subsequently, at the later stage, plants require light for development, and the most obvious change is characterized by the greening of cotyledons [12]. However, to date, there is a lack of research on phytotoxicity affecting plant growth in photomorphogenesis. That is, whether the chlorophyll content of cotyledons can aid in characterizing the changes in phytotoxicity to achieve a comprehensive evaluation of compost phytotoxicity. Chinese cabbage (*Brassica rapa* L.) was selected in this study because its seeds are medium in size (1–2 mm), germinate rapidly, and are sensitive to compost phytotoxicity [14,15].

This study, therefore, compared the effects of the two extraction methods on phytotoxicity evaluation by preparing compost water extracts based on the dry and wet weight of the samples. Moreover, a comprehensive evaluation of compost phytotoxicity was performed through a seedling establishment test of Chinese cabbage seeds.

2. Materials and Methods

2.1. Experimental Materials

The predominant raw material used was cow manure obtained from a dairy farm in Taigu District, Shanxi, China. The auxiliary materials used in composting are typical agricultural wastes in local areas. Corn straw was obtained from the vicinity of the dairy farm with a fragment of approximately 3 cm, and the spent mushroom substrate was collected from a mushroom production base in Lvliang District, Shanxi, China. Sawdust, the primary component, was shredded to 5–10 mm. The seeds of the Chinese cabbage (*Brassica rapa* L.) used in the phytotoxicity test were purchased from a local market. The characteristics of the raw materials used are listed in Table 1.

Table 1. Basic physicochemical properties of the raw materials.

Materials	Moisture Content (%)	TOC (%)	TN (%)	C/N
Cow manure	74.19	25.79	1.58	16.32
Corn straw	4.18	45.52	0.93	48.95
Spent mushroom substrate	0.72	43.55	0.82	53.11

Note: Moisture content was calculated based on wet basis; TOC: Total Organic Carbon; TN: Total Nitrogen. TOC and TN were acquired based on dry basis.

2.2. Experimental Designs and Sample Collection

The composting material comprised 90% cow manure, 8% corn straw, and 2% spent mushroom substrate on a wet weight basis, with the total weight of the pile being 25 kg. Depending on whether the agent was added, the experiment was divided into two treatments—CM (cow manure compost) and ACM (cow manure compost added with a microbial agent). An organic matter-decomposing microbial agent (Shanxi Kaisheng Fertilizer Co., Ltd., Yuncheng, China) was added to the ACM treatment with a weight ratio of 10% of the cow manure dry weight in a pile. The effective bacteria in the microbial agent are *Bacillus licheniformis* and *Bacillus subtilis*, with viable bacteria numbers over 5.0×10^7 cfu/g.

Composting trials were performed at the Experimental Station of the College of Resources and Environment at the Shanxi Agricultural University. Rainproofing and ventilation were in good condition. A plastic foam box (wall thickness, 3 cm; length × width × height, 54 × 39 × 28 cm) was used for composting for 70 days. During composting, the temperature at the center of the piles was measured daily using a digital needle thermometer. The composts were manually turned and sampled on days 1, 3, 5, 7, 10, 14, 21, 28, 35, 42, 49, 56, 63, and 70. Approximately 300 g of fresh sample was collected and divided into two parts at each sampling time. One part was determined for the moisture content of the compost and to prepare the compost water extract (4 °C), and the other part was air-dried and ground to pass through a 1-mm sieve to analyze the humic acid content. The samples were dried at 105 °C to determine the moisture content.

2.3. Measurement and Analysis Methods

2.3.1. Preparation of Compost Water Extract

Fresh samples and deionized water were mixed at a ratio of 1:10 (wet weight, w/v) and 1:20 (dry weight, w/v) at 150 rpm for 60 min in a horizontal shaker at room temperature and then centrifuged at 5000 rpm for 10 min to obtain the compost water extract.

2.3.2. Seedling Establishment Test

The phytotoxicity of compost was determined using a seedling establishment test based on the compost water extract, and the indexes included the relative growth of radicles and chlorophyll content of cotyledons. The Chinese cabbage seeds used in this experiment with a germination rate ≥85%, which is readily obtainable, easy to germinate, and sensitive to phytotoxicity. They were placed on filter paper in a Petri dish (Φ 90 mm), added with 5 mL of the compost water extract (or deionized water for the control), and put in an incubator under suitable conditions of 25 °C in darkness. A total of 20 seeds were assigned per dish, and the test was replicated three times. The radicle length (mm) of the seeds was measured, and relative radicle growth (RRG) was calculated. The calculation formula used is as follows:

$$RRG\% = \frac{average\ \text{radicle}\ lengthofallseedsinsample}{averageradiclelengthofallseeedsincontrol} \times 100 \quad (1)$$

The chlorophyll content was determined using the colorimetric method. After the first incubation in the dark for 48 h, the seeds were incubated under a light intensity of 6000 lux for 12 h. The cotyledons were then cut and ground in a mortar and added with 5 mL ethanol (96%, v/v). After centrifugation, the supernatant was diluted, and the absorbance values were recorded using a UV-vis spectrophotometer (N4S, INESA Instrument, Shanghai). The absorbance values were measured at 649 nm (A_{649}) and 665 nm (A_{665}) using 96% ethanol as the control. The relationships between the absorbance and the contents of chlorophyll a and b are as follows [16]:

$$C_a = 13.95A_{665} - 6.88A_{649} \quad (2)$$

$$C_b = 24.96A_{649} - 7.32A_{665} \quad (3)$$

2.3.3. Determination of Physicochemical Indexes of Compost Water Extract

The pH and electrical conductivity (EC) of the compost water extract were measured using a pH/EC meter (pHS-3C, Precision Scientific Instrument, Shanghai, China), and the nitrate-nitrogen (NO_3^--N) and ammonium-nitrogen (NH_4^+-N) contents with a segmented flow analyzer (AMS, Alliance, France). The total organic carbon (TOC) and total nitrogen (TN) contents were measured using a TOC/TN analyzer (Multi N/C 3100, Analytik Jena AG, Jena, Germany).

2.3.4. Determination of Humic Substances Contents and E_4/E_6

For the humus fractional composition, 5 g air-dried samples were placed in a centrifuge tube, adding 30 mL of NaOH (0.1 mol·L^{-1}) and $Na_4P_2O_7$ (0.1 mol·L^{-1}) mixture (1:1, v/v). The supernatant and residues were separated after shaking the extraction at 70 °C for 1 h and centrifugation at 3000 rpm for 20 min. The extracted humic substances were separated into humic acid (HA) and fulvic acid (FA) fractions by acidifying the extract to pH 1.3–1.5 using 0.5 M H_2SO_4 at 68–70 °C, after which the humic acids were separated by filtering. The separated humic acids were redissolved in a 0.1 M NaOH solution. The carbon contents of HA and FA were then determined using a TOC analyzer. E_4/E_6 is the ratio of the absorbance of the humic acid at 465 and 665 nm.

2.4. Statistical Analysis

The data were expressed as mean ± SD, and one-way analysis of variance (ANOVA) was performed between different extraction methods using SPSS 24.0 (IBM, Armonk, NY, USA).

3. Results and Discussion

The phytotoxicity of composts is influenced by several factors, including physicochemical parameters, phytotoxic substances, maturity parameters, and humification degree. As a bioassay, the seed germination test combines the effect of all factors to reflect phytotoxicity, which could be directly affected by the water-soluble matter. Plant growth includes both underground root growth and aboveground leaf growth. The relative radicle growth of seeds used as an evaluation index of phytotoxicity is, therefore, not comprehensive. The radicle is the organ with the greatest change observed during skotomorphogenesis, and cotyledons are the parts of seedlings sensitive to stress during photomorphogenesis. Dark-grown plants display an etiolated phenotype characterized by longer hypocotyl length and smaller yellowish cotyledons. In contrast, in the presence of light, photomorphogenesis is manifested by short hypocotyl lengths and open and expanded green cotyledons, thereby promoting greening to allow seedlings to adjust for optimal light-harvesting capacity and autotrophic growth [17]. Cotyledons are the most sensitive organs to the external environment during photomorphogenesis, their chlorophyll content characterizing their degree of stress. This study, therefore, aimed to comprehensively evaluate the phytotoxicity based on radicle growth and cotyledon chlorophyll content.

3.1. Phytotoxicity Indicators

3.1.1. Changes in Relative Root Growth during Composting

The RRG of Chinese cabbage in the two treatments increased gradually with the progression of the composting process (Figure 1) but decreased at the beginning of composting, which was related to the incomplete degradation of organic matter and high NH_4^+-N content. At the end of composting, the RRG of the ACM treatment was higher than that of

CM treatment. This indicates that the addition of a microbial agent accelerated the maturity of the compost and reduced the phytotoxic effects on the elongation of seed radicle. The RRG of Chinese cabbage demonstrated different changes under different extraction methods for the different treatments at the early stage of composting. However, the RRG of Chinese cabbage based on wet weight extraction was higher than that based on dry weight extraction. Therefore, the phytotoxicity of the compost may be underestimated when the moisture content of the sample is high. In the CM treatment, RRG under dry weight extraction was lower than that under wet weight extraction during the entire composting process. However, in the ACM treatment, no significant change was observed in the RRG under different extraction methods ($p > 0.05$). In the CM treatment, the different extraction methods demonstrated significant differences ($p < 0.05$).

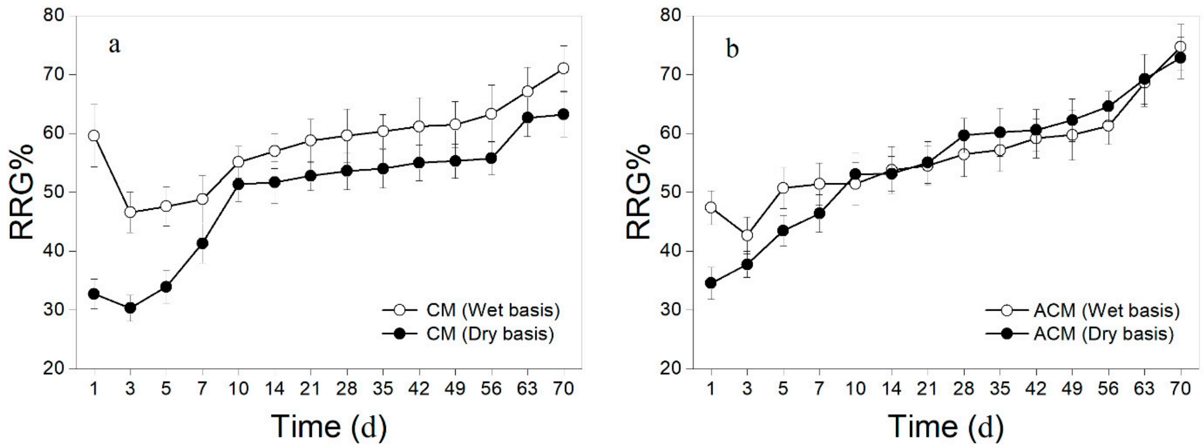

Figure 1. RRG in phytotoxicity tests of aqueous extracts of composts with (**b**) and without microbial agent (**a**).

3.1.2. Changes in Chlorophyll Content during Composting

The contents of chlorophyll a and chlorophyll b reflect the photosynthetic capacity and nitrogen content of leaves to a certain extent, which is crucial for plant growth [17]. The CM and ACM treatments enhanced the chlorophyll content of cotyledons at the end of composting compared to the control (deionized water). The chlorophyll a content increased by 51.67% and 62.87% based on the wet weight extraction of samples, and the chlorophyll b content increased by 38.13% and 15.78%, respectively. Based on the dry weight extraction method, the chlorophyll a content increased by 79.76% and 94.45%, and the chlorophyll b content increased by 44.89% and 107.29%, respectively (Figure 2). The chlorophyll content of the ACM treatment was greater than that of the CM treatment. Compost maturity, therefore, influenced the production of cotyledon chlorophyll. At the end of composting, the relative chlorophyll content of seedlings extracted based on dry weight was lower than that under wet weight extraction in the CM treatment. In contrast, after day 56 of the ACM treatment, the relative contents of chlorophyll a and b in cotyledons were higher under dry weight extraction than under wet weight extraction. In the CM treatment, the relative contents of chlorophyll a and b were significantly different under different extraction methods ($p < 0.05$). Additionally, under different treatments and extraction methods, because of the high NH_4^+-N content in the thermophilic phase of composting, the relative contents of chlorophyll a and b were lower before day 7 of composting and demonstrated a significant downward trend, then gradually increased, and varied substantially on days 21 and 42. This was related to the changes in TOC and TN during composting. Nitrogen is an indispensable element in chlorophyll synthesis. The relative contents of chlorophyll a and b increased after day 42, which might be attributed to an increase in the TN content.

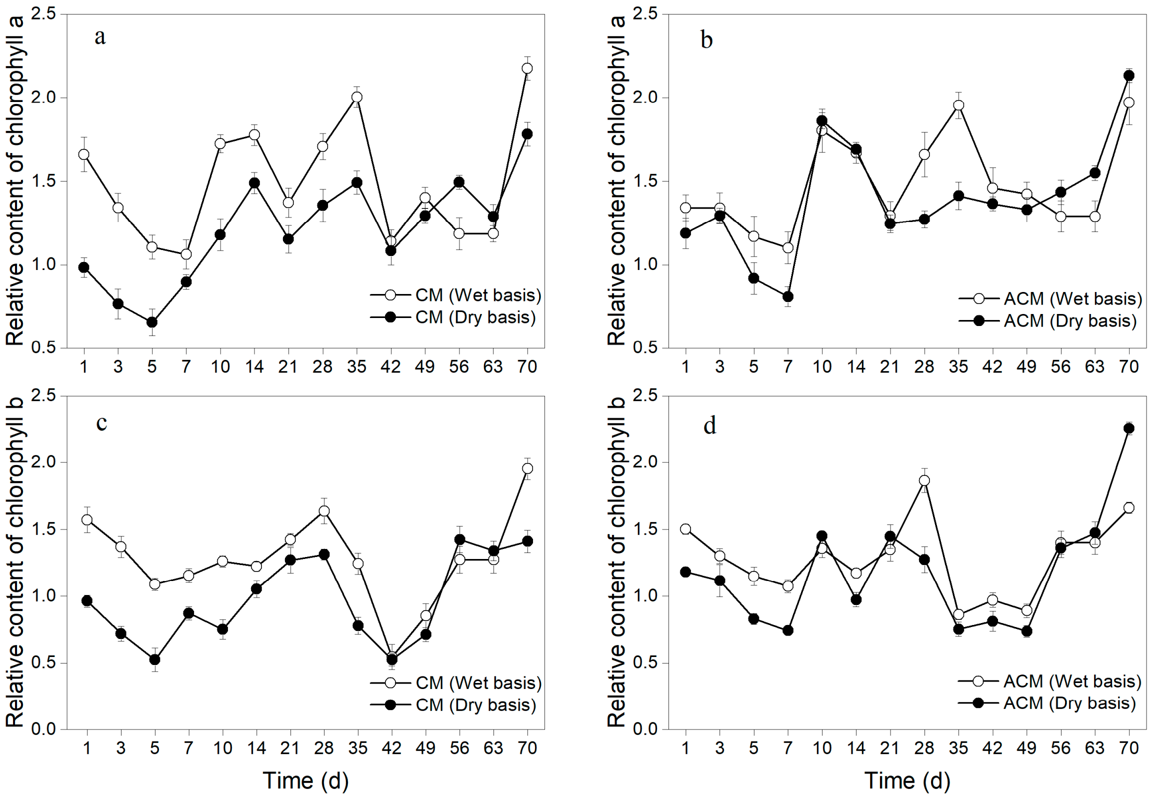

Figure 2. Changes in chlorophyll content in cotyledons under different treatments and extraction methods. (**a**) Changes in relative content of chlorophyll a in the CM treatment; (**b**) Changes in relative content of chlorophyll a in the ACM treatment; (**c**) Changes in relative content of chlorophyll b in the CM treatment; (**d**) Changes in relative content of chlorophyll b in the ACM treatment.

3.2. *Physicochemical Indicators of Composts*

3.2.1. Temperature and Moisture Content during Composting

The temperature of all the treatments increased rapidly and entered the thermophilic phase (>55 °C) on day 3 and lasted for 6 days (Figure 3a), which could meet the requirements for harmless sanitation of livestock and poultry manure. This was the result of intense microbial activity caused by the decomposition of organic matter in the composting substrates [18]. During the thermophilic phase, the temperature peaks in the ACM treatment and CM treatments were 69.1 °C (3 d) and 67.3 °C (4 d), respectively, indicating that the addition of microbial agents promoted an increase in the composting temperature and accelerated the heating process. Some increase in the temperature occurred after turning, which was related to better aeration. The temperature of the ACM treatment was higher than that of the CM treatment before day 14, suggesting that the addition of the microbial agent rapidly increased the temperature of the piles.

Moisture content has a significant impact on the composting process because it is directly related to composting efficiency and quality [19]. During the composting process, the moisture content of both the CM and ACM treatments decreased. As the composting temperature decreased, moisture evaporation slowed, and the moisture content of the compost stabilized in the CM treatment, as presented in Figure 3b. However, the water content of the CM treatment was notably higher than that of the ACM treatment in the late composting stage. The water content of the ACM treatment fluctuated dramatically over the entire composting process. This may be because the addition of the microbial agent rendered the temperature of the ACM treatment higher than that of CM treatment at the thermophilic phase, thus making the aerobic fermentation more thorough. This was more conducive to lowering the water content. At the end of composting, the moisture contents of the CM and ACM treatments were 63.78% and 46.94%, respectively, which decreased by 7.18% and 19.78% compared to the start of composting, respectively.

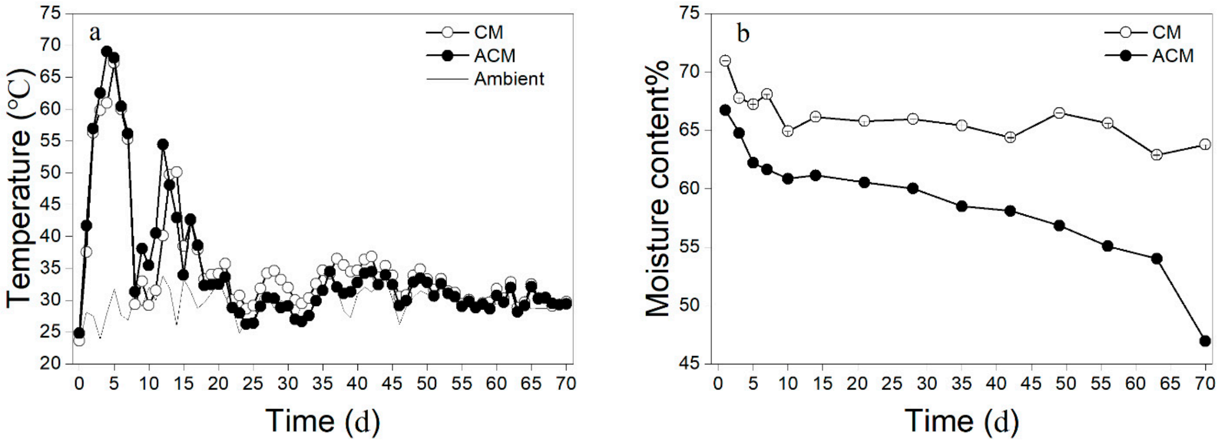

Figure 3. Changes in temperature (**a**) and moisture content (**b**) in different treatments during composting.

3.2.2. pH and EC

pH is not only a key environmental factor that affects the composting process but also an indicator of compost maturity [20]. The pH was changed by the moisture content of the samples, as presented in Figure 4a,b. The pH of the two treatments based on wet weight extraction of the sample changed substantially during the composting process. The rapid decrease in pH in the two treatments in the early stage of the composting process, followed by an increase in pH was consistent with the results of Nie et al. [21]. They attributed the decrease in pH to the formation of low molecular weight organic acids and the increase in pH to the rapid degradation of organic acids and proteins in the late stage of the composting process, releasing large amounts of NH_3 and producing large amounts of OH^- through ammonification. At the end of composting, the pH of the two treatments was close to 7.50, which was favorable for the enhancement of microbial activity and composting quality [22]. In ACM treatment, the pH under different extraction methods was significantly different ($p < 0.05$). The pH based on wet weight extraction was higher than that based on dry weight extraction before day 56, whereas the opposite results were observed after day 56. The CM treatment also demonstrated similar changes.

As presented in Figure 4c,d, the EC values of the two treatments steadily increased during late composting. This may be related to the production of inorganic compounds and the relative increase in the ion concentration caused by organic degradation and water evaporation. At the end of composting, the EC values of the CM and ACM treatments under the different extraction methods were lower than 4 mS·cm^{-1}, which did not represent a risk factor for plant growth [23]. The EC values in the two treatments differed significantly between the different extraction methods ($p < 0.05$). Furthermore, the EC values based on the dry weight extraction of the samples were higher than those based on wet weight extraction, especially in the CM treatment. Consequently, different extraction methods for composting with low salinity could exclude the influence of salinity on the phytotoxicity evaluation results.

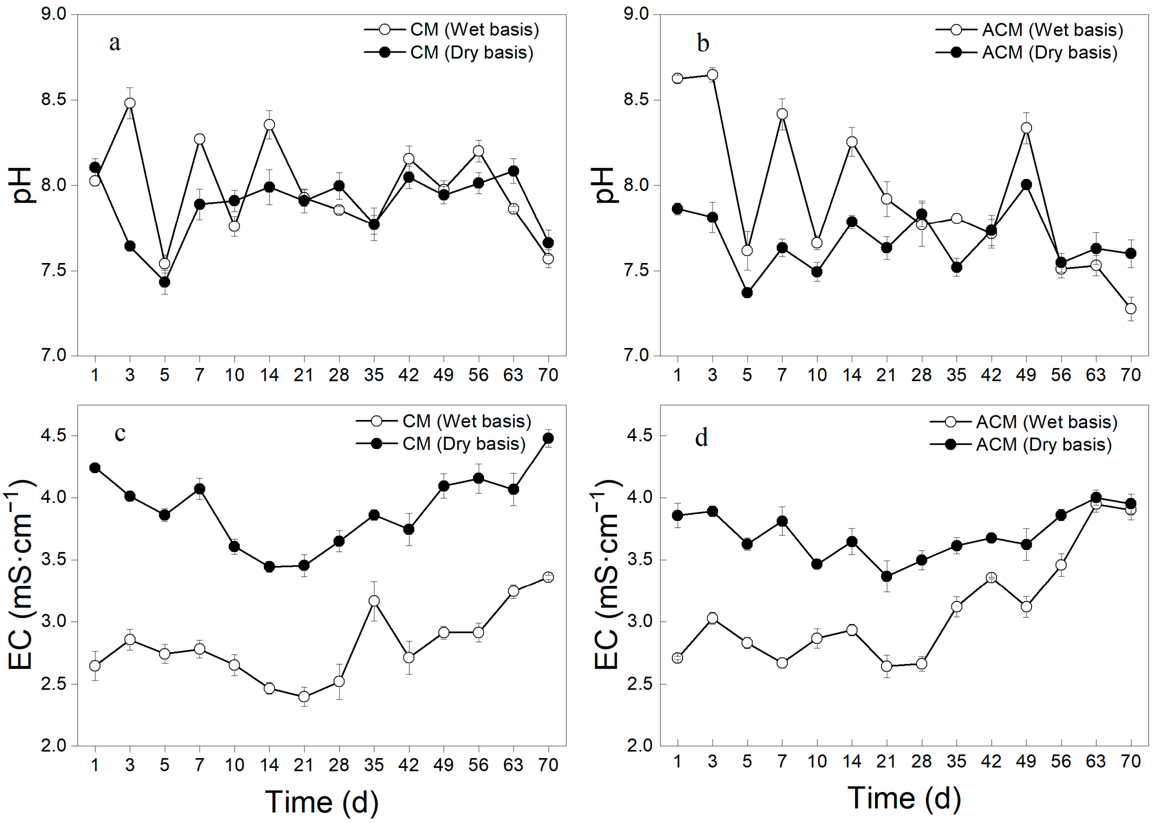

Figure 4. Changes of pH (**a**,**b**) and EC (**c**,**d**) in different treatments during composting.

3.3. Variations in Carbon and Nitrogen Contents

3.3.1. NO_3^--N and NH_4^+-N

Changes in the NO_3^--N and NH_4^+-N content during composting were analyzed (Figure 5). The NO_3^--N contents in the two treatments increased and reached a maximum at the end of composting. The NO_3^--N content in the ACM treatment was much higher than that in the CM treatment, indicating that the addition of the microbial agent to the composting process might promote the activity and proliferation of nitrifying bacteria. No significant difference was observed between the different extraction methods for the two treatments ($p > 0.05$). The NH_4^+-N contents in the two treatments initially increased and then decreased. As the primary source of composting phytotoxicity [24], the NH_4^+-N content was reduced to a minimum at the end of composting. Considering organic materials quickly decompose to generate NH_4^+-N at the start of composting and then with the composting entering the thermophilic phase, the NH_4^+-N content gradually reduces with a large quantity of NH_3 volatilization and nitrification. In the early stages of composting, the NH_4^+-N content was higher based on dry weight extraction than that based on wet weight extraction. The difference in the NH_4^+-N content caused by the two extraction methods was reduced after day 21. There were no significant differences in the extraction methods between the two treatments ($p > 0.05$).

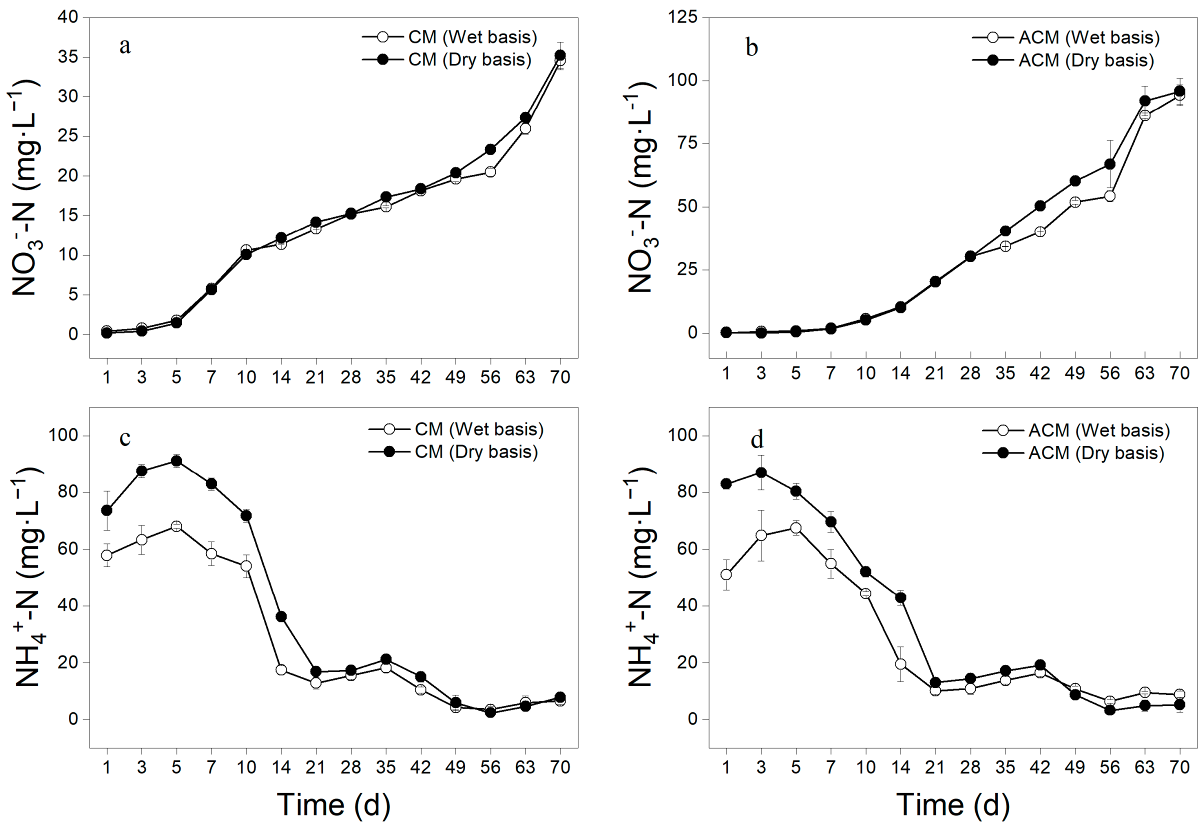

Figure 5. Changes of NO_3^--N (**a**,**b**) and NH_4^+-N (**c**,**d**) in different treatments during composting.

3.3.2. TOC and TN

Water-soluble carbon and nitrogen concentrations can better indicate material transformation during composting than solid composting samples, as organic matter is continuously decomposed and converted during composting [25]. On the one hand, carbon is rapidly decomposed, and on the other hand, carbon-containing substances, such as humus, form gradually. The TOC concentration increased in the first seven days of both treatments (Figure 6a,b) because the rate of production of soluble organic carbon in the biotransformation process was faster than the degradation rate. Subsequently, the microbial activity was enhanced, and soluble substances (i.e., sugar, amino acids, and peptides) were metabolized and assimilated, thereby resulting in a decrease in the TOC concentration [26]. After day 28 of composting, the TOC content recovered and stabilized due to further degradation of refractory components, such as lignocellulose. The TOC content based on the dry weight extraction of samples in the two treatments was high, and the different extraction methods demonstrated significant differences ($p < 0.05$).

The TN content initially increased and then decreased in the CM and ACM treatments, reaching a maximum on day 7 (Figure 6c,d). That is, amination bacteria were generated during the thermophilic phase of composting, and the NH_4^+-N content increased sharply because the ammoniating effect was strong, thereby increasing the TN content. The TN content decreased after the thermophilic phase of composting owing to the suppression of ammonifying bacteria and the loss of ammonia by volatilization. The TN content increased from day 28, during which the ammonia loss rate decreased and became lower than the nitrogen mineralization rate [27]. Microorganisms, therefore, influence nitrogen changes during composting. The dominant microorganisms change during different stages of composting, thereby influencing nitrogen transformation and form. The TN content gradually increased after day 42 owing to the concentration effect under different treatments and different extraction methods, with a similar change in TOC content. In the two treatments, the TN content based on the dry weight of the sample was higher, whereas the different extraction methods in the CM treatment demonstrated significant differences ($p < 0.05$).

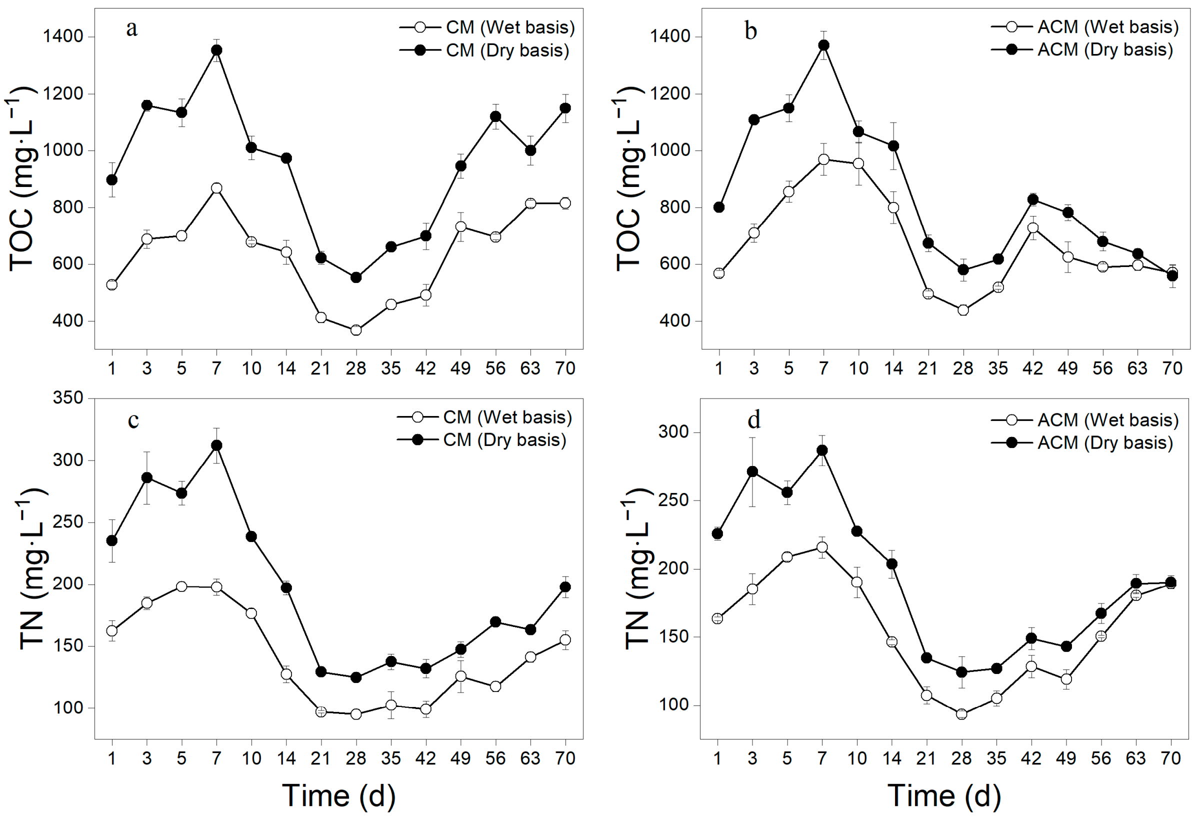

Figure 6. Changes in the TOC (**a**,**b**) and TN (**c**,**d**) content in different treatments during composting.

3.4. Changes in the Humus Content during Composting

The rapid growth of microorganisms during composting leads to the decomposition of unstable organic compounds in humus [28]. Humus is an organic polymer formed by composting microorganisms and enzymes, which affects the water and fertilizer retention capacity of composting products, as well as the movement and transformation characteristics of pollutants in the environment. Its composition and structure are important indicators of phytotoxicity reduction during composting [29]. HA and FA are the primary components of humus. HA is a macromolecular complex with relatively stable activity, whereas FA has a low molecular weight and high activity, making it easily decomposed and utilized by microorganisms [30]. The HA structure is complicated, and the increase in HA content occurs mostly during the thermophilic phase of composting when phytotoxic substances can be absorbed. The increasing trend of HA content, therefore, indicates the maturity of the compost and a decrease in phytotoxicity during composting [31]. In this experiment, the FA content of the two treatments declined progressively throughout the composting process, while the HA content increased relatively with a stable structure (Table 2). At the end of composting, the HA content in the ACM treatment was higher, indicating that the ACM treatment had a high degree of humification. When compared to the first day, the HA content in the CM and ACM treatments increased by 101.79% and 78.95%, respectively. The FA contents decreased by 22.03% and 46.43%, respectively. The primary reason for these differences is that although FA and HA are produced in the process of humification, FA can be used as an energy source and substrate by microorganisms to produce more stable HA in mature compost [32].

Table 2. Dynamics of humus carbon during composting.

Samples	CM		ACM	
	HA-C (mg·g^{-1})	FA-C (mg·g^{-1})	HA-C (mg·g^{-1})	FA-C (mg·g^{-1})
1 d	5.60	5.13	7.60	5.60
7 d	8.60	5.19	8.30	3.99
10 d	9.30	4.60	10.20	4.60
35 d	10.10	4.17	11.70	3.62
70 d	12.30	4.00	13.60	3.00

Note: HA-C, humic acid carbon; FA-C, fulvic acid carbon.

HA/FA is the ratio of humic acid to fulvic acid content. The HA/FA values of the different treatments were similar in the early stages of composting, and the HA/FA of the ACM treatment was greater than that of the CM treatment in the later stages. At the end of composting, the HA/FA of the two treatments were 3.08 and 4.53, respectively. A HA/FA > 1.9 generally indicates that the compost is completely decomposed [33]. Therefore, in this experiment, the two compost treatments met the maturity requirements, and the addition of the microbial agent promoted the synthesis of HA and reduced the phytotoxicity of the compost.

E_4/E_6 reflects the quality and degree of aromatization of HA in the compost. E_4/E_6 is a parameter for the rapid evaluation of the degree of compost humification, which decreases as the molecular weight or condensation degree of HA increases [34]. E_4/E_6, therefore, exhibited a general declining trend during composting. Similar results were observed in this study. After composting, the E_4/E_6 values of HA in the CM and ACM treatments decreased from 11.95 and 11.07 to 4.56 and 4.47, respectively, indicating that the mixed materials were converted into mature organic solids and that the addition of a microbial agent could accelerate the condensation and aromatization of compost humus.

3.5. Relation between Phytotoxicity and Extraction Method

3.5.1. Difference between Composting Water Extracts Prepared by Fresh Weight and Dry Weight

The phytotoxicity of compost depends on the concentration of the aqueous compost extract [35]. Considering the changes in moisture content during composting, it is possible to underestimate phytotoxicity when the moisture content is high in the early stage of composting and overestimate it when the moisture content decreases in the later stage. In this experiment, pH, EC, NO_3^--N, NH_4^+-N, TOC, and TN varied under different extraction methods for the CM and ACM treatments. Except for the pH value under the wet-weight extraction method, which was slightly higher than that under the dry-weight extraction method, other indexes in the composting process demonstrated opposite trends or were similar at the end of composting. The EC value is greatly affected by different extraction methods in different treatments, especially in the CM treatment. The EC value based on dry weight extraction is much larger than that based on wet weight extraction. Dry weight extraction based on samples can therefore reflect the salt stress on plants after composting more accurately. The highest NH_4^+-N content was attained in the CM and ACM treatments on day 5 and day 3, respectively, and the difference in the NH_4^+-N content under different extraction methods was 33.92–34.23%. As NH_4^+-N is the main source of composting phytotoxicity, the dry weight extraction method based on samples can provide a more accurate assessment of phytotoxicity. In addition, the TOC and TN content of the two treatments were significantly different when using different extraction methods during composting. Excluding the influence of water content based on the dry weight, the extraction method can, therefore, better reflect the changes in the TOC and TN content in the compost water extract. The phytotoxicity evaluation indexes RRG, and cotyledon chlorophyll demonstrated an upward trend under the two extraction methods. In the CM treatment, the RRG and chlorophyll contents of Chinese cabbage seeds were higher under the wet weight extraction method. At the end of composting, the RRG of

Chinese cabbage seeds reached 71.05% based on the wet weight extraction method and 63.23% under dry weight extraction. If the case that the RRG was greater than 70% as the application requirement of composting, it was considered that the composting did not meet the standard under dry weight extraction and that the composting time might need to be further extended. The moisture content of the ACM changed dramatically during composting because of the addition of microbial agent. The phytotoxicity of the compost was effectively reduced by microbial agent. Thus, the RRG of Chinese cabbage under the two extraction methods tended to be consistent in late composting. This rule is also reflected in the changes in chlorophyll content in the cotyledons. The results of single factor analysis of variance revealed that the indexes in the CM treatment demonstrated significant differences under different extraction methods, that is, the dry weight or wet weight extraction of samples would have a greater impact on the evaluation results of compost phytotoxicity without the addition of microbial agent.

3.5.2. Comparison between Seedling Establishment Test and Seed Germination Test

Based on the seed germination test, the seedling establishment test not only considered the potential effects of compost on the below-ground parts of plants but also explored the chlorophyll content in the above-ground parts of plants as a reflection of compost phytotoxicity. In this study, the RRG and chlorophyll content of Chinese cabbage under different treatments and different extraction methods were maintained at low levels or decreased at the beginning of composting under phytotoxic substance stress. The RRG gradually increased with composting and reached a maximum at the end. However, the contents of chlorophyll a and b still fluctuated after day 35 of composting, suggesting that the plant seedlings were subjected to toxic substance stress but steadily weakened. Simultaneously, plant seedlings would adapt to compensate for inhibition during the early stage [36]. The chlorophyll content in cotyledons of Chinese cabbage gradually increased with a decrease in composting phytotoxicity, indicating that chlorophyll content in cotyledons could characterize the change in composting phytotoxicity and further validate the evaluation results of phytotoxicity. Chlorophyll can therefore be used as an auxiliary or verification index to evaluate and provide a comprehensive understanding of phytotoxicity.

The ratio of raw materials to auxiliary materials determines the ventilation environment inside the reactor, including differences in moisture content and initial nutrients [37,38]. The addition of microbial agents can shorten the fermentation cycle, maintain nutrients, and accelerate the maturity of materials [39]. In this study, corn straw and spent mushroom substrates were used as conditioners in cow manure compost, and aerobic composting experiments were carried out by inoculating mature bacteria. The effects of different treatments on the physical, chemical, and biological indexes of compost were detected. The results demonstrated that throughout the composting process, the ACM treatment with the microbial agent rapidly increased the temperature to a high value and rapidly reduced the NH_4^+-N content. The NO_3^--N content was much higher than that of the CM treatment, and the RRG and chlorophyll content of Chinese cabbage seeds were high, indicating significantly reduced compost phytotoxicity. Moreover, by analyzing the changes in humus components during composting, it was discovered that the HA content increased while the FA content decreased. At the end of composting, the HA content, HA/FA ratio, and the value of E_4/E_6 demonstrated that the humification degree of the ACM treatment was higher, which is consistent with the results of a previous study [40]. The phytotoxicity of compost results from a combination of several factors, including temperature, pH, EC, TOC, TN, HA, small-molecular-weight organic compounds, and NH_4^+-N, all of which can affect seed germination and radicle elongation [41]. The results of previous studies suggest that seed germination is positively related to humification but negatively related to the NH_4^+-N content [42,43].

4. Conclusions

The preparation of the compost water extract based on the dry weight of the samples could reflect the phytotoxicity more accurately without the interference of moisture content. In this study, the characteristics and requirements of different stages of plant growth were considered, and it was found that the chlorophyll content of cotyledons was the same as the RRG of seeds, which could characterize the evolution of phytotoxicity during composting. The seedling establishment test, therefore, facilitated a comprehensive evaluation of compost phytotoxicity.

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