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Oyster shell-functionalized biochar enhanced compost humification during the co-composting of pig manure with rice straw

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Abstract

The lower humification efficiency during composting is an emerging challenge that attenuates compost quality. Although biochar has the potential to enhance humification, its effectiveness is limited by factors such as its alkaline pH and the absence of functional groups. Here, Ca-modified biochar was prepared via co-pyrolysis of coconut shell and oyster shell (1:1 ratio) at 600 °C and added to pig manure composting at a 10% (w/w) dose. The result of spectroscopy analysis of the developed compost revealed that carboxyl and carbonyl functional groups facilitated the adhesion of oyster shells to the biochar surface. The addition of Ca-modified biochar in compost not only increased the concentrations of humic acid (+ 8.6%) but also improved the seed germination index (GI) (+ 18.9%) compared to the control group. The excitation-emission matrix analysis indicated that the modified biochar facilitated the transformation of protein-like molecules, thereby enhancing the humification of organic matter by 4.92% compared to the control group. Microbial analysis indicated that *Proteobacteria* and *Bacteroidetes* were predominant in the modified biochar group, exhibiting a 107% higher prevalence, as compared to the control group. These enriched microbial taxa, known for their role in lignin degradation, are inferred to inhibit nitrogen volatilization, generate precursor substances, and catalyze the conversion of organic matter into stable humus. The correlation analysis further revealed a significant positive correlation between *Proteobacteria* and GI ($p < 0.05$). These findings demonstrated that Ca-modified biochar enhances physicochemical and microbiological processes, offering a scalable solution for improving humification and supporting sustainable compost production.

Highlights

- Efficiently converting oyster shells and coconut shells into practical compost enhancers.
- Modified biochar possesses abundant carboxyl/hydroxyl groups and facilitates a gradual release of Ca^{2+}.
- Modified biochar enriched key microbial genera (e.g., *Thermopolyspora*, *Niabella*) implicated in humification.
- Treatment increased the HA/FA ratio by 0.33 and the germination index (GI) by 22.59% compared to the control.

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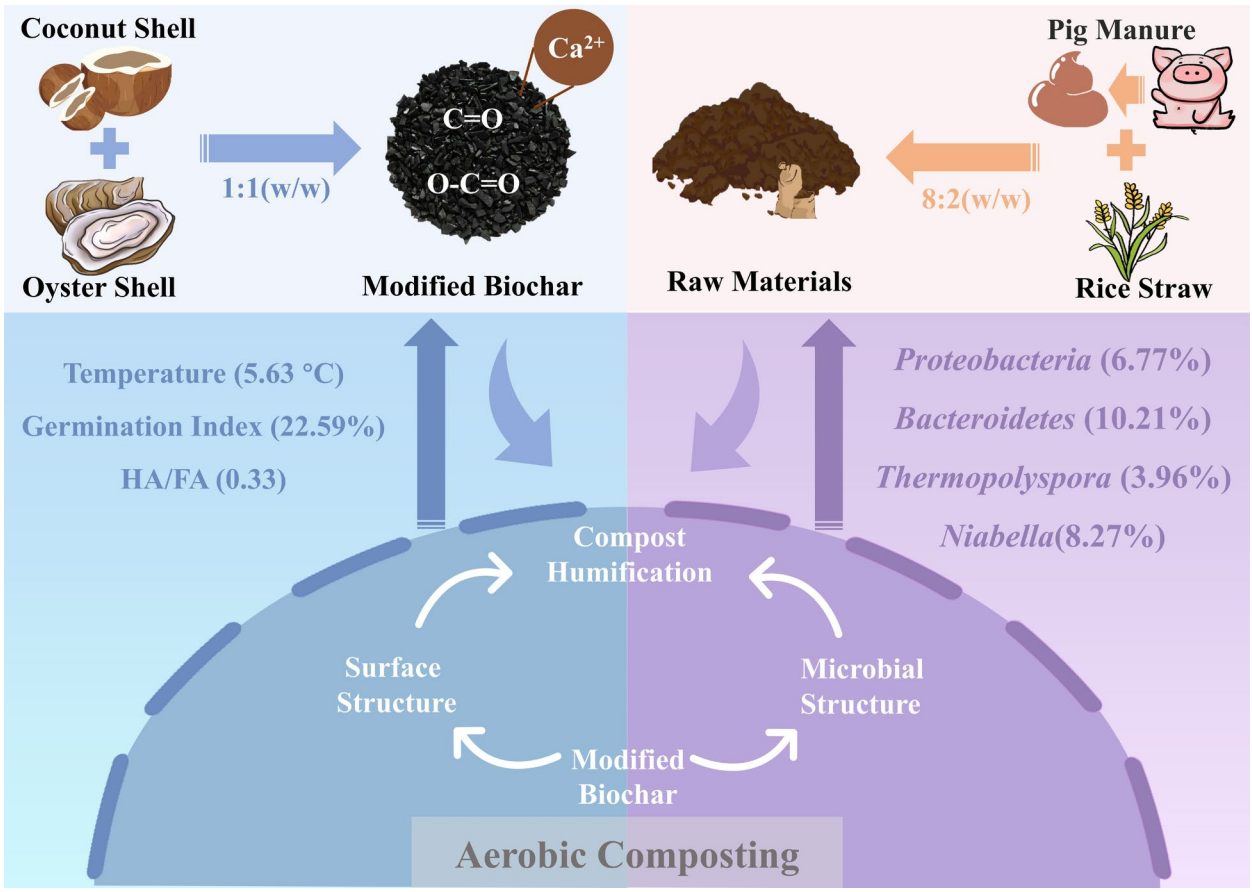
Keywords Composting, Ca-modified biochar, Humification, Microbial community

Graphical Abstract



1 Introduction

China is the largest producer of livestock in the world and the population is gradually increasing every year (Jin et al. 2020). Due to the increasing population of livestock, the manure management is becoming a challenge and conventional manure management techniques which are currently being used are resulting in about 4 billion tons of emissions per year, with pig manure (PM) accounting for 45.9% (Zhou et al. 2023). Improper disposal of PM is causing many environmental issues like carbon emissions, pathogens and heavy metals accumulation and eutrophication (Liu et al. 2023a). Consequently, the sustainable manure management technologies, such as composting, which not only treat the manure in an effective way but also do not cause any environmental issues, are the need of the hour (Bai et al. 2023). Composting effectively converts PM into humus (HS) through microbial and enzymatic processes (Tian et al. 2023). HS helps in reducing heavy metal pollution, accelerates antibiotic degradation, and improves nutrient retention in soils. It is primarily composed of aromatic, phenolic, and quinone compounds (Liu et al. 2023c). However, traditional composting is currently facing several challenges, such as slow biotransformation and complex physiological and biochemical processes, which hinder the efficient humus formation (Duan et al. 2021). Therefore, improved methods to accelerate humus formation during composting are urgently needed.

Recent studies have highlighted the effect of adding foreign chemicals and bacteria during composting to enhance humification and composting maturity, including biochar, minerals, and bacteria (Wang et al. 2025). Besides these additions, the adjustments to material ratios and reaction conditions have also been evaluated

for improving compost quality (Xu et al. 2022). Among all the above-mentioned materials, biochar has gained considerable attention as an effective compost amendment (Zhang et al. 2022a). Biochar improves composting by enhancing microbial activity, moisture retention, and nutrient availability (Osman et al. 2022). Additionally, it serves as a physical substrate for microbial colonization (Zhao et al. 2022). Notably, the combination of biochar and compost products is more effective in enhancing soil fertility and promoting carbon sequestration due to their corresponding physical and chemical properties (Mikajlo et al. 2024). Biochar derived from coconut shell pyrolysis has high carbon content and high specific surface area compared with that of agricultural and forestry wastes. This makes it a popular and environmentally beneficial adsorbent that is useful for cleaning up pollution (Fu et al. 2024). However, unmodified coconut shell biochar has low original pore structures and functional group content, leading to restricted adsorption efficiency (Gao et al. 2023). Therefore, enhancing the surface characteristics of biochar is a critical step for using it as an additive in compost to improve its humification efficiency.

Previous studies have shown that inorganic modification of biochar improves its structure and adsorptive capacity (Zhou et al. 2021). Various physical and chemical methods have been employed to improve the properties of biochar, including mechanical grinding, acid–base treatment, redox modification, metal ion loading, and surface functionalization (Tang et al. 2025). Among these methods, the modification of biochar with metals like calcium (Ca), iron (Fe), magnesium (Mg), and aluminum (Al) aids in reducing ammonia and greenhouse gas emissions by establishing cationic bridges, thereby maintaining nutrients inside the composting matrix (Gu et al. 2024). In particular, Ca is regarded as an ideal modifying agent due to its low cost and environmental sustainability (Zhang et al. 2024b). However, most previous Ca-modification studies rely on chemical-grade calcium sources (Liu et al. 2023b), which can be cost-prohibitive and may not facilitate the effective release of active Ca^{2+} during composting (Zhuo et al. 2022). In contrast to pure calcium, oyster shells are a cost-effective biomass waste, rich in calcium carbonate, that can be converted into valuable products through pyrolysis (Song et al. 2023a). Oyster shells, in addition to calcium carbonate, are a natural organic–inorganic composite material that contains organic components such as proteins, glycoproteins, and polysaccharides (Li et al. 2024b). Adding oyster shells into compost improves its quality by accelerating the breakdown of organic matter and minimizing nitrogen loss (Lu et al. 2020). Moreover, oyster shells provide slow-release calcium, enhancing microbial activity (Huang et al. 2022) without the cost or leaching risks of iron/potassium additives (Zhang et al. 2024b). However, oyster shells biochar produced through conventional high-temperature pyrolysis often shows poor calcium activation and agglomeration (Zhang et al. 2024b), resulting in a longer compost maturation period (Liu et al. 2023b). Considering the potential benefits of soluble Ca^{2+} and biochar for composting, modified biochar to adsorb and immobilize Ca^{2+} can enhance Ca^{2+} activation and improve carbon and nitrogen retention.

Therefore, this study investigated the effects of Ca-modified biochar on compost humification and revealed its potential microbial mechanism for promoting HS formation. The main objectives were (1) investigating the mechanism by which the release of Ca^{2+} and alkaline compounds from oyster shells enhances the physical and chemical properties of biochar, (2) examine the effects of Ca-modified biochar on composting humification and nutrient immobilization, and (3) elucidate the potential mechanisms driving microbial community behavior in the presence of Ca-modified biochar during composting. The results of this study provide theoretical support for using Ca-modified biochar as a novel composting additive to enhance humification during composting.

2 Materials and methods

2.1 Raw materials

Coconut shells and oyster shells were obtained from *Hainan Pingping Coconut Trading Co*. and a local market. The raw materials were washed thoroughly to remove dust and other impurities and then dried at 105 °C in an oven for 24 h, and the dried material was sieved through an 80-mesh filter. Oxygen-limited pyrolysis at a temperature of 600°C was used to produce coconut shell biochar (CB) and oyster shell-modified coconut shell biochar (CB1). A mixture of coconut shell powder and oyster shells, in a 1:1 mass ratio, was placed in a tube muffle furnace (KSL-1000X-M). The mixture was heated to 600 °C at a rate of 10 °C per minute, held at this temperature for 240 min, and then allowed to cool naturally in a vacuum desiccator to room temperature (Wang et al. 2023a).

2.2 Composting of pig manure

Fresh PM and rice straw were used as a feedstock for composting. The PM was collected from a pig farm in *Yazhou District, Sanya City, Hainan Province*, while the rice straw was obtained from the *Batou Base* test field and mechanically shredded into 1–2 cm fragments. The PM and rice straw were mixed in an 8:2 (w/w, dry weight basis) ratio to achieve a C/N ratio of 25–30. The physicochemical parameters of the feedstocks are provided in Table S1.

The composting of the PM and Rice straw was conducted in a custom-made lab-scale reactor with a volume

of 200 L, a height of 70 cm, an external diameter of 45 cm, and a tank of wall thickness 3 cm. This reactor has been previously described in detail (Yuan et al. 2016). The composting process was conducted for 36 days. Continuous ventilation at a ventilation rate of 0.48 L (kg dry matter)$^{-1}$ min^{-1} was provided for the initial 10 days, reduced to 0.32 L (kg dry matter)$^{-1}$ min^{-1} during 11–25 days, and no ventilation was provided during days 26–36. Each reactor was equipped with a temperature sensor that automatically recorded the temperature throughout the process.

2.2.1 Sample collection

Samples were collected on days 0, 4, 7, 11, 16, 22, and 36 of the experiment. On each sampling day, samples were taken from the upper, central, and lower sections of the composting pile and mixed to form a representative sample. The collected representative sample was then divided into three portions. One portion was stored at 4 °C for immediate analysis. The second portion was air-dried, ground, and sieved through a 0.25 mm mesh for physicochemical analysis. The third portion was stored at −20 °C for bacterial community composition analysis. All tests were performed in triplicate, and average results were reported.

2.2.2 Physicochemical parameters

A pH meter (METTLER TOLEDO) and a conductivity meter were used to measure pH and electrical conductivity (EC). The seed germination index (GI) was determined following the standard procedure NY 525–2022, with cabbage seeds incubated at 25 ± 2 °C in the dark for 48 h (Liu et al. 2022b). Total carbon (TC) content was quantified using the potassium dichromate method (Byers et al. 1978). Total nitrogen (TN), total phosphorus (TP), and total potassium (TK) were measured using a flame spectrophotometer, a UV spectrophotometer, and the Kjeldahl method, respectively. Ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) were quantified with an AA3 continuous flow analyzer (Li et al. 2023). Humic substances (HS), fulvic acid (FA), and humic acid (HA) were extracted using the method described by Liu et al. (2022b).

2.2.3 Spectroscopic examination

The morphology of the unmodified and Ca-modified biochars was analyzed using a scanning electron microscope (SEM, Hitachi Regulus 8100). For further characterization, X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha) was employed. Fluorescence intensity was measured using an F-7000 fluorescence spectrophotometer (Aqualog Horiba). Emission (Em) spectra were recorded from 250 to 600 nm in 2 nm increments while varying the excitation (Ex) wavelength between 200 and 500 nm. The spectral response of a blank water sample was subtracted from the spectra of each sample. The fluorescence characteristics of all dissolved organic matter (DOM) excitation-emission matrices (EEMs) were analyzed using parallel factor analysis (PARAFAC) modeling in MATLAB software with the DOM Fluor toolbox, following the methodology described by Xu et al. (2022).

2.2.4 DNA extraction

DNA was extracted from the compost samples utilizing the Soil DNA Sample Isolation Kit (Omega Bio-Tek) to evaluate the microbial community structure throughout the composting process. The sequencing data were processed, subjected to quality control, and separated to get optimal sequences. The optimized sequences were utilized for OTU clustering to generate OTU abundance tables for further study. The V3-V4 region of the bacterial 16S rDNA gene was amplified utilizing forward primer 341 F (ACTCCTACGGGAGGCAGCA) and reverse primer 806R (GGACTACHVGGGTWTCTAAT) (Cai et al. 2022). All bioinformatics data analysis was performed on an Illumina Novaseq 6000/Miseq platform (Guangdong Meige Gene Technology Co., Ltd., China). Illumina sequence data have been deposited in the NCBI database under the accession number PRJNA1235418 for the bacterial 16S rRNA gene.

2.3 Statistical analyses

Data are expressed as the mean ± standard deviation ($n = 3$). Microbial network analysis was performed in R (version 4.4.1) using the WGCNA and igraphic packages, with visualization in Gephi. Correlation heatmaps were also generated in R. All data underwent one-way analysis of variance (ANOVA), and means were compared utilizing Duncan's multiple range test ($p < 0.05$) in SPSS 24.0 (IBM Corp., USA).

3 Results and discussion

3.1 Characterization of biochar

Scanning electron microscopy images revealed that the surface of coconut shell biochar (CB) was relatively smooth and contained numerous micropores. In contrast, the surface of Ca-modified coconut shell biochar (CB1) was rough and uneven (Fig. 1). Energy-dispersive spectroscopy analysis confirmed that Ca was the predominant element in these deposits, constituting 38.54% by mass. These deposits primarily consisted of $CaCO_3$ and CaO, both of which gradually release Ca^{2+} during the composting process. This Ca^{2+} release helps in stabilizing pH by neutralizing organic acids, such as acetic and propionic acid (Zhang et al. 2024b), while also providing ionic bond sites that facilitate the cross-linking of humic

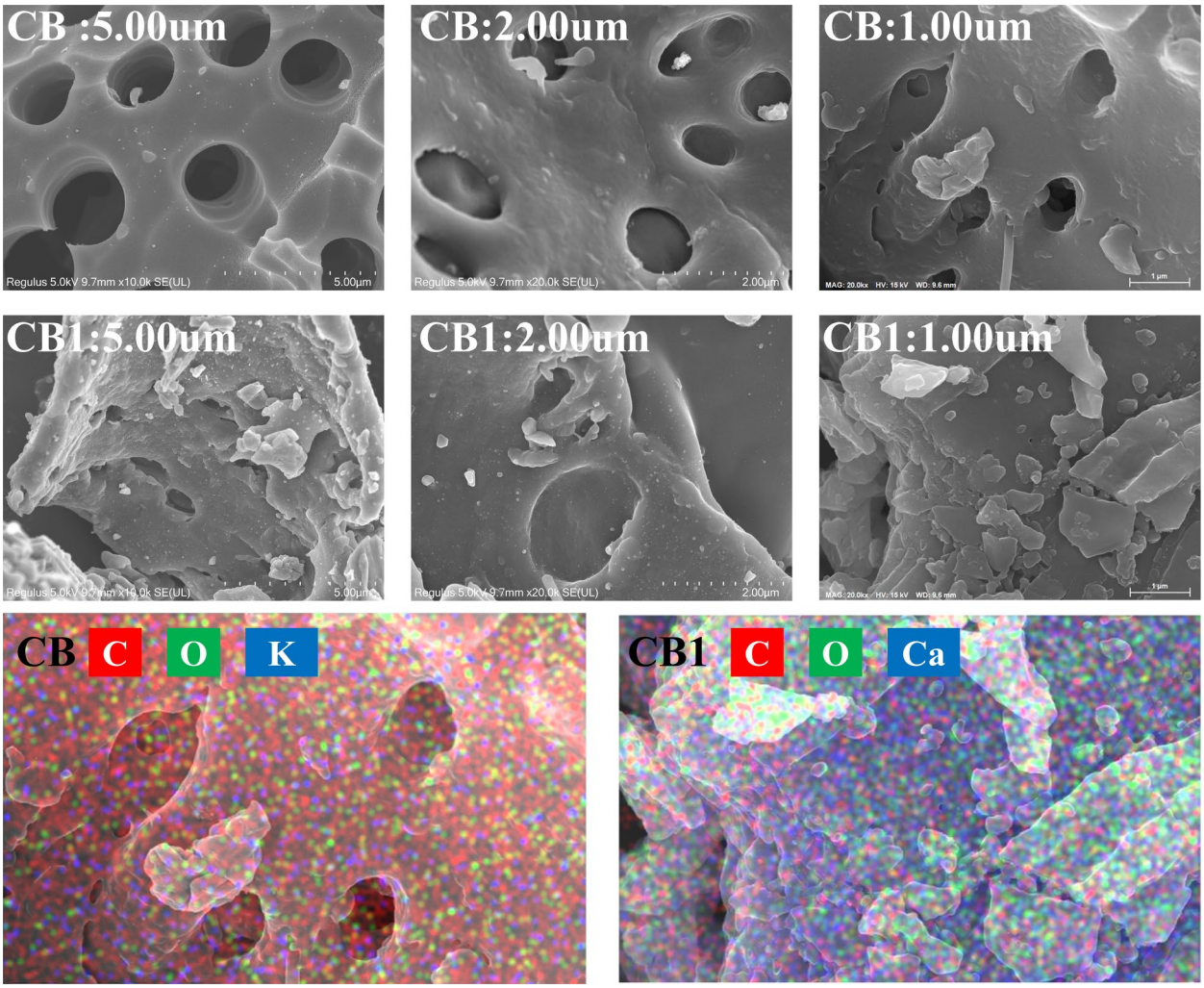


Fig. 1 SEM micrographs of coconut shell biochar (CB) and oyster shell-modified biochar (CB1) at 5.00 µm, 2.00 µm, and 1.00 µm scales

acid molecules (Wu et al. 2022). The elemental composition of biochar plays a crucial role in its structural stability (Egyir et al. 2022). Table S2 illustrates the percentage content of each element in the biochar before and after modification. In CB, the mass percentages of carbon (C), oxygen (O), and potassium (K) were 92.53%, 6.85%, and 0.62%, respectively. In contrast, the CB1 showed a significant shift in elemental composition, with mass percentages of 15.54% C, 45.91% O, and 38.54% Ca.

The surface characteristics of Ca-modified biochar were further examined using XPS analysis of the C 1 s and O 1 s spectra. As shown in Fig. 2, the C 1 s spectrum of CB exhibited a prominent peak at 284.8 eV, corresponding to the aliphatic C–C/C-H structure. In contrast, the C 1 s spectrum of CB1 showed significantly increased intensities at 286.4 eV (C–OH) (Chen et al. 2020) and 289.8 eV (C=O) (Saqib Rashid et al. 2022), indicating a substantial presence of oxygen-containing functional groups. The O 1 s spectra further revealed a notable increase in the relative abundance of O-C=O/-OH (532.4 eV) (Qiu et al. 2020) and O=C–OH (536.2 eV) (Saqib Rashid et al. 2022) in CB1 compared to CB, confirming the enhancement of carboxyl and hydroxyl groups. These polar functional groups can adsorb small organic molecules (e.g., phenols, carboxylic acids) via hydrogen bonding and electron donor–acceptor interactions. Moreover, they act as active sites for catalyzing oxidative condensation reactions (Song et al. 2020), which facilitate the synthesis of HA macromolecules (Zhou et al. 2022).

3.2 Changes in physicochemical parameters

Figure 3 illustrates the changes in temperature, pH, GI, TC, TN, C/N, EC, NH_4^+-N, and NO_3^- during the whole composting period. Initially, all treatment groups followed a four-stage composting temperature pattern: heating phase, thermophilic phase, cooling phase, and

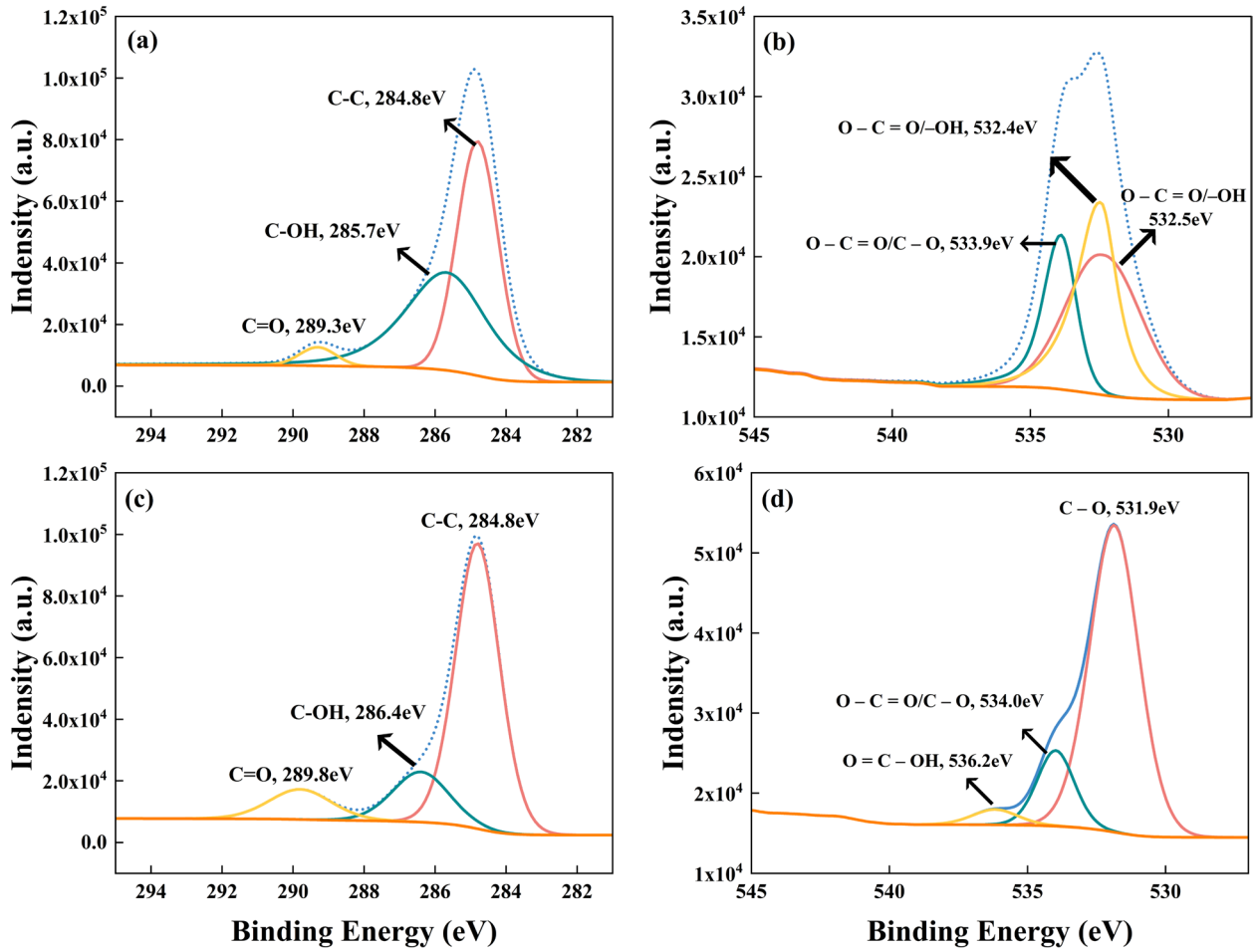


Fig. 2 High-resolution XPS spectra of C 1 s and O 1 s for (**a**, **b**) coconut shell biochar (CB) and (**c**, **d**) oyster shell-modified biochar (CB1)

maturity phase (Fig. 3a). Temperature dynamics across all treatment groups exhibited a similar trend. All treatments reached the thermophilic phase (> 50 °C) by day 2, driven by the rapid decomposition of organic matter, and maintained this phase for more than 10 days, meeting the criteria for high-temperature composting (Gu et al. 2024). A temporary temperature drop was observed from day 4 to day 7, likely due to the physical turning of the pile. The highest temperatures in the three groups were 63.84 °C (Control group), 66.39 °C (Biochar group), and 69.47 °C (Modified biochar group). This indicates that the addition of modified biochar resulted in an increase in temperature during the thermophilic phase (Wang et al. 2021b). This phenomenon aligns with previous studies incorporating modified biochar into composting (Manu et al. 2021).

Changes in pH are closely related to the conversion of organic matter during composting. At the start, the control treatment exhibited a pH of 5.92, which was notably different from the other treatment groups, largely due to the higher alkalinity of biochar (Fig. 3b). Throughout the heating phase, the pH of all treatment groups increased, peaking on day 4. This rise was associated with the proliferation of alkaline bacteria and the activity of thermophilic microorganisms (Xu et al. 2022). By day 36, the pH levels of the control, biochar, and modified biochar treatments stabilized around 7.5, with the modified biochar group slightly higher at 7.74. This increase in pH can be attributed to the enhanced activity of alkaline bacteria and the promotion of denitrification by the modified biochar (Wang et al. 2021b).

The GI is a reliable and widely used indicator of compost toxicity and maturity (Wang et al. 2021a). In this study, the GI values of the modified biochar and biochar groups reached 80% (the phytotoxicity threshold) by day 11, which was 7 days earlier than the control group. Liu et al. (2024) indicated that adding seafood shell powder into compost accelerates the decomposition of harmful chemicals, supporting the results of this study. The GI values for all treatments followed

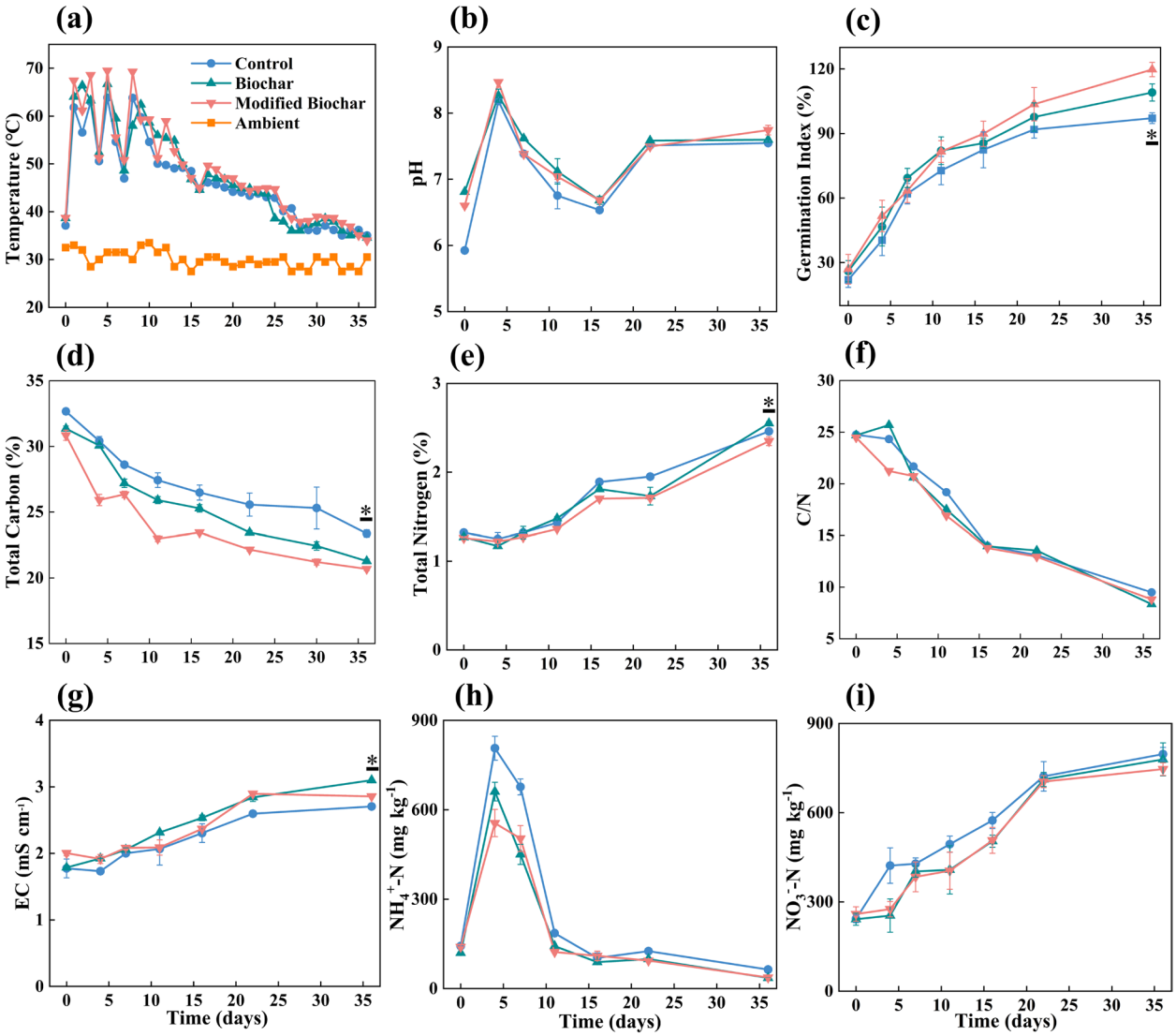


Fig. 3 Dynamic profiles of maturity indicators during the 36-day composting period: (**a**) temperature, (**b**) pH, (**c**) germination index (GI), (**d**) total carbon (TC), (**e**) total nitrogen (TN), (**f**) C/N ratio, (**g**) electrical conductivity (EC), (**h**) NH_4^+-N, and (**i**) NO_3^--N across treatments

a similar trend, increasing significantly during the thermophilic phase (0–11 days) and reaching values between 97.13% and 119.72% by the end of the composting process (Fig. 3c). After composting, the GI values for the modified biochar and biochar groups were 119.72% and 109.04%, respectively, significantly higher than the control group, which reached 97.13% ($p < 0.05$). These results indicate that modified biochar significantly reduces phytotoxicity during composting.

Figure 3d illustrates the temporal variations in TC content across the three treatments. The TC concentration gradually decreased due to the rapid degradation of low-molecular-weight compounds, such as lipids, proteins, and carbohydrates (Chen et al. 2021). Once these easily degradable compounds were depleted, cellulose, hemicellulose, and lignin became the predominant components of the organic matter. These compounds are notably resistant to degradation, which resulted in a slower rate of TC degradation after day 11 (Cai et al. 2018). The TC content of the modified biochar and biochar groups significantly decreased in comparison to the control group ($p < 0.05$) at the end of composting, probably due to the increased microbial activity facilitated by the modified biochar (Meng et al. 2024). The increased microbial activity enhanced the effective breakdown of organic matter, hence leading to higher temperatures in these treatments.

TN content, shown in Fig. 3e, exhibited a temporal pattern distinct from that of TC. TN levels initially decreased during the first three days, then increased steadily. This fluctuation likely reflects the differing decomposition rates of carbon and nitrogen. A similar pattern has been observed in previous studies (Kong et al. 2022). The end concentration of TN was significantly lower in the modified biochar group compared to the other groups ($p < 0.05$). This indicates that the modified biochar improved the mineralization and transformation of nitrogen during composting (Zhu et al. 2019). This happened because there was increased microbial activity and higher temperatures, which made it easier for organic nitrogen to break down and nitrogen to be lost. Additionally, the C/N ratio decreased from 24.48–24.75 to 8.34–9.50, meeting the standard for mature compost (C/N < 15). This decrease aligns with values observed in later stages of composting, confirming that the compost had reached a decomposed state (C/N < 15) (Aydın Temel 2023).

The EC value is an important indicator of compost salinity, influencing plant growth and seed germination (Fig. 3g). Initially, the EC value decreased due to the volatilization, utilization, or co-precipitation of NH_4^+, volatile fatty acids, NO_3^-, and nitrites with mineral ions (Cui et al. 2021). As gas volatilization slowed down, significant quantities of water-soluble salt ions, such as phosphate, sulfate, NH_4^+, sodium, and potassium, accumulated during the intense degradation of organic matter (Cui et al. 2021). This process caused a gradual increase in the EC value (Zhang et al. 2017). Eventually, the EC value stabilized. After the composting process, the EC values for the control, biochar, and modified biochar groups were 2.71, 3.10, and 2.86 mS cm^{-1}, respectively. The Biochar group exhibited a slightly higher EC value ($p < 0.05$), likely due to the leaching of soluble salts from the biochar. However, all treatment groups remained below the 4 mS cm^{-1} agricultural safety threshold, indicating that the salinity levels of the compost products were within acceptable limits (Xu et al. 2021).

The metabolic characteristics of the composting system were further analyzed through nitrogen transformation processes (Fig. 3h, i). The rapid increase in NH_4^+-N concentrations during the first phase of composting (0–7 days) was due to the intense mineralization of nitrogen-rich organic matter, such as proteins. As temperatures exceeded 50 °C, nitrifying bacterial activity was suppressed, leading to a temporary decline in NH_4^+-N accumulation (Ren et al. 2021). Figure 3h shows that all treatments initially exhibited a rise in NH_4^+-N content, which was followed by a gradual decrease over the composting period. This early increase is likely linked to the degradation of proteinaceous materials. However, the rapid temperature rise during the initial composting phase inhibited the growth of nitrifying bacteria, resulting in a significant drop in NH_4^+-N levels due to the reduction of organic nitrogen and the substantial production of NH_3 during the high-temperature phase (Yang et al. 2019). This was further confirmed by the observed increase in NO_3^--N concentration (Fig. 3i). During the first composting phase, NO_3^--N concentrations remained low and variable, but they gradually increased over time. This trend can be attributed to the elevated temperatures, which hindered the survival of nitrifying bacteria, the rapid depletion of NH_4^+-N during NO_3^--N synthesis, and the increase in pH, all of which affected the nitrification process and inhibited NH_4^+-N accumulation (Yang et al. 2019).

3.3 Humic acid and fulvic acid

HA, as the primary component of HS, serves as a direct indicator of compost breakdown and stability (Wang et al. 2022). Figure 4a shows a significant increase in HA content across all treatment groups during the initial composting phase (0–14 days). In the control group, HA content rose from 18.30 mg kg^{-1} to 73.72 mg kg^{-1}. The modified biochar treatment group reached a peak value of 80.63 mg kg^{-1}, surpassing the control group by 6.91%. This significant increase in HA content indicates fast humification, a key process in composting. Previous studies have shown that the accumulation of HA is closely linked to an increase in molecular weight and aromatic condensation (Kong et al. 2022), indicating that oxidative coupling processes progressively transform smaller molecular precursors in the waste into more complex aromatic structures (Li et al. 2024a). This transition is a crucial sign of the humification process (Zhang et al. 2019). In our study, adding modified biochar significantly improved this process. This is likely because it adsorbs reactive intermediates and provides a surface for electron transfer, which speeds up abiotic condensation reactions and makes HA synthetically proceed faster (Wu et al. 2020).

Regarding FA content, Fig. 4b shows the changes that reflect the chemical reconfiguration along the humification pathway. Composting reduced FA content from 57.93 mg kg^{-1} to 33.68 mg kg^{-1} in the control group, indicating a shift from aliphatic to aromatic components (Amir et al. 2010). After composting, the FA concentrations in the control, biochar, and modified biochar groups were 33.68, 31.10, and 32.03 mg kg^{-1}, respectively. The decrease in FA shows that the additives helped HA form, which confirms the observation that humification went more quickly when biochar was present (Gao et al. 2024). The modified biochar group exhibited a higher HA/FA ratio of 2.52, compared to 2.43 in the biochar

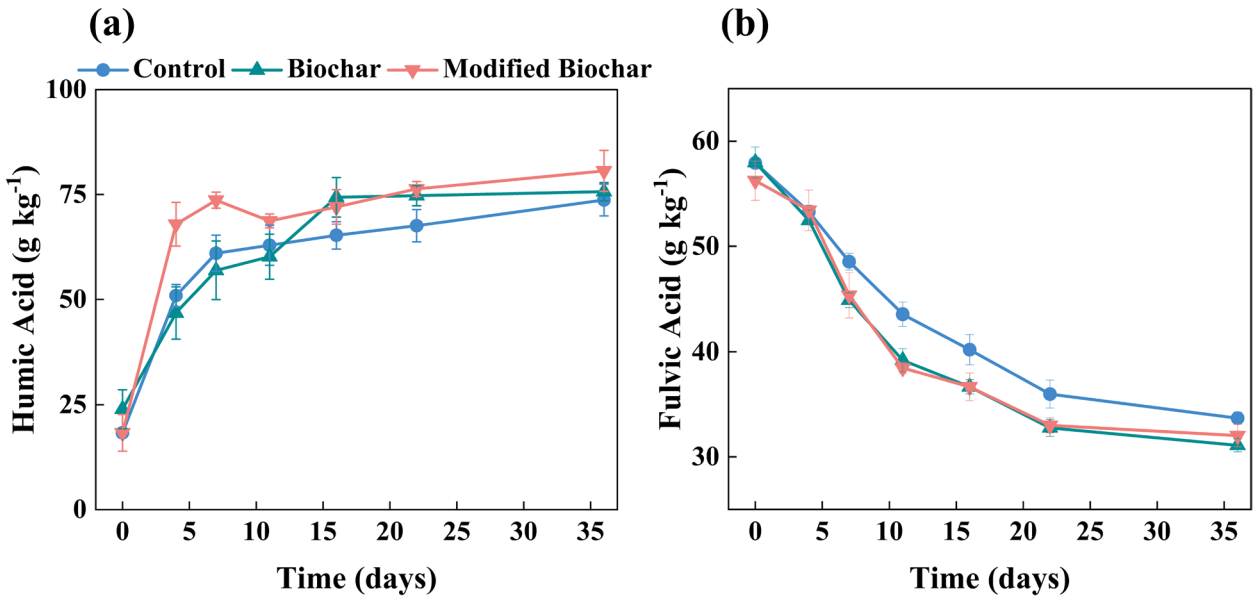


Fig. 4 Temporal evolution of humic substance fraction dynamics: (**a**) humic acid (HA) and (**b**) fulvic acid (FA) concentrations in different treatments

group. This shows that adding modified biochar had a more significant effect on the humification process, most likely because of the effect of Ca^{2+}. Previous studies have demonstrated that Ca^{2+} enhances the stability of HA polymerization by promoting ionic bonds between carboxyl groups in HA (Wang et al. 2015) and phenolic hydroxyl groups (Miao et al. 2018), thereby reducing the depletion of small-molecule precursors like FA (Xie et al. 2019).

3.4 Evolution of a fluorescent substance

Excitation-emission matrix fluorescence coupled with parallel factor analysis (EEM-PARAFAC) is a highly sensitive technique for characterizing dynamic changes in dissolved organic matter (Xu et al. 2022). Figure 5 illustrates the identification of three fluorescent components in the compost samples. C1 (342/418 nm) is attributed to humic acid-like compounds, exhibiting characteristic fluorescence peaks associated with aromatic ring condensation structures (Yang et al. 2024). C2 (370/420, 481 nm) represents a humic-like component, indicating medium molecular weight humic intermediates (Ishii and Boyer 2012). C3 (284, 352/472 nm) indicates proteins, primarily reflecting the fluorescence properties of amino acids and short peptides (Yamashita and Jaffé 2008).

The maximum fluorescence (Fmax) represents the percentage of maximum fluorescence relative to the total fluorescence, providing insight into the presence of organic components (Zhang et al. 2016). As shown in Fig. 5, the Fmax value of C1 consistently increased throughout the composting process, reaching 65.58% in the modified biochar treatment group during the degradation phase. This value was significantly higher than those of the control group (60.66%) and the biochar group (61.25%) ($p < 0.05$). Similar results were observed by Zhang et al. (2016), suggesting that modified biochar accelerated the specific production of humic acid-like compounds by promoting phenolic condensation and quinone group formation. The Fmax value of C2 demonstrated a declining trend. Xu et al. (2022) observed similar results, indicating the gradual conversion of humic-like intermediates into stable humic acid. Meanwhile, the Fmax value of C3 steadily declined in both the modified and biochar treatments, reaching its lowest point in the modified biochar group (15.02%). This represented a reduction of 5.6% and 2.84% compared to the control group (20.62%) and the biochar group (17.86%), respectively ($p < 0.05$). This finding further supports the idea that modified biochar enhances the degradation of protein-like small molecules, which contribute to humic acid synthesis through the mechanism of calcium ions with alkaline properties (Zhang et al. 2021). Modified biochar is proposed to accelerate the initial degradation of proteins and carbohydrates, increasing the pool of precursors (Zhou et al. 2022). Subsequent abiotic condensation of phenolic compounds into quinone structures, crucial for humic acid stability is likely enhanced (Hardie et al. 2009). Additionally, Ca^{2+} may promote the aggregation and stabilization of organic molecules, further facilitating HA formation (Xie et al. 2019).

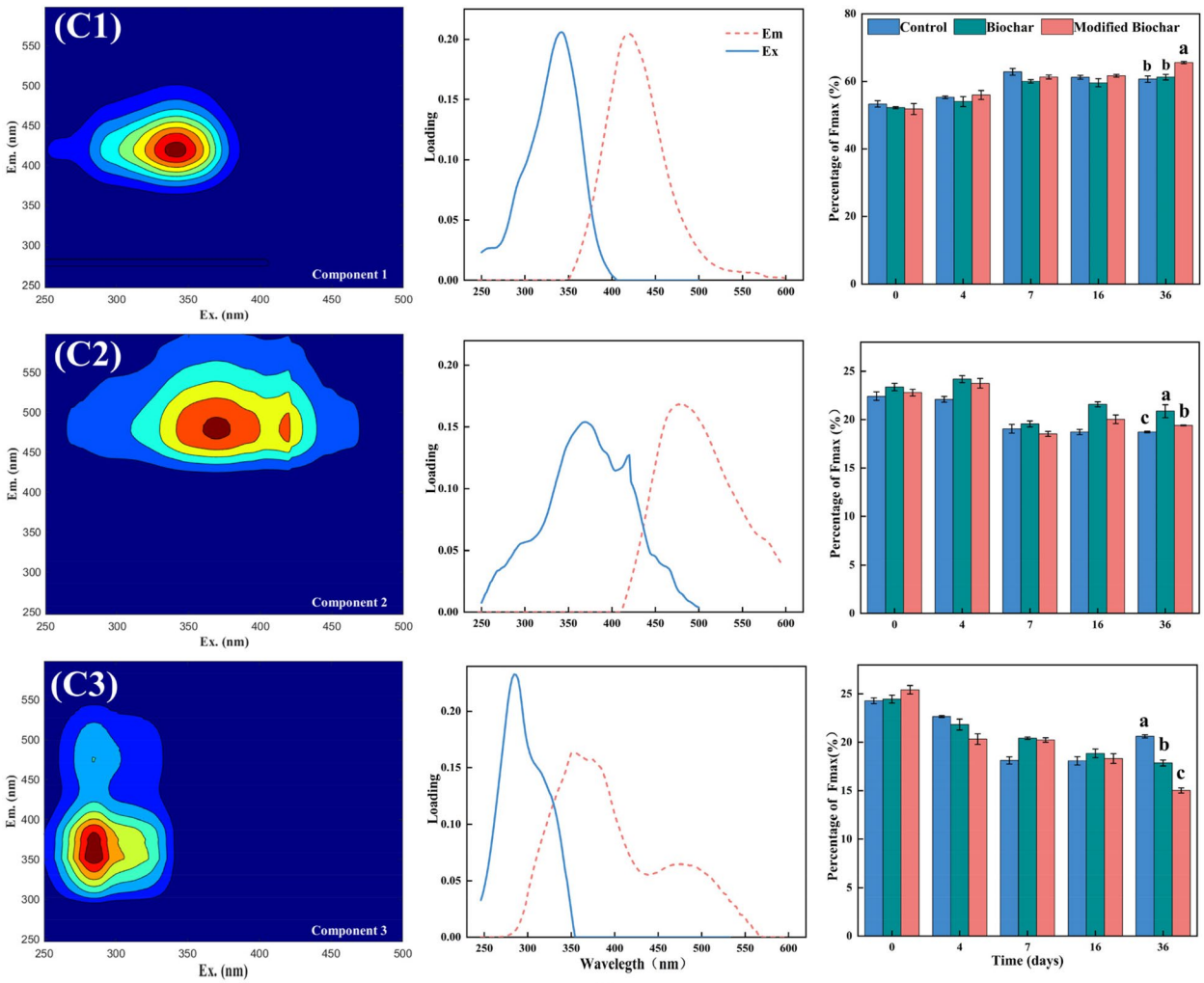


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Fig. 5 PARAFAC-derived fluorescent DOM components identified by EEM spectroscopy: Spectral signatures (left) and relative abundance (Fmax%, right) of C1 (humic-like), C2 (terrestrial humic-like), and C3 (protein-like) substances at 0, 4, 7, 16, and 36 days. The contour plots (left) illustrate the distinctive excitation-emission loadings of the three PARAFAC-derived components identified from the comprehensive set of all samples

3.5 Microbial community structure

Alpha diversity analyses (Fig. 6a, b) showed that the Richness indices and CHAO indices of both biochar and modified biochar treatment groups were significantly higher compared to the control group ($p < 0.05$). The CHAO (Liu et al. 2022a) and Richness (Chao et al. 2022) indices describe the richness and diversity of microbial communities, respectively, with the larger values indicating greater richness and diversity. This suggests that the incorporation of biochar substantially enhances microbial community richness and diversity (Li et al. 2024a). To further explore these differences, principal coordinate analysis (PCoA) was used to examine the compost samples. Figure 6c highlights a clear separation between the control and other treatment groups, indicating that biochar addition led to a notable shift in compost community composition. The control samples were dispersed, suggesting a less stable community structure, while the modified biochar groups exhibited tightly clustered sample points with a positive displacement along the PC1 axis. This suggests that biochar additions facilitated microbial community reorganization by enhancing the microbial habitat during composting (Zhou et al. 2023). The increase in diversity is likely attributed to the abundant oxygenated functional groups (C–OH, C = O) on the biochar surface, which serve as electron shuttles, thereby stimulating microbial metabolic networks (Zhang et al. 2022b).

The phylum and genus levels of bacterial community were detected by taxonomic analysis during composting (Fig. 6e, f). The dominant phyla were similar under

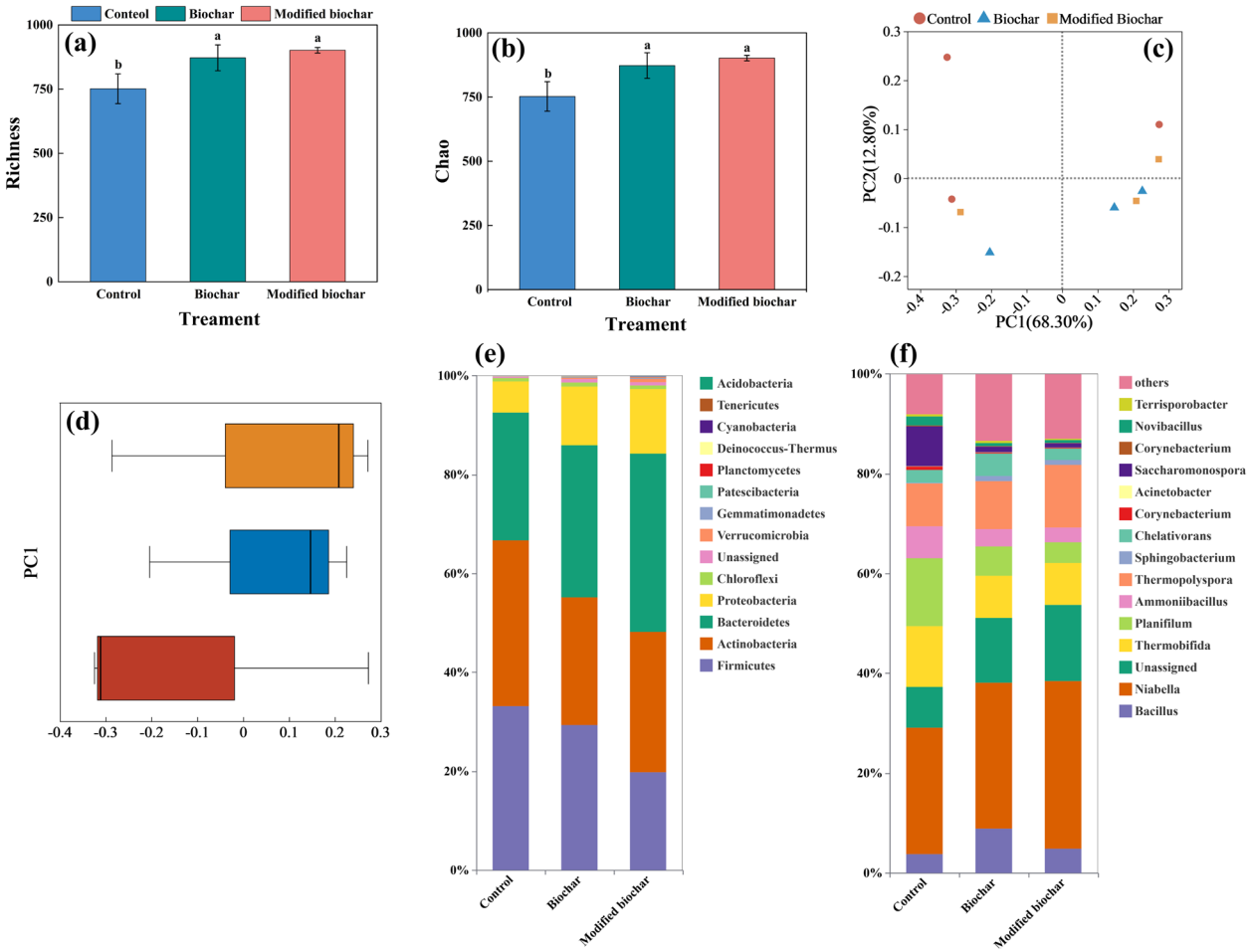

Fig. 6 Microbial community analysis: (**a**, **b**) *α*-diversity indices (Richness and Chao1); (**c**, **d**) *β*-diversity visualized by PCoA (PC1: 68.30%, PC2: 12.80%); (**e**) phylum-level and (**f**) genus-level taxonomic composition across treatments

the three treatments, mainly including *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*. These phyla are well-known for their significant contributions to organic biodegradation in compost (Wang et al. 2023b). *Proteobacteria* dominated during the composting, with a 107% higher prevalence compared to the control group. Research suggests that *Proteobacteria* are crucial to the breakdown of lignocellulose and nitrogen transformation (Ren et al. 2021). Additionally, *Bacteroidetes* increased by 39.50% relative to the control group, correlating with enhanced lignocellulose degradation and nitrogen transformation. The research discovered that *Bacteroidetes* decomposes macromolecules of organic matter to make humification precursors (Ye et al. 2021). These precursors led to more HA formation (80.63 mg kg^{-1}) and faster compost maturity (GI 119.72%).

Figure 6f shows the bacterial community composition at the genus level. In the modified biochar group, the dominant genera were *Niabella*, *Unassigned*, and *Thermopolyspora*, which together accounted for over 60% of the total bacterial population. Previous studies have shown that *Niabella* is crucial in decomposing nitrogenous organic waste by secreting proteases (Kim et al. 2007), which release NH_4^+-N and provide amino precursors for HA formation (Wang et al. 2009). Thai et al. (2024) reported that *Niabella* contains 166 genes, with approximately 8.9% involved in glucose metabolism. Meanwhile, *Thermopolyspora* produces lignin peroxidase and cellulase, enzymes that degrade the tough lignin structure, releasing aromatic monomers. These monomers are then used to form the HA macromolecular backbone via oxidative coupling (Zhang et al. 2014). The findings indicate that the complementary functions of *Niabella* and *Thermopolyspora* in C and N metabolism established a synergistic network. Consequently, there is a notable improvement in humification efficiency, evidenced by the HA/FA ratio of 2.52 in the modified

biochar group. Furthermore, the porous structure of biochar facilitated the colonization of *Niabella*, while its alkaline surface boosted the thermotolerant enzyme activity of *Thermopolyspora*, further strengthening the metabolic benefits of the microbial community (He et al. 2024). This analysis demonstrates that modified biochar accelerates compost humification by enriching specific functional microorganisms.

3.6 Analysis of the composting bacterial community network

The microbial community interaction networks provide insights into the effects of additives on microbial interactions (Fig. 7). This network analysis focuses on microorganisms with a relative abundance greater than 0.01 and a significant correlation. The networks for the control, biochar, and modified biochar treatment groups consisted of 22, 28, and 27 nodes, with 72, 141, and 181 edges, respectively. Notably, the modified biochar treatment group exhibited a significantly higher number of edges (181) and a greater average node degree (13.41) compared to the other groups. This suggests a denser microbial community with more frequent interactions in the modified biochar group (Chen et al. 2022). Analyzing the bacterial phyla, the microbial networks across all groups were primarily dominated by *Firmicutes*, *Actinobacteria*, and *Proteobacteria*. The modified biochar group showed the highest proportions of *Actinobacteria* and *Proteobacteria*, each at 25.93%, significantly higher than the control group, which recorded 22.73% and 9.09%, respectively. Previous studies have indicated that the abundance of *Actinobacteria* in compost correlates directly with the efficiency of cellulose and hemicellulose degradation (Zhao et al. 2016). *Actinobacteria* are essential for lignocellulose degradation, while the proliferation of *Proteobacteria* enhances C and N interactions, as evidenced by the transition from NH_4^+-N to NO_3^--N.

The intricate network in the modified biochar treatment group likely accelerates the humification process through a combination of metabolic complementarity and functional overlap. Bacteria such as *Thermopolyspora* and *Bacillus*, which break down cellulase and lignin, make the system more flexible when the temperature and pH change. This helps the breakdown of organic matter and production of HA to continue (Banerjee et al. 2016). Additionally, a strong positive correlation ($r > 0.7$) exists between the key bacterial genera *Thermopolyspora* (*Actinobacteria*) and *Niabella* (*Bacteroidetes*). This synergy facilitates HA formation through metabolic complementarity: *Thermopolyspora* degrades lignin to release aromatic monomers (Song et al. 2023b), while *Niabella* contributes amino precursors (Chen et al. 2024). The porous structure of biochar provides attachment sites for bacteria, supporting the colonization of these functional microorganisms. The addition of calcium (38.54%) likely enhances lignin peroxidase activity (He et al. 2024), further boosting interactions among these key microbes (Zhang et al. 2024a). The enhanced humification rate

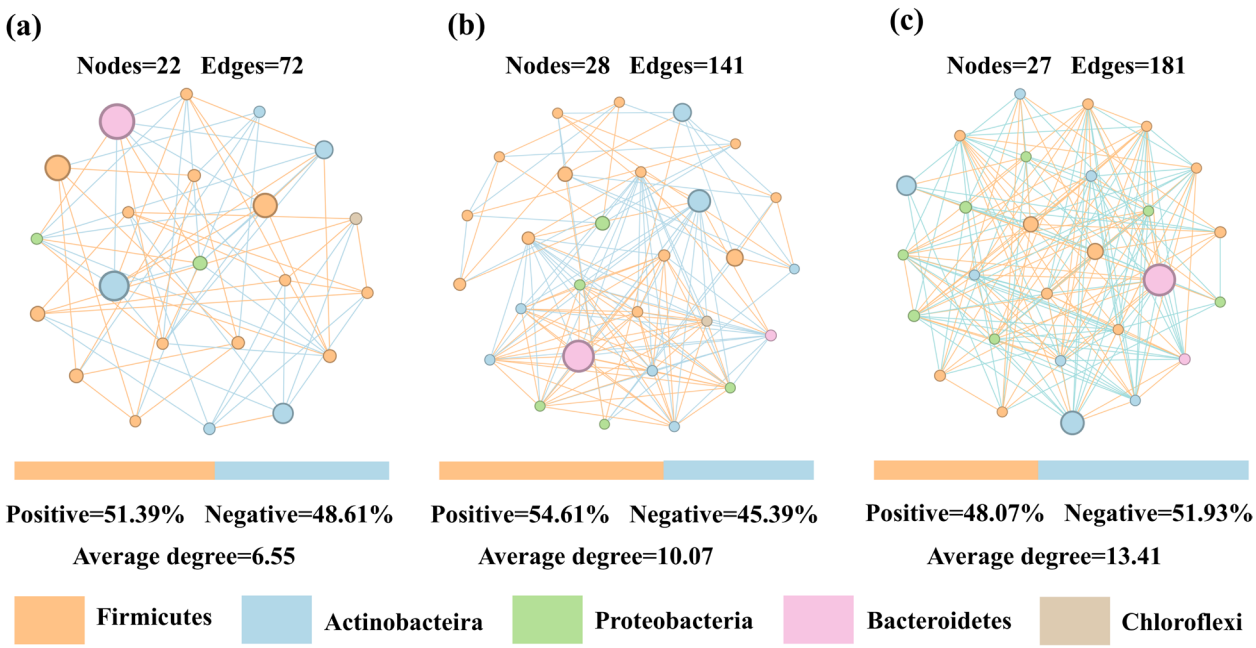

Fig. 7 Genus-level bacterial co-occurrence networks in (**a**) Control, (**b**) Biochar, and (**c**) Modified Biochar treatments, showing nodes/edges counts and positive/negative correlation edges

observed in the modified biochar treatment group (HA = 80.63 mg kg^{-1}, GI = 119.72%) aligns with this synergistic process, underscoring the importance of these microbial interactions in accelerating compost maturation. Similar results were observed by Yin et al. (2024), indicating that biochar altered the interaction patterns between the bacterial community and the humification process.

3.7 Correlations between bacterial communities and composting properties

Correlation heatmaps were used to assess the relationship between the microbial community and environmental factors in the modified biochar, as shown in Fig. 8. The analysis revealed a significant positive correlation between *Proteobacteria* and GI ($p < 0.05$). *Proteobacteria* were the dominant microbes in the modified biochar composting process, with their relative abundance 107% higher than the control group and 9.05% greater than the biochar group. This indicates that *Proteobacteria* are crucial in facilitating the humification of compost in our study. Previous research has highlighted that *Proteobacteria* contribute to humification by breaking down lignin (Ye et al. 2021) and influencing nitrogen dynamics (Ye et al. 2021). These findings are consistent with observed patterns in organic matter degradation and humus formation during modified biochar composting.

The study also found significant positive correlations between *Verrucomicrobia*, *Planctomycetes*, and *Cyanobacteria* with both pH ($p < 0.01$) and GI ($p < 0.01$). The modified biochar treatment group showed significantly higher abundances of these bacterial taxa compared to the control and biochar groups (*Verrucomicrobia*: 0.67%, *Planctomycetes*: 0.09%, and *Cyanobacteria*: 0.11%) ($p < 0.05$). Thus, our data indicate their enrichment following modified biochar treatment, with functional hypotheses primarily corroborated by recent research. Previous studies have shown that *Verrucomicrobia* contribute to HS synthesis by breaking down cellulose and hemicellulose into reducing sugars (Trivedi et al. 2016). *Planctomycetes*, including anaerobic ammonia-oxidizing bacteria, help in retaining nitrogen in the soil by

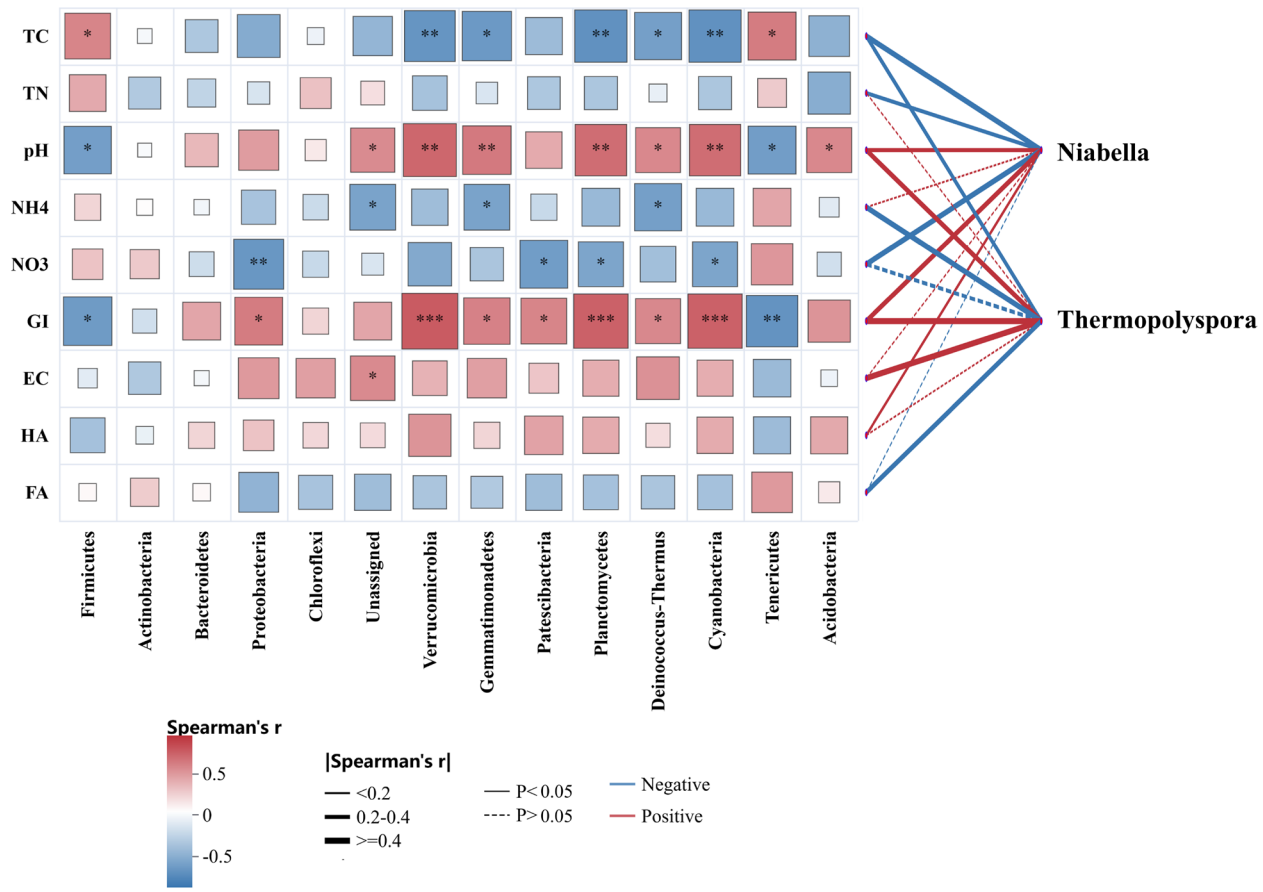

Fig. 8 Spearman correlations between dominant bacterial phyla/genera and environmental factors (*$p < 0.05$). Dashed lines: non-significant associations

preventing NH_3 loss, which promotes HA condensation (Kartal et al. 2011). *Cyanobacteria* break down proteins to generate amino acid precursors through heterotrophic metabolism in the absence of light (Xu et al. 2022) and also fix nitrogen, improving compost's nitrogen use efficiency (Zhang et al. 2022b). These results suggest that modified biochar enhances these functional microbial phyla by modifying the compost's acid–base conditions and introducing key functional components. This creates a multi-level functional network that boosts C and N metabolism, improves precursor availability, and accelerates humification.

At the genus level, *Niabella* (*Bacteroidetes*) and *Thermopolyspora* (*Actinobacteria*) were notably enriched in the modified biochar treatment group compared to the other controls. Both genera showed significant positive correlations with GI, pH, and HA content ($p < 0.05$) and significant negative correlations with TC ($p < 0.05$). Previous studies have shown that *Niabella* promotes HA condensation by releasing amino compounds (Chen et al. 2024), while *Thermopolyspora* facilitates the formation of humic precursors by degrading lignin into aromatic monomers such as vanillin (Song et al. 2023b). The modified biochar treatment group is more efficient in enhancing humification due to its greater abundance of functionally suitable microbial phyla and genera, as evidenced by the examination of microbial-environmental interactions. However, this study only used high-throughput sequencing to reveal the difference in the bacterial community. Future study should use more modern molecular biology techniques, such as quantitative PCR and metagenomic sequencing, to further systematically reveal the formation mechanism from the aspect of functional genes, enzymes and microbial community involved in humification production.

This study demonstrates that Ca-modified biochar acts as a multifunctional amendment to enhance humification efficiency in composting through microbially driven mechanisms. By incorporating Ca loading and regulating alkaline properties, Ca-modified biochar improves HA content by 6.1% and increases the GI by 8.9% compared to unmodified biochar. It also significantly enhances the presence of carboxyl and carbonyl functional groups, while positively influencing the microbial ecology. This treatment promotes C and N metabolism and supports small molecule precursor production by enhancing the functional genera *Thermopolyspora* and *Niabella*, while also fostering collaboration with auxotrophic groups such as Verrucomicrobia, Planctomycetes, and Cyanobacteria. The findings provide both theoretical support and practical solutions for improving composting efficiency through the use of modified biochar and microbial synergy.

4 Conclusion

This study demonstrates that Ca-modified biochar acts as a multifunctional amendment to enhance humification efficiency in composting through microbially driven mechanisms. By incorporating Ca loading and regulating alkaline properties, Ca-modified biochar improves HA content by 6.1% and increases the GI by 8.9% compared to unmodified biochar. It also significantly enhances the presence of carboxyl and carbonyl functional groups, while positively influencing the microbial ecology. This treatment promotes C and N metabolism and supports small molecule precursor production by enhancing the functional genera *Thermopolyspora* and *Niabella*, while also fostering collaboration with auxotrophic groups such as *Verrucomicrobia*, *Planctomycetes*, and *Cyanobacteria*. The findings provide both theoretical support and practical solutions for improving composting efficiency through the use of modified biochar and microbial synergy.

Supplementary Information

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Supplementary Material 1.

Authors' contributions

All authors contributed to the study conception and design. Jinfeng He and Li Li contributed equally to this work. Software, Investigation, Writing—original draft, and Writing—review & editing were performed by Jinfeng He. Formal analysis was performed by Li Li. Writing—review & editing and Software were performed by Yulin Shi, Keke Wang, Jiayu He, Yunze Ruan, Huanyu Bao, Muhammad Usman Khan, and De-qiang Li. Conceptualization, Methodology, Resources, Writing—review & editing, Project administration, and Funding acquisition were performed by Shanshuai Chen and Pingshan Fan. The first draft of the manuscript was written by Jinfeng He, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

Raw data have been deposited in the National Center for Biotechnology Information (NCBI) under the BioProject number: PRJNA1235418. (http://www.ncbi.nlm.nih).

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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